QTRACE and DigitalTRACE Analysis System Operator's Manual

Copyright © 2024 by JETA Molecular BV. All Rights Reserved.

Table of contents

Welcome	
Product Use Limitations	. 3
Limited License Agreement	. 4
Disclaimer	
Copyright and Trademarks	
Introduction	
QTRACE and DigitalTRACE System Workflow	. 9
Materials	
Key to Symbols	11
Materials Provided	12
Materials Sold Separately	16
DNA Sample Requirements	
Warnings	
Shipping Conditions	20
Storage and Handling Requirements	
Technical Support	
Genotyping Test	
Protocol	25
Genotyping Data Analysis and Report	37
Multiple Donor Analysis Using QTRACE Plates	
Custom Genotyping Panels	
Virtual Typing	50
Monitoring Test	
Assigning Informative Markers	
Protocol	
Monitoring Data Analysis and Report	61
Assay Filtering from Monitoring Reports	
External Reference Functions	
Simultaneous Genotyping and Monitoring	68
Software Overview	
Preferences	70
Software Access And User Profile Management	78
File Management	80
Data Exports - csv and xlsx	82
HPRIM Data Export	
Software Buttons	
Drag and Drop Plate Layouts	
Anonymized Reporting	91
Modification of Existing Data	
Data Analysis Algorithms	
Bibliography	
Glossary	
End User Software License Agreement	103

Welcome

Welcome to the QTRACE[®] and DigitalTRACE[™] Analysis System Operator's Manual and Help System. This file serves as both the QTRACE[®] System Operator's Manual (PN 3 31047, JETA Molecular), the DigitalTRACE[™] Operator's Manual (PN 331307) and the help system found within the TRACE Analysis[™] Software package.

Navigate to your subject of interest and find the solution to your question. If your topic of interest is not listed or you would like to receive additional information, do not hesitate to contact us. The content of this help system is regularly updated. We encourage you to inform us on inaccuracies or suggestions. We do our utmost to implement your suggestions swiftly, such that you and other QTRACE[®] and DigitalTRACE[™] System users may benefit from it.



Product Use Limitations

This version of the TRACE Analysis[™] Software is for Research Use Only. It is not intended for use in diagnostic procedures.

No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the QTRACE[®] and DigitalTRACE[™] Analysis System kits or components with the following terms:

The QTRACE[®] and DigitalTRACE[™] Kits may be used solely in accordance with the QTRACE[®] and DigitalTRACE[™] Analysis System Operator's Manual and for use with components contained in the kits only. JETA Molecular grants no license under any of its intellectual property to use or incorporate the enclosed components of these kits with any components not included within these kits except as described in the QTRACE[®] and DigitalTRACE[™] Analysis System Operator's Manual and additional protocols available at www.jetamolecular.com.

Other than expressly stated licenses, JETA Molecular makes no warranty that these kits and/or their use(s) do not infringe the rights of third-parties.

The QTRACE[®] and DigitalTRACE[™] Kits and their components are licensed for one-time use and may not be re-used, re-furbished, re-sold or reverse engineered.

JETA Molecular specifically disclaims any other licenses, expressed or implied other than those expressly stated.

The purchaser and user of the QTRACE[®] and DigitalTRACE[™] Kits agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. JETA Molecular may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

Disclaimer

JETA Molecular makes every effort to ensure that this manual is accurate. JETA Molecular disclaims liability for any inaccuracies or omissions that may have occurred. Information in this manual is subject to change without notice.

JETA Molecular assumes no responsibility for any inaccuracies that may be contained in this manual.

JETA Molecular reserves the right to make improvements to this manual and/or to the products described in this manual, at any time without notice.

If you find information in this manual that is incorrect, misleading, or incomplete, we would appreciate your comments and suggestions. Please send them to info@jetabv.com.

Copyright and Trademarks

This publication, including all photographs, illustrations, is protected under international copyright laws, with all rights reserved. Neither this manual, nor any of the material contained herein, may be reproduced without written consent of JETA Molecular.

JETA Molecular and its logo design are registered trademarks of JETA Molecular BV in the U.S. and/or certain other countries.

QTRACE[®] and DigitalTRACE[™] are registered trademarks of ElsworthMolecular Holding BV in the U.S. and/or certain other countries. QTRACE[®] Products and Software are licensed exclusively to JETA Molecular BV from ElsworthMolecular Holding BV.

MicroAmp[®] is a registered trademark of Thermo Fisher.

Moq Copyright © 2007. Clarius Consulting, Manas Technology Solutions, InSTEDD http://www.moqthis.com/ All rights reserved.

NUnit

Portions Copyright © 2002-2013 Charlie Poole or Copyright © 2002-2004 James W. Newkirk, Michael C. Two, Alexei A. Vorontsov or Copyright © 2000-2002 Philip A. Craig

PDFsharp Copyright © 2005-2007 empira Software GmbH, Cologne (Germany)

SharpZipLib

WPF Toolkit

All other trademarks, copyrights, patents, service marks, logos and trade names are the sole property of their respective owners.

Introduction

Background Information

The QTRACE[®], DigitalTRACE[™] and MultiTRACE[™] Genotyping Plates, QTRACE[®] and DigitalTRACE[™] Monitoring Assays and the TRACE Analysis[™] Software meet the needs of any research application that requires highly sensitive detection and quantification of the genome of one individual in the background of another individual or individuals. A genetic chimera is an organism with two or more genetically distinct cell populations, *i.e.*, cell populations with different genomes.

Chimerism can arise in humans through a variety of means, such as inheritance, maternal-fetal stem cell trafficking during gestation, blood vessel sharing in fraternal twin gestation, blood transfusions, bone marrow transplantation, cord blood transplantation, and solid organ transplantation. The presence of two distinct human genomes in a sample can also occur simply through the mixing of human cells from more than one individual, for example, when two cell lines are cross-contaminated, or in forensic tissue samples.

Product Overview

The QTRACE[®] and the DigitalTRACE[™] Analysis System consist of DNA genotyping plates, individual quantification assays, one reference assay, and the TRACE Analysis[™] Software.

The QTRACE[®] INDEL Assays are a set of 80 genetic markers that are able to differentiate, and then quantify, the contributors to a human-mixed DNA sample using quantitative polymerase chain reaction (qPCR). Each of the 80 assays is designed to a distinct bi-allelic insertion/deletion (INDEL) or copy number polymorphisms in the human genome. The QTRACE[®] INDEL assays are based upon self-quenched, hydrolysis probe chemistry. In a qPCR, a dye labeled oligonucleotide probe enables the detection of a specific PCR product as it accumulates during PCR cycling. qPCR has been shown in various studies to quantify target DNA samples over an 11-log dynamic range in optimized conditions (100 billion-fold differences in starting copy number; Nolan, *et al*, 2006). Comparison of cycle thresholds (CTs) from different samples can be used to determine the relative amounts of DNA in two different samples (Livak and Schmittgen, 2001). The high sensitivity is the result of the very large dynamic range of the real-time amplification method and is limited essentially by the input copy number of total genomic DNA that can be added to the PCR reaction. The RNase P assay serves as reference assay for the quantification.

The DigitalTRACE[™] INDEL assays are a set of 70 digital polymerase chain reaction (dPCR) assays based upon self-quenched, hydrolysis probe chemistry. Each of the assays is designed to a distinct bi-allelic insertion/deletion (INDEL) or copy number polymorphisms in the human genome. In a dPCR reaction, a dye-labeled oligonucleotide probe enables the detection of a specific PCR product at the end PCR cycling. The high sensitivity is the result of the very large dynamic range of the real-time amplification method and is limited essentially by the input copy number of total DNA that can be added to the dPCR reaction. In the Monitoring test, the assays are formulated with the target in channel FAM and the reference assay (RNase P) in channel HEX.

The QTRACE[®] and DigitalTRACE[™] HLA Assays are qPCR or dPCR assays based upon selfquenched, hydrolysis probe chemistry. These assays are designed to detect the loss of HLA heterozygosity after haploidentical HSCT. The TRACE Analysis[™] Software was designed specifically for the QTRACE[®] and DigitalTRACE[™] INDEL Assay Set. The software provides a streamlined workflow for both the genotyping and quantification tests. The software guides the user through assay setup, performs data analysis, generates results reports and stores the data collected for samples over time.

The procedure for determining the level of a genome of interest in a sample consists of two parts: a genotyping test and a quantification test.

Genotyping Test

In the initial genotyping test, the DNAs that comprise a mixed DNA sample are analyzed using a QTRACE[®], DigitalTRACE[™] or MultiTRACE[™] Genotyping Plate, to identify all of the informative assays for the samples. An informative assay is an assay for a marker allele that is present (positive) in one individual genome *and* absent (negative) in the other genome.

The QTRACE[®] Genotyping plate contains a duplicate set of assays: the 46 quantification assays and the reference (RNaseP) assay that serves as both a positive control and a No Template Control (NTC).

The QTRACE[®] Extended Panel Genotyping Plate, containing additional 34 markers, is available for rare cases where more markers may be needed, including patients receiving a second transplant or highly similar siblings.

The DigitalTRACE[™] Genotyping Plate contains a set of 43 quantification assays and the reference (RNase P) assay that serves as both a positive control and a No Template Control (NTC).

The DigitalTRACE[™] EP QIAcuity Genotyping Plate represents an extended panel of dPCR markers that can be used for dPCR monitoring in the case of need for additional markers allowing to distinguish between donor and recipient DNA. This plate contains a set of 27 quantification assays and the reference (RNase P) assay that serves as both a positive control and a No Template Control (NTC).

The MultiTRACE[™] Genotyping Plate contains a set of 45 quantification assays and the reference (RNase P) assay that serves as both a positive control and a No Template Control (NTC).

Monitoring Test

In the monitoring (quantification) test, two or more of the informative assays identified in the genotyping test is used to quantify the DNA of interest in an unknown sample relative to a reference sample (calibrator). Any of the informative assays identified in the genotyping test can be used to perform a quantification test. The amount of the genome positive for the informative allele in the unknown sample is determined relative to the amount of that same genome in the reference sample, and the result is expressed as a percentage (ratio). For example, a result of 5% indicates that there is 5% of genome A in the unknown sample relative to the reference sample. The simplest case assumes that the reference sample has 100% of genome A.

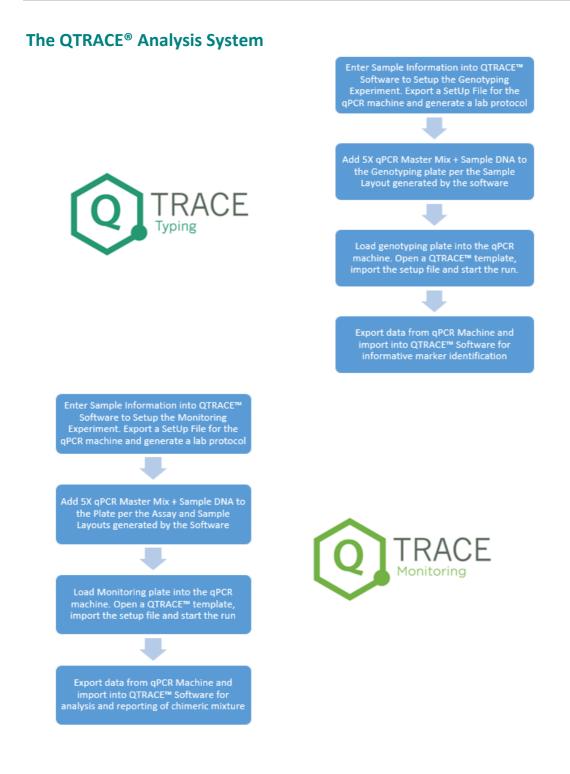
The informativeness of a multi-locus genotyping panel is a measure of the probability of finding at least one informative assay between two individual genomes (or DNA samples). Informativeness is calculated from the population frequency estimates of the alleles used to

make up a multi-locus genotyping panel, and thus differs between ethnic populations. In addition, the informativeness of any panel of polymorphic loci is higher in unrelated individuals than in related individuals.

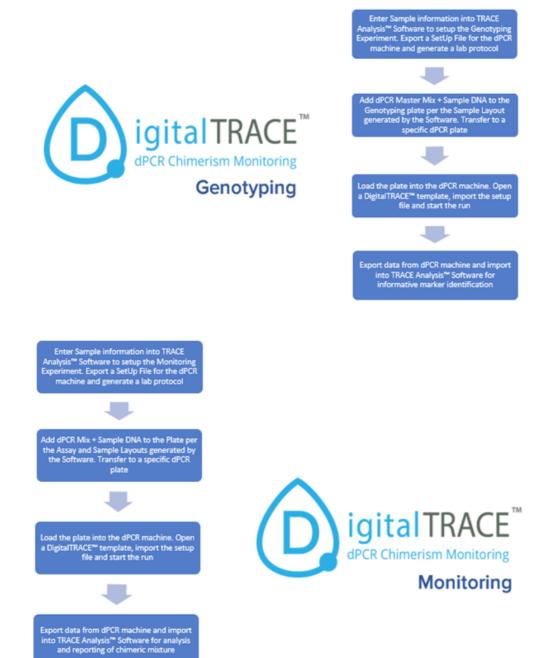
Note: Estimates are based upon both public and proprietary allele frequency data for the 80 assay panel.

The performance of the QTRACE[®] and DigitalTRACE[™] Analysis System has been verified to a level of 0.1% minor component DNA in 150ngs total DNA.

QTRACE and DigitalTRACE System Workflow



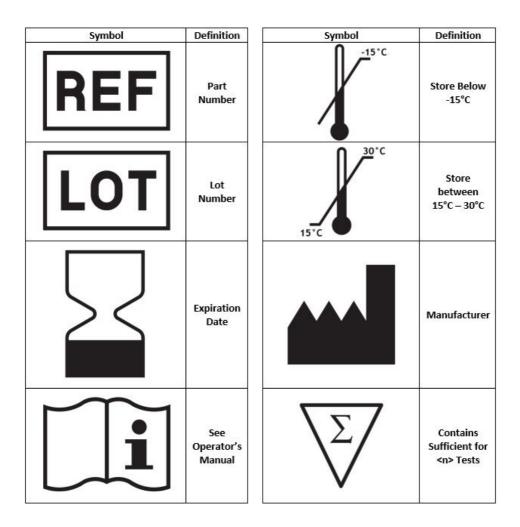
The DigitalTRACE[™] Analysis System



Materials

Key to Symbols

The following symbols appear within the labeling of the QTRACE[®] and DigitalTRACE[™] System Products:



Materials Provided

REF	Name	Description	Storage Conditions	Unit
121045	QTRACE [®] Genotyping Plates, 4-Pack	Four ABI MicroAmp® Optical 96-well plates pre-arrayed with INDEL qPCR Assays; individually sealed	30°C	Вох
121056	QTRACE [®] Genotyping Plates - FAST	Four ABI FAST MicroAmp [®] Optical 96-well plates pre- arrayed with INDEL qPCR Assays; individually sealed	30°C	Вох
121066	QTRACE [®] Genotyping Plates – Roche 480	Four Roche 480 Optical 96-well plates pre-arrayed with INDEL qPCR Assays; individually sealed	30°C	Вох
121129	MultiTRACE™ Genotyping Plate Pack – ABI 0.2mL v2	Four ABI MicroAmp [®] Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed	30°C	Вох
121220	MultiTRACE™ Genotyping Plate Pack - ABI 0.1mL v2	Four ABI MicroAmp [®] Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed	30°C	Вох
121221	MultiTRACE™ Genotyping Plate Pack - LC480 v2	Four Roche 480 Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed	30°C	Вох
121226	MultiTRACE™ Genotyping Plate Pack – ABI 0.2mL v3	Four ABI MicroAmp [®] Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed	30°C	Вох
121285	MultiTRACE™ Genotyping Plate Pack – ABI 0.1mL v3	Four ABI MicroAmp [°] Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed	30°C	Вох
121228	MultiTRACE™ Genotyping Plate Pack – LC480 v3	Four Roche 480 Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed	30°C	Вох
121139	QTRACE [®] Extended Panel Genotyping Plate - ABI 0.2mL	Four ABI MicroAmp [®] Optical 96-well plates pre-arrayed with INDEL qPCR Assays; individually sealed	30°C	Вох
121045	DigitalTRACE™ QIAcuity Genotyping Plate	Two ABI MicroAmp [®] Optical 96- well plates pre-arrayed with INDEL dPCR Assays; individually sealed	30°C	Вох

121056	DigitalTRACE™ EP QIAcuity Genotyping Plate	Two ABI MicroAmp [®] Optical 96- well plates pre-arrayed with INDEL dPCR Assays; individually sealed	30°C	Вох	
--------	---	--	------	-----	--

REF	Name	Description	Storage Conditions	Unit
311044	QTRACE [®] qPCR Master Mix	1.6ml Buffered solution of dNTPs, a DNA Polymerase, a passive reference dye and MgCl ₂	-200	Tube
311043	QTRACE [®] RNaseP Assay	1.1ml buffered solution containing a mix of primers and probe for detecting the RNaseP gene	-200	Tube
	QTRACE [®] INDEL Assays	375µl buffered solution containing a mix of primers and probe for detecting the variant of interest	-200	Tube
	DigitalTRACE™ INDEL Assays	26μl buffered solution containing a mix of primers and probe for detecting the variant of interest in FAM and a reference assay in HEX	-200	Tube
	QTRACE [®] HLA Assays	100µl buffered solution containing a mix of primers and probe for detecting the variant of interest	-200	Tube
	DigitalTRACE™ HLA Assays	26μl buffered solution containing a mix of primers and probe for detecting the variant of interest in FAM and a reference assay in HEX	-200	Tube
711295	QTRACE [®] Universal Positive Control	360µl buffered solution containing synthetic DNA serving as positive control	-200	Tube
711294	DigitalTRACE™ Universal Positive Control	360µl buffered solution containing synthetic DNA serving as positive control	-200	Tube

QTRACE[®] Reference Numbers:

REF	Name	REF	Name
211140	QTRACE [®] INDEL Assay 102	211030	QTRACE [®] INDEL Assay 748
211141	QTRACE [®] INDEL Assay 113	211053	QTRACE [®] INDEL Assay 755
211142	QTRACE [®] INDEL Assay 120	211031	QTRACE [®] INDEL Assay 768
211310	QTRACE [®] INDEL Assay 126	211167	QTRACE [®] INDEL Assay 777
211001	QTRACE [®] INDEL Assay 137	211032	QTRACE [®] INDEL Assay 784
211002	QTRACE [®] INDEL Assay 148	211168	QTRACE [®] INDEL Assay 795

211143	QTRACE [®] INDEL Assay 157	211033	QTRACE [®] INDEL Assay 803
211311	QTRACE [®] INDEL Assay 161	211052	QTRACE [®] INDEL Assay 819
211144	QTRACE [®] INDEL Assay 176	211034	QTRACE [®] INDEL Assay 824
211145	QTRACE [®] INDEL Assay 183	211035	QTRACE [®] INDEL Assay 832
211146	QTRACE [®] INDEL Assay 198	211036	QTRACE [®] INDEL Assay 840
211003	QTRACE [®] INDEL Assay 209	211037	QTRACE [®] INDEL Assay 854
211312	QTRACE [®] INDEL Assay 216	211317	QTRACE [®] INDEL Assay 861
211147	QTRACE [®] INDEL Assay 222	211169	QTRACE [®] INDEL Assay 874
211147	QTRACE® INDEL Assay 222	211105	QTRACE® INDEL Assay 884
211004	QTRACE® INDEL Assay 240	211038	QTRACE® INDEL Assay 888
211148	QTRACE [®] INDEL Assay 252	211171	QTRACE [®] INDEL Assay 892
211006	QTRACE® INDEL Assay 267	211039	QTRACE® INDEL Assay 907
211149	QTRACE [®] INDEL Assay 275	211035	QTRACE® INDEL Assay 916
-		211040	QTRACE® INDEL Assay 910
211313	QTRACE [®] INDEL Assay 284		
211007	QTRACE [®] INDEL Assay 291	211173	QTRACE [®] INDEL Assay 936
211008	QTRACE [®] INDEL Assay 305	211041	QTRACE [®] INDEL Assay 948
211009	QTRACE [®] INDEL Assay 312	211042	QTRACE® INDEL Assay 954
211010	QTRACE [®] INDEL Assay 326	211174	QTRACE [®] INDEL Assay 962
211150	QTRACE [®] INDEL Assay 333	211175	QTRACE [®] INDEL Assay 971
211011	QTRACE [®] INDEL Assay 345	211176	QTRACE [®] INDEL Assay 987
211012	QTRACE [®] INDEL Assay 356	211177	QTRACE [®] INDEL Assay 990
211013	QTRACE [®] INDEL Assay 359	211078	QTRACE [®] HLA Assay H005
211014	QTRACE [®] INDEL Assay 361	211080	QTRACE [®] HLA Assay H007
211055	QTRACE [®] INDEL Assay 373	211081	QTRACE [®] HLA Assay H009
211064	QTRACE [®] INDEL Assay 386	211083	QTRACE [®] HLA Assay H017
211151	QTRACE [®] INDEL Assay 396	211130	QTRACE [®] HLA Assay H020
211015	QTRACE [®] INDEL Assay 408	211085	QTRACE [®] HLA Assay H022
211314	QTRACE [®] INDEL Assay 411	211087	QTRACE [®] HLA Assay H025
211016	QTRACE [®] INDEL Assay 425	211088	QTRACE [®] HLA Assay H028
211017	QTRACE [®] INDEL Assay 434	211131	QTRACE [®] HLA Assay H029
211152	QTRACE [®] INDEL Assay 441	211091	QTRACE [®] HLA Assay H036
211153	QTRACE [®] INDEL Assay 450	211092	QTRACE [®] HLA Assay H038
211315	QTRACE [®] INDEL Assay 457	211093	QTRACE [®] HLA Assay H039
211018	QTRACE [®] INDEL Assay 469	211094	QTRACE [®] HLA Assay H041
211154	QTRACE [®] INDEL Assay 472	211095	QTRACE [®] HLA Assay H043
211155	QTRACE [°] INDEL Assay 482	211096	QTRACE [®] HLA Assay H045
211156	QTRACE [®] INDEL Assay 493	211098	QTRACE® HLA Assay H045
211130	QTRACE® INDEL Assay 504	211098	QTRACE® HLA Assay H051 QTRACE® HLA Assay H052
211015	QTRACE® INDEL Assay 504	211099	QTRACE® HLA Assay H052
211020	QTRACE [®] INDEL Assay 520	211100	QTRACE® HLA Assay H055
211021	QTRACE [®] INDEL Assay 531	211132	QTRACE® HLA Assay H101
211022	QTRACE [®] INDEL Assay 548	211132	QTRACE® HLA Assay H101
211157	QTRACE [®] INDEL Assay 555	211134	QTRACE® HLA Assay H103
211158	QTRACE [®] INDEL Assay 567		
		211279	QTRACE® HLA Assay H104
211159	QTRACE® INDEL Assay 574	211280	QTRACE [®] HLA Assay H105
211160	QTRACE [®] INDEL Assay 585	211281	QTRACE [®] HLA Assay H106
211161	QTRACE [®] INDEL Assay 597	211282	QTRACE [®] HLA Assay H107
211023	QTRACE [®] INDEL Assay 601	211283	QTRACE [®] HLA Assay H108
211024	QTRACE [®] INDEL Assay 615	211284	QTRACE [®] HLA Assay H109
211025	QTRACE [®] INDEL Assay 626	211290	QTRACE [®] HLA Assay H110
211026	QTRACE [®] INDEL Assay 634	211291	QTRACE [®] HLA Assay H111

QTRACE and DigitalTRACE Analysis System Operator's Manual

211316	QTRACE [®] INDEL Assay 647	211292	QTRACE [®] HLA Assay H112
211027	QTRACE [®] INDEL Assay 650	211293	QTRACE [®] HLA Assay H113
211162	QTRACE [®] INDEL Assay 663	211296	QTRACE [®] HLA Assay H114
211164	QTRACE [®] INDEL Assay 678	211297	QTRACE [®] HLA Assay H115
211165	QTRACE [®] INDEL Assay 681	211298	QTRACE [®] HLA Assay H116
211166	QTRACE [®] INDEL Assay 694	211299	QTRACE [®] HLA Assay H117
211028	QTRACE [®] INDEL Assay 706	211300	QTRACE [®] HLA Assay H118
211065	QTRACE [®] INDEL Assay 710	211301	QTRACE [®] HLA Assay H119
211051	QTRACE [®] INDEL Assay 721	211319	QTRACE [®] HLA Assay H120
211029	QTRACE [®] INDEL Assay 736		

DigitalTRACE[™] Reference Numbers:

REF	Name	REF	Name
811140	DigitalTRACE™ INDEL Assay 102	811051	DigitalTRACE™ INDEL Assay 721
811141	DigitalTRACE™ INDEL Assay 113	811029	DigitalTRACE™ INDEL Assay 736
811142	DigitalTRACE [™] INDEL Assay 120	811030	DigitalTRACE™ INDEL Assay 748
811310	DigitalTRACE™ INDEL Assay 126	811053	DigitalTRACE™ INDEL Assay 755
811001	DigitalTRACE™ INDEL Assay 137	811031	DigitalTRACE™ INDEL Assay 768
811002	DigitalTRACE™ INDEL Assay 148	811167	DigitalTRACE™ INDEL Assay 777
811143	DigitalTRACE™ INDEL Assay 157	811032	DigitalTRACE™ INDEL Assay 784
811311	DigitalTRACE™ INDEL Assay 161	811168	DigitalTRACE™ INDEL Assay 795
811144	DigitalTRACE™ INDEL Assay 176	811033	DigitalTRACE™ INDEL Assay 803
811145	DigitalTRACE™ INDEL Assay 183	811052	DigitalTRACE™ INDEL Assay 819
811146	DigitalTRACE™ INDEL Assay 198	811034	DigitalTRACE™ INDEL Assay 824
811003	DigitalTRACE™ INDEL Assay 209	811035	DigitalTRACE™ INDEL Assay 832
811312	DigitalTRACE™ INDEL Assay 216	811036	DigitalTRACE™ INDEL Assay 840
811147	DigitalTRACE™ INDEL Assay 222	811037	DigitalTRACE™ INDEL Assay 854
811004	DigitalTRACE™ INDEL Assay 235	811317	DigitalTRACE™ INDEL Assay 861
811005	DigitalTRACE™ INDEL Assay 240	811169	DigitalTRACE™ INDEL Assay 874
811148	DigitalTRACE™ INDEL Assay 252	811170	DigitalTRACE™ INDEL Assay 884
811006	DigitalTRACE™ INDEL Assay 267	811038	DigitalTRACE™ INDEL Assay 888
811149	DigitalTRACE™ INDEL Assay 275	811171	DigitalTRACE™ INDEL Assay 892
811313	DigitalTRACE™ INDEL Assay 284	811039	DigitalTRACE™ INDEL Assay 907
811007	DigitalTRACE™ INDEL Assay 291	811040	DigitalTRACE™ INDEL Assay 916
811008	DigitalTRACE™ INDEL Assay 305	811172	DigitalTRACE™ INDEL Assay 923
811009	DigitalTRACE™ INDEL Assay 312	811173	DigitalTRACE™ INDEL Assay 936
811010	DigitalTRACE™ INDEL Assay 326	811041	DigitalTRACE™ INDEL Assay 948
811150	DigitalTRACE™ INDEL Assay 333	811042	DigitalTRACE™ INDEL Assay 954
811011	DigitalTRACE™ INDEL Assay 345	811174	DigitalTRACE™ INDEL Assay 962
811012	DigitalTRACE™ INDEL Assay 356	811175	DigitalTRACE™ INDEL Assay 971
811013	DigitalTRACE™ INDEL Assay 359	811176	DigitalTRACE™ INDEL Assay 987
811014	DigitalTRACE™ INDEL Assay 361	811177	DigitalTRACE™ INDEL Assay 990
811055	DigitalTRACE [™] INDEL Assay 373	811078	DigitalTRACE™ HLA Assay H005
811064	DigitalTRACE [™] INDEL Assay 386	811080	DigitalTRACE [™] HLA Assay H007
811151	DigitalTRACE [™] INDEL Assay 396	811083	DigitalTRACE [™] HLA Assay H017
811015	DigitalTRACE [™] INDEL Assay 408	811084	DigitalTRACE [™] HLA Assay H020
811314	DigitalTRACE [™] INDEL Assay 411	811085	DigitalTRACE [™] HLA Assay H022
811016	DigitalTRACE [™] INDEL Assay 425	811087	DigitalTRACE™ HLA Assay H025
811017	DigitalTRACE™ INDEL Assay 434	811088	DigitalTRACE [™] HLA Assay H028
811152	DigitalTRACE™ INDEL Assay 441	811131	DigitalTRACE [™] HLA Assay H029
811153	DigitalTRACE [™] INDEL Assay 450	811091	DigitalTRACE™ HLA Assay H036
811315	DigitalTRACE™ INDEL Assay 457	811092	DigitalTRACE™ HLA Assay H038

811018 DigitalTRACE [™] INDEL Assay 469 811093 DigitalTRACE [™] HLA Assa	y H039
811154 DigitalTRACE [™] INDEL Assay 472 811094 DigitalTRACE [™] HLA Assa	y H041
811155 DigitalTRACE™ INDEL Assay 482 811095 DigitalTRACE™ HLA Assa	y H043
811156 DigitalTRACE [™] INDEL Assay 493 811096 DigitalTRACE [™] HLA Assa	y H045
811019 DigitalTRACE [™] INDEL Assay 504 811098 DigitalTRACE [™] HLA Assa	y H051
811054 DigitalTRACE™ INDEL Assay 519 811099 DigitalTRACE™ HLA Assa	y H052
811020 DigitalTRACE™ INDEL Assay 520 811100 DigitalTRACE™ HLA Assa	y H053
811021 DigitalTRACE [™] INDEL Assay 531 811101 DigitalTRACE [™] HLA Assa	y H054
811022 DigitalTRACE [™] INDEL Assay 548 811133 DigitalTRACE [™] HLA Assa	y H102
811157 DigitalTRACE [™] INDEL Assay 555 811134 DigitalTRACE [™] HLA Assa	y H103
811158 DigitalTRACE™ INDEL Assay 567 811279 DigitalTRACE™ HLA Assa	y H104
811159 DigitalTRACE [™] INDEL Assay 574 811280 DigitalTRACE [™] HLA Assa	y H105
811160 DigitalTRACE [™] INDEL Assay 585 811281 DigitalTRACE [™] HLA Assa	y H106
811161 DigitalTRACE [™] INDEL Assay 597 811282 DigitalTRACE [™] HLA Assa	y H107
811023 DigitalTRACE [™] INDEL Assay 601 811283 DigitalTRACE [™] HLA Assa	y H108
811024 DigitalTRACE [™] INDEL Assay 615 811284 DigitalTRACE [™] HLA Assa	y H109
811025 DigitalTRACE [™] INDEL Assay 626 811290 DigitalTRACE [™] HLA Assa	y H110
811026 DigitalTRACE [™] INDEL Assay 634 811291 DigitalTRACE [™] HLA Assa	y H111
811316 DigitalTRACE [™] INDEL Assay 647 811292 DigitalTRACE [™] HLA Assa	y H112
811027 DigitalTRACE [™] INDEL Assay 650 811293 DigitalTRACE [™] HLA Assa	y H113
811162 DigitalTRACE [™] INDEL Assay 663 811296 DigitalTRACE [™] HLA Assa	y H114
811163 DigitalTRACE [™] INDEL Assay 670 811297 DigitalTRACE [™] HLA Assa	y H115
811164 DigitalTRACE [™] INDEL Assay 678 811298 DigitalTRACE [™] HLA Assa	y H116
811165 DigitalTRACE [™] INDEL Assay 681 811299 DigitalTRACE [™] HLA Assa	y H117
811166 DigitalTRACE [™] INDEL Assay 694 811300 DigitalTRACE [™] HLA Assa	y H118
811028 DigitalTRACE [™] INDEL Assay 706 811319 DigitalTRACE [™] HLA Assa	y H120
811065 DigitalTRACE [™] INDEL Assay 710	

REF	Name	Description	
341048	TRACE Analysis™ Software	Minimum System Requirements: Windows 7, 2 GB RAM, 250 MB free disk space, Network connection allowing TCP/IP traffic to and from port 3500, Microsoft .NET framework 4.5. Recommended System Requirements: Windows 7, 2 GB RAM, Internet connection for license validation and automatic updates, 500 MB free disk space, Microsoft .NET framework 4.5	

REF	Name	Description
331047	QTRACE [®] System Operator's Manual	Operator's Manual for QTRACE [®] Analysis System
331307	DigitalTRACE™ Operator's Manual	Operator's Manual for DigitalTRACE [™] Analysis System

Materials Sold Separately

Additional Reagents Required but not Provided

Item name	Catalog number
Modified TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) (also called TE 0.1 Buffer or TE-4 Buffer)	Not applicable

Molecular grade water (DNase and RNase free)	Not applicable
QIAcuity Probe PCR Kit (Qiagen)	250102
ddPCR Supermix for Probes (Biorad)	1863023

Additional Consumables Required but not Provided

Item name	Catalog number
Pipette Tips, disposable, sterile, aerosol-resistant, filtered, capable of dispensing up to 20, 200, and 1000 μL	Not applicable
1.5 mL microcentrifuge tubes	Not applicable
Lint-free tissue	Not applicable
Gloves, powder-free	Not applicable
96-well plates or strip tubes	Not applicable
Plate seals	Not applicable
QIAcuity Nanoplate 26k 24-well (Qiagen)	250001
QIAcuity Nanoplate 26k 8-well (Qiagen)	250031
QIAcuity Nanoplate 8.5k 96-well (Qiagen)	250021
QIAcuity Nanoplate 8.5k 24-well (Qiagen)	250011
DG8 Cartridges (Biorad)	1864008
DG8 Gaskets (Biorad)	1863009
ddPCR 96-well PCR Plates (Biorad)	12001925
PCR Plate Heat Seal, foil, pierceable (Biorad)	1814040
Droplet Generation Oil for Probes (Biorad)	1863005
ddPCR Droplet Reader Oil (Biorad)	1863004

Additional Equipment Required but not Provided

Item name	Catalog number
Adjustable single channel pipettes (0.5-1000 μL capacity)	Not applicable
Adjustable multi-channel, multi-dispensing pipettes (0.5-200 μL capacity)	Not applicable
Vortex mixer with flat rubber platform head	Not applicable
Centrifuge	Not applicable
Centrifuge with microtiter plate assembly	Not applicable
PC for the installation of TRACE Analysis [™] Software	Not applicable

DNA Sample Requirements

Purified DNA should have an A_{260}/A_{280} ratio between 1.7 and 2.0.

We recommend using a fluorometric method to accurately quantify DNA.

If necessary, DNA should be diluted in 10mM Tris, pH 8.0; 0.1mM EDTA (TE) or nuclease-free H_2O before use.

Blood samples should be collected in ACD or EDTA anticoagulation tubes. <u>Do NOT use</u> <u>heparinized samples</u>, as this has an inhibitory effect on a PCR

The optimal amount of template DNA to use in genotyping is 5 ng per well for the QTRACE[®] Genotyping plates and 10 ng per well for the DigitalTRACE[™] Genotyping plates. To streamline the process, validate your DNA purification procedure so you can use a set volume corresponding to 1-10 ng DNA.

The optimal amount of template DNA to use in chimeric mixture analysis depends on the desired sensitivity detection limit.

The following chart shows the relationship between input DNA amounts and sensitivity. These calculations assume at least 10 copies of the minor component DNA in the total amount of DNA.

Input DNA (ng)	# Cells	Sensitivity %
150	22 727	0.04%
100	15 151	0.07%
50	7 576	0.13%
25	3 788	0.26%
12.5	1 894	0.53%
6.25	947	1.06%

Sensitivity %	# Cells	Input DNA (ng)
0.05%	20 000	132
0.10%	10 000	66
1.00%	1 000	7
2.00%	500	3
5.00%	200	1

Warnings

For Research Use Only. Not for use in diagnostic procedures.

Use good laboratory practices for sample handling and tracking.

Use only recommended materials, procedures, and equipment.

Use sterile disposable pipettes and filtered pipette tips.

Wear appropriate personal protective equipment (*e.g.*, safety glasses, disposable gloves, and protective clothing) when handling samples and reagents.

Clean and disinfect all work surfaces with a 10% bleach (0.525% sodium hypochlorite) solution and follow with 70% ethanol, ensuring that all bleach residue is removed.

Assays should be run by individuals experienced in good laboratory practices and who have been previously trained to use the equipment by the original equipment manufacturer (OEM). Operate, calibrate and maintain all instruments and equipment according to procedures provided by the manufacturers.

To reduce the risk of contamination, the area where amplified DNA is handled must be physically isolated from the work areas for sample preparation and qPCR setup. Do not use components past their expiration date.

Do not dilute reagents.

Visually inspect wells or tubes after pipetting steps to detect operator errors with pipetting, sample transfer, etc.

To prevent repeated freeze/thaw cycling of reagents during frozen storage, do not store reagents within freezers that use an automatic defrost function (*i.e.*, frost-free).

Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent tubes.

To prevent contamination, after aliquots are removed do not return the remaining volume to the original tube.

Comply with all local, state, or national laws and regulations related to chemical storage and disposal.

CHEMICAL HAZARD. Ethanol is a flammable liquid and vapor. Exposure can cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact can dry the skin. Exposure can cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

CHEMICAL HAZARD. Bleach (sodium hypochlorite) is a corrosive liquid and vapor. Exposure can cause severe irritation or damage to eyes, skin and the respiratory system. Harmful if swallowed. Prolonged or repeated contact can lead to sensitization (*e.g.,* irritation) if skin damage occurs during exposure. Medical conditions that can be aggravated by exposure to high concentrations of vapor or mist include heart conditions or chronic respiratory problems such as asthma, emphysema, chronic bronchitis or obstructive lung disease. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

TRACE Analysis[™] Software does not provide a mechanism to edit data files obtained from the qPCR and dPCR System or result files once they are created.

Shipping Conditions

The QTRACE[®], DigitalTRACE[™] and MultiTRACE[™] Genotyping Plates are shipped at ambient temperature. The QTRACE[®] PCR Master Mix, QTRACE[®] INDEL Assays, QTRACE[®] HLA Assays, DigitalTRACE[™] INDEL Assays and DigitalTRACE[™] HLA Assays are shipped frozen.

Examine the shipment upon receipt and if the integrity of the products has been compromised during shipment, immediately contact your local customer support representative.

Storage and Handling Requirements

Upon receipt, the individual components should be stored according to the temperatures listed on the labels.

Note: When storage recommendations are observed, both unopened and opened/recapped tubes are stable until the expiration date indicated on the label. genotyping plates are stable when stored in the air-tight pouch containing a desiccant bag. Do not use any component after the expiration date.

Do not use any component that visibly shows signs of having been compromised (*e.g.*, particulate matter, presence of foreign debris, cloudy appearance, discoloration).

Technical Support

For technical assistance and more information:

Please contact your local distributor

France bionobis 7 rue Nicolas Copernic 78280 Guyancourt - SQY France

Tél : 01 30 43 01 55 Fax : 01 30 43 01 15 Email: <u>info@bionobis.com</u> https://www.bionobis.com

<u>Iberia</u> Werfen Plaza Europa, 21-23 08908 L'Hospitalet de Llobregat Barcelona, Spain

Tel: +34 93 401 01 01 Email: <u>customerservice-es@werfen.com</u> https://www.werfen.com/es/es

Estonia Quantum Eesti AS Tiigrisilma 8 Räni alevik Kambja vald 61708 Tartu, Estonia

Tel: +372 7 301 321 Email: <u>quantum@quantum.ee</u> https://quantum.ee/

<u>Turkiye</u> ATC Genomics Galip Erdem Cad. 607. Sok. No:9 06550 Çankaya - Ankara, Turkiye

Tel: +90 312 496 43 14 Email: bilgi@atc.com.tr http://www.atc.com.tr or **JETA Molecular** Krommewetering 101C 3543 AN, Utrecht The Netherlands

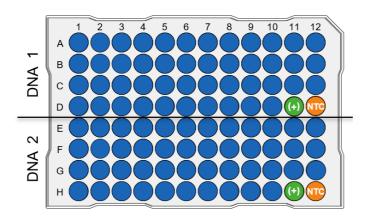
Tel: +31 (0)6 54 13 66 97 Email: <u>info@jetabv.com</u> https://www.jetamolecular.com

Genotyping Test

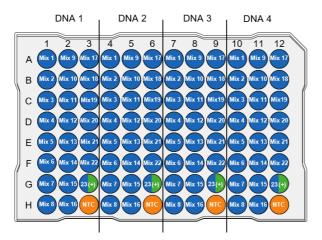
The QTRACE[®] Analysis System

The DNAs that comprise a mixed DNA sample are analyzed using a QTRACE[®] Genotyping Plate and QTRACE[®] qPCR Master Mix, to identify all of the informative assays for the samples. An informative assay is an assay for a marker allele that is present (positive) in one individual genome *and* absent (negative) in the other genome.

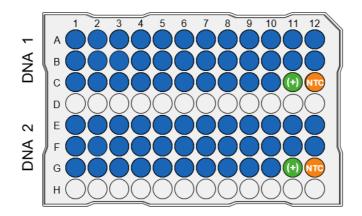
The genotyping plate contains a duplicate set of assays: the 46 quantification assays and the reference (RNaseP) assay that serves as both a positive control and a No Template Control (NTC), pre-dispensed and dried in an optical qPCR plate. The QTRACE[®] qPCR Master Mix comes supplied with dUTP and UNG for built-in carryover contamination control.



The multiplexed version of the genotyping plate (MultiTRACE[™]) contains a quadruplicate set of assays: the 45 quantification assays and the reference (RNaseP) assay that serves as both a positive control and a No Template Control (NTC), pre-dispensed and dried in an optical qPCR plate.



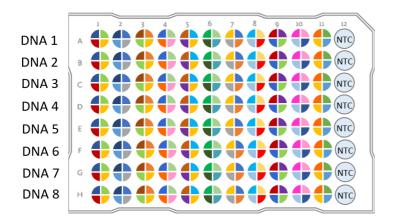
The Extended Panel genotyping plate can be used in rare cases including patients receiving a second transplant or highly similar siblings, where there may be need for additional markers to the standard QTRACE genotyping plate. It contains a duplicate set of 34 quantification assays and the reference (RNaseP) assay that serves as both a positive control and a No Template Control (NTC), pre-dispensed and dried in an optical qPCR plate.



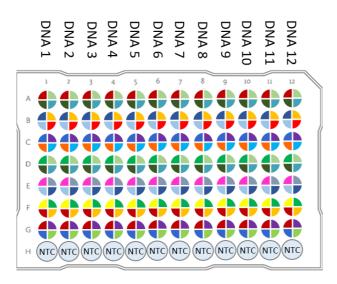
The DigitalTRACE[™] Analysis System

The DNAs that comprise a mixed DNA sample are analyzed using a DigitalTRACE[™] or MultiTRACE[™] Genotyping Plate, to identify all of the informative assays for the samples. An informative assay is an assay for a marker allele that is present (positive) in one individual genome and absent (negative) in the other genome.

The DigitalTRACE[™] Genotyping Plate contains a set of 43 quantification assays and the reference (RNase P) assay that serves as both a positive control and a No Template Control (NTC). The assay sets are pre-arrayed in rows.



The DigitalTRACE[™] EP QIAcuity Genotyping Plate represents an extended panel of dPCR markers that can be used for dPCR monitoring in the case of need for additional markers allowing to distinguish between donor and recipient DNA. This plate contains a set of 27 quantification assays and the reference (RNase P) assay that serves as both a positive control and a No Template Control (NTC). The assay sets are pre-arrayed in columns.



Alternatively, the MultiTRACE[™] Genotyping Plates can be used for a dPCR genotyping. The MultiTRACE[™] Genotyping Plates contain the quantification assays in FAM and the reference (RNase P) assay that serves as both a positive control and a No Template Control (NTC) in HEX.

Protocol

Before setting up an experiment, select the instrument as well as the type and version of the genotyping plate used.



button and choose from the available instruments and

The QTRACE® Analysis System

Press the "Preferences" genotyping plate types.

To generate a new record in TRACE Analysis[™] Software, in the Section labeled "Recipient" enter the Recipient Name, Recipient Identifier and a unique Sample Identifier. While "Date of Birth" is an optional field for all samples, a "Date of Transplant" must be entered, if you ultimately want to have the data stored and reported in a temporal manner.

Re	cipient 🜏
Recipient Name	
Recipient ID	
Sample ID	
Concentration ng/ul	20
Date of Birth	XXX-XXX-XXXXX 15
Date of Transplant	XX-XX-XXXX IS
Gender	© Male © Female
Comment	
Disease Type	•
D	lonor 🛛 🖌 🗙
Donor Name	
Donor ID	
Sample ID	
Concentration ng/ul	20
Gender	© Male ⊚ Female
Comment	

For a sample which should be genotyped against the Recipient sample, enter the Donor Name, Donor Identifier and unique Sample Identifier. You can comparatively genotype up to two samples on a single standard plate or up to four samples on a single multiplexed plate using TRACE Analysis™ Software. TRACE Analysis™ Software also allows you to virtually compare multiple samples, independent of when they were genotyped. Based on the data from verification studies, JETA Molecular recommends the use of 5ng DNA input per well for genotyping. (DNA inputs are customized in the software's <u>Preferences</u> for Concentrations).

Once all samples to be comparatively genotyped are entered into the Sample window, press the

"Add Typing Samples" button to add the samples to the plate set up file. You will see the samples now added to the 96 well plate in the middle of the screen.

When using a multiplexed version of the QTRACE[®] Genotyping Plate, you may add the four DNA samples to be genotyped as two couples Recipient/Donor (added to the plate in two steps, the second couple after the first one). In this case, TRACE Analysis[™] Software generates a separate genotyping report for each couple Recipient/Donor.

Alternatively, the four DNA samples may be added to the plate (in one single step) as one Recipient and three Donors, using the Add Donor button:

Add Donor 🗧 🕇

In this case, TRACE Analysis[™] Software generates a single genotyping report containing all four DNA samples genotyped.

Sample Layout View for 2 Samples being genotyped in the standard QTRACE[®] genotyping plate (left) and in the multiplexed genotyping plate (right):

A	James 1234 240	James 1234 601	James 1234 768	James 1234 312	James 1234 832	James 1234 948	James 1234 137	James 1234 469	James 1234 531	James 1234 386	James 1234 736	James 1234 548		
3	James 1234 425	James 1234 361	James 1234 710	James 1234 148	James 1234 803	James 1234 907	James 1234 520	James 1234 345	James 1234 267	James 1234 784	James 1234 634	James 1234 434		
2	James 1234 209	James 1234 706	James 1234 326	James 1234 721	James 1234 626	James 1234 840	James 1234 359	James 1234 954	James 1234 854	James 1234 615	James 1234 408	James 1234 824	ſ	
)	James 1234 504	James 1234 650	James 1234 235	James 1234 916	James 1234 748	James 1234 356	James 1234 819	James 1234 755	James 1234 519	James 1234 373	James 1234 RNaseP	NTC RNaseP		
	Julie 4567 240	Julie 4567 601	Julie 4567 768	Julie 4567 312	Julie 4567 832	Julie 4567 948	Julie 4567 137	Julie 4567 469	Julie 4567 531	Julie 4567 386	Julie 4567 736	Julie 4567 548		
	Julie 4567 425	Julie 4567 361	Julie 4567 710	Julie 4567 148	Julie 4567 803	Julie 4567 907	Julie 4567 520	Julie 4567 345	Julie 4567 267	Julie 4567 784	Julie 4567 634	Julie 4567 434	ļ	
3	Julie 4567 209	Julie 4567 706	Julie 4567 326	Julie 4567 721	Julie 4567 626	Julie 4567 840	Julie 4567 359	Julie 4567 954	Julie 4567 854	Julie 4567 615	Julie 4567 408	Julie 4567 824		
ł	Julie 4567 504	Julie 4567 650	Julie 4567 235	Julie 4567 916	Julie 4567 748	Julie 4567 356	Julie 4567 819	Julie 4567 755	Julie 4567 519	Julie 4567 373	Julie 4567 RNaseP	NTC RNaseP		

ł	Brian	Brian	Brian	Cindy	Cindy	Cindy	Jim			AL .		
١	4321	4321	4321	7654	7654	7654	9876	5876	9876	6543	6543	6543
	Mix028	Mix036	Mix044	Mix028	Mix036	Mix044	Mix028	Mix036	Mix044	Mix028	Mix036	Mix044
3	Brian	Brian	Brian	Cindy	Cindy	Cindy	Jim	Jim	Jim	.111	Jill	Jill
	4321	4321	4321	7654	7654	7654	9876	9876	9876	6543	6543	6543
	Mix029	Mix037	Mix045	Mix029	Mix037	Mix045	Mix029	Mix037	Mix045	Mix029	Mix037	Mix045
,	Brian	Brian	Brian	Cindy	Cindy	Cindy	Jim	Jim	Jim	.HI	Jill	Jill
	4321	4321	4321	7654	7654	7654	S876	S076	SE76	6543	6543	6543
	Mix030	Mix038	Mix046	Mix030	Mix038	Mix046	Mix030	Mix038	Mix046	Mix030	Mix038	Mix046
)	Brian	Brian	Brian	Cindy	Cindy	Cindy	Jim	Jim	Jim	АП	,511	ЯІ
	4321	4321	4321	7654	7654	7654	9876	S876	9876	6543	6543	6543
	Mix031	Mix039	Mix047	Mix031	Mix039	Mix047	Mix031	Mix039	Mix047	Мін031	Mix439	Мінф47
	Brian	Brian	Brian	Cindy	Cindy	Cindy	Jim	Jim	Jim	.fil	Jil	,81
	4321	4321	4321	7654	7654	7654	9876	5876	9876	6543	6543	6543
	Mix032	Mix040	Mix048	Mix032	Mix040	Mix048	Mix032	Mix040	Mix048	Mix032	Mix040	Mix648
	Brian	Brian	Brian	Cindy	Cindy	Cindy	Jim	Jim	Jim	.68	JEE	Jil
	4321	4321	4321	7654	7654	7654	S876	5876	SE76	6543	6543	6543
	Mix033	Mix041	Mix049	Mix033	Mix041	Mix049	Mix033	Mix041	Mix049	Min033	Miss041	Mix649
3	Brian	Brian	Brian	Cindy	Cindy	Cindy	Jim	Jim	Jim	581	Jil	Jill
	4321	4321	4321	7654	7654	7654	S876	5876	SE76	6543	6543	6543
	Mix034	Mix042	Mix050	Mix034	Mix042	Mix050	Mix034	Mix042	Mix050	Mix034	Mix042	Mix050
ł	Brian 4321 Mix035	Brian 4321 Mix043	NTC RNaseP	Cindy 7654 Mix035	Cindy 7654 Mix043	NTC RNaseP	Jim 5876 Mix035	5m 5876 Miy043	NTC RNaseP	38 6543 Mix035	ЛІ 6543 Мін043	NTC RNaseP

The colors in the small plate images (Sample View and Assay View) at the bottom of the window are enabled in the large plate image, by touching the colored plate image of interest. By touching the same image again, the large plate colors disappear. (For Genotyping, the assays are already in the wells and are not added by the operator).

Once the samples have been added to your plate, and the experiment name and operator-ID

have been entered, press the "Export Setup to PCR" button.

Browse to the location where you want the file saved and name it as you wish. This file can then be imported into your qPCR machine's QTRACE[®] template to execute the qPCR analysis.

Once the file is saved, TRACE Analysis[™] Software generates a protocol, based on the experimental inputs and the settings in the preferences menu.

The following protocol is an example output from TRACE Analysis[™] Software for the Genotyping procedure using a <u>standard genotyping plate</u>:

Set up all reactions in a pre-PCR lab, under ambient conditions without ice.

Open a QTRACE[®] Genotyping Plate Pack and remove the genotyping plate.

Label the genotyping plate with the genotyping test name.

Collect the two DNA samples to be screened, as well as QTRACE[®] qPCR Master Mix and deionized H_2O . Briefly vortex and centrifuge all tubes before opening

Label three, 1.5 mL microcentrifuge tubes:

Sample 1 Mix Sample 2 Mix NTC Mix

For each sample to be genotyped, prepare a qPCR Master Mix containing sample DNA, deionized H_2O and QTRACE[®] qPCR Master Mix as suggested by QTRACE[®] Software in Table 1. A No Template Control (NTC) Mix is prepared with de-ionized H_2O and QTRACE[®] qPCR Master Mix.

Table 1. qPCR Master Mix Composition

	1	
Sample 1 qPCR Mix	<u>1X</u>	<u>50X</u>
QTRACE [®] qPCR Master Mix	5.0ul	250.0ul
"recipient ID" DNA	0.8ul	40.0ul
H ₂ O	<u>19.2ul</u>	<u>960.0ul</u>
	25.0ul	1250.0ul
Sample 2 qPCR Mix	<u>1X</u>	<u>50X</u>
QTRACE [®] qPCR Master Mix	5.0ul	250.0ul
"Donor ID" DNA	0.1ul	5ul
H ₂ O	<u>19.9ul</u>	<u>955ul</u>
	25.0ul	1250.0ul
NTC qPCR Mix	<u>1X</u>	<u>3X</u>
QTRACE [®] qPCR Master Mix	5.0ul	15.0ul
H ₂ O	<u>20.0ul</u>	<u>60.0ul</u>
	25.0ul	75.0ul

Vortex each tube to thoroughly mix the contents and centrifuge briefly to collect the reaction mix at the bottom of the tube.

Remove the adhesive cover from the genotyping plate.

Refer to the DNA Sample Layout Plate Layout at the end of the protocol for pipetting the following:

Dispense 25 μ l of the Sample 1 Mix into Wells A1-D6 and in Well D11 of the genotyping plate.

Dispense 25 μ l of the Sample 2 Mix into Wells E1-H6 and in Well H11 of the genotyping plate. Dispense 25 μ l of the 5X PCR Master Mix/NTC mixture to wells D12 and H12. **A repeat pipettor is recommended to minimize pipetting repetition and increase accuracy.**

Visually inspect plate wells from the sides and bottom to confirm consistent volume. - *For use with qPCR machines which do not accept standard ABI MicroAmp Optical Plates:* Transfer the genotyping assay reactions to a 96-well plate for your instrument using a multichannel pipette.

Seal the plate completely with MicroAmp[®] Optical Adhesive Film using the MicroAmp[®] Adhesive Film Applicator.

IMPORTANT! Vortex the plate to mix the contents of each well. Centrifuge the plates briefly using a plate centrifuge to collect the contents at the bottom of the wells. Load the plate into your qPCR machine. Open a pre-configured QTRACE[®] qPCR template and save the file with a unique name. (If you don't have a template, please see thermal cycling profiles below.

Import the Sample Setup sheet generated by TRACE Analysis[™] Software.

Save the file and start the qPCR run.

The following protocol is an example output from TRACE Analysis[™] Software for the Genotyping procedure using a <u>multiplexed genotyping plate</u>:

Set up all reactions in a pre-PCR lab, under ambient conditions without ice.

Open a MultiTRACE[™] Genotyping Plate Pack and remove the genotyping plate. Label the genotyping plate with the genotyping test name. Collect the four DNA samples to be screened, as well as QTRACE[®] qPCR Master Mix and deionized H2O. Briefly vortex and centrifuge all tubes before opening Label five, 1.5 mL microcentrifuge tubes:

Sample 1 Mix Sample 2 Mix Sample 3 Mix Sample 4 Mix NTC Mix

For each sample to be genotyped, prepare a qPCR Master Mix containing sample DNA, deionized H₂O and QTRACE[®] qPCR Master Mix as suggested by TRACE Analysis[™] Software in Table 2. A No Template Control (NTC) Mix is prepared with de-ionized H₂O and QTRACE[®] qPCR Master Mix.

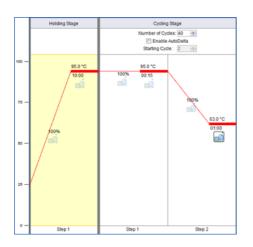
Table 2. qPCR Master Mix Composition – MultiTRACE[™] plates

Sample 1 qPCR Mix	<u>1X</u>	<u>25X</u>
QTRACE [®] qPCR Master Mix	5.0ul	125.0ul
"Recipient 1 ID"		
DNA	0.3ul	7.5ul
H ₂ O	<u>19.7ul</u>	<u>492.5ul</u>
	25.0ul	625.0ul

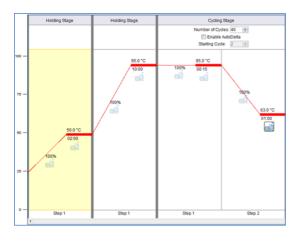
Sample 2 qPCR Mix	<u>1X</u>	<u>25X</u>
QTRACE [®] qPCR Master Mix	5.0ul	125.0ul
"Donor 1 ID" DNA	0.2ul	5.4ul
H ₂ O	<u>19.8ul</u>	<u>494.6ul</u>
	25.0ul	625.0ul
Sample 3 qPCR Mix	<u>1X</u>	<u>25X</u>
QTRACE [®] qPCR Master Mix "Recipient 2 ID"	5.0ul	125.0ul
DNA	0.4ul	8.9ul
H ₂ O	<u>19.6ul</u>	<u>491.1ul</u>
	25.0ul	625.0ul
Sample 2 qPCR Mix	<u>1X</u>	<u>25X</u>
QTRACE [®] qPCR Master Mix	5.0ul	125.0ul
"Donor 2 ID" DNA	0.1ul	2.5ul
H ₂ O	<u>19.9ul</u>	<u>497.5ul</u>
	25.0ul	625.0ul

Thermal Cycling Protocol for QTRACE® System Products

The QTRACE[®] System will deliver optimal results when the following thermal profile is used in qPCR.



For labs which require use of molecular tests with built-in carryover contamination control, the QTRACE[®] qPCR Master Mix contains dUTP and Uracil N-Glycosylase (UNG) enzyme. In order to use this enhanced capability of the master mix, add a 2 minute hold at 50°C, prior to the enzyme activation/initial denaturation step.



The DigitalTRACE[™] Analysis System

Genotyping Test Protocol - QIAcuity

Change the instrument type in the Preferences of the TRACE Analysis[™] Software to QIAcuity and Plate type to QIAcuity, v1.

To generate a new record in TRACE Analysis[™] Software, in the Section labeled "Recipient" enter the Recipient Name, Recipient Identifier and a unique Sample Identifier. While "Date of Birth" is an optional field for all samples, a "Date of Transplant" must be entered, if you ultimately want to have the data stored and reported in a temporal manner.

Re	cipient 🖌 🤶
Recipient First Name	
Recipient Last Name	
Recipient ID	
Sample ID	
Concentration ng/ul	100
Date of Birth	XX-XX-X000X 15
Date of Transplant	XX-XX-XXXX 15
Gender	O Male O Female
Comment	
Disease Type	~
D)onor 🗸 🗙
Donor First Name	
Donor Last Name	
Donor ID	
Sample ID	
Concentration ng/ul	100
Date of Birth	XX-XX-X000X 15
Gender	O Male O Female
Comment	

For a sample which should be genotyped against the Recipient sample, enter the Donor Name, Donor Identifier and unique Sample Identifier. You can genotype up to eight samples on a single plate using TRACE Analysis[™] Software. TRACE Analysis[™] Software also allows you to virtually compare multiple samples, independent of when they were genotyped. Based on the data from verification studies, JETA Molecular recommends the use of 10 ng DNA input per well for genotyping. (DNA inputs are customized in the software's <u>Preferences</u> for Concentrations). Enter the concentrations of your samples to let the software calculate with.

After all samples to be comparatively genotyped are entered into the Sample window, press the

Screen "Add Typing Samples" button to add the samples to the plate set up file. You will see the samples now added to the 96-well plate in the middle of the screen.

Sample Layout View for 8 Samples being genotyped in the DigitalTRACE[™] QIAcuity plate:

L.	Tom 001 QIAMix	Tom 001 QIAMix	Tom 001 QIAMixt	Tom 001 QIAMix(001			Tom 001 QIAMixt	001		Tom 001 QIAMixt	NTC NTC Mis
	Alex 002 QIAMix	Alex 002 QIAMixt	Alex 002 QIAMixt	Alex 002 QIAMix(002	Alex 002 QIAMixt	002	Alex 002 QIAMix(002	002	Alex 002 QIAMix(NTC NTC Mi>
	Julie 003 QIAMix	Julie 003 QIAMix	Julie 003 QIAMixt	Julie 003 QIAMix(003		003	003	003	003	Julie 003 QIAMix(NTC NTC Mib
)	004	James 004 QIAMixt	James 004 QIAMixt	James 004 QIAMix(004		004	004	004		James 004 QIAMix(NTC NTC Mis
	Peter 005 QIAMix	Peter 005 QIAMixt	Peter 005 QIAMixt	Peter 005 QIAMixt	005	Peter 005 QIAMixt	005	Peter 005 QIAMix(005	005	Peter 005 QIAMixt	NTC NTC Mis
	006	005	006	Barbara 006 QIAMix(006	006	006	006	006		006	NTC Mb
	John 007 QIAMix	John 007 QIAMix	John 007 QIAMixi	John 007 QIAMix(007		007	007	007	007	John 007 QIAMix(NTC NTC Mb
	008	Ashley 008 QIAMix	008	Ashley 008 QIAMix(008		800	008	008	008	Ashley 008 QIAMixt	NTC NTC Mis

The colors in the small plate images (Sample View and Assay View) at the bottom of the window are enabled in the large plate image, by touching the colored plate image of interest. By touching the same image again, the large plate colors disappear. (For Genotyping, the assays are already in the wells and are not added by the operator).

Once the samples have been added virtually to your plate, and the experiment name have been



Browse to the location where you want the file saved and name it as you wish. This file can then be imported into your dPCR machine's DigitalTRACE template to execute the dPCR analysis.

Once the file is saved, TRACE Analysis[™] Software generates a protocol, based on the experimental inputs and the settings in the Preferences menu. Print out this protocol.

- 1. Set up all reactions in a pre-PCR lab, under ambient conditions without ice. 2. Collect all DNA samples to be screened, as well as QIAcuity typing plate together with 4x Probe PCR Master Mix and
- de-ionized H₂O.
- ueronnee vnpo. Briefly vortex and centrifuge all tubes before opening. For each sample to be genotyped, lable a tube and a Mix containing sample DNA, de-ionized H₂O and 4x Probe PCR Master Mix as suggested by TRACE Analysis^w Software in Table 1. A No Template Control (NTC) Mix is prepared with

de-ionized H₂O and 4x Probe PCR Master Mix. * - Make a Ten-Fold Dilution (1:10) of Sample

Table 1. Master Mix Composition										
Sample 1 dPCR Mix	1 x	13 x								
4x Probe PCR Master Mix	3,0 µl	39,0 µl								
001 DNA	0,5 µl	*6,5 µl								
H₂O	8,5 µl	110,5 µl								
	12,0 µl	156,0 µl								
Sample 2 dPCR Mix	1 x	13 x								
4x Probe PCR Master Mix	3,0 µl	39,0 µl								
002 DNA	0,5 µl	*6,5 µl								
H₂O	8,5 µl	110,5 µl								
	12,0 µl	156,0 µl								
NTC dPCR Mix	1 x	10 x								
4x Probe PCR Master Mix	3,0 µl	30,0 µl								
H ₂ O	9,0 µl	90,0 µl								
	12.0.1	420.0.1								

- 5. Vortex each tube to thoroughly mix the contents and centrifuge briefly to collect the reaction mix at the l the tube.
- 6. Remove the adhesive cover from the genotyping plate
- 7. Deliver 13.2 µl of each Sample Mix and NTC mix to the Typing plate as defined in TRACE Analysis™ Software's Assa Layout view
- 8. An automated multi nannel pipette is recommended in this step to minimize pipetting repet
- accuracy.
- Visually inspect plate wells from the sides and bottom to confirm consistent volur
 Seal the plate with an Adhesive Film.
- 11. IMPORTANT! Vortex the plate to mix the contents of each well. Centrifuge the plates briefly using a plate centrifuge
- to collect the contents at the bottom of the wells
- 12. Remove the Adhesive cover very carefully. 13. Transfer 12 µl of each prepared reaction mix into a single column of a 96-well 8.5K Nanoplate. Seal the Nanop
- with the compatible plate sealer.
- 14. Load the Nanoplate into the QJAcuity digital PCR system 15. Launch the QIAcuity Software Suite.
- 16. Open the DigitalTRACE typing template and import the Sample Setup sheet generated by TRACE Analysis™ Software
- 17 Save the file and start the ru

Experiment Setup in QIAcuity Suite Software, v2.5

Create a new QIAcuity Plate by selecting "New Plate".

Load a template by selecting "Plate templates..." and import the appropriate DigitalTRACE Genotyping template:

Plate templates
Import template
Save as template

Press "Save changes". The new plate will appear in the main window of the QIAcuity Software Suite:



Click on the plate name to open the plate configuration procedure. Type in a new plate name and save the changes.

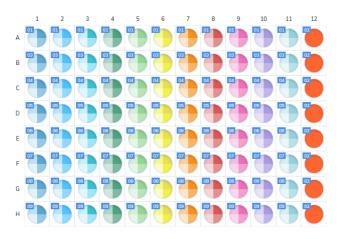
Import the Sample Setup sheet (.csv) generated by TRACE Analysis[™] Software by selecting "Plate layout" tab and "CSV import/export".

Select "Import from CSV".

Pate List	(*) CSV Insertilescen	
1 2 3 4 5 6 7 8 9 30 11 12	Active selectors: - Selected wells: 0	
• • • • • • • • • • • • • • • • • • •		
	Import from CSV	\otimes
	Warning: Importing a CSV file will overwrite the existing layout configuration including all reaction mixes and samples	
· 00000000000		
• <u> </u>	Choose a file or drag it here	
	Concel Im	nport

Import the plate setup file by clicking on "Import".

Inspect if all your selected samples are represented on the Plate figure.



Select "Done" to finish the setup. Start the run.

Thermal Cycling and Imaging Protocol for QIAcuity

The DigitalTRACE[™] System will deliver optimal results when the following thermal profile is used in dPCR:

Number of cycles	Temperature	Time
1	95 °C	3 min
40	95 °C	15 s
40	60 °C	30 s

Use the following imaging parameters:

Channel	Exposure duration	Gain
Green	500 ms	6
Yellow	500 ms	6
Orange	Off	Off
Red	300 ms	4
Crimson	400 ms	4

Genotyping Test Protocol - Biorad

Change the instrument type in the Preferences of the TRACE Analysis[™] Software to Biorad QX-200 and Plate type to MultiTRACE, v3.

To generate a new record in TRACE Analysis[™] Software, in the Section labeled "Recipient" enter the Recipient Name, Recipient Identifier and a unique Sample Identifier. While "Date of Birth" is an optional field for all samples, a "Date of Transplant" must be entered, if you ultimately want to have the data stored and reported in a temporal manner.

Re	cipient 🗸 🗠
Recipient First Name	
Recipient Last Name	
Recipient ID	
Sample ID	
Concentration ng/ul	100
Date of Birth	XX-XX-XXXX 15
Date of Transplant	XX-XX-XXXX IS
Gender	O Male O Female
Comment	
Disease Type	~
D	lonor 🗸 🗙
Donor First Name	
Donor Last Name	
Donor ID	
Sample ID	
Concentration ng/ul	100
Date of Birth	XX-XX-XXXX 15
Gender	O Male O Female
Comment	

For a sample which should be genotyped against the Recipient sample, enter the Donor Name, Donor Identifier and unique Sample Identifier. You can comparatively genotype up to four samples on a single plate using TRACE Analysis[™] Software. TRACE Analysis[™] Software also allows you to virtually compare multiple samples, independent of when they were genotyped. Based on the data from verification studies, JETA Molecular recommends the use of 10 ng DNA input per well for genotyping. (DNA inputs are customized in the software's Preferences for Concentrations). Enter the concentrations of your samples to let the software calculate with. Once all samples to be comparatively genotyped are entered into the Sample window, press the "Screen" button to add the samples to the plate set up file. You will see the samples now added to the 96-well plate in the middle of the screen.

Sample Layout View for 4 Samples being genotyped in the multiplexed plate:

		<u> </u>										
	1	2	3	4	5	6	7	8	9	10	11	12
A	Tim	Tim	Tim	Bert	Bert	Bert	Ken	Ken	Ken	Jenny	Jenny	Jenny
	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	Mix028	Mix036	Mix044	Mix028	Mix036	Mix044	Mix028	Mix036	Mix044	Mix028	Mix036	Mix044
B	Tim	Tim	Tim	Bert	Bert	Bert	Ken	Ken	Ken	Jenny	Jenny	Jenny
	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	Mix029	Mix037	Mix045	Mix029	Mix037	Mix045	Mix029	Mix037	Mix045	Mix029	Mix037	Mix045
С	Tim	Tim	Tim	Bert	Bert	Bert	Ken	Ken	Ken	Jenny	Jenny	Jenny
	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	Mix030	Mix038	Mix046	Mix030	Mix038	Mix046	Mix030	Mix038	Mix046	Mix030	Mix038	Mix046
D	Tim	Tim	Tim	Bert	Bert	Bert	Ken	Ken	Ken	Jenny	Jenny	Jenny
	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	Mix031	Mix039	Mix047	Mix031	Mix039	Mix047	Mix031	Mix039	Mix047	Mix031	Mix039	Mix047
E	Tim	Tim	Tim	Bert	Bert	Bert	Ken	Ken	Ken	Jenny	Jenny	Jenny
	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	Mix032	Mix040	Mix048	Mix032	Mix040	Mix048	Mix032	Mix040	Mix048	Mix032	Mix040	Mix048
F	Tim	Tim	Tim	Bert	Bert	Bert	Ken	Ken	Ken	Jenny	Jenny	Jenny
	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	Mix033	Mix041	Mix049	Mix033	Mix041	Mix049	Mix033	Mix041	Mix049	Mix033	Mix041	Mix049
G	Tim	Tim	Tim	Bert	Bert	Bert	Ken	Ken	Ken	Jenny	Jenny	Jenny
	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	Mix034	Mix042	Mix050	Mix034	Mix042	Mix050	Mix034	Mix042	Mix050	Mix034	Mix042	Mix050
Η	Tim 1001 Mix035	Tim 1001 Mix043		Bert 1002 Mix035	Bert 1002 Mix043	NTC RNaseP		Ken 1003 Mix043	NTC RNaseP		Jenny 1004 Mix043	NTC RNaseP

Once the samples have been added virtually to your plate, and the experiment name have been

entered, press the "Export Setup to PCR" button.

Browse to the location where you want the file saved and name it as you wish. This file can then be imported into your dPCR machine's DigitalTRACE template to execute the dPCR analysis. Once the file is saved, TRACE Analysis[™] Software generates a protocol, based on the experimental inputs and the settings in the preferences menu. Print out this protocol.

2x ddPCR Supermix

- Set up all reactions in a pre-PCR lab, under ambient conditions without ice.
 Open a MultTRACE® Genotyping Plate Pack and remove the genotyping plate.
 Label the genotyping plate with the genotyping test name.
 Collect the four DNA samples to be screened, as well as 2x ddPCR Supermix Master Mix and de-ionized H₂O. Sriefly vortex and centrifuge all tubes before opening.
 Label five LS ml microcentrifuge tubes:
- - a) Sample 1 Mix b) Sample 1 Mix c) Sample 2 Mix c) Sample 3 Mix d) Sample 4 Mix e) NTC Mix
- 6. For each sample to be genotyped, prepare a ddPCR Mix containing sample DNA, de-ionized H₂O and 2x ddPCR Supermix as suggested by TRACE Analysis⁴⁴ Software in Table 1. A No Template Control (NTC) Mix is prepared with de-ionized H₂O and 2x ddPCR Supermix.

Table 1. Master Mix Composition Sample 1 dPCR Mix 1 x 27 x 2x ddPCR Supermix 1001 DNA ermix 11,0 μl 297,0 μl 0,5 μl 13,5 μl Ногона 0,5 µг 13,5 µг H₂O 10,5 µг 283,5 µг 22,0 µг 594,0 µг Sample 2 dPCR Mix 1 x 27 x 2x ddPCR Supermix 11,0 μl 297,0 μl 1002 DNA 0,5 μl 13,5 μl H₂O 10,5 µl 283,5 µl 22,0 µl 594,0 µl Sample 3 dPCR Mix 1 x 27 x 2x ddPCR Supermix 11,0 µl 297,0 µl 1003 DNA 0,5 µl 13,5 µl H₂O 10,5 µl 283,5 µl 22,0 µl 594,0 µl Sample 4 dPCR Mix 1 x 27 x 2x ddPCR Supermix 1004 DNA 11,0 µl 297,0 µl 0,5 µl 13,5 µl H₂O 10.5 ul 283.5 ul 22,0 µl 594,0 µl NTC dPCR Mix 1x 5x

	H ₂ O	11,0 µl	55,0 µl
		22,0 µl	110,0 µl
7.	Vortex each tube to thoroughly mix the con-	tents and cent	trifuge briefly to collect the reaction mix at the
	bottom of the tube.		
8.	Remove the adhesive cover from the genoty	/ping plate.	
9.	Dispense 22 μl of the Sample 1 Mix into We	lls A1-G3 by co	olumns of the genotyping plate.
10.	Dispense 22 µl of the Sample 2 Mix into We	lls A4-G6 by co	olumns of the genotyping plate.
11.	Dispense 22 μl of the Sample 3 Mix into We	lls A7-G9 by co	olumns of the genotyping plate.
12.	Dispense 22 µl of the Sample 4 Mix into We	ls A10-G12 by	y columns of the genotyping plate.
13.	Dispense 22 µl of the 5X PCR Master Mix/NT	C mixture to v	wells H3, H6, H9, H12.
14.	A repeat pipettor is recommended to minin	nize pipetting	g repetition and increase accuracy.
15.	Refer to the DNA Sample Layout Plate Layou	it at the end of	of the protocol.
16.	Visually inspect plate wells from the sides an	nd bottom to c	confirm consistent volume.
17.	Seal the plate completely with MicroAmp® (Optical Adhesiv	ive Film using the MicroAmp® Adhesive Film
	Applicator.		
18.	IMPORTANT! Vortex the plate to mix the co	ontents of eac	ch well.Centrifuge the plates briefly using a plate
	centrifuge to collect the contents at the bott	tom of the wel	ells.
19.	Remove the Adhesive cover very carefully.		
20.	!!!The following steps from 21 to 26 are on	ly for manual (droplet generator users:
21.	Transfer 20 µl of each prepared sample to the	ne sample well	lls (middle row) of the DG8 cartridge.
22.	Add 70 µl of droplet generation oil to each o	il well of the D	DG8 cartridge.
23.	Hook the gasket over the cartridge holder u	sing the holes	on both sides.
24.	Load the cartridge in the QX200 droplet gen	erator.	
25.	When droplet generation is complete, remo	ve the disposa	able gasket from the holder and discard it.
26.	Pipet 40 µl of the contents of the droplets in	nto a single col	olumn of a 96-well PCR plate.
27.	Seal the PCR plate with foil plate seals that a	are compatible	e with the PX1 PCR plate sealer and the needles
	in the QX200 droplet reader.		
28.	Place the plate into the thermal cycler for Pl	CR amplificatio	on.
29	Load the plate after amplification into QX20	0 droplet read	der.
	Import the Sample Setup sheet generated b	y TRACE Analy	ysis™ Software.

11,0 µl 55,0 µl

Thermal Cycling Protocol for Biorad

The DigitalTRACE[™] System will deliver optimal results when the following thermal profile is used in dPCR:

Number of cycles	Temperature	Time	Ramp Rate
1	95 °C	10 min	2 °C/s
40	94 °C	30 s	2 °C/s
40	59 °C	60 s	2 °C/s
1	98 °C	10 min	2 °C/s

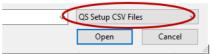
Biorad Droplet Reader Setup in QuantaSoft

To read the signal after the PCR cycling was completed, setup an experiment in QuantaSoft. Load a template by clicking on Load

QTRACE and DigitalTRACE Analysis System Operator's Manual

QuantaSoft Version 1.	7.4.0	917						
BIO RAD				0	ptions			
BIOTAD	l r F	tup Plate	Experiments					
1		🚰 Load 🚽 Save As	ABS					
Setup	i Fi	lemplate	CNV1 CNV2					
	6	New Load	ABS RED CNV1 CNV2 CNV2 CNV3 CNV4 GEX					
000		Save As	New Edit Remove					
Run	•	01	02	03	04	05	06	
Analyze	*	8	Β	8		8		8
C	_							
About	в	8	9	9		9	9	H
Contact Support	-							
	с	8	8	8		8		8
XX	D	8	8	8	8	8		8
YY								
	E	8	8	8		8		8
Y								

Select QS Setup CSV Files as file format



												– 0 ×
	230000 Biorad typing		c	Optiona								
BIO RAD	Setup Plate	Experiments										
Setup	Save As Template New	ABS RED CNV1 CNV2 CNV3 CNV4 GEX										
Bun	😭 Load 📓 Save As	CNV4 GEX New Edt Remove										
10211	* 01 1001	02	03	04	05	06	07	08	09	10	1004	12
Analyze	A85 923 A 10 167	A85 128 1982	A85 678 1777	1002 ▲ A85 923 107	A85 158 158 162	1002 ▲ A85 ● 678 ■ 777	AB5 222 1017	AB5 128 138	A85 421 1777	A85 923 917	A85 158 192	ADS ↓ 122 ↓ 777
Ċ	1001	1001	1001	1002	1002	1002	1003	1003	1003	1004	1004	1004
	B U 252	■ 485 ■ 233 ■ 222	A88 985 997	A85 900 252	203 222	NAA2 A85 € 505 € 901	A85 900 1212	A65 233 222	A&S 555 0 971	A65 900 102	A65 233 222	A68 505 101
Contact Support	1001 433 C U 006	1001 A&S 037 037	1001 AES 102 004	1002 Alis 433 530	1002 Alasi 537 630	1002 Add 102 102 024	1003 ABS 433 100 200	1003 Alis 557 U 470	1003 Add 102 U 024	1004 AEG 453 256	1004 Alia 397 100	1004 ABS 102 454
	1001	1001	1001	1002	1002	1902	1003	1903	1003	1004	1004	1004
	D 120	235 211 2215	176 176 177	ABS 254 251 250	205 721	₩445 178 178 178 567	A2G 834 120	₩445 235 211	ALG 138 138 137	ADG 851 120	AEG 233 721	Ang 138 138
AA	1001 A85 176 E 137	1001 ABS 450 275	1001 ADS 884 482	1002 ABS 2115 115 1157	1002 ABS 450 275	1002 ABS 884 482	1003 ABS 785 137	1003 ABS 450 273	1003 ABS 884 482	1004 A05 216 117	1004 ABS 450 1273	1004 ADS 884 482
A												
S	F U 681	1001 A85 110 1748	1001 A55 112 155 155	1002 A55 112 123	1002 A85 710 745	1002 A55 112 135	1003 AB5 112 112 681	1003 A55 710 1745	1003 A55 112 155	1004 A05 182 031	1004 A855 213 145	1004 A85 112 133 1355
4	1001 A85 002 01 01 02	1001 A85 472 134	1001 A88 127 RNaseP	1002 A85 922 396	1002 A85 472 574	1002 ASS 127 RNaseP	1003 A85 822 196	1003 A85 472 534	1003 A85 137 RotaseP	1004 A85 02 105	1004 A85 422 536	1004 A85 117 PhaseP
									1			M
D	1001 ABS 663 H 441	1001 A85 824 916	NTC ABS RhaseP	1002 ABS 063 U 441	1002 Alas 204 205	NTC AES RolaseP	1003 ABS 053 H1	1003 A65 024 024	NTC AES RNaseP	1004 ABS 653 441	1004 A60 004 919	ABS PrimeP

Start the droplet reading run.

Genotyping Data Analysis and Report

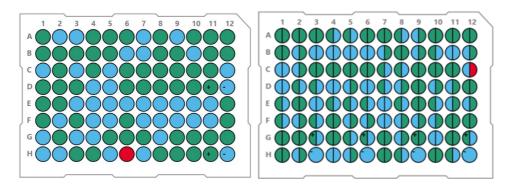
The QTRACE[®] Analysis System

Import qPCR data by clicking the "Import qPCR Data" button .

Browse to the location of your exported qPCR data file and select it. TRACE Analysis[™] will import the data.

TRACE Analysis[™] will perform a quality analysis on the data and will present the data in the plate view. The image below shows a genotyping result for a standard (left) and multiplexed (right) genotyping plate. There are three quality scores given to genotyping data: 1) positive, 2) negative and 3) atypical. These values are represented in the plate image by three different





Dark green sections represent data scored as truly positive for a sample.

Light blue sections represent data scored as truly negative for a sample.

Red sections represent data scored as atypical for a sample. (Atypical assay results will exclude the assay from consideration as a potentially informative assay for all samples grouped in the analysis).

For more information on the Genotyping Algorithm used by TRACE Analysis[™] Software, go to the <u>Data Analysis Algorithms</u> Section of this manual.

After inspecting the quality of the data, pressing the "Calculate" Calculate button makes TRACE Analysis[™] perform comparative genotyping analysis. It will determine and display markers which are informative for all samples in a group.

TRACE Analysis[™] displays each assay which was informative for a single sample in the group being compared.

TRACE Analysis[™] also displays the chromosomal location of the informative assays, as well as the positive or negative status of the assays for visual inspection.

The report generated from a QTRACE[®] or DigitalTRACE[™] Genotyping experiment may be sorted to provide a custom view of the data.

							Eve	vori	ment r	eno				
								2611	internet	epo				
cope:	 Entir 	e experiment										Column	Order	
	O Tran	plantation	Samuel	Haskell							Sort by:	Informative ~	Descendin	n ·
	Full											Marker	Ascending	
Format:											Then by:	Locus	Ascending	9
	O Sum	marized										Informative for		
												InfoCq	4	
Save as												Delta Cg	I	
												Detta Cq	_	
🖌 🗋 🔍	. 🔍 🛄	8 🛛 🖾												
												_		-
		· · ·	Used Ass	ioys										
		1	Rasary Lo	cus informative for			Recipient 1			Cq				
				2q Samuel Haskell		-0.2		27,5						
				4g Samuel Haskell 7p Samuel Haskell		0.75	Positive Positive	28,4		40,0				
				7p Samuel Haskell g Samuel Haskell		2.26	Positive	30,0		40,0				
				Do Mud Haskel	26.5		Negative	40.0		26.9				
		I L				0.64				-				
			145 1	7g Mud Haskell	27.2		Negative	40,0	Positive	27,3				
		I - F	854 1	20 Mud Haskel		0.23	No. of Co.	40,0	Positive	27,7				
				2g Mud Haskell g Mud Haskell		0.17	Negative Negative	40.0		25,7				
			469 7			0.57	Negative	40,0		28,1				
			768 1	Sq Mud Haskell		0.75	Negative	40,0		28,3				
				Sq Mud Haskel		0.79	Negative	40,0		28,3				
				Sq Mud Haskell		0.9	Negative	40,0		28,4				
				Mud Haskel Mud Haskel		0.96	Negative Negative	40,0		28,5				
			235 2			1.49	Negative	40,0		29,0				
			137 1			1.63		40,0		29,2				
			240 8				Negative	40,0		40,0				
				3g None	- t-	· .	Negative	40,0		40,0				
				p None 7p None			Positive Negative	27,8		28,4				
				7p None	1	1	Negative	40,0		40,0				
				p None	1.		Negative	40,0		40,0				
				g None			Negative	40.0		40.0				

The DigitalTRACE[™] Analysis System

Genotyping Data Analysis and Report - QIAcuity

After the QIAcuity run has finished, check in the QIAcuity Software Suite if the automatic thresholds are correct. Adjust the thresholds manually if needed.

A1	81	CI	D1	61	F1	G1	6 S
		· Sauce As		1.1.1.1.2.5			
		和资料和新闻		a station water		and the second	
				Charles and		122 1 1 2	
			-				
					1.000	1.041	
ust la d	And Street Street		and interesting		di minidati		checks with 1992
			whether it is a second of the				

Select all wells on the plate and analyze per target (not per channel), export data by selecting Export to CSV.

List	Signalmap Heatmap	Histogram 1D Scatterplot 2D	Scatterplot Concentrati	on diag	ram						
								Add to repor	t 🗌 Show mean	values for replicates (Export to CSV
	Sample/NTC/Control	Reaction Mix	Target	IC	Control type	Concentration * copies/µL	CI (95%)	Partitions valid	positive	negative	Threshold
			d681		-	0.000		8191	0	8191	30.86
A1	01 c105 221010	QIAMix001	d971	+		0.372	147.5%	8191	1	8190	53.81
		- dominant	d113			0.000		8191	0	8191	20.66
			d597			149.4	9.9%	8191	392	7799	24.86
			d777			0.376	147.5%	8271	1	8270	21.93
A2	01 c105 221010	QIAMix002	d396			0.000		8271	0	8271	34.68
	N	An damagar	d892			0.000		8271	0	8271	21.93
			d333			180.2	9.1%	8271	466	7805	24.23
											cla

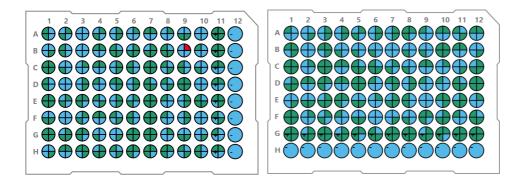
Import dPCR data to TRACE Analysis[™] Software by clicking the "Import PCR Data" [™] button. Browse to the location of your exported dPCR data file and select it.

TRACE Analysis[™] Software will perform a quality analysis on the data and will present the data in the plate view.

There are three quality scores given to genotyping data: 1) positive (green), 2) negative (blue) and 3) atypical (red).

These values are represented accordingly in the plate image by three different colors:





An atypical assay results will exclude the assay from consideration as a potentially informative assay for all samples grouped in the analysis.

After inspecting the quality of the data, pressing the "Calculate" ^{Calculate} button makes TRACE Analysis™ perform comparative genotyping analysis. It will determine and display markers which are informative for all samples in a group.

Press the "Report" Report... button to generate the Genotyping Report

TRACE Analysis[™] Software displays each assay which was informative for a single sample in the group being compared, and it also displays the chromosomal location of the informative assays, as well as the positive or negative status of the assays for visual inspection.

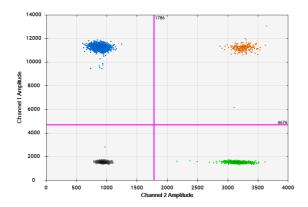
The report generated from a TRACE Analysis[™] Genotyping experiment may be sorted to provide a custom view of the data.

ReportWindow																-		2
						Exper	ime	nt re	port									
Kope:	Entire experi							_							Colum		Order	_
																-		
0	Transplantati	on Tom												Sort by:	Locus	w	Descend	dine
Format:	Full													Then by:	Marke	r	Ascendi	na
	Summarized													· · · · · · · · · · · · · · · · · · ·	Locus			
<u> </u>	Summarized														Inform	native for	1	
-															InfoC	9		
Save as															Delta	Cq		
0.0.0																-		
015 51		Recipient			Don											_		-
		Name:	Tom		Nam		Ale											
		ID:	45458		ID:			4654										
		Gender:	Unknown		Gen	der		known										
		Disease ty				of birth:												
		Date of bi			Corr	ments:	-											
		Transplant																
		date:																
		Comment	F: -															
		Used Assoy Assay Locu	s Informative for	Recipient	ow	Concen- P tration t		Partition (valid)	Donor 1	aw	Concen-		Partitions (valid)					
		d721 Xq	None	Positive	0.9	163,40	424		Positive	1 10	221.70	545	(want) 8207					
		d333 9q		Positive		180,20	466	8271	Negative	0,0		0						
		6472 99	Tom	Positive		141,90	369	\$247	Negative	0,0		2	8240					
		d113 8q d252 8q	None	Negative	0,0		0		Negative Negative	0,0		0						
		d252 Bq d936 Bp	None	Negative Negative	0,0		0	8224	Negative	0.0		0	8256					
		d198 7q	Tom	Positive		187,80	482	\$260	Negative	0,0		0						
		d990 7q	Tom	Positive	1,1		487	\$239	Negative	0,0		0	8283					
		d567 7p d892 6q	None	Positive Negative	1,1	0.00	500 0	8259 8271	Positive Negative	1,1		623	8266					
		d971 6g	Alex	Negative	0.0		1	8191	Positive	1.0		\$76	8224					
		d222 6p	Tom	Positive	1,0	172,20	443	\$242	Negative	0,0	0,00	0	8283					
		d795 5q	None	Negative	0,0		1	8205	Negative	0,0		3	8275					
		d176 5p d694 4p	None Alex	Positive Negative	0,8	141,70	369	8277 8214	Positive Positive	0,9	205,90	\$10 1193	8204 8253					
		d120 20	Tom	Positive		290,10	976	8224	Negative	0.0		1193	8253					
			Alex	Negative	0,0		0	8259	Positive		233,50	\$76	8266					
		d574 2g																
		d574 2q d678 2q d585 2p	Alex	Negative Positive	0,0		0 460		Positive Negative	1,1	245,50	596	8234 8256					

To create an anonymized report, go to the <u>Anonymized Reporting</u> Section.

Genotyping Data Analysis and Report - Biorad

After the reading process has finished, check in the QuantaSoft Software if the automatic thresholds are correct. Adjust the thresholds manually if needed.



Select all wells on the plate and export data by selecting Export CSV.

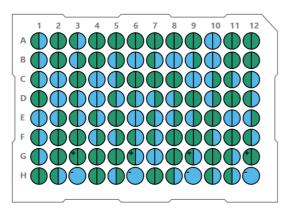
Import dPCR data to TRACE Analysis[™] Software by clicking the "Import PCR Data" [™] button. Browse to the location of your exported dPCR data file and select it.

TRACE Analysis[™] Software will perform a quality analysis on the data and will present the data in the plate view.

There are three quality scores given to genotyping data: 1) positive (green), 2) negative (blue) and 3) atypical (red).

These values are represented accordingly in the plate image by three different colors:





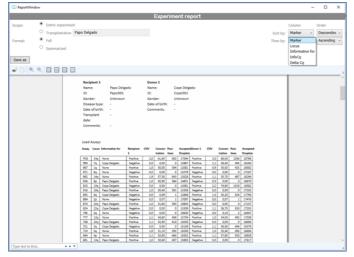
An atypical assay results will exclude the assay from consideration as a potentially informative assay for all samples grouped in the analysis.

After inspecting the quality of the data, pressing the "Calculate" Calculate button makes TRACE Analysis™ perform comparative genotyping analysis. It will determine and display markers which are informative for all samples in a group.

Press the "Report" Report... button to generate the Genotyping Report

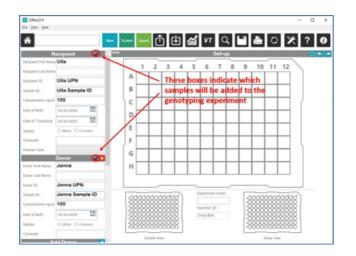
TRACE Analysis[™] Software displays each assay which was informative for a single sample in the group being compared, and it also displays the chromosomal location of the informative assays, as well as the positive or negative status of the assays for visual inspection.

The report generated from a TRACE Analysis[™] Genotyping experiment may be sorted to provide a custom view of the data.

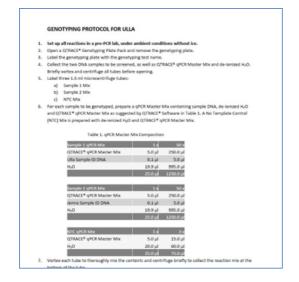


Multiple Donor Analysis Using QTRACE Plates

Enter Recipient and Donor 1 Sample Information, as you would normally. Note: the check boxes always indicate which samples will be added to the experiment.



Following the normal setup and export steps, TRACE Analysis[™] will make a protocol for typing both samples on one plate.



When the data is imported and approved,



TRACE Analysis[™] generates a report for the first pair.

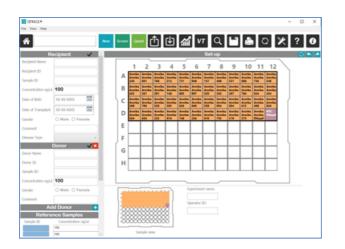
				Chi	meri	sm G	enotypin	g - F	ull Repo	ort
Experin	nent name	e:	Recipient	and Do	nor 1 G	ienotyp	ing			
Experin	nent date:		24 March	2017						
Data fo	Ider:		C:\Users\	Doug\D	esktop'	QTRAC	E\Transplan	tation		
Operati	or name:		Doug							
Recipie	nt				Donor					
Name:		Ulla			Name:	-	Jonna			
ID:		Ulla UF	N		ID:		Jonna UP	N		
Gender		Female			Gende	-	Female			
Disease		- subarc			Comm		remare			
Date of		- 10 July	2017		comm	ensi				
Comme	lant date:	10 July	201/							
Used A		Informat	ine for	halas a		Ma	Recipient	0	Over 1	64
Assay 1	Locus	Informat	ive for	InfoCq		ACe	Recipient	_	(Donor 1	Cq
Assay 1 548	Locus Χρ	Ulla Ulla	ive for	InfoCq 26.64 26.89		0 0.25	Recipient Positive Positive	26.6	Negative	6q 40.0
548 148	Locus	Ulla	ive for	26.64		0	Positive	26.0		40.0
Assay 548 148 601	Xp 17q	Ulla	ive for	26.64		0	Positive Positive	26.0 26.1 27.1	Negative	40.0
Assay 548 148 601 504 408	Xp 17q 14q 17p 2q	Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19		0 0.25 0.44 0.51 0.56	Positive Positive Positive Positive Positive	26.0 26.1 27.1 27.1 27.1 27.1	Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 408 948	tocus Xp 17q 14q 17p 2q 1p	Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29		0 0.25 0.44 0.51 0.56 0.66	Positive Positive Positive Positive Positive	26.0 26.1 27.1 27.1 27.1 27.1	Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 408 948 425	tecus Xp 17q 14q 17p 2q 1p 6q	Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32		0 0.25 0.44 0.51 0.56 0.66 0.69	Positive Positive Positive Positive Positive Positive	26.0 26.1 27.1 27.1 27.1 27.1 27.1	Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 405 948 425 634	tecus Xp 17q 14q 17p 2q 1p 6q 11q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78	Positive Positive Positive Positive Positive Positive Positive	26.4 26.1 27.1 27.1 27.1 27.1 27.1 27.1 27.1	Negative Negative Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 548 548 601 504 408 548 425 634 784	xp 17q 14q 17p 2q 1p 6q 11q 10q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32 27.41 27.57		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.78 0.94	Positive Positive Positive Positive Positive Positive	26.4 26.1 27.1 27.1 27.1 27.1 27.1 27.1 27.1 27	Negative Negative Negative Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 4 548 148 601 504 408 948 425 634 784 240	tecus Xp 17q 14q 17p 2q 1p 6q 11q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78	Positive Positive Positive Positive Positive Positive Positive Positive Positive	26.0 26.1 27.1 27.1 27.1 27.1 27.1 27.1 27.1 27	Negative Negative Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 548 601 504 405 948 425 634 784 240 768	Xp 17q 14q 17p 2q 1p 6q 11q 10q 8q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32 27.41 27.57 27.62		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.94 0.39	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive	26.4 26.1 27.1 27.1 27.1 27.1 27.1 27.1 27.4 27.4 27.4 27.4 27.4 27.4 27.4 27.4	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 408 948 425 634 425 634 240 768 356 515	tocus xp 17q 14q 17p 2q 1p 6q 11q 10q 8q 18q 18q 18q 18q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.32 27.32 27.57 27.62 28.19 29.15 25.09		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.54 0.59 0.78 0.54 0.59 1.56 2.52 -0.7	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Negative Negative	26.4 26.1 27.1 27.1 27.1 27.1 27.1 27.1 27.1 27	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 408 948 425 634 425 634 784 240 768 356 519 520	tocus xp 17q 17q 14q 17p 6q 11q 10q 8q 18q 20q 20q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32 27.41 27.57 27.62 28.19 29.15 29.15 25.09 25.29		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.54 0.59 1.56 2.52 -0.7 -0.51	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Negative Negative	26.4 26.1 27.1 27.1 27.1 27.1 27.1 27.1 27.4 27.4 27.4 27.4 27.4 28.1 29.1 29.1 40.0 40.0	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 408 948 425 634 425 634 240 784 356 519 520 226	totus xp 17q 17q 14q 17p 2q 1p 6q 11q 10q 8q 18q 18q 18q 6q 6q 6q 6q 6q 6q 6q 6q 6q 6	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32 27.41 27.57 27.62 28.19 29.15 25.09 25.29 25.77		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.94 0.99 1.56 2.52 -0.7 -0.51 -0.02	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Nagative Nagative	26.4 26.1 27.1 27.1 27.1 27.1 27.1 27.4 27.4 27.4 27.4 27.4 27.4 27.4 27.4	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Positive Positive	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 405 948 425 634 240 768 356 515 515 520 326 721	totus xp 17q 14q 17p 2q 1p 6q 11q 10q 8q 18q 18q 18q 20q 6q 20q 6q 23q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	28.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32 27.32 27.41 27.57 27.62 28.19 29.15 25.09 25.29 25.77 26.27		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.99 1.56 2.52 -0.7 -0.51 -0.02 0.48	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Negative Negative Negative	264 261 273 273 273 273 273 273 274 274 274 274 283 293 400 400 400 400	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Positive Positive Positive	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 405 948 425 634 425 634 280 768 356 515 515 515 520 126 721 819	torus Xp 17q 14q 17p 2q 1p 6q 11q 10q 10q 10q 10q 20q 20q 20q 20q 20q 20q 21q 14q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	ive for	28.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32 27.32 27.41 27.57 27.62 28.19 29.15 25.09 25.29 25.29 25.77 26.27 26.59		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.94 0.94 0.99 1.56 2.52 -0.7 -0.51 -0.02 0.48 0.79	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Negative Negative Negative Negative	26.6 27.3 27.3 27.3 27.3 27.3 27.3 27.4 27.4 28.2 29.3 40.0 40.0 40.0 40.0 40.0	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Positive Positive Positive	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 148 601 504 408 348 425 634 284 784 284 784 284 784 286 515 520 326 515 520 326 515 520 326 515 520 326 523 326	terus Xp 17q 24 17p 24 17p 64 17p 64 18q 18q 18q 18q 20q 64 20q 64 20q 20q 214 214 20q 20q 214 214 20q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	live for	26.64 26.89 27.07 27.14 27.19 27.32 27.32 27.41 27.57 27.62 28.19 29.15 25.29 25.29 25.29 25.29 25.59 25.59 26.68		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.99 1.56 0.99 1.56 2.52 -0.7 -0.51 -0.02 0.48 0.79 0.88	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Negative Negative Negative Negative Negative	26.6 26.5 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Positive Positive Positive Positive Positive	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 1 548 548 601 504 408 948 425 634 784 240 768 356 515 520 326 520 326 723 835 803 748	torus Xp 17q 14q 17p 2q 1p 6q 11q 10q 10q 10q 10q 20q 20q 20q 20q 20q 20q 21q 14q	Ulla Ulla Ulla Ulla Ulla Ulla Ulla Ulla	live for	28.64 26.89 27.07 27.14 27.19 27.29 27.32 27.32 27.32 27.41 27.57 27.62 28.19 29.15 25.09 25.29 25.29 25.77 26.27 26.59		0 0.25 0.44 0.51 0.56 0.66 0.69 0.78 0.94 0.94 0.99 1.56 2.52 -0.7 -0.51 -0.02 0.48 0.79	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Negative Negative Negative Negative	26.6 26.5 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Positive Positive Positive	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0
Assay 548 148 600 504 408 948 425 948 425 948 425 524 784 240 784 240 768 356 515 515 515 515 515 515 515 515 515 5	terus Xp 17q 14q 17p 2q 1p 2q 1p 6q 11q 10q 8q 18q 20q 20q 20q 20q 20q 21q 21q 20q 20q 20q 20q 20q 20q 20q 20q 20q 20	Ulta Ulta Ulta Ulta Ulta Ulta Ulta Ulta	ive for	26.64 26.89 27.07 27.14 27.19 27.29 27.32 27.41 27.57 27.62 28.19 29.15 25.29 25.29 25.29 25.29 25.57 26.68 26.81		0 0.25 0.44 0.51 0.56 0.56 0.56 0.56 0.59 0.78 0.54 0.59 1.56 2.52 -0.7 -0.51 -0.51 0.48 0.79 0.88 1.01	Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Positive Negative Negative Negative Negative Negative Negative	26.4 26.5 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Positive Positive Positive Positive Positive Positive	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0

When the second donor is to be analyzed, select the recipient's record in the sample search window next to the home button. The samples which have already been genotyped will appear as they did when initially entered into TRACE Analysis[™] Software. Notice that the check boxes by default are not checked, as these samples have already been genotyped by the software.

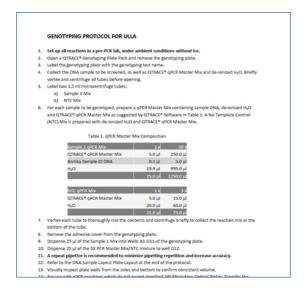
If you click the Add Donor 🛨 button, a new set of fields appear for anew sample record to be associated with the same recipient sample.

CRACET								-
in You links								
4		score Ourt	dî l	t) 🚮 🗸		-		2
•							<u> </u>	<u>`</u>
		- 1090		Set	up		_	
Incipient First Name	Ulla	1	2 3 4	5 6	7 8	9 10	11 12	,
technierit Last Name	Ulla UPN	A				1		ì.
belgier# (D	Ulla Sample ID							
iangia ID		B		Boxes are				1
Secondarian regist			defau	lt, as their	sampl	e typin	-	4
Nex of Dethi		C	data is	approve	d			L
Not of Transplant.					++	\rightarrow	\rightarrow	1
iender	O Male O Female	D	1					L
annest		E T						1
Sistace Type			Only t	nis sample	will be	e added	to	
(econd Date of Transplant	xa-xa-xaxax 🕅 🍃	F	/ the ge	otyping e	experin	nent, bi	ıt	1
	Donor Of			a will be				4
bosor Parst Marine	Jonna	G	this re			~		L
Dovor Last Name		н	this re	pient	++	\rightarrow	-	1
CE tored	Jonna UPN	7						
ampio 3D	Jonna Sample ID							
Concentration regist								_
ten of liters	x0x-30x-30000x	-0000	10000000000	Expensed as	ane:	-00000	100000	50
Sender	O Mole O Female		88888888 /	Operator 10x		12000	*****	8
Comment			888888888	Deup Bout		38888	*****	X
	Donor Q	· 2000	. 2000000			133333		8
Donor First Name			Sangle vev					_
Notor Last Name	Last Name		President reser				nay ven	

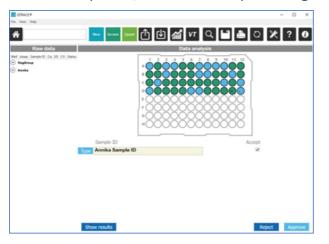
Enter the new sample information for the second donor, as well as the Date of the Second Transplant. When the Screen button is clicked, only the new sample is added to the experiment.



TRACE Analysis[™] will now generate a genotyping protocol for only the second donor.



When data is imported, one half of the plate image will be colored.



After data Approval, TRACE Analysis[™] will display informative assays for the combinations of samples associated with the recipient sample.

Marker 240 356	Chr. location	Informative for			
	0		Ulla	Jonna	Annika
356	8q	Ulla	Positive	Negative	Negativ
	18q	Ulla	Positive	Negative	Negativ
469	7p	Jonna	Negative	Positive	Negativ
504	17p	Ulla	Positive	Negative	Negativ
520	20q	Jonna	Negative	Positive	Negativ
531	17p	Jonna	Negative	Positive	Negativ
634	11q	Ulla	Positive	Negative	Negativ
736	Xp	Annika	Negative	Negative	Positive
768	18q	Ulla	Positive	Negative	Negativ
819	14q	Jonna	Negative	Positive	Negativ
854	12q	Annika	Negative	Negative	Positive
954	6q	Annika	Negative	Negative	Positive

Clicking the data overview button shows the genotyping results for all assays.

QTRACE®						
^		New Screen	Quant			
P	ecipient			Туре		
Recipient Name	Ulla	Chr. location	Informative for	Ulla	Jonna	Annika
Recipient ID	Ulla UPN	10	None	Negative	Negative	Negativ
		170	None	Positive	Negative	Positive
Sample ID	Ulla Sample ID	59	None	Positive	Positive	Positive
Concentration ng/ul	100	29	None	Positive	Positive	Positive
Date of Birth	7/10/2017	8g	Ulla	Positive	Negative	Negativ
Date of Transplant	7/10/2017	4q	None	Negative	Positive	Positive
Gender	O Male @ Female	Sq	None	Positive	Positive	Negative
Comment		20q	None	Positive	Positive	Positive
Disease Type		18q	None	Negative	Negative	Negativ
	Donor x	6q	None	Negative	Positive	Positive
Donor Name	Jonna	1p	None	Negative	Negative	Negative
	Jonna UPN	18q	Ulla	Positive	Negative	Negative
Donor ID		11p	None	Negative	Positive	Positive
Sample ID	Jonna Sample ID	12q	None	Negative	Positive	Positive
Concentration ng/ul	100	18q	None	Positive	Positive	Positive
Gender	O Male @ Female	2q	None	Positive	Negative	Positive
Comment		6q	None	Positive	Negative	Positive
	Donor ×	3q	None	Positive	Positive	Positive
Donor Name	Annika	7p	Jonna	Negative	Positive	Negativ
Donor ID	Annika UPN	17p	Ulla	Positive	Negative	Negative
Sample ID	Annika Sample ID	8q	None	Negative	Positive	Positive
Concentration ng/ul	100	20q	Jonna	Negative	Positive	Negativ
Gender	O Male @ Female	17p	Jonna None	Negative	Positive	Negative

Custom Genotyping Panels

With TRACE Analysis[™] Software version 1.08, users of TRACE Analysis[™] Software can perform customized genotyping. They can choose which assays they want to use for genotyping experiments. They are able to create, store and use custom genotyping assay panels to perform genotyping using TRACE Analysis[™] Software. This additional functionality requires special license keys to be issued from JETA Molecular. Please contact your local sales representative or JETA Molecular directly for access.

TRACE Analysis[™] will generate unique protocols, based on the experimental set up and it will score the data, as it does for standard QTRACE Genotyping Plates manufactured by JETA. Users who perform off-line genotyping and then assign informative assays in the software, will no longer have to do this.

Genotyping Panels are created using entire rows or columns of a 96-well plate. By default, each panel must contain one positive control and one negative control reaction. The possible number of INDEL assays in a genotyping panel are 6, 10, 14, 22, 30, 34, 38 and 46.

Orientation	Available INDEL Assay Panel Sizes	Plate Throughput
1 Row	10 Assays	8 Samples
2 Rows	22 Assays	4 Samples
3 Rows	34 Assays	2 Samples
4 Rows	46 Assays	2 Samples
1 Column	6 Assays	12 Samples
2 Columns	14 Assays	6 Samples
3 Columns	22 Assays	4 Samples
4 Columns	30 Assays	3 Samples
5 Columns	38 Assays	2 Samples
6 Columns	46 Assays	2 Samples

Under File, there is an option called 'Plate editor'

File	View Help					
	New	Ctrl+N				
	Home	Alt+H		New	Screen	Quan
	Preferences	Alt+P				
	Plate editor	Alt+E				
	Data management	Alt+D				
	Migrate data	Alt+M	R			
	Custom reports	Alt+R				
	Exit					

New plate configurations are created by pressing the 'Add new' button. Give the plate a name.

Choose the desired plate orientation and pick from the available assays to create a custom panel.

PlateEditorWindow				- 0	х
Plate configuration	a la Vincent	 Add ne 	w Delete		
Orientation Rows	v				
102	275	469	650	✔ 819	
113	291	472	663	824	
120	305	482	670	✔ 832	
137	312	493	678	✔ 840	
148	326	504	681	854	
157	333	519	694	874	
176	✓ 345	520	706	884	
183	356	531	710	888	
198	359	548	721	892	
209	361	555	736	907	
222	373	567	748	916	
235	386	574	755	923	
240	396	585	768	936	
252	✔ 408	597	777	✓ 948	
267	425	601	784	✓ 954	
267 N00470	434	615	795	962	
267 010210	441	626	803	971	
267 510450	450	634	803-A3	987	
Save changes	Cancel				

Once a Plate has been saved using the Plate editor, the plate appears as an option in the Plate Configuration menu in the Preferences.

late setup	Custom types	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Machine	for genotypi	ABI 7500	v2.0.6	v				
Machine	for monitorin	ABI 7500	v2.0.6	v				
Layout t	уре			~				
Replicate	25	qPCR Trip	licates ~					
Color mo	ode	Pastel	v Pla	ate for genoty	ping a la Vinc	ent	*	
			Pla	ate for monito	96-low o	density	~	

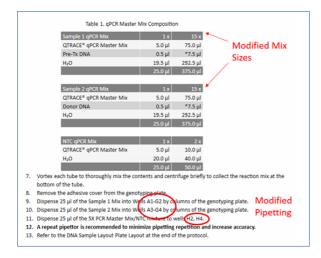
The following is an example of a single donor:patient pair being tested by a 14 member panel with a column configuration:

Sample View:



Assay View:





The software can also combine multiple templates, if needed.

The following is an example of using 3 different column templates in one experiment. The first 2 samples added use 2 columns, the next 2 use 3 column and the last pair use 1 column.

The protocol instructs the user to make 7 different mixes for typing. The mixes vary on size, depending on how many assays are in the panel

Sample 1 qPCR Mix	1 x	15 x	Sample 5 qPCR Mix	1 x	7 x
QTRACE [®] qPCR Master Mix	5.0 μl	75.0 μl	QTRACE [®] qPCR Master Mix	5.0 μl	35.0 μl
180425R6-3 DNA	0.5 µl	*7.5 μl	180425R6-5 DNA	0.5 μl	*3.5 μl
H₂O	19.5 μ Ι	292.5 µl	H₂O	19.5 μl	136.5 µl
	25.0 μl	375.0 μl		25.0 µl	175.0 µl
Sample 2 qPCR Mix	1 x	15 x	Sample 6 qPCR Mix	1 x	7 x
QTRACE [®] qPCR Master Mix	5.0 μl	75.0 μl	QTRACE [®] gPCR Master Mix	5.0 µl	35.0 µl
180425D6-3 DNA	0.5 μl	*7.5 μl	180425D6-5 DNA	0.5 μl	*3.5 µl
H₂O	19.5 μ Ι	292.5 µl	H ₂ O	19.5 μl	136.5 µl
	25.0 µl	375.0 μl	1120	25.0 μl	175.0 μl
Sample 3 qPCR Mix	1 x	23 x			
QTRACE [®] qPCR Master Mix	5.0 μl	115.0 μl	NTC qPCR Mix	1 x	6 x
180425R6-4 DNA	0.5 µl	*11.5 μl	QTRACE [®] qPCR Master Mix	5.0 μ Ι	30.0 µl
H₂O	1 9.5 μl	448.5 μl	H₂O	20.0 µl	120.0 μl
	25.0 µl	575.0 μl		25.0 µl	150.0 µl

and the pipetting instruction respond to the templates being used:

8. Remove the adhesive cover from the genotyping plate.

- 9. Dispense 25 μl of the Sample 1 Mix into Wells A1-G2 by columns of the genotyping plate.
- 10. Dispense 25 μ l of the Sample 2 Mix into Wells A3-G4 by columns of the genotyping plate.
- 11. Dispense 25 μ l of the Sample 3 Mix into Wells A5-G7 by columns of the genotyping plate.
- 12. Dispense 25 μ l of the Sample 4 Mix into Wells A8-G10 by columns of the genotyping plate.
- 13. Dispense 25 µl of the Sample 5 Mix into Wells A11-G11 by columns of the genotyping plate.
- 14. Dispense 25 μl of the Sample 6 Mix into Wells A12-G12 by columns of the genotyping plate.
- 15. Dispense 25 μl of the 5X PCR Master Mix/NTC mixture to wells H2, H4, H7, H10, H11, H12.
- 16. A repeat pipettor is recommended to minimize pipetting repetition and increase accuracy.
- 17. Refer to the DNA Sample Layout Plate Layout at the end of the protocol.

Here is the layout:

	1	2	3	4	5	6	7	8	9	10	11	12
ł	180425R6-3 180425R6-3 291	180425R6-3 180425R6-3 519	180425D6-3 180425D6-3 291		180425R6-4 180425R6-4 137	180425R6-4 180425R6-4 408	180425R6-4 180425R6-4 710	180425D6-4 180425D6-4 137	180425D6-4 180425D6-4 408	180425D6-4 180425D6-4 710	180425R6-5 180425R6-5 137	180425D6-5 180425D6-5 137
3	180425R6-3 180425R6-3 305	180425R6-3 180425R6-3 634	180425D6-3 180425D6-3 305	180425D6-3 180425D6-3 634	180425R6-4 180425R6-4 148	180425R6-4 180425R6-4 519	180425R6-4 180425R6-4 721	180425D6-4 180425D6-4 148	180425D6-4 180425D6-4 519	180425D6-4 180425D6-4 721	180425R6-5 180425R6-5 148	180425D6-5 180425D6-5 148
2	180425R6-3 180425R6-3 312		180425D6-3 180425D6-3 312	180425D6-3 180425D6-3 824	180425R6-4 180425R6-4 209	180425R6-4 180425R6-4 520	180425R6-4 180425R6-4 736	180425D6-4 180425D6-4 209	180425D6-4 180425D6-4 520	180425D6-4 180425D6-4 736	180425R6-5 180425R6-5 209	180425D6-5 180425D6-5 209
C	180425R6-3 180425R6-3 326		180425D6-3 180425D6-3 326		180425R6-4 180425R6-4 345	180425R6-4 180425R6-4 601	180425R6-4 180425R6-4 748	180425D6-4 180425D6-4 345	180425D6-4 180425D6-4 601	180425D6-4 180425D6-4 748	180425R6-5 180425R6-5 235	180425D6-5 180425D6-5 235
	180425R6-3 180425R6-3 345	180425R6-3 180425R6-3 854	180425D6-3 180425D6-3 345	180425D6-3 180425D6-3 854	180425R6-4 180425R6-4 356	180425R6-4 180425R6-4 615	180425R6-4 180425R6-4 832	180425D6-4 180425D6-4 356	180425D6-4 180425D6-4 615	180425D6-4 180425D6-4 832	180425R6-5 180425R6-5 240	180425D6-5 180425D6-5 240
	180425R6-3 180425R6-3 434		180425D6-3 180425D6-3 434	180425D6-3 180425D6-3 907	180425R6-4 180425R6-4 359	180425R6-4 180425R6-4 626	180425R6-4 180425R6-4 840	180425D6-4 180425D6-4 359	180425D6-4 180425D6-4 626	180425D6-4 180425D6-4 840	180425R6-5 180425R6-5 267	180425D6-5 180425D6-5 267
3	180425R6-3 180425R6-3 469	180425R6-3 180425R6-3 RNaseP	180425D6-3 180425D6-3 469	180425D6-3 180425D6-3 RNaseP	180425R6-4 180425R6-4 373	180425R6-4 180425R6-4 634	180425R6-4 180425R6-4 RNaseP	180425D6-4 180425D6-4 373	180425D6-4 180425D6-4 634	180425D6-4 180425D6-4 RNaseP	180425R6-5 180425R6-5 RNaseP	180425D6-5 180425D6-5 RNaseP
ł	180425R6-3 180425R6-3 504	NTC RNaseP	180425D6-3 180425D6-3 504	NTC RNaseP	180425R6-4 180425R6-4 386	180425R6-4 180425R6-4 706	NTC RNaseP	180425D6-4 180425D6-4 386	180425D6-4 180425D6-4 706	NTC RNaseP	NTC RNaseP	NTC RNaseP

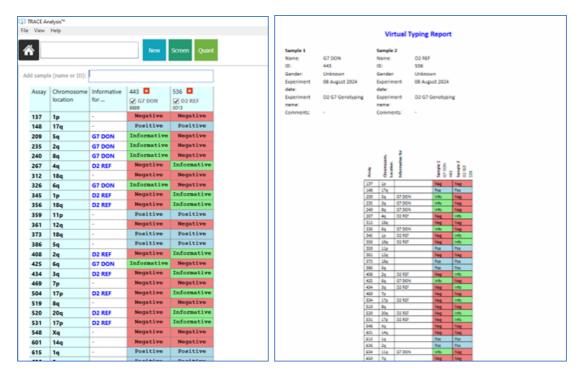
Virtual Typing

ντ

Virtual Typing functionality was added in TRACE Analysis[™] version 1.5. Virtual Typing allows the user to compare any DNA genotyped against any other using the same genotyping plate configuration. This functionality is helpful for validation of new lots of plates, as well as for research purposes. Reports can also be generated from any custom view created in virtual typing.

Validation of a new lot of plates may require verification of the typing patterns within a plate as well as with multiple known DNA samples. Inspecting 46 assays many times over against a spreadsheet or printed list of known genotypes is extremely tedious and prone to error. With Virtual Typing functionality it is easy to quickly see whether a new lot of genotyping plates gives the expected results.

Similarly, if users need to make control mixtures, the VirtualTyping function allows easy comparison of controls which can be made for specifc assays of interest.



Monitoring Test

Once recipient specific markers have been found, quantification is performed to monitor engraftment. In the monitoring test, two or more of the informative assays is used to quantify the DNA of interest in an unknown sample relative to a reference sample. Any of the informative assays identified in the genotyping can be used to perform monitoring.

The QTRACE[®] Analysis System

The fraction of DNA positive for the informative marker in the unknown composition is determined relative to the pure reference sample DNA and the result is expressed as a percentage (ratio).

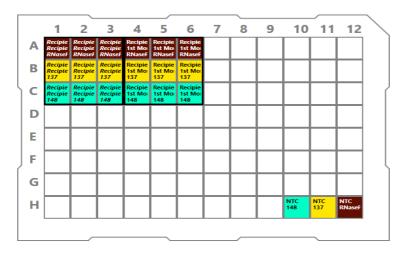
Monitoring Sample View

Monitoring Samples are arranged into groups byTRACE Analysis[™] Software. The NTC reactions for each assay are in the lower right hand corner of the plate.

	4		2	4		~	7	~	0	10		40	$\overline{}$
	1	_	_	-	_	_	/	8	9	10	11	12	
Α	Recipie	Recipie Recipie RNaseł	Recipie	1st Mo	Recipie 1st Moi RNaseF	1st Moi							
В	Recipie Recipie 137	Recipie Recipie 137	Recipie Recipie 137	Recipie 1st Moi 137	Recipie 1st Moi 137	Recipie 1st Moi 137							
) C	Recipie Recipie 148	Recipie Recipie 148	Recipie Recipie 148		Recipie 1st Moi 148								
D													
E													
F													
G													
н										NTC 148	NTC 137	NTC RNaseF	
											_		

Monitoring Assay View

TRACE Analysis[™] Software adds the assays to the plate in rows.

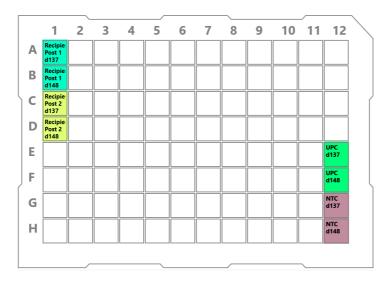


The DigitalTRACE[™] Analysis System

The fraction of DNA positive for the informative marker in the unknown composition is determined relative to the reference gene (RNase P) signal and the result is expressed as a percentage (ratio).

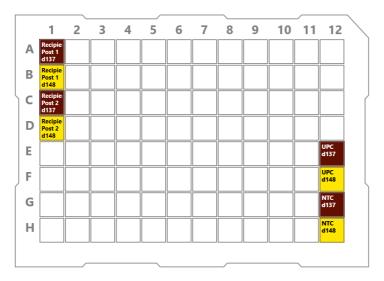
Monitoring Sample View

Monitoring Samples are arranged into groups byTRACE Analysis[™] Software. The NTC and UPC (Universal Positive Control) reactions for each assay are in the lower right hand corner of the plate.



Monitoring Assay View

TRACE Analysis[™] Software adds the assays to the plate in columns.



See section <u>Drag and Drop Plate Layouts</u> to move a well from one location to another location.

Assigning Informative Markers

TRACE Analysis[™] Software allows you to perform sample monitoring and leverage all the features of TRACE Analysis[™], without the need to genotype the sample in advance. If you know that an assay is informative for your sample, you can designate its informative status and then use it as you normally would.

In order to use this feature of TRACE Analysis™:

Enter all the information necessary about the Recipient and the Donor samples and choose an appropriate Reference Sample.

Enter information related to the Post Sample(s) being tested.

In the Informative Markers section, click the "Add Marker" 🖸 button.

When this button is clicked, a window opens with all Assays available for assignment.

0 312 0 326 0 333 0 345 0 356 0 359		493 504 519 520 531		678 681 694 706		854 874 884		
326 333 0 0 345 0 0 356 359		504 519 520		681 694	X O O	874	XOO	
0 333 0 345 0 356 0 359		519 520		694	XOO		ALC: NOT THE OWNER OF THE OWNER OWNER OF THE OWNER	
345 356 0 0 359		520	XOO		Second Second	884		
356	XOO		Annual	706				
359	tion of the second seco	531				888	× O O	
-				710	XOO	892	\times \circ \circ	
		548	X O O	721	\times \odot	907	$\mathbf{X} \odot \mathbf{O}$	
361	XOO	555	X O O	736	\times \odot	916	$\mathbb{X} \bigcirc \odot$	
373	XOO	567	X O O	748	\odot	923	XOO	
386	XOO	574	X O O	755	× •	936	XOO	
396	× OO	585	X O O	768	× O O	948	$\mathbf{X} \bigcirc \mathbf{O}$	
408	× O •	597	X O O	777	× O O	954	\times \odot	
425	XOO	601	X O O	784	× O ●	962	\times \circ \circ	
434	XOO	615	× O O	795	× O O	971	× O O	
441	XOO	626	× • •	803	× O O	987	× O O	
450	XOO	634	XOO	819	× • •	990	XOO	
469	× O O	650	× O O	824	X • •			
472	XOO	663	× O O	832	× O O			
482	× O O	670	× O O	840	× • •			
	396 408 425 434 441 450 469 472	396 第 ○ 408 第 ○ 408 第 ○ 425 第 ○ 434 第 ○ 441 第 ○ 450 第 ○ 459 第 ○ 469 第 ○ 482 第 ○	296 № ○ 585 408 № ○ 597 425 № ○ 601 434 № ○ 615 441 № ○ 634 459 № ○ 634 0 450 № ○ 634 0 459 № ○ 650 442 № ○ 663	396 ⋈ ○ 585 ⋈ ○ 408 ⋈ ○ 597 ⋈ ○ 423 ⋈ ○ 601 ⋈ ○ 434 ⋈ ○ 601 ⋈ ○ 441 ⋈ ○ 634 ⋈ ○ 450 ⋈ ○ 634 ⋈ ○ 472 ⋈ ○ 650 ⋈ ○ 482 ⋈ ○ 670 ⋈ ○	396 第 ○ 585 第 ○ 776 408 第 ○ 597 第 ○ 777 425 第 ○ 601 第 ○ 774 ● 434 第 ○ 615 第 ○ 441 第 ○ 634 第 ○ 819 ○ 450 第 ○ 634 第 ○ 469 第 ○ 653 第 ○ 824 ○ 482 第 ○ 670 第 ○	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	296 ₩ ○ 585 ₩ ○ 768 ₩ ○ 948 408 ※ ○ 597 ೫ ○ 777 ೫ ○ 954 425 ೫ ○ 601 ೫ ○ 784 ೫ ○ 954 ● 434 ೫ ○ 615 ೫ ○ 975 ೫ ○ 971 ○ 436 ೫ ○ 615 ೫ ○ 803 ೫ ○ 971 ○ 430 ೫ ○ 634 ೫ ○ 819 ೫ ○ 997 ○ 459 ೫ ○ 650 ೫ ○ 819 № ○ 990 ○ 472 ೫ ○ 650 ೫ ○ 822 ※ ○ 848 ○ 848 ○ 0 990 990 990 990 948 ○ 948 ○ 0 0 0 0 0 0 0 0 0 0	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$

Assign the informative assays by clicking the circle and sample combination which is appropriate.

Press the 'X' to reset the choice for that assay.

In case of DigitalTRACE[™] Assays, the assay name begins with the "d" symbol.

Markers								-		×
d102	X O O	d312	X O O	d493	× 00	d678	XOO	d854	×o	0
d113	XOO	d326	× O O	d504	× O O	d681	× O O	d874	×O	0
d120	XOO	d333	× O O	d519	XOO	d694	× O O	d884	×O	0
d137	X O O	d345	× O O	d520	× O O	d706	X O O	d888	×O	0
d148	XOO	d356	X O O	d531	XOO	d710	× O O	d892	×O	0
d157	XOO	d359	× O O	d548	× O O	d721	× O O	d907	×O	0
d176	× O O	d361	× O O	d555	XOO	d736	× O O	d916	×O	0
d183	× OO	d373	× O O	d567	XOO	d748	× O O	d923	×O	0
d198	XOO	d386	× OO	d574	XOO	d755	× O O	d936	×O	0
d209	XOO	d396	× O O	d585	× O O	d768	× O O	d948	×O	0
d222	× O O	d408	× O O	d597	XOO	d777	× O O	d954	×O	0
d235	× O O	d425	× O O	d601	× O O	d784	× O O	d962	×O	0
d240	XOO	d434	× O O	d615	XOO	d795	× O O	d971	×O	0
d252	XOO	d441	×OO	d626	XOO	d803	× O O	d987	×O	0
d267	× OO	d450	× O O	d634	× O O	d819	× O O	d990	×O	0
d275	× O O	d469	× O O	d650	× O O	d824	× O O			
d291	XOO	d472	× O O	d663	XOO	d832	XOO			
d305	×OO	d482	× O O	d670	× O O	d840	× O O			
DELs HLA	Custom									
Cancel	1								0	k

In the HLA tab, you can select for HLA TRACE[™] Assays designed for HLA Loss of Heterozygosity monitoring.

Markers								-		×
H005	X O O	H039	XOO	H104	X OO	H115	× OO			
H007	XOO	H041	XOO	H105	XOO	H116	XOO			
H009	X O O	H043	XOO	H106	XOO	H117	XOO			
H017	× OO	H045	\times \circ \circ	H107	XOO	H118	× O O			
H020	× O O	H051	XOO	H108	XOO	H119	× O O			
H022	XOO	H052	XOO	H109	XOO					
H025	× O O	H053		H110	XOO					
H028	XOO	H054	XOO	H111	XOO					
H029	XOO	H101	XOO	H112	XOO					
H036	XOO	H102	XOO	H113	XOO					
H038	XOO	H103	X 0 0	H114	XOO					
	INDELS HLA	Custom								
Cancel									0	k

In the Custom tab, custom assays can be added.

3 Markers	-		х
3A-10.1 🕱 🔿 🖉 JBex15 🕱 🔿 🔿			
JA-11.0 🕱 🔾 🔾			
JA-8.1 🕱 🔿 🔿			
0.0-AC			
38-13.1 🕱 🔿 🔿			
J8-14.1 🕱 🔿 🔿			
38-16.1 🕱 🔾 🔾			
38-17.0 🕱 🔾 🔾			
J8-18.1 🕱 🔿 🔿			
38-19.2.0 🕱 🔾 🔿			
J8-20.1 🗷 🔿 🔿			
La seconda de la constante de la const			
INDELS HLA Custom			
Cancel		0	
Concer			

Once the marker(s) have been assigned, click ok.

Now the markers are selectable for the sample in the Informative Markers window.

TRACE Analysis[™] Software will save this information, so it only has to be entered once for a given sample.

Protocol

In order to perform a monitoring experiment with TRACE Analysis[™] Software, start typing the name of your Recipient Sample in the Sample Entry window. TRACE Analysis[™] Software will start searching for the record as you are entering the name. The name can also be chosen from the drop down menu which appears when you click in the sample entry box.

The QTRACE[®] Analysis System

Once the Recipient Sample name is found and selected, the identifying information initially entered for the samples and the genotyping data results will appear.

(For samples wherein genotyping data was not determined using TRACE Analysis[™] Software, please see section on <u>Assigning Informative Markers</u>).



The Reference Sample(s) and its/their concentration(s) for a group of samples in the experiment must be defined. This is typically a pure sample of the recipient's DNA.

In the "Post Sample" entry window, a unique Sample ID must be entered in the Sample ID field.

A sample Date must be entered.

Check the Sample type which is being tested - Sample Types can be defined by the User in the

Preferences Menu.

Click on the name(s) of the Assays you wish to use for the experiment. Once all the information about the sample has been entered, click the "Add Quant Samples"

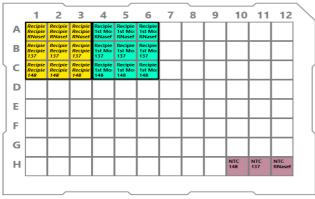
^{Quant} button and the sample will be added to the plate.

The reactions for a particular recipient sample are grouped together. In the example below, all reactions are performed in triplicate with two informative markers.

You can toggle between coloring for the Sample View and the Assay View by pressing the appropriate small plate at the bottom of the window.

One View (see "Sample Layout view") shows the placement of Pure (pre-transfer) and Mixed Samples (Post-Transfer). Pre samples go in wells A1-A3, B1-B3 and C1-C3. Post samples go in A4-A6, B4-B6 and C4-C6.

Sample Layout view



The other view shows the placement of the Assays. The Reference Gene Assay is used in A1-A6 and H12. Assay 014 is used in B1-B6 and H11. Assay 016 is used in C1-C6 and H10

Assay Layout view

		~											
	1	2	3	4	5	6	7	8	9	10	11	12	\backslash
Α	Recipie Recipie RNaseF	Recipie	Recipie	Recipie 1st Moi RNaseF	1st Mo	Recipie 1st Mo RNaseF							
В	Recipie Recipie 137		Recipie Recipie 137	Recipie 1st Mo 137	Recipie 1st Mo 137	Recipie 1st Mo 137							
C		Recipie Recipie 148			Recipie 1st Moi 148								ſ
D													
E													
F													
G													
н										NTC 148	NTC 137	NTC RNaseF	
											_	_	
					_						\sim		

The reactions in Row H constitute No Template Controls (NTCs) and have deionized water added in place of the sample.

Proceed to add more experiments to fill the plate as per you needs.

Once all samples have been added to your plate, press the "Export Setup to PCR" button. Browse to the location where you want the file saved and name it as you wish. This file can then be imported into your qPCR machine's QTRACE[®] template to execute the qPCR analysis. Once the file is saved, TRACE Analysis[™] generates a protocol, based on the experimental inputs and the settings in the preferences menu.

The DigitalTRACE[™] Analysis System

For Post samples wherein genotyping data was either determined using qPCR or not determined using TRACE Analysis[™] Software, please see section on <u>Assigning Informative Markers</u>.

In order to perform a Monitoring experiment with TRACE Analysis[™] Software using both the QIAcuity or Biorad dPCR instrument, select the name of your Recipient Sample in the Sample Entry window from the drop-down menu.

Once the Recipient Sample name is found and selected, choose from a pop-up window to use monitoring plate format which must be selected before in the Preferences. The selected sample's identifying information initially entered will appear.

Type in the Post sample information:

- A unique Sample ID must be entered in the Sample ID field.
- A sample Date must be entered.
- Check the Sample type which is being tested Sample Types can be defined by the User in the Preferences menu.
- Click the plus sign next to the "Add Sample" tab

In the Informative Assays window, choose the loci to be tested by selecting assays from the list based on the screening test results.

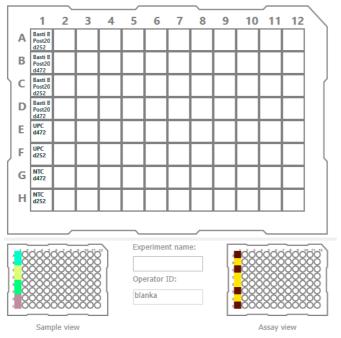
Once all the information about the sample has been entered, click the "Add Quant Samples"

button and the sample will be added to the plate.

A negative control (NTC) is automatically added to the plate layout. A positive control (UPC) is added if it is enabled in the dPCR Data and Reports tab in the <u>Preferences</u>. The use of UPC is optional.

You can toggle between coloring for the Sample View and the Assay View by pressing the appropriate small plate at the bottom of the window. One View shows the placement of monitoring and control samples. The other view shows the placement of the Assays.

Monitoring Samples are arranged into groups by TRACE Analysis[™] Software, based on the preferences set for the monitoring machine. You can drag the wells to rearrange the samples within the plate. In this case, both controls were placed to the first column.



Proceed to add more samples to fill the plate as per your needs.

Once all samples have been added to your plate, press the "Export Setup to PCR" button. Browse to the location where you want the file saved and name it as you wish. This file can then be imported into your dPCR machine's template file to execute the dPCR analysis.

Once the file is saved, TRACE Analysis[™] Software generates a protocol, based on the experimental inputs and the settings in the preferences menu.

Monitoring Test Protocol - QIAcuity

The following protocol is an example output from TRACE Analysis[™] Software for a Monitoring test using QIAcuity dPCR:

1.	Set u	p all reactions in	a pre-PCR lab, unde	r ambient condition	s without ice.				
2.	Brief	ly vortex and cent	rifuge all tubes befo	re opening.					
3.	Prep	are DNA dilutions	as specified in Table	1:					
	_			Table 1. DNA di					
	No.	Name	Sample ID	Concentration	Sample	Water	Total	Amount pe	
					Volume	Volume	Volume	Reaction	
	1	Post Sample	Post_test	26 ng/µl	12,69 µl	48,91 µl	61,60 µl	150 nj	
	2	Post Sample	Post2_test	41 ng/μl	8,05 µl	53,55 µl	61,60 µl	150 n	
4.	Prep	are for chosen inf	ormative dPCR Assa	the following mixtu	re:				
	Table 2. Master Mix + dPCR Assay mixture								
	Assa	y Mix		dPCR 20x assay	Total Volume				
Master Mix mix [µ]									
	d359)	46,00 µl	9,20 µl	55,20 µl				
	d626	;	46,00 μl	9,20 µl	55,20 µl				
5.	IMP	ORTANT! Vortex a	it least 5 seconds an	d spin briefly each p	repared Maste	r Mix + dPCF	R Assay mixto	ire.	
6.				lix + dPCR Assay Mix	to a 96-well pla	ite as define	d in TRACE A	nalysis™	
	Softy	vare's Assay Layoi							
							t Miour Mibo	re NTC and	
7.	Add	1 A A	mple DNA dilution a						
7.	Add UPC	are indicated add	30,8 µl water for NT	C and 30,8 µl from U	niversal Positiv	e Control for	UPC.		
	Add UPC	are indicated add	30,8 µl water for NT		niversal Positiv	e Control for	UPC.		
	Add UPC	are indicated add DRTANT! Vortex a	30,8 µl water for NT	C and 30,8 μl from U he 96-well plate to n	niversal Positiv	e Control for	UPC.		
8.	Add UPC IMPC well	are indicated add DRTANT! Vortex a plate briefly using	30,8 μl water for NT it least 15 seconds t g a microcentrifuge.	C and 30,8 μl from U he 96-well plate to n	niversal Positiv	e Control for s of each rea	UPC. action. Centri	ifuge the 96-	
8.	Add UPC IMPC well Trans	are indicated add DRTANT! Vortex a plate briefly using sfer 40 µl of each	30,8 μl water for NT it least 15 seconds t g a microcentrifuge.	C and 30,8 µl from U he 96-well plate to n nix into a Nanoplate.	niversal Positiv	e Control for s of each rea	UPC. action. Centri	ifuge the 96-	
8. 9.	Add UPC IMPC well Trans Load	are indicated add DRTANT! Vortex a plate briefly using sfer 40 µl of each	30,8 μl water for NT it least 15 seconds t g a microcentrifuge. prepared reaction m to the QIAcuity digit	C and 30,8 µl from U he 96-well plate to n nix into a Nanoplate.	niversal Positiv	e Control for s of each rea	UPC. action. Centri	ifuge the 96-	
8. 9. 10.	Add UPC IMPC well Trans Load	are indicated add DRTANT! Vortex a plate briefly using sfer 40 µl of each the Nanoplate in ch the QIAcuity So	30,8 µl water for NT it least 15 seconds t g a microcentrifuge. prepared reaction m to the QIAcuity digit oftware Suite.	C and 30,8 µl from U he 96-well plate to n nix into a Nanoplate.	Iniversal Positiv nix the content Seal the Nanop	e Control for s of each rea late with the	r UPC. action. Centri	ifuge the 96- plate sealer.	

Experiment Setup in QIAcuity Suite Software, v2.5

Create a new QIAcuity Plate by selecting "New Plate".

Similarly as for the Genotyping, load a template by selecting "Plate templates..." and import the appropriate DigitalTRACE Monitoring template.

Press Save Plate. The new plate will appear in the main window of the QIAcuity Software Suite.



Click on the plate name to open the plate configuration procedure. Type in a new plate name and save the changes.

Import the Sample Setup sheet (.csv) generated by TRACE Analysis[™] Software by selecting "Plate layout" tab and "CSV import/export".

Select "Import from CSV".

Plate List				Telp	
				 CSV import/export 	
	1	2	3	Active selection:	
	A 🐌 🦲			Selected wells: 0	
	• 🐌 🦳				
	c 🐌 🦳				
	• 🐌 🦳				
	· 🐌 🦳				
	F 🐌 🦳				
	۰ 🐌				
	н 🐌 🦳				
				CSV successfully imported.	
				C Sove changes	() Dor

Inspect if all your selected samples are represented on the Plate figure. Select "Done" to finish the setup. Start the run.

Thermal Cycling and Imaging Protocol for QIAcuity

The DigitalTRACE[™] System will deliver optimal results when the following thermal profile is used in dPCR:

Number of cycles	Temperature	Time
1	95 °C	3 min
	95 °C	15 s
40	60 °C	30 s

Channel	Exposure duration	Gain
Green	500 ms	6
Yellow	500 ms	6
Orange	Off	Off
Red	Off	Off
Crimson	Off	Off

After the QIAcuity run has finished, check in the QIAcuity Software Suite if the automatic thresholds are correct. Adjust the thresholds manually if needed.

Select all wells on the plate and analyze per target (not per channel), export data by selecting Export to CSV.

Monitoring Test Protocol - Biorad

The following protocol is an example output from TRACE Analysis[™] Software for a Monitoring test using Biorad ddPCR:

	Set up all reactions in a pre-PCR lab, under ambient conditions without ice.								
2.	Briefly vortex an	d centrifuge all tubes b	efore opening.						
3.	Prepare DNA dil	utions as specified in Ta	ble 1:						
			Table 1. DNA dilu	_					
	Name	Sample ID	Concentration	Sample	Water	Total	Amount pe		
				Volume	Volume	Volume	Reactio		
1	Basti Bob	Post201	20 ng/µl	17,25 µl	5,52 µl	22,77 µl	150 n		
2	Basti Bob	Post202	20 ng/µl	17,25 µl	5,52 µl	22,77 µl	150 n		
4.	Prepare for chos	en informative dPCR As	say the following m	ixture:					
	-		dPCR Supermix + dF						
	Assay	ddPCR supermix	dPCR assay [uL]	Total V	/olume				
		for probes (no							
		dUTPs) [uL]							
	d252	46,00 μl	4,60 µl	5	0,60 µl				
	d472	46,00 μl	4,60 µl	5	0,60 µl				
5.	Analysis [™] Softwa	are's Assay Layout view							
	Analysis [™] Softwa	are's Assay Layout view th Sample DNA dilution							
6.	Analysis™ Softwa Add 9.9 μl of ead Software's Layou	are's Assay Layout view th Sample DNA dilution	and water for NTC	wells as indic	cated by TRAC	CE Analysis™	tubes		
6.	Analysis™ Softwa Add 9.9 μl of ead Software's Layou	are's Assay Layout view ch Sample DNA dilution ut View. ortex the 8 strip tube to	and water for NTC	wells as indic	cated by TRAC	CE Analysis™	tubes		
6.	Analysis [™] Softwa Add 9.9 µl of ead Software's Layou IMPORTANT! Vo briefly using a m	are's Assay Layout view ch Sample DNA dilution ut View. ortex the 8 strip tube to	and water for NTC	wells as indic	cated by TRAC	CE Analysis™	tubes		
6. 7.	Analysis [™] Softwa Add 9.9 µl of eac Software's Layou IMPORTANT! Vo briefly using a m !!!The following	are's Assay Layout view ch Sample DNA dilution ut View. ortex the 8 strip tube to nicrocentrifuge.	and water for NTC mix the contents o only for manual dra	wells as indic of each react oplet genera	cated by TRAG tion. Centrifu tor users:	CE Analysis™ ge the 8 strip	tubes		
6. 7. 8. 9.	Analysis [™] Softwa Add 9.9 µl of ead Software's Layou IMPORTANT! Vo briefly using a m !!!The following Transfer 20 µl of	are's Assay Layout view ch Sample DNA dilution ut View. ortex the 8 strip tube to nicrocentrifuge. steps from 9 to 14 are	and water for NTC mix the contents of only for manual dru to the sample wells	wells as indic of each react oplet genera (middle row	cated by TRAC tion. Centrifu itor users: v) of the DG8	CE Analysis™ ge the 8 strip	tubes		
6. 7. 8. 9. 10.	Analysis [™] Softwi Add 9.9 µl of ead Software's Layou IMPORTANT! Vo briefly using a m !!!The following Transfer 20 µl of Add 70 µl of dro	are's Assay Layout view th Sample DNA dilution ut View. ortex the 8 strip tube to hicrocentrifuge. steps from 9 to 14 are each prepared sample	and water for NTC on the contents of only for manual dra to the sample wells ach oil well of the Do	wells as indic of each react oplet genera (middle row G8 cartridge	cated by TRAC tion. Centrifu itor users: v) of the DG8	CE Analysis™ ge the 8 strip	tubes		
6. 7. 8. 9. 10. 11.	Analysis [™] Softwi Add 9.9 µl of ead Software's Layou IMPORTANT! Vo briefly using a m !!!The following Transfer 20 µl of Add 70 µl of dro Hook the gasket	are's Assay Layout view ch Sample DNA dilution ut View. ortex the 8 strip tube to nicrocentrifuge. steps from 9 to 14 are <i>i</i> each prepared sample plet generation oil to each	and water for NTC o mix the contents of only for manual dru to the sample wells ach oil well of the Di er using the holes o	wells as indic of each react oplet genera (middle row G8 cartridge	cated by TRAC tion. Centrifu itor users: v) of the DG8	CE Analysis™ ge the 8 strip	tubes		
6. 7. 8. 9. 10. 11. 12.	Analysis [™] Softwi Add 9.9 µl of ead Software's Layou IMPORTANT! Vo briefly using a m !!!The following Transfer 20 µl of Add 70 µl of droj Hook the gasket Load the cartridg	are's Assay Layout view ch Sample DNA dilution ut View. ortex the 8 strip tube to nicrocentrifuge. steps from 9 to 14 are each prepared sample plet generation oil to er over the cartridge hold	and water for NTC on mix the contents of only for manual drives to the sample wells ach oil well of the Difer using the holes of generator.	wells as indic of each react oplet genera (middle row G8 cartridge n both sides	cated by TRAC tion. Centrifu ntor users: v) of the DG8	CE Analysis™ ge the 8 strip cartridge.			
6. 7. 8. 9. 10. 11. 12. 13.	Analysis [™] Softwi Add 9.9 µl of ead Software's Layou IMPORTANT! Vo briefly using a m !!!The following Transfer 20 µl of Add 70 µl of droj Hook the gasket Load the cartridg When droplet ge	are's Assay Layout view th Sample DNA dilution ut View. ortex the 8 strip tube to icrocentrifuge. steps from 9 to 14 are each prepared sample plet generation oil to ea over the cartridge hold ge in the QX200 droplet	and water for NTC on mix the contents of only for manual drives of the sample wells such oil well of the Dire using the holes of generator.	wells as indic of each react oplet genera (middle row G8 cartridge n both sides ole gasket fro	cated by TRAC tion. Centrifu ator users: v) of the DG8	CE Analysis™ ge the 8 strip cartridge. r and discard i			
 6. 7. 8. 9. 10. 11. 12. 13. 14. 	Analysis ³⁶ Software's Layou Add 9.9 µl of ead Software's Layou IMPORTANT! Voc briefly using a m !!!The following Transfer 20 µl of Add 70 µl of drop Hook the gasket Load the cartridg When droplet gg Pipet 40 µl of thm	are's Assay Layout view th Sample DNA dilution it View. view. the 8 strip tube to incrocentrifuge. steps from 9 to 14 are each prepared sample plet generation oil to ea over the cartridge hold ge in the QX200 droplet eneration is complete, r.	and water for NTC on mix the contents of only for manual drive to the sample wells ach oil well of the Di er using the holes of generator. emove the disposat ets into a single colu	wells as indic of each react oplet genera (middle row G8 cartridge, n both sides ole gasket fro mn of a 96-v	cated by TRAC cion. Centrifu ator users: v) of the DG8	CE Analysis™ ge the 8 strip cartridge. r and discard i	t.		
 6. 7. 8. 9. 10. 11. 12. 13. 14. 	Analysis ³⁶ Software's Layou Add 9.9 µl of ead Software's Layou IMPORTANT! Voc briefly using a m !!!The following Transfer 20 µl of Add 70 µl of drop Hook the gasket Load the cartridg When droplet gg Pipet 40 µl of thm	are's Assay Layout view th Sample DNA dilution it View. ortex the 8 strip tube to icrocentrifuge. steps from 9 to 14 are each prepared sample plet generation oil to er over the cartridge hold ge in the QX200 droplet eneration is complete, r. e contents of the drople te with foil plate seals t	and water for NTC on mix the contents of only for manual drive to the sample wells ach oil well of the Di er using the holes of generator. emove the disposat ets into a single colu	wells as indic of each react oplet genera (middle row G8 cartridge, n both sides ole gasket fro mn of a 96-v	cated by TRAC cion. Centrifu ator users: v) of the DG8	CE Analysis™ ge the 8 strip cartridge. r and discard i	t.		
 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 	Analysis ³⁵ Softwa Add 3-9 µl of eac Software's Layou IMPORTANT! Vo briefly using a m !!!The following Hook the gasket Load the cartrid <u>i</u> When droplet gg Pipet 40 µl of the Seal the PCR plate in the QX200 droc Place the plate in	are's Assay Layout view th sample DNA dilution to View. vrtex the 8 strip tube to incroentrifuge. steps from 9 to 14 are each prepared sample plet generation oil to e over the cartifide hold ge in the QX200 droplet eneration is complete, ri- contents of the drople te with foil plate seals t spolet reader.	and water for NTC on mix the contents of only for manual dri to the sample wells be oil well of the D er using the holes or generator. emove the disposat ets into a single colu hat are compatible or PCR amplification	wells as indic oplet genera (middle row G8 cartridge n both sides ole gasket fro mn of a 96-v with the PX1 n.	cated by TRAC cion. Centrifu ator users: v) of the DG8	CE Analysis™ ge the 8 strip cartridge. r and discard i	t.		
6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17.	Analysis ¹¹⁰ Softw. Add 3-9 µl of eaa Software's Layou IMPORTANT! Vo briefly using a m IIIThe following Transfer 20 µl of Add 70 µl of dror Hook the gasket Load the cartridg When droplet ga Pipet 40 µl of th Seal the PCR plai in the QX200 dri Place the plate ai	are's Assay Layout view th Sample DNA dilution to view. The the Strip tube to increentrifuge. steps from 9 to 14 are each prepared sample teget generation oil to e: over the cartridge hold ge in the QX200 droplet generation is complete, re- contents of the droplet te with hold plate seals to splet reader.	and water for NTC ' mix the contents of only for manual dr to the sample wells ch oil well of the D or generator. emove the disposat tes into a single colu tes into a single colu or PCR amplification XX200 droplet reade	wells as indic of each react oplet genera (middle row G8 cartridge in both sides ole gasket fro min of a 96-w with the PX1 n.	cated by TRAC ion. Centrifu itor users: v) of the DG8	CE Analysis™ ge the 8 strip cartridge. r and discard i	t.		
6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17.	Analysis ¹¹⁰ Softw. Add 3-9 µl of eaa Software's Layou IMPORTANT! Vo briefly using a m IIIThe following Transfer 20 µl of Add 70 µl of dror Hook the gasket Load the cartridg When droplet ga Pipet 40 µl of th Seal the PCR plai in the QX200 dri Place the plate ai	are's Assay Layout view th sample DNA dilution to View. vrtex the 8 strip tube to incroentrifuge. steps from 9 to 14 are each prepared sample plet generation oil to e over the cartifide hold ge in the QX200 droplet eneration is complete, ri- contents of the drople te with foil plate seals t spolet reader.	and water for NTC ' mix the contents of only for manual dr to the sample wells ch oil well of the D or generator. emove the disposat tes into a single colu tes into a single colu or PCR amplification XX200 droplet reade	wells as indic of each react oplet genera (middle row G8 cartridge in both sides ole gasket fro min of a 96-w with the PX1 n.	cated by TRAC ion. Centrifu itor users: v) of the DG8	CE Analysis™ ge the 8 strip cartridge. r and discard i	t.		

Use the same cycling parameters as for a Genotyping test:

Number of cycles	Temperature	Time	Ramp Rate
1	95 °C	10 min	2 °C/s
40	94 °C	30 s	2 °C/s
40	59 °C	60 s	2 °C/s
1	98 °C	10 min	2 °C/s

The droplet reading process is the same as for the Genotyping test. Start the droplet reading run.

After the reading process has finished, check in the QuantaSoft Software if the automatic thresholds are correct. Adjust the thresholds manually if needed. Select all wells on the plate and export data by selecting Export CSV.

Monitoring Data Analysis and Report

Import qPCR data by clicking the "Import PCR Data" button, and browse to the location of your exported qPCR data file and select it.

The QTRACE[®] Analysis System

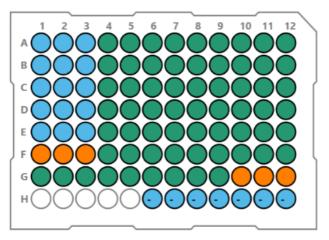
TRACE Analysis[™] Software uses a green/amber/red color coding for well highlighting.



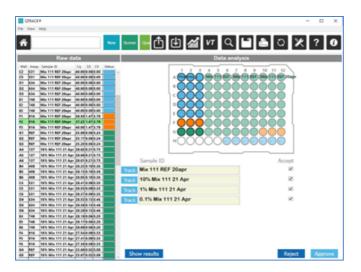
Green wells always mean positive wells (Go). Red wells are data which has been rejected, manually or automatically (Stop). Amber wells highlight the need for data inspection (Caution).

Light blue wells **O**remain as negative wells.

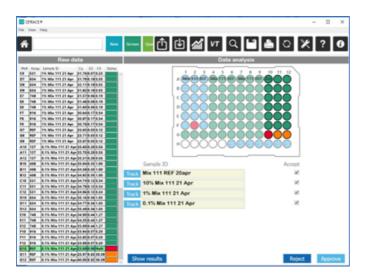
Monitoring plates contain green and light blue colors, indicating true positives or negatives. Amber colored wells highlight replicates which violate user-defined replicate highlighting rules, based on %CV, SD or Cq Range.



Replicate wells in Monitoring which violate the replicate highlighting preferences set by the user will first be highlighted in Amber.



The user can then Accept/Reject individual wells in an attempt to conform to the acceptable variance allowed. Rejected wells turn Red and the remaining wells will either turn green, light blue or remain Amber - if the Rejected well does not cause the remaining replicates to meet acceptable variance.



Once all the data has been inspected, pressing the "Calculate" ^{Calculate} button allows TRACE Analysis[™] to perform the calculations necessary to determine the proportion of the mixed sample which contains the sample of interest.

The percentage of the total sample which contains the sample is displayed in the right hand Result window.



As more data is collected for a particular sample over time, TRACE Analysis[™] Software provides a longitudinal view.

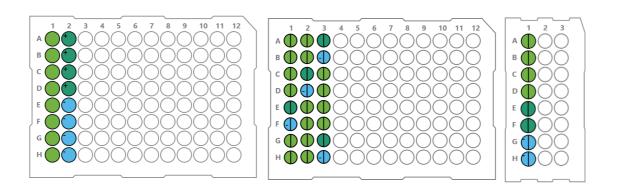
To view the composite set of data for an individual sample, press the "Overview" 🌌 button.

Pushing the Overview button takes you to a screen showing all the information input for a particular group of samples as well as all of their genotyping and monitoring data.

TRACE Analysis**													- 1	
View Help														
~						1	at la	n la la	107				N 2	
~		New St	veen Quant				Ľ	¥]∭		4	ш	<u> </u>	℀ ?	14
Re	ecipient		Genotypin	a					Mon	itoring				
lecipient First Name	Luke	Harker	Chr. location	InfoCq	ACc.									
ecipient Last Name	Skywalker	504	17p			Sample 1	Type 0	late	Target	Chr.	ddCq	DNA (%)	Reference PI	in
	L\$123	504	17p					1-12-2020	504	17p	9.88	0.11	PreTx DNA	Lui
lecipient ID	L8123							1-12-2020	504	17p	6.61	1.02	PreTx DNA	5.0
ample ID	PreTx DNA	721	Xq		0			1-12-2020	504	17p Xq	3.39	9.54	Prets DNA Prets DNA	Lu Lu
	100	721	Xq		0			1-12-2020	721	Xq	3.28	10.27	Prets DNA	
oncentration ng/ul	100	854	120					1 13 3555	721	×-	0.74	0.13	Burger Para	
ute of Birth	31/12/2020	854	120				Los	noitudinal	chimeria	cm resu	It of Lui	ke Skow	alker	
			124		0	100 -	Lor	ngitudinal	chimeris	sm resu	It of Lu	ke Skywa	alker	_
ute of Transplant	31/12/2020		129		•	100	Loi	ngitudinal	chimeris	sm resu	lt of Lu	•	DNA S04, PreTx D	
ute of Transplant			124		•	100	Lor	ngitudinal	chimeri	sm resu	lt of Lu	•		
ate of Transplant	31/12/2020		12q		•	100	Lor	ngitudinal	chimeri	sm resu	lt of Lu	•	DNA S04, PreTx D	
ate of Transplant	31/12/2020		12q		•		Lor	ngitudinal	chimeri	sm resu	lt of Lu	•	DNA S04, PreTx D	
ate of Transplant ender omment	31/12/2020		12q		•	100	Lor	ngitudinal	chimeri	sm resu	lt of Lu	•	DNA S04, PreTx D	
ute of Transplant iender omment koease Type	31/12/2020		124		0		Lor	ngitudinal	chimeri	sm resu	lt of Lu	•	DNA S04, PreTx D	
late of Transplant lender lomment ksease Type	31/12/2020 55 O Male O Female		124		•		Loi	ngitudinal	chimeri	sm resu	lt of Lu	•	DNA S04, PreTx D	
lomment Xoease Type Ionor First Name	31/12/2020 (1) O Male O Female Donor (1)		129		•	10 -	Loi	ngitudinal	chimeri	sm resu	lt of Lu	•	DNA S04, PreTx D	
aate of Transplant lender lomment ksease Type lonor First Name lonor Last Name	S1/12/2020 5 Male O Female Donor 5 Darth		124		•	ndelma 2	Loi	ngitudinal	chimeri	sm resu	It of Lu	•	DNA S04, PreTx D	
ute of Transplant ionment ionment issase Type ionor First Name ionor Last Name ionor ID	St/12/2020 5 Male O Female Donor Darth Vader		124		•	10 administration	Lor	ngitudinal	chimeri	sm resu	It of Lu	•	DNA S04, PreTx D	
iender comment tonore First Name honor First Name honor ID ample ID	31/12/2020 (1) O Made O Female Donior C Darth Vader DV123 DV Sample ID		124		•	10 administration	Lor	ngitudinal	chimeri	sm resu	It of Lu	•	DNA S04, PreTx D	
iender iender iomment isease Type bonor First Name ionor Last Name ionor ID ample ID ioncentration ng/ul	Stri2/22/2 (1) O Male O Female Donor D Darth Vader DV123 DV123 DV Sample ID 100		124		•	10 sefuencial yright	Lor	ngitudinal	chimeri	sm resu	It of Lu	•	DNA S04, PreTx D	
Inte of Transplant Ionment Ionment Ionor First Name Ionor First Name Ionor Lost Name Ionor ID Ioncentration ng/ul Iute of Birth	Stri2/2020 (1) O Male O Female Darth Vader DV123 DV Sample ID 100 Stri2/2020 (1)		124		•	10 sefuencial yright	Lor	ngitudinal	chimeri	sm resu	It of Lu	•	DNA S04, PreTx D	
Inte of Transplant Ionment Ionment Ionor First Name Ionor First Name Ionor Lost Name Ionor ID Ioncentration ng/ul Iute of Birth	Stri2/22/2 (1) O Male O Female Donor D Darth Vader DV123 DV123 DV Sample ID 100		124		•	10 sefuencial yright	Lou	ngitudinal	chimeri	sm resu	It of Lu	•	DNA S04, PreTx D	

The DigitalTRACE[™] Analysis System

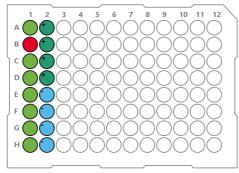
TRACE Analysis[™] Software uses a light green/dark green/red color coding for well highlighting.



Light green wells represent post sample wells. Dark green wells represent the positive control DNA, Red wells are data which has been rejected.

Light blue wells represent non-template control (NTC).

The user can then Accept/Reject individual wells in an attempt to conform to the acceptable variance allowed. Rejected wells turn Red.



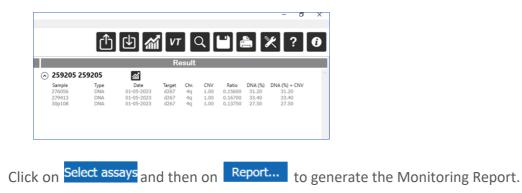
When necessary, for example in case that no CNV information is available from the genotyping, TRACE Analysis[™] Software allows the user to change the CNV information for each sample by

selecting 1 or 2 in the indicated area under the Rawdata view Show rawdata in order to obtain an accurate quantification result:

ED TAGE Analysis" New York: Indo		- 0 ×
A	(中)	v7 Q 🖬 🏝 🗶 ? O
Ree data	Data analysis	
Mail Ang, Yang E, Mailan Kawan, Sur, 201, 201, 201, 201, 201, 201, 201, 201		
	Sample ID	Accest
	276056	8
	279413	×
	11 30p108	2
	POS	×
	These locations are subjected on the spectrum.	
	Show results	Reject Calculate

Once all the data has been inspected, pressing the "Calculate" Calculate button allows TRACE Analysis[™] to perform the calculations necessary to determine the proportion of the mixed sample which contains the sample of interest.

The percentage of the total sample which contains the sample is displayed in the right hand Result window.



As more data is collected for a particular sample over time, TRACE Analysis[™] Software provides a longitudinal view. For more details, see the <u>The QTRACE[®] Analysis System</u> part of this section.

Assay Filtering from Monitoring Reports

Assay Filtering is a feature which allows a user to selectively remove all data from a particular assay in the final report. This may be important, for example, if one pipette tip from a multichannel pipette did not properly dispense the necessary reagents to a set of reactions. While the data may appear to have good precision - all negatives in this example - it may not be accurate. In the following image, there is a failure of Assay 721 for all samples:

CTRACE*		- 🗆 X
Ä	New Screen Quant 🕕 🕁 📶 V1	7 Q 💾 🚔 C 🗶 ? 🖸
Raw data	Data	a analysis
Weil Assay Sample ID Cq SD CV Status	1 2 3 4 5	6 7 8 9 10 11 12
NegGroup		
Assay Filtering R		
A1 434 R Control 29.32 0.01 0.03		
A2 434 R Control 29.33 0.01 0.03		
B1 721 R Control 40.00 0.00 0.00		
82 721 R Control 40.00 0.00 0.00		
C1 824 R Control 27.97 0.02 0.07	F 0 0 0 0 0	
C2 824 R Control 27.94 0.02 0.07	E000000	
D1 REF R Control 27.26 0.03 0.10		
D2 REF R Control 27.30 0.03 0.10		
A3 434 R Sample 1 28.87 0.07 0.25	©00000	
A4 434 R Sample 1 28.97 0.07 0.25	+00000	
B3 721 R Sample 1 40.00 0.00 0.00		
B4 721 R Sample 1 40.00 0.00 0.00 C3 824 R Sample 1 27,60 0.03 0.11		
C3 824 R Sample 1 27.60 0.03 0.11		
D3 REF R Sample 1 25,20 0.01 0.04	Sample ID	Accept
D4 REF R Sample 1 25.20 0.01 0.04	Track R Control	×
A5 434 R Sample 2 37.90 0.55 1.47		
A6 434 R Sample 2 37.12 0.55 1.47	Track R Sample 1	8
85 721 R Sample 2 40.00 0.00 0.00	D. Committee C.	3
86 721 R Sample 2 40.00 0.00 0.00	Track R Sample 2	
C5 824 R Sample 2 35.15 0.28 0.80	Track R Sample 3	×.
C6 824 R Sample 2 35.55 0.28 0.80		
D5 REF R Sample 2 24.79 0.04 0.17	Track R Sample 4	×
D6 REF R Sample 2 24.85 0.04 0.17		2
A7 434 R Sample 3 34.21 0.00 0.00	Track R Sample 5	20
A8 434 R Sample 3 34.21 0.00 0.00		
87 721 R Sample 3 40.00 0.00 0.00		
88 721 R Sample 3 40.00 0.00 0.00		
C7 824 R Sample 3 32.80 0.02 0.05		
C8 824 R Sample 3 32.78 0.02 0.05	Show results	Reject Approve
D7 REF R Sample 3 24.75 0.03 0.13	SHOW RESULTS	Reject Approve

If the plate is approved, under the Results pane, there is 0% reported for Assay 721, while the other assays detec chimerism levels well above zero.

			Res	ult		
ng R	สมั					
	Date 17-08-2018	Target 434	Chr. 3q	ddCq 1.67	DNA (%) 31.32	Reference R Control
	17-08-2018	721	Xq	0.00	0.00	R Control
	17-08-2018	824	22q	1.70	30.76	R Control
	17-08-2018	434	3q	10.65	0.06	R Control
	17-08-2018	721	Xq	0.00	0.00	R Control
	17-08-2018	824	22q	9.85	0.11	R Control
	17-08-2018	434	3q	7.39	0.60	R Control
	17-08-2018	721	Xq	0.00	0.00	R Control
	17-08-2018	824	22q	7.34	0.62	R Control
	17-08-2018	434	3q	3.80	7.16	R Control
	17-08-2018	721	Xq	0.00	0.00	R Control
	17-08-2018	824	22q	3.96	6.45	R Control
	17-08-2018	434	3q	8.80	0.22	R Control
	17-08-2018	721	Xq	0.00	0.00	R Control
	17-08-2018	824	22q	9.09	0.18	R Control

If a report is made at this point, the data from Assay 721 will be included in the calculations and the result will underestimate the actual level of recipient in the same.

Sample	Туре	Date	Target	Chr.	ΔΔCq	DNA (%)	Reference	Informative for
R Sample 1	BM	17-Aug-2018	434	Зq	1.67	31.32	R Control	R Control
R Sample 1	BM	17-Aug-2018	721	Xq	0	0	R Control	R Control
R Sample 1	BM	17-Aug-2018	824	22q	1.7	30.76	R Control	R Control
					Mean:	20.69		
R Sample 2	BM	17-Aug-2018	434	Зq	10.65	0.06	R Control	R Control
R Sample 2	BM	17-Aug-2018	721	Xq	0	0	R Control	R Control
R Sample 2	BM	17-Aug-2018	824	22q	9.85	0.11	R Control	R Control
					Un Transate	0.00		
					Mean:	0.06		
2					Mean:	0.00		
R Sample 3	BM	17-Aug-2018	434	3q	Mean:	<i><i>b</i>:</i>	R Control	R Control
R Sample 3 R Sample 3	BM BM	17-Aug-2018 17-Aug-2018	434 721	3q Xq		0.6	20	R Control R Control
2X.08			-	1		0.6 0	R Control	

In order to remove the data for Assay 721 from the Report, after approving the data, press the 'Select Assays' Select assays button.

The 'FilterAssaysWindow' will appear. In this window, users can choose to exclude the data from an entire assay from reporting.

FilterAssaysWindo			
Select assays used			
	for the report		
Unselect All			
434			
721			
✓ 824			
Save changes	Cancel		
oure changes	Cancer	 	

In this example, once Assay 721 is de-selected and the changes saved, once the 'Report' button is pressed, the data is no longer present in the report and the average chimerism levels reflect only the included data.

Cell fractions

Sample	Туре	Date	Target	Chr.	ΔΔCq	DNA (%)Reference	Informative for
R Sample 1	BM	17-Aug-2018	434	3q	1.67	31.32R Control	R Control
R Sample 1	BM	17-Aug-2018	824	22q	1.7	30.76R Control	R Control
					Mean:	31.04	
R Sample 2	BM	17-Aug-2018	434	3q	10.65	0.06R Control	R Control
R Sample 2	BM	17-Aug-2018	824	22q	9.85	0.11 R Control	R Control
					Mean:	0.09	
R Sample 3	BM	17-Aug-2018	434	3q	7.39	0.6 R Control	R Control
R Sample 3	BM	17-Aug-2018	824	22q	7.34	0.62R Control	R Control
					Mean:	0.61	

External Reference Functions

The External Reference sample within TRACE Analysis[™] Software has been enhanced in version 2.0. In addition to being able to specifty and use an external reference sample in a qPCR experiment, now the sample may be an impure sample (e.g., an earlier timepoint from the recipient) or a Universal Positive Control (Ref. number 711295).

Typically, the reference sample in qPCR is pure donor or pre-transplant, recipient DNA. In both cases, the reference sample is considered 100% and the calculation of % DNA, based on ddCq, assumes this.

A sample which is less than 100% recipient may be used as a reference, but the software needs to correct the % DNA calculation, based on the starting % of the sample.

TRACE Analysis[™] Software now provides this flexibility and automatic correction in monitoring data.

Refere	ence Sampl	es	
Sample ID	Concentration ng/ul	% reference	
	100	100	
	100	100	
10% Recipient	10	10	

Here is an example of processing the same data wherein the reference sample is either a mixture or a pure sample.

Cell fractions

Sample	Туре	Date	Target	Chr.	ΔΔCq	DNA (%)
15	DNA	21-Jan-2020	548	Xq	2.88	1.36
15	DNA	21-Jan-2020	361	12q	2.72	1.52
15	DNA	21-Jan-2020	916	10q	2.73	1.5
					Mean:	1.46
15	DNA	21-Jan-2020	916	10q	2.73	15.04
in the second	and the second se	CONTRACTOR CONTRACTOR			0.70	
15	DNA	21-Jan-2020	361	12q	2.72	15.16
15 15	DNA DNA	21-Jan-2020 21-Jan-2020	361 548	12q Xq	2.72	15.16 13.6

10% Reference Sample

Pure Reference Sample

Simultaneous Genotyping and Monitoring

With the use of Custom Genotyping Panels, TRACE Analysis[™] Software has the potential to allow concurrent genotyping and monitoring experiments on a single 96-well plate.

This example shows the use of a genotyping panel of 10 assays for a new recipient and also monitoring a different recipient on the same plate.

After setting up a custom panel based on one Row in the Plate editor, choose the name of the custom panel in the 'Plate configuration' drop down menu (Plate setup tab of Preferences).

late setup	Custom types	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Machine	for genotypi	ABI 7500	v2.0.6	v				
Machine	for monitorir	ABI 7500	v2.0.6	v				
Layout ty	уре			v				
Replicate	25	qPCR Dup	olicates ~					
Color mo	ode	Pastel	v Pl;	ate for genoty	ping 1 Row		¥	
			Pla	ate for monito	96-low of	density	~	

Set up a genotyping test, followed by a monitoring test. When the 'Export' button is pressed, a unique, combined protocol is generated by TRACE Analysis[™] Software. The combined protocol is consistent with the normal workflow for a monitoring experiment. That is, all reagent additions are performed manually, with master mix, assays and DNA dilutions being added to the plate.

		Technical Support											
	JETA Molecular												
	Krommewetering 101C												
				3	543AN, Utrec	ht							
				+	31.654.136.6	97							
	GENOTYPING P	ROTOCOL FOR	NEW TYPING R										
	QUANTIFICATI	ON PROTOCOL F	OR RETURNING	PATIENT									
1.	Set up all reaction	s in a pre-PCR lab, u	nder ambient condi	tions withou	t ice.								
	Briefly vortex and centrifuge all tubes before opening.												
2.	Briefly vortex and	centrifuge all tubes b	before opening.										
2.		centrifuge all tubes to ons as specified in Ta	1 0										
-	Prepare DNA diluti	0	able 1:										
-	Prepare DNA diluti	ons as specified in T	able 1:										
-	Prepare DNA diluti	ons as specified in T	able 1:	itions									
-	Prepare DNA diluti	ons as specified in T	able 1: Sample	itions Sample	Water	Total	Amount pe						
3.	Prepare DNA diluti * - Make a Ten-Fol	ons as specified in Ta d Dilution (1:10) of S	able 1: ample Table 1. DNA dilu		Water Volume	Total Volume	Amount pe Reaction						
3. No.	Prepare DNA diluti * - Make a Ten-Fol	ons as specified in Ta d Dilution (1:10) of S	able 1: ample Table 1. DNA dilu	Sample									
3. No.	Prepare DNA diluti * - Make a Ten-Fol Name	ons as specified in T d Dilution (1:10) of S Sample ID	able 1: iample Table 1. DNA dilu Concentration	Sample Volume	Volume	Volume	Reaction						
3. No. 1 2	Prepare DNA diluti * - Make a Ten-Fol Name New Typing R	ons as specified in T d Dilution (1:10) of S Sample ID 10831 13 Aug	able 1: iample Table 1. DNA dilu Concentration *10 ng/µl	Sample Volume 6.05 µl	Volume <mark>175.45</mark> μl	Volume 181.50 µl	Reaction 5 n 5 n						
3.	Prepare DNA diluti * - Make a Ten-Fol Name New Typing R New Typing D	ons as specified in T. d Dilution (1:10) of S Sample ID 10831 13 Aug 12560 13 Aug	able 1: Table 1. DNA dilu Concentration *10 ng/µl *10 ng/µl	Sample Volume 6.05 µl 6.05 µl	Volume 175.45 μl 175.45 μl	Volume 181.50 μl 181.50 μl	Reaction 5 n						
3. No. 1 2 3	Prepare DNA diluti * - Make a Ten-Fol Name New Typing R New Typing D Returning Patient	ons as specified in T. d Dilution (1:10) of S Sample ID 10831 13 Aug 12560 13 Aug DNA 103 13 Aug	able 1: Table 1. DNA dilu Concentration *10 ng/µl *10 ng/µl *10 ng/µl	Sample Volume 6.05 µl 6.05 µl 13.20 µl	Volume 175.45 μl 175.45 μl 184.80 μl	Volume 181.50 μl 181.50 μl 198.00 μl	Reaction 5 m 5 m 10 m						

After the results from the qPCR machine are imported into TRACE Analysis[™] Software, the analysis algorithms appropriate for the samples are applied and the results displayed in the Result pane of the software.

TRACE Analysis [™] e View Help									-		×
ñ	New Screen Quar	e.	Ċ U	<u>۱</u>	πQ		à	×	?	1	Ð
	Data analysis					Res	ılt				
[1 2 3 4 5 6 7 8	9 10 11 12		New Ty	ping R	14	1				
	AOOOOOOOO			Marker	Chr. location	InfoCq	ΔCq	Informatik	ive for	New Ty	rpin
	• 0000000			137	1.0	31.56	2,2758	New Typi	ing D	Nega	χtγ
	00000000() 000C		209	54	31.72	2,388	New Typi	ing R	Posit	tive
	· 0000000			305	20q	30.13	0,8462	New Typi	ing D	Nega	<i>a</i>
	00000000			520	200			New Typi		Posit	
	00000000			710	54			New Typi		Nega	
				907	11q 10q			New Typi		Posit	
	+00000000	0000						New 1994	ang D	reega	124
l				 unname Sample 	ed transplan	tation		Terpet	Dr.	ddCq	
Sample ID		Accept		Post 2 13 Aug Post 1 13 Aug	EM EM		-	148	170 170	7.22	
10831 13 Aug	1	×.		Post 3 13 Aug Post 1 13 Aug	EM			148	170	10.71 4.13	
ype 12560 13 Aug	1	1		Post 3 13 Aug Post 2 13 Aug	EM EM			386	50	10.23	
rack DNA 103 13 A	lug	V		Post 3 13 Aug Post 2 13 Aug	EM			748	220	11.73	
rack Post 1 13 Aug	1	×.		Post 1 13 Aug	EM			748	220	3.98	
rack Post 2 13 Aug	1	1									
rock Post 3 13 Aug	1	×									
				C					_		3
									Selec	t assa	γS
Show rawdata		Reject	Calculate								

TRACE Analysis[™] Software also generates a report for both recipients being tested on the single plate.

Software Overview

Use of TRACE Analysis[™] Software facilitates setting up qPCR and dPCR based tests for multiple platforms, analyzes data, calculates and displays analyzed data and stores sample-specific information for easy retrieval or exporting to a laboratory information management system. All data files generated by the software are stored in .xml format, for ease of data transfer.



Preferences

The Preferences Menu of TRACE Analysis[™] Software allows for customization of many experimental parameters.

Through the Preferences window, you can choose default settings for common variables, as well as enter information which may be unique to their testing regime or laboratory.

There are four main tabs within the window which enable changes to the experimental plate setup, manual entry of sample types, disease states and reference samples, experimental protocol variables and data storage customization. These customized parameters are found on the Plate Setup, Custom Types, Concentrations and Data Locations tabs.

Plate Setup Preferences

Tab 1: Plate Setup Preferences

late setup	Custom types	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Machine	for genotypir	QIAcuity		v				
Machine	for monitorin	QIAcuity		v				
Layout ty	уре			v				
Replicate	25	dPCR Sing	gletons ~					
Color mo	de	Pastel	v Pla	ate for genoty	QIAcuity	/, v1, 96	۷	
			Pla	ate for monito	ring 96-low o	density	×	

"Machine for genotyping":

Define the default genotyping analysis platform by clicking the radio button appropriate for the machine in use. TRACE Analysis[™] Software will generate the appropriate sample setup file for the machine, as well as be able to analyze the results exported from that machine.

"Machine for monitoring":

Define the default monitoring analysis platform by clicking the radio button appropriate for the machine in use. TRACE Analysis[™] Software will generate the appropriate sample setup file for the machine, as well as be able to analyze the results exported from that machine.

"Layout type":

Define whether the samples are placed onto the plate in groups or in rows. We strongly recommend organizing samples by groups, as this makes the pipetting scheme very easy to follow.

"Replicates":

Define whether to perform quantitative analysis using singletons, duplicates or triplicates. For a qPCR monitoring, JETA Molecular recommends the use of triplicates, as low concentration DNA samples may exhibit stochastic variance in Cq.

"<u>Color mode</u>":

The color highlights which appear on the software interface and the protocols can be modified to use a scheme which is preferred by the user.

"<u>Plate for genotyping</u>":

This preference selects the plate chemistry version in use for genotyping. Different plate versions have slightly different assay panel members. If you have created Custom Genotyping Panels, they will appear here.

"<u>Plate for monitoring</u>":

This preference selects the plate version in use for monitoring. This allows you to select among different plate types used in quantitative analysis by digital PCR.

Custom Types Preferences

In the Custom types tab of the Preferences Menu, users may define the cell types being used, the diseases associated with the samples as well as any external reference materials which may be used.

Tab 2: Custom Types Preferences

late setup	Custom typ	pes	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Cell Types	Diseases	Ext	ernal references						
Custom s	ample type	s ma	ay be added or d	leleted using this n	nenu				
DNA			×	Ad	bi				
B cells			×						
T cells			×						
Blood			×						
BM			×	Re	set				

Cell Types tab of the Custom types tab of the Preferences Menu:

Define the origin of the materials being tested. You can type a sample material in the empty box near the Add button, then press Add, and the sample type will now appear as an option in TRACE Analysis[™]. The small "x" on the same line as the sample type is used to remove that type from the software. It is best to add all known types from the earliest use of TRACE Analysis[™].

Cell Types Diseases External references Custom disease types may be added or deleted using this menu AML * Add Reset	late setup	Custom typ	Concentra	tions	Data and Reports	Data locations	Material tracking	Users	Language	
AML 🗶 Add	Cell Types	Diseases	External refere	nces						
	Custom d	isease type	s may be adde	d or d	eleted using this n	nenu				
Reset	AML		×		Ad	bb				
Ne Jek					Re	cot				
					- No.					

Diseases tab of the Custom types tab of the Preferences Menu:

Define diseases associated with the samples to be tested.

Type the disease name in the empty box near the Add button, then press Add, and the disease will now appear as an option in TRACE Analysis[™] Software. The small "x" on the same line as the disease name can be used to remove that disease from the software.

It is best to add all known diseases from the earliest use of TRACE Analysis[™] Software.

late setup	Custom typ	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Cell Types	Diseases	External references						
Custom r	eference sa	mples may be adde	d or deleted using t	this menu				
Cell lin	e 101		×	Add				
Univers	sal Positive	Control	×	Reset				
]					

External references tab of the Custom types tab of the Preferences Menu:

Define external reference DNAs to be used in the monitoring using qPCR.

Type the reference name in the empty box near the Add button, then press Add, and the reference will now appear as an option in TRACE Analysis™ Software. The small "x" on the same

line as the reference name can be used to remove that reference from the software. It is best to add all known references from the earliest use of TRACE Analysis[™] Software.

Concentrations Preferences

Tab 3: Concentration Preferences

late setup	Custom types	Concentrations	Data and Reports	Data loca	ations	Material tracking	Users	Language	
Correctio	on for excess	master mix		15	%				
Correctio	on for excess	DNA dilution		15	%				
Sample 1	Input for Gen	otyping		10	ng				
Reference	ce Sample Inp	out for Monito	ring (qPCR)	10	ng				
PostTx S	Sample Input	for Monitoring	J	150	ng				
Default	concentration	of samples		50	ng/	μL			
						Reset			

Concentrations tab of the Preferences Menu:

Define the variables used in protocol generation and experimental execution.

Define how much excess master mix and how much excess DNA dilution to use in the experimental protocol.

TRACE Analysis[™] calculates the volumes needed, based on the experimental setup and then adds these additional factors to provide more than enough of each solution to execution the experiment.

Define the sample input for genotyping using different amplification methods.Based on the data from verification studies, JETA Molecular recommends the use of 5ng DNA input per well for genotyping using qPCR and 10ng for genotyping using dPCR.

Define the amount of reference sample used for monitoring using qPCR

Define the sensitivity to achieve in the experiment by entering the target DNA input per well for the monitoring samples.

While sensitivity may be thought of in terms of cell numbers or percentages or grams of DNA, the software uses nanograms of input to generate an appropriate protocol.

Define the default concentrations of samples.

This number will appear for all samples and can be altered if necessary.

Data and Reports Preferences

Tab 4: Data and Report Preferences

<u>qPCR tab:</u>

late setup	Custom types	Concentrations	Data an	d Reports	Data locations	Material tracking	Users	Language
qPCR dP	PCR				Laboratory	Information		
Replicat	e Highlightin	g Method			Department	Technical Supp	ort	
Method	Value				Institution	JETA Molecula	r	
○ % CV	2				Address	Krommeweter	ing 101	c
SD	1				Postal code, 0	City 3543 AN, Utre	cht	
Cq Ran	ge 0.5				Telephone	+316 5413 669	97	
Report I	Highlighting				Comment			
Method			Low	High	Anonymous	Reporting		
Replica	te Highlighting				- · ·	nymous Reporting		
Reference	nce Sample Cq R	ange	24	28	HPRIM Rep			
Referen	nce Sample ∆Cq	[-1.5	1.5	Allow HPRI	3		
Monito	ring Sample RNa	aseP Cq Range	20	24				

In order to achieve more customized data analysis,TRACE Analysis[™] has a tab called Data and Reports in the Preferences section of the software.

There is a Replicate Highlighting Section and a Report Highlighting Section.

In Replicate Highlighting, users can select between three methods to highlight variance in replicate samples. Replicates displaying variance in %CV, SD or Cq range may be user defined, based on the lab's practices. The chosen method is applied to all replicates in monitoring, whether it is the reference sample or the monitoring sample.

In Report Highlighting, users can select to have data highlighted on the experimental reports, if the data falls outside of input ranges. The user defines which ranges are critical and these will be readily apparent when data reports are reviewed

In Report Highlighting, users have the ability to choose to have portions of their report highlighted, if values generated fall outside an expected range. The user can choose up to four different data quality inspections to be performed on monitoring data, with values outside of the input ranges highlighted automatically on the report

Protocols and Reports can be customized by adding institutional information under the Laboratory Information section. Any information entered here will appear at the top of all protocols and reports generated by TRACE Analysis[™] Software.

dPCR tab:

Preferences	×	(a) Preferences	×
Plate setup Custom types Concentrations Data and Reports	Data locations Material tracking Users Language	Plate setup Custom types Concentrations Data and Reports Data locations Material tracking Users Language	
GPCR dPCR Report Highlighting Low Total valid droplets/partitions 6000 Post transplant RNaseP 200 concentration (copies/µl) 200 NTC concentration (copies/µl) 0 UPC concentration 5 Other UPC	Laboratory Information Department Technical Support Institution JETA Molecular Address Kommexetering 101C Postal code, City 3543 AA, Utrecht Telephone -1316 5413 6697 Comment Anonymous Reporting Allow Anonymous Reporting HPRIM Reporting Allow HPRIM Reporting	Image: Constraint of Constraints Laboratory Information Report Highlighting Department Technical Support Total valid droplets/partitions 3000 9000 Address Post transplant RNaseP 200 5000 Postal code, City concentration (copies/µI) 0 0.3 Comment UPC concentration [copies/µI] 5 1000 Anonymous Reporting Other HPRIM Reporting Use UPC MIRK Reporting	
	Ok Cancel Apply	Ok Cancel /	Apply

The number of valid droplets/partitions, post transplant RNase P concentration, UPC and NTC concentration can be defined here. If the actual droplet/partition number or RNaseP, NTC or UPC concentration falls outside of the defined range, data will be highlighted in the report.

In the report highlighting settings, the total valid droplet/partitions values would be different for plate-based digital PCR (example on the right) and for droplet-based digital PCR (example on the left).

The use of a Universal Positive Control in a dPCR monitoring can be enabled or disabled here.

Data Location Preferences

Tab 5: Data locations Preferences

		concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Choose th	e location whe	re the software s	stores your data fil	es				
Data stor	re location		C:\Users\jeroen\D	ropbox (JETA)\;	ETA Team Folder	JETA)	02 📜	Default

Data locations tab of the Preferences Menu:

Define where TRACE Analysis[™] Software stores the data it generates.

For labs using multiple copies of TRACE Analysis[™] Software, this location is likely best set as a shared location on a server. When all local copies of TRACE Analysis[™] Software point to the same data storage location, all copies can read and write to the same data files, eliminating the need to transfer files between computers.

Reagent Tracking Preferences

This tab is where reagent lot numbers and expiration dates may be entered into TRACE Analysis[™] Software. This information will populate protocols and reports, eliminating the need for the operator to write the information each time.

qPCR Reagent tracking tab

This tab enables to enter reagent lot numbers and expiration dates for qPCR reagents.

late setup	Custom types	Concent	trations	Data a	and Reports	Data locatio	ons	Material tracking	Users	Language	
qPCR Reag	gent tracking	dPCR Rea	gent trac	king	Equipment &	consumable	es ti	racking			
Name			Lot N	umber	Expiry Date			Comment			
QTRACE	INDEL Assay 6	i34	P10	700	31/07/202	23 15	5				^
QTRACE	INDEL Assay 6	i50	NOO	580	31/03/202	2 15	,				
QTRACE	INDEL Assay 6	i63	Q10	490	30/09/202	24 15	,				
QTRACE	INDEL Assay 6	570	P104	150	28/02/202	23 15	,				
QTRACE	INDEL Assay 6	78	R11	370	31/05/202	25 15	,				
QTRACE	INDEL Assay 6	81	R11	380	31/05/202	25 15	,				
QTRACE	INDEL Assay 6	i94	R11	390	31/05/202	25 15	,				
QTRACE	INDEL Assay 7	06	NOO	060	31/12/202	21 15	,				
QTRACE	INDEL Assay 7	10	N01	910	31/08/202	2 15	,				
QTRACE	INDEL Assay 7	21	N01	920	31/08/202	2 15	a				~

dPCR Reagent tracking tab

This tab enables to enter reagent lot numbers and expiration dates for dPCR reagents.

late setup	Custom types	Concentrations	Data and Reports	Data locations	Material trac	king Users	Language	
qPCR Rea	gent tracking	dPCR Reagent trac	king Equipment	& consumables tr	acking			
Name			Lot Number	Expiry Date	Cor	nment		
d102			T10580	31/03/2026	15			^
d113			S10670	30/06/2026	15			
d120			T10870	31/05/2027	15			
d137			S10680	30/06/2026	15			
d148			S10230	31/03/2026	15			
d157			T10210	31/03/2027	15			
d176			T10590	30/04/2027	15			
d183			S11300	30/11/2026	15			
d198			S11100	31/10/2026	15			
d209			S10110	31/01/2026	15			

Equipment & consumables tracking tab

This tab enables to enter serial number and location information for equipment and consumables.

Plate setup	Custom 1	types	Conce	entrations	Data	and Repo	orts Data locations	Material	tracking	Users	Language	
qPCR Reage	ent tracki	ing dF	PCR R	eagent tra	cking	Equipme	nt & consumables tr	acking				
Name	Lot N	umber		Expiry Da	te		Comment		Prin	t		
ABI 750	0 SN 2	7500)	xx	21/10/2	021	15	Genetic Analysis I	.ab	-			
P10	SN Y	xx		25/02/2	021	15	DNA Lab		~			
P100	SN X	xxx		24/03/2	021	15	PCR Setup Lab		-			
Ad	d		De	lete	021	15						
Ad	d		De		021	15						

Users Preferences

This tab is where User profiles are managed by an individual with 'Supervisor' rights in TRACE Analysis™ Software.

ate setup	Custom types	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Jser Man	agement							
		Name		Role	^			
Technici	an		Anal	yst				
Advance	ed User		Adva	ancedUser				
Lab Dire	ector		Supe	ervisor				
Technici	an 2		Anal	yst				
					\sim			
Change	Password	Add User	Delete Use	er				

Language Preferences

This tab allows the users to select among available language versions.

late setup	Custom types	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
Language				Engli	sh			
				Englis	h			

Software Access And User Profile Management

TRACE Analysis[™] Software restricts access to the software and well as functions within the software.

When TRACE Analysis[™] launches for the fist time, User Profiles need to be established.

Once logged in with JETA's credentials, a Supervisor account needs to be created.

In the Preferences, under the new User tab, there is an option for creating New Users.

ate setup	Custom types	Concentrations	Data and Reports	Data locations	Material tracking	Users	Language	
User Man	agement							
	(j	Name		Role				
Changes	Descurred	Add How	Delate Use					
Change	Password	Add User	Delete Use	er				
Change	Password	Add User	Delete Use	er				
Change	Password	Add User	Delete Use	er				

By clicking 'Add User' a dialog box opens wherein the details of the new user can be added.

Add User		8 <u>-10</u>	Х
Name	Lab Director		
Password	•		
And the second sec			
Confirm password			

After the Supervisor has created an account, multiple additional "Supervisor," "Advanced User," and "Analyst" accounts can be made.

late setup	Custom types	Concentrations	Data and Reports	Data locations	Reagent tracking	Users	Language	
User Man	agement							
	1	Name		Role	· ^ ·			
Technicia	an		Analy	/st				
Advance				ncedUser				
Lab Dire				rvisor				
Technicia	an 2		Analy	/st				
Change	Password	Add User	Delete Use	er	~			

Analysts and Advanced Users are allowed to change their passwords, and to edit the Reagent tracking Preferences information. All other Preferences settings are controlled by the Supervisor. Advanced Users inspect and can validate a result.

	Edit all tabs in Preferences	Results Validation	Add or Delete Users/Passwords	Edit Reagent Tracking Data
Supervisor	Yes	Yes	Modify All Users and Passwords	Yes
Advanced User	No	Yes	Modify Personal Password	Yes
Analyst	No	No	Modify Personal Password	Yes

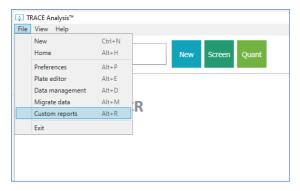
File Management

There are a few files that TRACE Analysis[™] stores to enable tracking of data, logging of errors and supply a list of assays from JETA Molecular's kits.

Data storage and retrieval in TRACE Analysis[™] Software version 2.0 is enhanced through the introduction of a relational database data storage format. Previous versions of software relied on generation of .xml files which were stored in various folders, making the information captured in the software difficult to access.

Information is now captured and stored in a database, making SQL queries of the data possible. This also facilitates enhanced performance of the software, allowing users more ability to modify or update experiments and sample records, as needed.

In order to query records in the database, under 'File-->Custom reports' leads to a search interface for TRACE Analysis[™] Software.



Search queries can be built, executed and stored. The results of the queries can also be easily exported to .csv and .xls format files. The image below shows the results for the number of times an assay is informative for recipients in the database.

TRACE Analysis"						-		×
File View Help								
ñ		New Screen Quant	<u>1</u>	±] М́ [∨т	۹ 💾 🚔	×	?	0
<u>Count</u> of	UMNS WellPrimers Name None OffsetControl as WellPrimers dd a new column)	OffsetControl Count None		Columns Sort [Click here to add a n	5		Ciea Save Q Load Q Execute (uery uery
 ✓ 1 Sample ✓ 2 WellPri ✓ 3 Sample 	nditions wds where all of the following as Otraceholode starts with Gr mera InformativeForSample in as SampleType is equal to Br dd a new column]	enotyping a not null						
Query Re					[Export to Csv] [Expo	rt to Excel]		
	WellPrimers OffsetControl Count							
137	375					^		
	281					_		
157	47 64					_		
176	64 53					_		
198	60					_		
	329					_		
	50					~		
666	30							

For the logging of errors, files are created in your temporary directory (C:\Users\Your Profile\AppData\Local\Temp), named QTRACE.log(n).

These file store per session in TRACE Analysis[™] Software what actions the user performs. In the case of an error these log files can be send to support to detect the cause and solve the issue.

The list of assays from JETA's kits is stored by default at C:

\ProgramData\QTRACE\QTRACEKit\JETAMarkers.txt. This file is created during installation and must at all times be present and unmodified for TRACE Analysis[™] Software to work.

Experimental Files Saved in TRACE Analysis[™] Software

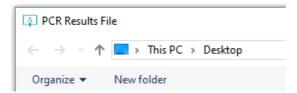
TRACE Analysis[™] Software automates the storage of all files associated with a recipient's testing history by automatically creating folders which take the form: recipient Name_recipient ID

\rightarrow QTRACE \rightarrow Data Folders \rightarrow	Gregor Mendel_7458	
Name	Date modified	Туре
PCR Results Files	16/01/2025 09:14	File folder
PCR Run Files	16/01/2025 09:13	File folder
PCR Setup Files	11/11/2024 08:35	File folder
Protocols	11/11/2024 08:37	File folder
ᡖ Reports	16/01/2025 09:14	File folder

Within each of these folders are all the files used to track the recipient. When PCR data is imported into TRACE Analysis[™], the user also has the ability to identify the location of the original PCR machine file.

_	5
k	6

After clicking the "Import PCR Data" button, TRACE Analysis™ first asks the user to identify the location of the exported results file. The imported file is automatically saved to the PCR Results File folder, while the data from the file is extracted and analyzed in TRACE Analysis[™].



After selecting the results file, TRACE Analysis[™] prompts the user to identify the location of the data collection file from the PCR machine. The imported file is automatically saved to the PCR Runs File folder.

PCR Machine	File
← → • ↑	🔜 > This PC > Desktop
Organize 🔻	New folder

The selection of the data file is optional, but if it is selected, TRACE Analysis™ will copy this file

and put it into the Run Files folder for each patient in the same experiment. With the reports that are generated from each experiment, they are differentially saved – only the report for the specific patient goes into that patients file, while the other patient sample has a separate report, and it is saved in their separate folder.

TRACE Analysis[™] Software also creates a folder called Exports. The data from every experiment is automatically converted into an xml file which is then easily available to integrate into a LIMS.

QTRACE	
Name	
🌏 Data Folders	
🚽 Experiment	
Exports	
on Sample	
🛃 Transplantati	on

Data Exports - csv and xlsx

In addition to the previous .xml export files which were automatically created by TRACE Analysis[™] Software, TRACE Analysis[™] Software v1.08 now automatically generates .csv and .xlsx files containing the data from the experimental reports.

QTRACE > Exports		~ Ū	Search Exports	5
Name	Date modified	Туре	1	Size
180614 L06300 UCLA801 T0.xlsx	08/04/18 4:33 PM	Microsoft Excel Worksheet		6 KB
180614 L06300 UCLA801 T0.csv	08/04/18 4:33 PM	Microsoft Excel Comma Separat	ed Values File	3 KB
180614 L06300 UCLA801 T0.xml	08/04/18 4:33 PM	XML Document		14 KB

These new automated exports are stored in the Exports folder within the QTRACE folder.

The following is an example of the data found in the xlsx file from a Custom Genotyping experiment.

This file has recipient and donor IDs, informative assays, dCq values, assay status (positive, negative or atypical) and the mean Cq values for assay replicates.

	10831 21 Aug	2018-08-	10831 21 Aug					
1	12560 21 Aug	12560 21	Aug					
1	916	10q	12560 21 Aug	0.52	Negative	40	Positive	29.8002
2	305	20q	12560 21 Aug	0.85	Negative	40	Positive	30.1296
3	710	5q	12560 21 Aug	1.06	Negative	40	Positive	30.3468
4	137	1p	12560 21 Aug	2.28	Negative	40	Positive	31.5592
5	907	11q	10831 21 Aug	0.45	Positive	29.777	Negative	40
6	520	20q	10831 21 Aug	0.78	Positive	30.1095	Negative	40
7	209	5q	10831 21 Aug	2.39	Positive	31.7184	Negative	40
8	361	12q	None	2	Negative	40	Negative	40
9	706	16p	None	-	Positive	30.3095	Positive	30.2039
10	755	11q	None	2	Negative	40	Negative	40
11	NTC	14q	None	-	Negative	40	Negative	40
12	POS	14g	None	14	Positive	29.3304	Positive	29.2834

The following is an example of an xlsx export from a monitoring experiment.

Again, samples are identified, the mean Cq values and the %DNA value for each sample and

assay combination are exported, in addition to the Mean % DNA for all assays tested on a single sample.

RECIPIENT	DNA 103 21 Aug	DNA 103 21 Aug	2018-08-	DNA 103 21 Aug
DONOR	2	DNA 103 21 Augr	DNA 103	21 Augr
SAMPLE	1	DNA 103 21 Aug	Referen	ce
MARKER	DNA 103 21 Aug	1	148	30.03
MARKER	DNA 103 21 Aug	2	386	30.99
MARKER	DNA 103 21 Aug	3	748	29.48
MARKER	DNA 103 21 Aug	4	RNaseP .	29.85
SAMPLE	2	Post 1 21 Aug	Sample	
MARKER	Post 1 21 Aug	1	148	29.68
MARKER	Post 1 21 Aug	2	386	30.83
MARKER	Post 1 21 Aug	3	748	29.17
MARKER	Post 1 21 Aug	4	RNaseP	25.57
SAMPLE	3	Post 2 21 Aug	Sample	
MARKER	Post 2 21 Aug	1	148	31.82
MARKER	Post 2 21 Aug	2	386	33.04
MARKER	Post 2 21 Aug	3	748	31.48
MARKER	Post 2 21 Aug	4	RNaseP	24.41
SAMPLE	4	Post 3 21 Aug	Sample	
MARKER	Post 3 21 Aug	1	148	37.63
MARKER	Post 3 21 Aug	2	386	38.1
MARKER	Post 3 21 Aug	3	748	38.1
MARKER	Post 3 21 Aug	4	RNaseP .	26.74
CELL_FRACTION	1	Post 1 21 Aug	148	6.58
CELL_FRACTION	2	Post 1 21 Aug	386	5.72
CELL_FRACTION	3	Post 1 21 Aug	748	6.36
CELL_FRACTION	4	Post 2 21 Aug	148	0.67
CELL_FRACTION	5	Post 2 21 Aug	386	0.56
CELL_FRACTION	6	Post 2 21 Aug	748	0.58
CELL_FRACTION	7	Post 3 21 Aug	148	0.06
CELL_FRACTION	8	Post 3 21 Aug	386	0.08
CELL_FRACTION	9	Post 3 21 Aug	748	0.03
CELL_FRACTION_MEAN	1	Post 1 21 Aug	6.22	
CELL_FRACTION_MEAN	2	Post 2 21 Aug	0.6	
CELL_FRACTION_MEAN	3	Post 3 21 Aug	0.06	

HPRIM Data Export

Fonction d'export HPRIM

 a) Activer cette fonction dans 'Preference\Data and Reports', en cochant 'Allow HPRIM Reporting'

late setup	Custom types	Concentrations	Data a	nd Reports	Data locations	Material tracking	Users	Language
qPCR dP	CR				Laboratory 1	Information		
Replicat	e Highlightin	g Method			Department	Technical Supp	ort	
Method	Value				Institution	JETA Molecula	r	
○ % CV	2				Address	Krommeweteri	ing 101	С
SD	1				Postal code, C	ity 3543 AN, Utre	cht	
🔾 Cq Ran	ge 0.5				Telephone	+316 5413 669	97	
Report I	lighlighting				Comment			
Method			Low	High	Anonymous	Reporting		
Replica	te Highlighting					ymous Reporting		
Referen	ice Sample Cq R	ange	24	28	HPRIM Repo	orting		
Referer	ice Sample ∆Cq		-1.5	1.5	Allow HPRI	~		
Monito	ring Sample RNa	aseP Cq Range	20	24		in hepotting		
Monito	ring Sample RNa	aseP Cq Range	20	24				
						Ok	Can	icel A

b) Créer un nouveau couple donneur- receveur

Les nouveaux champs sont encadrés en rouge.

Re	cipient	1
Recipient First Name	2	
Recipient Last Name		
Recipient ID		
Sample ID		
DNA ID		
G-LIMS ID		
Concentration ng/ul	100	
Date of Birth	XX-XX-XXXX	15
Date of Transplant	XX-XX-XXX-XXXXX	15
Gender	O Male O Fem	ale
	○ Male ○ Ferm	ale
Comment	O Male O Fem	ale
Comment Disease Type	O Male O Fem	ale v
Comment Disease Type		ale v
Comment Disease Type Donor First Name		ale ~
Comment Disease Type Donor First Name Donor Last Name		ale V
Gender Comment Disease Type Donor First Name Donor Last Name Donor ID Sample ID		ale V
Comment Disease Type Donor First Name Donor Last Name Donor ID Sample ID		√ ×
Comment Disease Type Donor First Name Donor Last Name Donor ID		v √ >
Comment Disease Type Donor First Name Donor Last Name Donor ID Sample ID DNA ID Concentration ng/ul		15 ■
Comment Disease Type Donor First Name Donor Last Name Donor ID Sample ID DNA ID)onor	v V X

Remarque importante:

Le numéro 'DNA ID' est un champ ajouté pour faciliter la préparation de la plaque. Le personnel technique du laboratoire peut y indiquer le numéro de son choix, celui-ci est affiché uniquement sur le protocole et sur le plan de plaque. Si celui-ci n'est pas renseigné lors de la création du patient, c'est le 'Sample ID' qui sera affiché sur le protocole.

c) Renseigner le nouveau champ '**Q-LIMS ID**'. Il apparait dans le bloc 'Recipient' après avoir sélectionné l'échantillon de référence pour le test de quantification.

Recipient First Name		
Recipient Last Name		
Recipient ID		
Sample ID		
DNA ID		
G-LIMS ID		
Concentration ng/ul	100	
Date of Birth	XX-XX-XX00X	15
	xx-xx-xxxx	15
Date of Transplant		
Date of Transplant Gender	O Male O Fem	ale
	O Male O Fem	ale

d) Renseigner le nouveau bloc 'Prior Sample'.

Celui-ci permet de quantifier l'échantillon précédent. La préparation de cet échantillon sur la plaque ainsi que le calcul du résultat sont effectués comme un échantillon 'Post Sample'. Le % ADN est exporté dans le fichier HPRIM et il est imprimé sur le rapport.

Attention: Le résultat de l'échantillon 'Prior Sample' n'est pas affiché sur le graphique de suivi.

Refere	nce Sam	ples
Sample ID	Concentration ng/ul	n % reference
	100	100
	100	100
Pavel example	10	100
Ref Sample	10	100
Pric	or Sample	e 🗙
Prior Sample ID		
DNA ID		
Concentration ng/u	100	
Prior Sample Date	XX-XX-XXX	x 15
Prior Sample Type	O BM	O Blood
	O T Cells	O B Cells
	O DNA	
	rior Sam	
Pos	t Sample	× ×
Sample ID		
DNA ID		
Concentration ng/u	100	
Sample Date	XX-XX-XXX	x 15
Sample Type	O BM	O Blood
	O T Cells	O B Cells
	O DNA	
	-	

e) Valider les résultats

- Après avoir importé les résultats de qPCR de votre patient et après avoir vérifié les données de

votre plaque dans la fenêtre « Data analysis », cliquer sur le bouton 'Calculate'

- Le logiciel affiche alors les résultats de votre génotypage ou de votre quantification dans la fenêtre 'Result'.

Exemple: résultat de génotypage

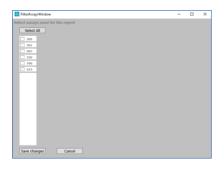
				Result						
RECEVEUR 210 RECEVEUR 210										
Marker	Chr. location	InfoCq	∆Cq	Informative for	RECEVEUR 210 RECEVEUR 210	DONNEUR 210 DONNEUR 210				
209	5q	31.46	2,6465	RECEVEUR 210 RECEVEUR 210	Positive	Negative				
235	2q	31.03	2,2197	RECEVEUR 210 RECEVEUR 210	Positive	Negative				
267	4q	30.7	1,6455	DONNEUR 210 DONNEUR 210	Negative	Positive				

Exemple: résultat de quantification

Result									
4083384	FIRST 408	3384 LAST 🕋	í						
Sample	Type	Date	Target	Chr.	ddCq	DNA (%)	Reference		
4081082	Blood	05-05-2019	356	18q	4.45	4.59	4083384		
4081082	Blood	05-05-2019	721	Xq	4.77	3.67	4083384		
4099253	B Cells	09-05-2019	356	18q	6.52	1.09	4083384		
4099253	B Cells	09-05-2019	721	Xq	3.97	6.37	4083384		

- Cliquer ensuite sur le bouton 'Select assays' Select assays'. Fonction autorisée ou non selon les droits de l'utilisateur.

- Une fenêtre s'ouvre pour sélectionner les marqueurs. Vous devez sélectionner les marqueurs de votre choix.



- Cliquer ensuite sur 'Save changes' pour valider vos résultats. Les boutons 'HPRIM Export' et 'Report' apparaissent alors.



f) Exporter un fichier HPRIM

- Cliquer sur le bouton 'HPRIM Export'. La fenêtre ci-dessous s'ouvre, puis choisir le dossier dans lequel le fichier HPRIM sera enregistré:

echer	cher un dossier	×
Bu	reau	^
> 📑	OneDrive	
> 2	User	
- 🗖	Ce PC	
_	E Bureau	
~	Documents	
	> Blocs-notes OneNote	
	> Bluetooth Folder	
	EXPERIMENT OTRACE	
		~
	Bluetooth Folder EXPERIMENT QTRACE	

Software Buttons



Home Screen Return to the home screen of TRACE Analysis[™] Software



Add New Sample

Add a new recipient record to the database. A record will always require a recipient name, unique recipient identifier and a sample identifier.



Add Typing Samples

After entering recipient and donor specific information, choosing Screen will start creating a genotyping experiment by placing your samples on the plate.



Add Quant Samples

After selecting a recipient to monitor and entering sample specific information, the Quant button adds your sample to a monitoring experiment



Export Setup to PCR Export an experiment sample setup file for use with a PCR instrument.



Import PCR Data Import the .txt or .csv results file from your PCR instrument to review the data collected.



Overview Shows all available data for a transplantation: informative markers and quantitative analyses.



Virtual Typing

Enables side-by-side comparison of genotypes of selected samples.



Browse Experiments

Browse all experiments previously created in TRACE Analysis[™] Software. By pressing the open button, you can re-open the imported data files.



Save

Update information about the recipients or donors.

Do not update information about the recipient or donor if there is PCR data waiting to be analyzed for them.



Print

Print all typing and monitoring results from the currently selected recipient data.



Preferences

Set your preferred instrument, sample types, number of replicates and disease types.



Help

Review this manual directly via TRACE Analysis[™] Software to search for useful tips, tricks and troubleshooting.



About

Technical information about TRACE Analysis[™] Software, such as version, license, contact information.

÷

Add Sample Add another sample.

×

Remove Remove a sample.



Reset Reset the experiment plate completely.



Undo

Reset the last placement onto the experimental plate.

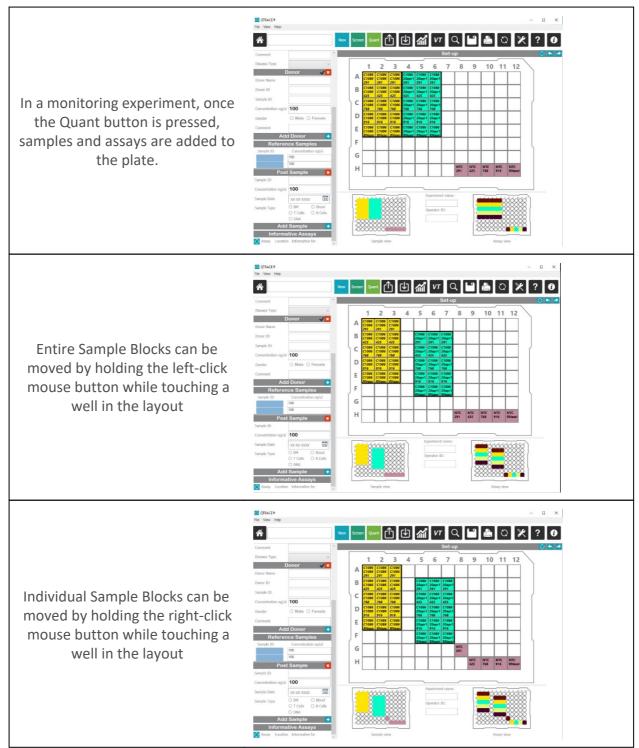


Redo

Reset the previous "Undo" action onto the experimental plate.

Drag and Drop Plate Layouts

TRACE Analysis[™], Version 1.5 introduced movable wells for customized plate layout capability. Once monitoring samples are placed onto the plate, the user has the ability to move a well from one location to another location. Whole sample groups can be moved by left-clicking and holding the mouse button. This action treats all the wells in a particular sample group the same, moving the entire block of wells. Right-clicking and holding the mouse button allows a user to move one well at a time.



Anonymized Reporting

The ability to generate anonymized reports from TRACE Analysis[™] Software is an option in the Preferences.

On the 'Data and Reports' tab of the Preferences, there is a check box which allows for reports to be generated without the names of the recipient and donor(s) appearing on them.

Plate setup	Custom types	Concentrations	Data a	nd Reports	Data locations	Material tracking	Users	Language	
qPCR dP0	CR				Laboratory 1	Information			
Replicate	e Highlightin	g Method			Department	Technical Supp	ort		
Method	Value				Institution	JETA Molecula	r		
○ % CV	2				Address	Krommeweter	ing 101	C	
SD	1				Postal code, C	City 3543 AN, Utre	cht		
Cq Rang	e 0.5				Telephone	+316 5413 669	97		
Report H	ighlighting				Comment				
Method			Low	High	Anonymous	Reporting			
Replicat	e Highlighting								
Referen	ce Sample Cq R	ange	24	28					
Referen	ce Sample ∆Cq		-1.5	1.5		5			
Monitor	ing Sample RN	aseP Cq Range	20	24		in nepoting			
Replicat Reference Refere	ce Sample Cq R ce Sample ΔCq	-	24 -1.5	28	Anonymous Allow Anon HPRIM Repo	nymous Reporting			

With the 'Allow Anonymous Reporting' option checked, Reports generated from TRACE Analysis[™] Software will have the Recipient ID or the Donor ID replacing the name of the individuals.

The following is an example of a Transplantation Report (Recipient data overview) generated with the normal settings and the same report when 'Allow Anonymous Reporting' is checked. In this example, the recipient's name is "Receveur" and the donor's name is "Donneur." "EPT2017R" is the sample ID of the recipient pre-transplant sample.

	Chim9-:	2	DNA	03-Nov-2017	13	7 1p	1	10.31	0.08EPT2017R	Receveur
	Chim9-	2	DNA	03-Nov-2017	23	5 2q	1	10.43	0.07EPT2017R	Receveur
	Chim9-:	2	DNA	03-Nov-2017	43	1 3q	1	10.32	0.08EPT2017R	Receveur
							N	lean:	0.08	
	Chim10	-2	DNA	03-Nov-2017	13	7 1p		9.35	0.15 EPT2017R	Receveur
	Chim10	-2	DNA	03-Nov-2017	23	5 2q		9.54	0.13EPT2017R	Receveur
	Chim10	-2	DNA	03-Nov-2017	43	1 3q		9.58	0.13 EPT2017R	Receveur
							N	lean:	0.14	
Assay	Locus	Informativ	ve for	InfoCq	∆Cq	Recipient	Cq	Donor 1	Cq	
1			ve for	InfoCq	ΔCq	Recipient	Cq	Donor 1	Cq	
ssay				InfoCq 31.69	∆Cq 3.2	Recipient Positive		Donor 1 Negative	Cq 40.0	
ssay	Locus	Informativ					31.7			
ssay 907 854	Locus 11q	Receveur		31.69	3.2	Positive	31.7	Negative	40.0	
907 854 840	Locus 11q 12q	Informativ Receveur Receveur		31.69 29.32	3.2 0.83	Positive	31.7 29.3 39.0	Negative Negative	40.0	
307 307 854 840 824	Locus 11q 12q 9q	Receveur Receveur Donneur		31.69 29.32 29.54	3.2 0.83 1.71	Positive Positive Negative	31.7 29.3 39.0 28.8	Negative Negative Positive	40.0 40.0 29.5	
ssay 907 854 840 824 768	Locus 11q 12q 9q 22q	Receveur Receveur Donneur Receveur		31.69 29.32 29.54 28.76	3.2 0.83 1.71 0.28	Positive Positive Negative Positive	31.7 29.3 39.0 28.8 40.0	Negative Negative Positive Negative	40.0 40.0 29.5 38.9	
ssay 907 854 840 824 768 736	Locus 11q 12q 9q 22q 18q	Informativ Receveur Receveur Donneur Receveur Donneur		31.69 29.32 29.54 28.76 30.87	3.2 0.83 1.71 0.28 3.04	Positive Positive Negative Positive Negative	31.7 29.3 39.0 28.8 40.0 40.0	Negative Negative Positive Negative Positive	40.0 40.0 29.5 38.9 30.9	
3554 907 854 840 824 768 736 710	Locus 11q 12q 9q 22q 18q Xp	Information Receveur Donneur Receveur Donneur Donneur Donneur		31.69 29.32 29.54 28.76 30.87 29.15	3.2 0.83 1.71 0.28 3.04 1.32	Positive Positive Negative Positive Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0	Negative Negative Positive Negative Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2	
ssay 907 854 840 824 768 736 736 710 706	Locus 11q 12q 9q 22q 18q Xp 5q	Information Receveur Donneur Receveur Donneur Donneur Donneur Donneur		31.69 29.32 29.54 28.76 30.87 29.15 30.43	3.2 0.83 1.71 0.28 3.04 1.32 2.6	Positive Positive Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0	Negative Negative Positive Negative Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4	
35589 907 854 840 824 768 736 710 706 550	Locus 11q 12q 9q 22q 18q Xp 5q 16p	Information Receveur Donneur Receveur Donneur Donneur Donneur Donneur Donneur		31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79	Positive Positive Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0	Negative Negative Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6	
assay 907 854 840 824 768 736 710 706 650 634	Locus 11q 12q 9q 22q 18q Xp 5q 16p 7q	Information Receveur Donneur Receveur Donneur Donneur Donneur Donneur Donneur Donneur		31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62 29.96	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79 2.13	Positive Positive Negative Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0 40.0	Negative Negative Positive Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6 30.0	
4558ay 907 854 840 824 768 736 710 706 650 634 548	Locus 11q 12q 9q 22q 18q Xp 5q 16p 7q 11q	Informativ Receveur Donneur Receveur Donneur Donneur Donneur Donneur Donneur Donneur Donneur		31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62 29.96 28.99	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79 2.13 1.16	Positive Positive Negative Positive Negative Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0 40.0 40.0 4	Negative Negative Positive Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6 30.0 29.6 30.0 29.0	
-	Locus 11q 12q 9q 22q 18q Xp 5q 16p 7q 11q Xq	Informativ Receveur Donneur Receveur Donneur Donneur Donneur Donneur Donneur Donneur Donneur		31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62 29.96 28.99 28.33	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79 2.13 1.16 0.5	Positive Positive Negative Positive Negative Negative Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0 40.0 40.0 4	Negative Negative Positive Positive Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6 30.0 29.6 30.0 29.0 28.3	

The following is the same report with anonymization of the individuals in the report. Where names existed before, now the sample ID of the individual is displayed.

	Chim9-2	2	DNA	03-Nov-2017	137	1p	1	0.31	0.08EPT2017R	[ID:EPT2017R
	Chim9-2	2	DNA	03-Nov-2017	235	2q	1	0.43	0.07 EPT2017R	[ID:EPT2017R
	Chim9-2	2	DNA	03-Nov-2017	434	3q	1	0.32	0.08 EPT2017R	[ID:EPT2017R
							M	lean:	0.08	
	Chim10	-2	DNA	03-Nov-2017	137	1p		9.35	0.15 EPT2017R	[ID:EPT2017R
	Chim10	-2	DNA	03-Nov-2017	235	2q		9.54	0.13EPT2017R	[ID:EPT2017R]
	Chim10	-2	DNA	03-Nov-2017	434	3q		9.58	0.13 EPT2017R	[ID:EPT2017R]
							M	lean:	0.14	
Infon	Locus	ssays Informat	ive for	InfoCq	ACq	Recipient	Cq	Donor 1	Cq	
-										
ssay	Locus	Informat								
ssay	Locus 11q	Informat	017R]	31.69	3.2	Positive	31.7	Negative	40.0	
ssay 107	Locus 11q 12q	Informat [ID:EPT2 [ID:EPT2	017R]	31.69 29.32	3.2 0.83	Positive Positive	31.7	Negative Negative	40.0	
07 54 40	Locus 11q 12q 9q	Informat (ID:EPT2 (ID:EPT2 (ID:EPT2	017R] 017R] 017D]	31.69 29.32 29.54	3.2 0.83 1.71	Positive Positive Negative	31.7 29.3 39.0	Negative Negative Positive	40.0 40.0 29.5	
ssay 907 354 340 324	Locus 11q 12q 9q 22q	Informat [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2	017R] 017R] 017D] 017R]	31.69 29.32 29.54 28.76	3.2 0.83 1.71 0.28	Positive Positive Negative Positive	31.7 29.3 39.0 28.8	Negative Negative Positive Negative	40.0 40.0 29.5 38.9	
ssay 107 154 140 124	Locus 11q 12q 9q	Informat (ID:EPT2 (ID:EPT2 (ID:EPT2	2017R] 2017R] 2017D] 2017R] 2017D]	31.69 29.32 29.54	3.2 0.83 1.71	Positive Positive Negative	31.7 29.3 39.0 28.8 40.0	Negative Negative Positive	40.0 40.0 29.5	
558ay 907 854 840 824 768 736	Locus 11q 12q 9q 22q 18q	Informat [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2	017R] 2017R] 2017D] 2017R] 2017D] 2017D]	31.69 29.32 29.54 28.76 30.87	3.2 0.83 1.71 0.28 3.04	Positive Positive Negative Positive Negative	31.7 29.3 39.0 28.8 40.0 40.0	Negative Negative Positive Negative Positive	40.0 40.0 29.5 38.9 30.9	
ssay 907 354 340 324 768 736 710	Locus 11q 12q 9q 22q 18q Xp	(ID:EPT2 (ID:EPT2 (ID:EPT2 (ID:EPT2 (ID:EPT2 (ID:EPT2 (ID:EPT2	2017R] 2017R] 2017D] 2017D] 2017D] 2017D] 2017D]	31.69 29.32 29.54 28.76 30.87 29.15	3.2 0.83 1.71 0.28 3.04 1.32	Positive Positive Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0	Negative Negative Positive Negative Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2	
ssay 907 354 340 324 768 736 736 710 706	Locus 11q 12q 9q 22q 18q Xp 5q	Informat [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2	017R] 017R] 017D] 017D] 017D] 017D] 017D] 017D] 017D]	31.69 29.32 29.54 28.76 30.87 29.15 30.43	3.2 0.83 1.71 0.28 3.04 1.32 2.6	Positive Positive Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0	Negative Negative Positive Negative Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4	
ssay 107 154 140 124 168 136 106 106 150	Locus 11q 12q 9q 22q 18q Xp 5q 16p	Informat [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2	017R] 017R] 017D] 017D] 017D] 017D] 017D] 017D] 017D] 017D]	31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79	Positive Positive Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0	Negative Negative Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6	
07 54 40 24 68 36 10 06 50 34	Locus 11q 12q 9q 22q 18q Xp 5q 16p 7q	Informat [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2	2017R] 2017R] 2017D] 2017D] 2017D] 2017D] 2017D] 2017D] 2017D] 2017D] 2017D]	31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62 29.96	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79 2.13	Positive Positive Negative Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0 40.0	Negative Negative Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6 30.0	
ssay 907 854 840 824 768 736 736 736 710 706 650 634 548	Locus 11q 12q 9q 22q 18q Xp 5q 16p 7q 11q	Informat [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2	017R] 017R] 017D] 017D] 017D] 017D] 017D] 017D] 017D] 017D] 017D] 017D]	31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62 29.96 28.99	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79 2.13 1.16	Positive Positive Negative Positive Negative Negative Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0 40.0 40.0	Negative Negative Positive Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6 30.0 29.6 30.0 29.0	
	Locus 11q 12q 9q 22q 18q Xp 5q 16p 7q 11q Xq	Informat [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2 [ID:EPT2	1017R] 1017R] 1017D] 1017D] 1017D] 1017D] 1017D] 1017D] 1017D] 1017D] 1017D] 1017D] 1017D] 1017D]	31.69 29.32 29.54 28.76 30.87 29.15 30.43 29.62 29.96 28.99 28.33	3.2 0.83 1.71 0.28 3.04 1.32 2.6 1.79 2.13 1.16 0.5	Positive Positive Negative Positive Negative Negative Negative Negative Negative Negative Negative Negative	31.7 29.3 39.0 28.8 40.0 40.0 40.0 40.0 40.0 40.0 40.0 4	Negative Negative Positive Positive Positive Positive Positive Positive Positive Positive	40.0 40.0 29.5 38.9 30.9 29.2 30.4 29.6 30.0 29.6 30.0 29.0 28.3	

Modification of Existing Data

TRACE Analysis[™] Software v2.0 allows for modification of some data associated with recipients, samples, transplantation dates and experiments.

To make changes to existing files, select the Data Management item from the File Menu

File	View Help					
	New	Ctrl+N				
	Home	Alt+H		New	Screen	Qu
	Preferences	Alt+P				
	Plate editor	Alt+E				
	Data management	Alt+D	1			
	Migrate data	Alt+M	R			
	Custom reports	Alt+R	n.			
	Exit					

Selecting from the four different buttons allows users to edit some information related to these topics.



The 'D/R Info' button allows editing of Donor and Recipient information.

(a)				-	>
		D	/R Info		
Donor or Recipient ID					
FirstName					
LastName					
Date of Birth	XX-XX-XX0XX	15			
Gender		~			
Comments					
			Cancel	Save	

Sample Information may be edited by pushing the 'Samples' button.



The 'Transplantations' button allows edits to Date of Transplant.

ф.				-		×
	Trar	nsplant	tation			
Recipient ID						
Date of Transplant	XX-XX-XXXXX	15				
Date of Second Transplant	XX-XX-XXXXX	15				
Export			Cancel	Sav	е	
Anonymize export						

The 'Experiments' button allows experiments to be edited and deleted.

(J)				-		×
	Experi	ment				
Name						
Operator ID						
Date of experiment	XX-XX-XXXX 15					
		Cancel		Save		
Delete	Warning! This will delete the exp	periment with all its rela	ated data	a with n	o returr	1.
Export						
Anonymize export						

Data Analysis Algorithms

The algorithms implemented in the TRACE Analysis[™] Software are designed to provide reliable analysis results based upon specific data criteria. Quality measurements, based upon the same data criteria, provide the operator with valuable information regarding data integrity.

The algorithms and quality measurements are invoked when the operator initiates data analysis through the TRACE Analysis[™] Software. Separate algorithms and quality measurements exist for both the genotyping and quantitation tests. Warning messages generated from the quality measurements are provided when calculated results fall outside established data criteria ranges.

The QTRACE[®] Analysis System

The data criteria used in the algorithms and quality measurements are not operator configurable. Cq data will need to be exported from the qPCR Software and manipulated in a spreadsheet program if another method of data analysis is desired.

Genotyping Test Algorithm

The genotyping test algorithm determines the marker state for each assay/sample reaction followed by the informative state for each assay. Quality measurements are performed after the algorithm has been applied to the data. There are three possible marker states for each reaction in regards to amplification: positive, negative, or atypical.

Marker States

Positive

An Amplification Control reaction (positive control with QTRACE[®] RNaseP Assay; wells D11 and H11 in the standard QTRACE[®] Genotyping Plate) is positive for amplification with a Cq value less than 34.0.

A reaction with any QTRACE[®] INDEL Assay is positive for amplification if the Δ Cq (the difference in Cq values between the reaction and the Amplification Control) is within an acceptable range. If the Amplification Control is positive, then each of the reactions on the plate must have a Δ Cq of -2.0 to +3.5 to be considered positive.

Note: An NTC reaction (negative control with QTRACE[®] RNaseP Assay; wells D12 and H12) can only be negative or atypical.

Negative

An Amplification Control reaction is negative for amplification with a Cq value greater than or equal to 34.0.

All other reactions including the NTC reaction are negative with a Cq value greater than or equal to 38.0.

Atypical

An NTC reaction is classified as atypical if the Cq value is less than 38.0. A "+" sign for an NTC reaction represents the atypical result in the Analysis View. This is assigned to atypical NTC reactions when quality measurements are assessed.

A reaction with any QTRACE[®] INDEL Assay will have atypical amplification if the Δ Cq (the difference in Cq values between the reaction and the Amplification Control) is outside the acceptable ranges defined in the algorithm settings outlined above under the Marker States – Positive section. The exception to this rule is if the assay has already been classified as negative for amplification in which case the marker state will remain as negative. A large number of atypical reactions for a sample is usually indicative of low input copy number (DNA quantity) for that particular sample.

Note: An Amplification Control reaction can only be positive or negative.

Informative States

Once marker state has been determined, the genotyping test algorithm compares the marker states between two DNA samples on a plate for each assay to determine informativity. There are three possible informative states for any QTRACE[®] INDEL Assay that can be applied to each assay result:

informative, not informative, or undetermined. There are two possible informative states for the control assays using QTRACE[®] RNaseP Assay: pass or fail.

Informative

An assay is informative for a pair of DNA samples if one of the samples is positive for amplification and the other sample is negative.

Not Informative

An assay is not informative for a pair of DNA samples if both samples are positive for amplification or if both samples are negative for amplification.

Undetermined

An assay is undetermined for a pair of DNA samples if one of the two samples has an atypical reaction result. Assays with an undetermined result are not informative and should not be selected for use in quantitation for the associated pair of DNA samples.

Pass

The Amplification Control reactions in wells D11 and H11 will be labeled as pass if they are positive for amplification. The NTC reactions in wells D12 and D12 will be labeled as pass if they are negative for amplification.

Fail

The Amplification Control reactions in wells D11 and H11 will be labeled as fail if they are negative for amplification. The NTC reactions in wells D12 and H12 will be labeled as fail if they are atypical for amplification.

Genotyping Test Quality Measurements

There are two types of quality measurements provided in the genotyping test analysis: Amplification Control and NTC. Genotyping test quality measurements are not used during application of the algorithm to the data. They are presented in the Report View to inform the operator of data integrity. A warning message providing more detail accompanies each quality measurement that fails.

Amplification Control

The Amplification Control quality measurement serves as a positive PCR control for each sample.

The monomorphic RNase P locus for QTRACE[®] RNaseP Assay is present in all samples. The Amplification Control reactions are located in wells D11 and H11: one reaction for each sample. The result for this control is determined to be either pass or fail.

The Cq threshold for the Amplification Control is 34.0. If the Amplification Control has a Cq value of less than 34.0 then the Amplification Control is determined as pass. If the Amplification Control has a Cq value of greater than or equal to 34.0 then the Amplification Control is determined as fail.

If the Amplification Control for a particular sample fails, then all reactions for that sample that have a Cq value less than 38.0 will have an atypical marker state. This occurs because no reliable Δ Cq can be calculated for any of the reactions run with this sample. It follows that the resulting informative state for each assay will be labeled as undetermined.

NTC

The NTC (No Template Control) quality measurement is determined as pass or fail per sample. The NTC reactions are located in wells D12 and H2: one reaction for each sample. In addition to the Report View.

The Cq threshold for the NTC is 38.0. If the NTC has a Cq value greater than or equal to 38.0 then the NTC is determined as pass. If the NTC has a Cq value less than 38.0 then the NTC is determined as fail.

Quantification Test Algorithm

The quantification test algorithm performs the steps necessary to generate percent determinations for the DNA of interest in a mixed sample.

Relative Quantification Background

Four possible combinations of samples and assays comprise a single quantification determination.

There will always be a reference sample and one unknown sample referred to generically as "sample". The reference sample is most commonly a DNA specimen from the individual whose DNA is to be quantified in the mixed unknown sample. Relative quantification requires that a reference sample mean Cq value is compared to the sample mean Cq value. There will always be a reference assay (RNaseP Assay) and one informative quantification assay referred to generically as "assay".

The reference assay is used to normalize for total DNA input amount among all reactions. To obtain the four possible combinations of samples and assays, the reference sample and unknown sample are each amplified with the reference assay and informative quantitative assay: Reference Sample/Reference Assay, Reference Sample/Assay, Sample/Reference Assay, and Sample/Assay. These reaction combinations all belong to a common Transplantation. A Transplantation contains a unique reference sample and all associated samples to be quantified in relation to the reference sample.

Quantification is performed using an established relative quantification method for real-time PCR also known as the $\Delta\Delta$ Cq method (Livak and Schmittgen, 2001). A sample calculation is outlined in the table below.

QTRACE[®] INDEL Assay 854 is the Assay and QTRACE[®] RNaseP Assay is the Reference Assay.

Well	Sample Name	Sample Type	Assay	Cq	Mean Cq	∆Cq	ΔΔCq	2 ^(-ΔΔCq)	Mean % DNA
B4	Sample 1	Post Sample	854	30.23	30.19		7.17	0.0069	0.69%
B5	Sample 1	Post Sample	854	30.12					
B6	Sample 1	Post Sample	854	30.23					
A4	Sample 1	Post Sample	RNaseP	21.72	21.74	8.46			
A5	Sample 1	Post Sample	RNaseP	21.63					
A6	Sample 1	Post Sample	RNaseP	21.86					
B1	Sample 2	100% Reference Sample	854	23.67	23.67				
B2	Sample 2	100% Reference Sample	854	23.72					
B3	Sample 2	100% Reference Sample	854	23.63					
A1	Sample 2	100% Reference Sample	RNaseP	22.34	22.39	1.29			
A2	Sample 2	100% Reference Sample	RNaseP	22.38					
A3	Sample 2	100% Reference Sample	RNaseP	22.44					

The ΔΔCq is calculated with the following formula: (Mean Cq, Sample/Assay – Mean Cq, Sample/ReferenceAssay) – (Mean Cq, ReferenceSample/Assay – Mean Cq, ReferenceSample/ReferenceAssay)

The sample and reference sample are each normalized for total DNA input by comparing the mean Cq value of the assay reactions to the mean Cq value of the reference assay reactions.

Next, the normalized sample reaction value is compared to the normalized reference sample reaction value to generate a relative quantification value or $\Delta\Delta$ Cq. This term is then converted into a linear term which represents the fold change in DNA input amount between the sample and reference sample with the following formula: 2-($\Delta\Delta$ Cq). The fold change term is multiplied by the proportion of the reference sample that is purely reference sample (usually 100%) to generate the final percent determination for the DNA of interest in the unknown sample.

Algorithm Steps

The mean and standard deviation of the Cq values for replicate reactions are first calculated. The standard deviation calculation requires at least three replicate values. The Reference Sample/Reference Assay quality measurement is applied next. If the Reference Sample/Reference Assay quality measurement passes, the $\Delta\Delta$ Cq, and DNA percents are calculated, displayed and stored. The Reference Sample/Reference Assay quality measurement requires that the mean Cq for the Reference Sample/Reference Assay must be \leq 32.0. Samples which have a Cq \geq 39.0 are designated as negative.

The mean and standard deviation of the Cq values for replicate reactions are first calculated. The Reference Sample/Reference Assay quality measurement is applied next. If the Reference Sample/Reference Assay quality measurement passes, $\Delta\Delta$ Cq, fold change, DNA percent, and mean DNA percent (if multiple quantification assays were used for the same unknown sample) are calculated.

The DigitalTRACE[™] Analysis System

The correct placement of the threshold is crucial for an accurate data analysis. Positive droplets/partitions (above the threshold) are scored as "1", and negative droplets/partitions (below the threshold) are scored as "0". This digital signal is used to calculate the starting DNA concentration by statistically analyzing the numbers of positive and negative droplets in a sample. The users are strongly advised to check thoroughly all the thresholds after each dPCR run, before importing the run results into the TRACE Analysis[™] Software. Incorrect thresholding will lead to wrong results.

The values gained from the Universal Positive Control (UPC) amplification are not used in the calculations.

Genotyping Test Algorithm

Based on the CNV value, a marker can be scored as positive, negative or atypical.

Note: An NTC reaction (negative control containing the RNaseP Assay) can only be negative or atypical.

Calculation of CNV

CNV = (Concentration Marker / Concentration Reference)*2

Marker States

Positive

For CNV values between 0.55 and 3.2, a marker is considered positive.

Negative

For CNV values lower than 0.02, a marker is considered negative.

Atypical

For CNV values between 0.02 and 0.55, between 1.4 and 1.6 and higher than 3.2, a marker is considered atypical.

Quantification Test Algorithm

The ratio (CNV Marker/CNV Reference) gained from the dPCR software (QuantaSoft or QIAcuity Software Suite) is used to calculate the DNA percentage. The ratio is based on the number of positive and negative partitions and is refined automatically by the use of Poisson statistics.

DNA % = (Concentration Marker / Concentration Reference)*100*2 for assays with CNV=1 DNA % = (Concentration Marker / Concentration Reference)*100 for assays with CNV=2

Number of Partitions

During the partitioning step, the sample is divided into thousands of individual partitions that serve both for the calculation of the sample concentration (positive partitions), but also for the accurate statistical refinement of the calculated result (empty partitions). Problems with the partitioning process can result in lower partition numbers and, therefore, inaccurate quantification results.

Copy Number Adjustment

TRACE Analysis[™] Software allows the user to change the CNV information for each sample. See the <u>Monitoring Data Analysis and Report</u> section for details.

Using the correct CNV is necessary to obtain the accurate quantification result. An incorrect CNV will lead to an underestimated or overestimated percentage value.

Bibliography

Alizadeh, et al. Quantitative Assessment of Hematopoietic Chimerism after Bone Marrow Transplantation by Real-Time Quantitative Polymerase Chain Reaction. Blood, 2002; 99: 4618-4625.

George D, et al. Detection and Quantification of Chimerism by Droplet Digital PCR. Chimerism. 2013 Jul-Sep;4(3):102-8.

Jimenez-Velasco, et al. Reliable Quantification of Hematopoietic Chimerism after Allogeneic Transplantation for Acute Leukemia using Amplification by qPCR of Null Alleles and Insertion/Deletion Polymorphisms. Leukemia, 2005; 19: 336-423.

Koldehoff, et al. Quantitative Analysis of Chimerism after Allogeneic Stem Cell Transplantation by Real-Time Polymerase Chain Reaction with Single Nucleotide Polymorphisms, Standard Tandem Repeats, and Y-Chromosome-Specific Sequences. Am J Hematol, 2006; 81: 735-746.

Thiede, et al. Strategies and Clinical Implications of Chimerism Diagnostics after Allogeneic Hematopoietic Stem Cell Transplantation. Acta Haematol, 2004; 112: 16-23.

Verhoeven, et al. A Novel High-Throughput Droplet Digital PCR-Based Indel Quantification Method for the Detection of Circulating Donor-derived Cell-free DNA After Kidney Transplantation. Transplantation. 2022 Sep 1;106(9):1777-1786.

Willasch, et al. Sequence Polymorphism Systems for Quantitative Real-Time Polymerase Chain Reaction to Characterize Hematopoietic Chimerism-High Informativity and Sensitivity As Well As Excellent Reproducibility and Precision of Measurement. Laboratory Hematology, 2007; 13: 73-84.

Glossary		

Bi-allelic	an allele which exists in two variant forms - a major and minor allele. Individuals may be homozygous for either variant or heterozygous	
Calibrator	a sample used as the basis for comparative results	
Chimerism	A biological state where two or more genetically distinct cell populations exist within a single individual	
Comparative C_T ($\Delta\Delta C_T$) method	Method for determining relative target quantity in samples. The relative quantity of target in each sample is determined by comparing the Cq in each sample to the Cq in the reference sample.	
Cq	Quantification Cycle; The fractional PCR cycle used for quantification; also referred to as the threshold cycle (C_T), or crossing point (Cp) and take-off point (TOP)	
Experiment	a collection of genotyping and monitoring reactions that are carried out simultaneously (i.e, on a single plate or in a single set tubes for RGQ cyclers).	
INDEL	Abbreviation for insertion/deletion polymorphism; a class of DNA mutation characterized by the loss or gain of genetic material at a specific locus	
Informative assay	An assay capable of distinguishing between genetic material from two or more sources; An informative assay is an assay for a marker allele that is present (positive) in one individual genome and absent (negative) in the other genome(s).	
Monitoring assay	an assay designed for use in quantification of a specific marker, allele, or analyte. The assay must demonstrate high specificity for accurate quantification and high sensitivity to achieve a desirable limit of detection	
Reference assay	an assay designed specifically to detect the total amount of DNA in a reaction; used in quantification to normalize the amount of input DNA between reactions; also referred to as an endogenous control assay	
Reference Gene	Gene used for normalization and relative quantification	

Reference Sample	a control sample possessing the target DNA (typically 100% target DNA) to be quantified in the experimental sample by relative quantification; also referred to as a calibrator
Recipient Sample	a control sample possessing the target DNA (typically 100% target DNA) to be quantified in the experimental sample by relative quantification; also referred to as a calibrator
Relative Quantification	a method of quantification where quantity of an unknown sample is derived by comparison to a reference sample
Sample	A sample is a unique donor or recipient
Passive reference	A dye that produces fluorescence signal. Because the passive reference signal should be consistent across all wells, it is used to normalize the reporter dye signal to account for non-PCR related fluorescence fluctuations caused by minor well-to-well differences in concentrations or volume. Normalization to the passive reference signal allows for high data precision.
Normalized reporter (Rn)	Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference.
Baseline-corrected normalized reporter (ΔRn)	The magnitude of normalized fluorescence signal generated by the reporter. In experiments that contain data from qPCR, the magnitude of normalized fluorescence signal generated by the reporter at each cycle the PCR amplification. Δ Rn (cycle) = Rn (cycle) – Rn (baseline), where Rn = normalized reporter
Threshold	The intensity of fluorescence that must be exceeded for each reaction to be seen as positive
Partition	A compartment where the PCR reaction takes place
Nanoplate	A compartment where the PCR reaction takes place
CNV	Copy Number Variation (CNV) refers to a type of genetic variation in which the number of copies of a particular segment of DNA differs between individuals
UPC	Universal Plasmid Control (UPC). A synthetic control sample possessing the targets for Assays in the DigitalTRACE™panel (typically 100% target DNA)

End User Software License Agreement

JETA MOLECULAR BV END-USER SOFTWARE LICENSE AGREEMENT AND LIMITED PRODUCT WARRANTY

NOTICE TO USER: PLEASE READ THIS DOCUMENT CAREFULLY. THIS IS THE CONTRACT BETWEEN YOU AND JETA MOLECULAR BV (JETA) REGARDING THIS SOFTWARE PRODUCT. THIS AGREEMENT CONTAINS WARRANTY AND LIABILITY DISCLAIMERS AND LIMITATIONS. YOUR INSTALLATION AND USE OF THIS JETA SOFTWARE IS SUBJECT TO THE TERMS AND CONDITIONS CONTAINED IN THIS END-USER SOFTWARE LICENSE AGREEMENT, AND YOU WILL BE BOUND BY THESE TERMS AND CONDITIONS IF YOU INSTALL AND USE THE SOFTWARE. IF YOU DO NOT AGREE TO THESE TERMS AND CONDITIONS, YOU SHOULD SELECT THE DECLINE BUTTON BELOW, WHEREUPON INSTALLATION WILL STOP, AND PROMPTLY RETURN THIS SOFTWARE, TOGETHER WITH ALL PACKAGING, TO JETA AND YOUR PURCHASE PRICE WILL BE REFUNDED

This JETA End-User License Agreement accompanies a JETA software product ("Software") and related explanatory materials ("Documentation"). The term "Software" includes all executable files and applications included as part of the software product, and any upgrades, modified versions, updates, additions and copies of the Software licensed to you by JETA. The term "JETA" as used in this License, means JETA Corporation. The term "License" or "Agreement" means this End-User Software License Agreement. The term "you", "Customer" or "Licensee" means the purchaser of this license to the Software. A copy of this End-User Software License Agreement can be printed by copying the text and pasting it into word processing software that provides printing capabilities.

Third-party Products

This Software includes the following third-party software products (hereinafter collectively referred to as the "Third-Party Products"): Microsoft - .NET Framework version 4.5 Moq Copyright (c) 2007. Clarius Consulting, Manas Technology Solutions, InSTEDD http://www.moqthis.com/ All rights reserved.

NUnit

Portions Copyright © 2002-2013 Charlie Poole or Copyright © 2002-2004 James W. Newkirk, Michael C. Two, Alexei A. Vorontsov or Copyright © 2000-2002 Philip A. Craig

PDFsharp

Copyright (c) 2005-2007 empira Software GmbH, Cologne (Germany)

SharpZipLib

WPF Toolkit

Title

Title, ownership rights and intellectual property rights in and to the Software and Documentation shall at all times remain with JETA Corporation and its subsidiaries. Title, ownership rights and intellectual property rights in and to the Third-Party Product shall at all times remain with their respective licensors. All rights not specifically granted by this License, including Federal and international copyrights, are reserved by JETA and its subsidiaries.

Copyright

The Software, including its structure, organization, code, user interface and associated Documentation, is a proprietary product of JETA Corporation or its suppliers, and is protected by international laws of copyright, as well as other intellectual property laws and treatises. The law provides for civil and criminal penalties for anyone in violation of the laws of copyright.

License

Use of the Software

1 Subject to the terms and conditions of this Agreement, JETA grants you a non-exclusive, nontransferable license only to install and use the Software on a single computer for the sole purpose of using the Software that you

have purchased. You must use the Software in accordance with all applicable laws and regulations. You may transfer the Software to another single computer (or network, if a network version), but the Software may never be installed on more than one computer (or more than one network, if a network version) at any one time.

2 If the Software uses registration codes, access to the number of licensed copies of Software is controlled by a registration code. For example, if you have a registration code that enables you to use three copies of Software simultaneously, you cannot install the Software on more than three separate computers.

3 YOU MAY MAKE ONLY ONE COPY OF THE SOFTWARE IN MACHINE-READABLE FORM SOLELY FOR BACKUP OR ARCHIVAL PURPOSES, PROVIDED YOU REPRODUCE ON ANY SUCH COPY ALL COPYRIGHT NOTICES AND ANY OTHER PROPRIETARY LEGENDS FOUND ON THE ORIGINAL. JETA SHALL NOT BE RESPONSIBLE FOR THE QUALITY, INTEGRITY, FUNCTIONALITY, OR PERFORMANCE OF ANY COPY OF THE SOFTWARE. YOU MAY NOT MAKE ANY OTHER COPIES OF THE SOFTWARE.

Restrictions

1 You may not copy, transfer, rent, modify, use, merge, or translate the Software, or the associated Documentation, in whole or in part, except as expressly permitted in this Agreement or in the license agreements covering the Third-Party Product.

2 You may not reverse assemble, decompile, or otherwise reverse engineer the Software.

3 You may not remove any proprietary, copyright, trade secret or warning legend from the Software or any Documentation.

4 You agree to comply fully with all export laws and restrictions and regulations of the United States and applicable foreign agencies or authorities, and you agree that you have the sole responsibility to obtain such licenses to export, re-export or import as may be required after delivery of the Software to you. You agree that you will not export or re-export, directly or indirectly, the Software into any country prohibited by the United States Export Administration Act and the regulations thereunder or other applicable United States law.

5 Subject to the respective license terms and conditions applicable to the Third-Party Product included in this Software, you may not modify, sell, rent, transfer (except temporarily in the event of a computer malfunction), resell for profit, or distribute this license or the Software, or create derivative works based upon the Software, or any part thereof or any interest therein.

Trial

If this license is granted on a trial basis, you are hereby notified that license management software may be included to automatically cause the Software to cease functioning at the end of the trial period.

Termination

You may terminate this Agreement by discontinuing use of the Software, removing all copies from your computers and storage media, and returning the Software and Documentation, and all copies thereof, to JETA. JETA may terminate this Agreement if you fail to comply with all of its terms, in which case you agree to discontinue using the Software, remove all copies from your computers and storage media, and return the Software and Documentation, and all copies thereof, to JETA.

U.S. Government End Users

The Software is a "commercial item," as that term is defined in 48 C.F.R. 2.101 (Oct. 1995), consisting of "commercial computer software" and "commercial computer software documentation," as such terms are used in 48 C.F.R. 12.212 (Sept. 1995). Consistent with 48 C.F.R. 12.212 and 48 C.F.R. 227.7202-1 through 227.7202-4 (June 1995), all U.S. Government end users acquire the Software with only those rights set forth herein.

European Community End Users

If this Software is used within a country of the European Community, nothing in this Agreement shall be construed as restricting any right available under the European Community Software Directive, O.J. Eur. Comm. (No. L. 122) 42 (1991).

LIMITED WARRANTY AND LIMITATION OF REMEDIES

Limited Warranty. JETA warrants that for a period of ninety days from the beginning of the applicable warranty period (as described below) the Software will function substantially in accordance with the functions and features described in the Documentation delivered with the Software when properly installed, and that for a period of ninety days from the beginning of the applicable warranty period (as described below) the tapes, CDs, diskettes or other media bearing the Software will be free of defects in materials and workmanship under normal use.

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation outside of the environmental or use specifications, or not in conformance with the instructions for any instrument system, Software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with Software or products not supplied or authorized by JETA and modification or repair of the products not authorized by JETA. Warranty Period Commencement Date. The applicable warranty period for the Software installed by JETA's personnel or authorized representative begins on the earlier of: the date of installation; or three (3) months from the date of shipment for the Software. For Software installed by you or anyone other than JETA, the warranty period begins on the date the Software is delivered to you. The applicable warranty period for media begins on the date the media is delivered to you.

JETA MAKES NO OTHER WARRANTIES OF ANY KIND WHATSOEVER, EXPRESS OR IMPLIED, WITH RESPECT TO THE SOFTWARE OR DOCUMENTATION, INCLUDING, BUT NOT LIMITED TO, WARRANTIES OF FITNESS FOR A PARTICULAR PURPOSE OR MERCHANTABILITY OR THAT THE SOFTWARE OR DOCUMENTATION IS NON-INFRINGING. ALL OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED. WITHOUT LIMITING THE GENERALITY OF THE FOREGOING, JETA MAKES NO WARRANTIES THAT THE SOFTWARE WILL MEET YOUR REQUIREMENTS, THAT OPERATION OF THE LICENSED SOFTWARE WILL BE UNINTERRUPTED OR ERROR FREE OR WILL CONFORM EXACTLY TO THE DOCUMENTATION, OR THAT JETA WILL CORRECT ALL PROGRAM ERRORS. JETA'S SOLE LIABILITY AND RESPONSIBILITY FOR BREACH OF WARRANTY RELATING TO THE SOFTWARE OR DOCUMENTATION SHALL BE LIMITED, AT JETA'S SOLE OPTION, TO (1) CORRECTION OF ANY ERROR IDENTIFIED TO JETA IN A WRITING FROM YOU IN A SUBSEQUENT RELEASE OF THE SOFTWARE, WHICH SHALL BE SUPPLIED TO YOU FREE OF CHARGE, (2) ACCEPTING A RETURN OF THE PRODUCT, AND REFUNDING THE PURCHASE PRICE UPON RETURN OF THE PRODUCT AND REMOVAL OF ALL COPIES OF THE SOFTWARE FROM YOUR COMPUTERS AND STORAGE DEVICES, (3) REPLACEMENT OF THE DEFECTIVE SOFTWARE WITH A FUNCTIONALLY EQUIVALENT PROGRAM AT NO CHARGE TO YOU, OR (4) PROVIDING A REASONABLE WORK AROUND WITHIN A REASONABLE TIME. JETA'S SOLE LIABILITY AND RESPONSIBILITY UNDER THIS AGREEMENT FOR BREACH OF WARRANTY RELATING TO MEDIA IS THE REPLACEMENT OF DEFECTIVE MEDIA RETURNED WITHIN 90 DAYS OF THE DELIVERY DATE. THESE ARE YOUR SOLE AND EXCLUSIVE REMEDIES FOR ANY BREACH OF WARRANTY. WARRANTY CLAIMS MUST BE MADE WITHIN THE APPLICABLE WARRANTY PERIOD.

LIMITATION OF LIABILITY

IN NO EVENT SHALL JETA OR ITS SUPPLIERS BE RESPONSIBLE OR LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY OR UNDER ANY STATUTE (INCLUDING WITHOUT LIMITATION ANY TRADE PRACTICE, UNFAIR COMPETITION OR OTHER STATUTE OF SIMILAR IMPORT) OR ON ANY OTHER BASIS FOR SPECIAL, INDIRECT, INCIDENTAL, MULTIPLE, PUNITIVE, OR CONSEQUENTIAL DAMAGES ARISING OUT OF THE POSSESSION OR USE OF, OR THE INABILITY TO USE, THE SOFTWARE OR DOCUMENTATION, EVEN IF JETA IS ADVISED IN ADVANCE OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING WITHOUT LIMITATION DAMAGES ARISING FROM OR RELATED TO LOSS OF USE, LOSS OF DATA, DOWNTIME, OR FOR LOSS OF REVENUE, PROFITS, GOODWILL OR BUSINESS OR OTHER FINANCIAL LOSS. IN ANY CASE, THE ENTIRE LIABILITY OF JETA AND ITS SUPPLIERS UNDER THIS LICENSE, OR ARISING OUT OF THE USE OF THE SOFTWARE, SHALL NOT EXCEED IN THE AGGREGATE THE PURCHASE PRICE OF THE SOFTWARE, IF PURCHASED INDEPENDENTLY, OR THE FAIR MARKET VALUE OF THE SOFTWARE, IF PURCHASED AS PART OF A JETA IN VITRO DIAGNOSTICS KIT. SOME STATES, COUNTRIES OR JURISDICTIONS LIMIT THE SCOPE OF OR PRECLUDE LIMITATIONS OR EXCLUSION OF REMEDIES OR DAMAGES, OR OF LIABILITY, SUCH AS LIABILITY FOR GROSS NEGLIGENCE OR WILLFUL MISCONDUCT, AS OR TO THE EXTENT SET FORTH ABOVE, OR DO NOT ALLOW IMPLIED WARRANTIES TO BE EXCLUDED. IN SUCH STATES, COUNTRIES OR JURISDICTIONS, THE LIMITATION OR EXCLUSION OF WARRANTIES, REMEDIES,

DAMAGES OR LIABILITY SET FORTH ABOVE MAY NOT APPLY TO YOU. HOWEVER, ALTHOUGH THEY SHALL NOT APPLY TO THE EXTENT PROHIBITED BY LAW, THEY SHALL APPLY TO THE FULLEST EXTENT PERMITTED BY LAW. YOU MAY ALSO HAVE OTHER RIGHTS THAT VARY BY STATE, COUNTRY OR OTHER JURISDICTION.

CONFIDENTIALITY

Except as expressly allowed by this License, you will not use or disclose any Software, Documentation or related technology, idea, algorithm or information except to the extent you can document that it is generally available for use and disclosure by the public without charge or license. You recognize and agree that there is no adequate remedy at law for a breach of this provision, that such a breach would irreparably harm JETA and that JETA is entitled to equitable relief (without need to post a bond) with respect to any such breach or potential breach in addition to any other remedies.

INDEMNITY

You shall defend, indemnify and hold harmless JETA and its officers, directors, employees and agents (the "JETA Indemnitees"), from and against all suits, claims, demands, losses, liabilities, damages and expenses (including reasonable attorneys' fees and costs) that the JETA Indemnitees may suffer or incur in connection with: (i) any third-party claim arising from your breach of this License; and (ii) any infringement by you of JETA's or its licensors' intellectual property rights in the Software or the Third-Party Product.

BUSINESS OBJECTS SUBLICENSE GRANT, TERMS AND CONDITIONS

You agree not to alter, disassemble, decompile, translate, adapt or reverse-engineer the Business Objects Software or the report file (.RPT) format;

You agree not to distribute the Business Objects Software with any general-purpose report writing, data analysis or report delivery product or any other product that performs the same or similar functions as Business Objects' product offerings;

You agree not to use the Business Objects Software to create for distribution a product that is generally competitive with Business Objects' product offerings;

You agree not to use the Business Objects Software to create for distribution a product that converts the report file (.RPT) format to an alternative report file format used by any general-purpose report writing, data analysis or report delivery product that is not the property of Business Objects; You agree not to use the Business Objects Software on a rental or timesharing basis or to operate a service bureau facility for the benefit of third-parties; BUSINESS OBJECTS AND ITS SUPPLIERS DISCLAIM ALL WARRANTIES, EXPRESS OR

IMPLIED, INCLUDING WITHOUT LIMITATION THE WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NONINFRINGEMENT OF THIRD PARTY RIGHTS. BUSINESS OBJECTS AND ITS SUPPLIERS SHALL HAVE NO LIABILITY WHATSOEVER UNDER THIS AGREEMENT OR IN CONNECTION WITH THE BUSINESS OBJECTS SOFTWARE.

GENERAL

This Agreement shall be governed by laws of the Netherlands, exclusive of its conflict of laws provisions. This Agreement shall not be governed by the United Nations Convention on Contracts for the International Sale of Goods. You hereby agree that the courts located in the Utrecht Netherlands, will constitute the sole and exclusive forum for the resolution of any and all disputes arising out of or in connection with this Agreement and you hereby irrevocably consent to the personal jurisdiction and venue of such courts and irrevocably waive any objections thereto. This Agreement contains the complete agreement between the parties with respect to the subject matter hereof, and supersedes all prior or contemporaneous agreements or understandings, whether oral or written. If any provision of this Agreement is held by a court of competent jurisdiction to be contrary to law, that provision will be enforced to the maximum extent permissible and the remaining provisions of this Agreement will remain in full force and effect. The controlling language of this Agreement, and any proceedings relating to this Agreement, shall be English. You agree to bear any and all costs of translation, if necessary. The headings to the sections of this Agreement are used for convenience only and shall have no substantive meaning. All questions concerning this Agreement shall be directed to: JETA Molecular BV, Krommewetering 101C, 3543AN,

Utrecht, Netherlands, Attention: Legal Department.

Unpublished rights reserved under the copyright laws of the United States. JETA Molecular BV, Krommewetering 101C, 3543AN, Utrecht, Netherlands.

JETA and its logo design are registered trademarks of JETA Molecular BV in the U.S.

and/or certain other countries.

 $\ensuremath{\mathsf{QTRACE}}\xspace^{\ensuremath{\mathsf{BV}}}$ is a registered trademark of ElsworthMolecular Holding BV in the U.S. and/or certain other countries.

All other trademarks, copyrights, patents, service marks, logos and trade names are the sole property of their respective owners.