QTRACE and DigitalTRACE Analysis System Operator's Manual

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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.

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Disclaimer

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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.

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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 self-quenched, 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 QTRACE® Analysis System

Enter Sample Information into QTRACE™ Software to Setup the Genotyping Experiment. Export a SetUp File for the qPCR machine and generate a lab protocol

Add 5X qPCR Master Mix + Sample DNA to the Genotyping plate per the Sample Layout generated by the software

Load genotyping plate into the qPCR machine. Open a QTRACE™ template, import the setup file and start the run.

Export data from qPCR Machine and import into QTRACE™ Software for informative marker identification

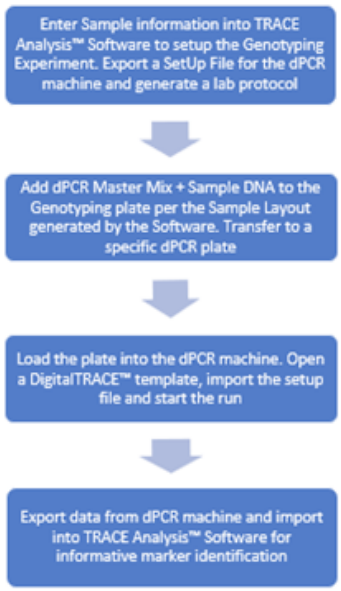
Enter Sample Information into QTRACE™ Software to Setup the Monitoring Experiment. Export a SetUp File for the qPCR machine and generate a lab protocol

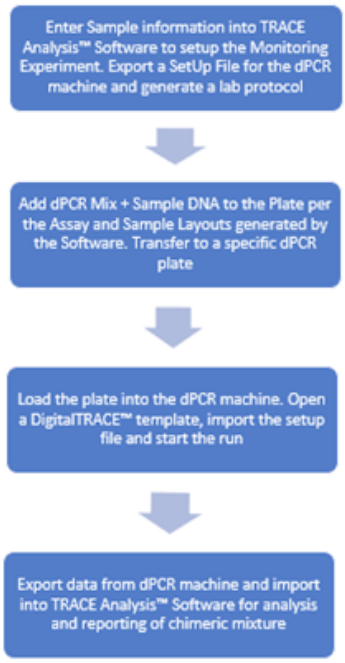
Add 5X qPCR Master Mix + Sample DNA to the Plate per the Assay and Sample Layouts generated by the Software

Load Monitoring plate into the qPCR machine. Open a QTRACE™ template, import the setup file and start the run

Export data from qPCR Machine and import into QTRACE™ Software for analysis and reporting of chimeric mixture

The DigitalTRACE™ Analysis System





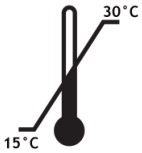
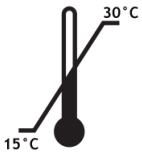
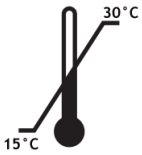
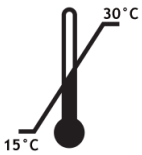
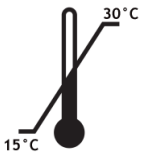
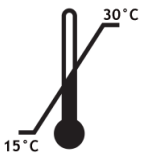
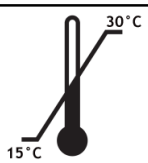
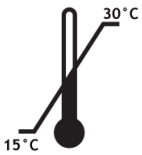
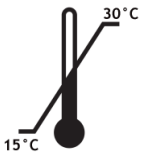
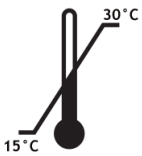
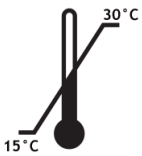
Materials

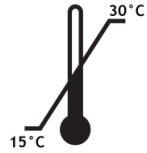
Key to Symbols

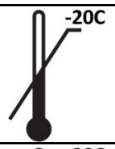
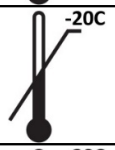
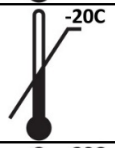
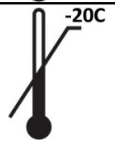
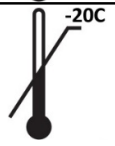
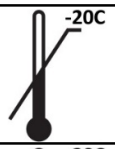
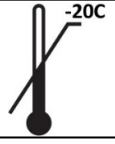
The following symbols appear within the labeling of the QTRACE® and DigitalTRACE™ System Products:

Symbol	Definition	Symbol	Definition
	Part Number		Store Below -15°C
	Lot Number		Store between 15°C – 30°C
	Expiration Date		Manufacturer
	See Operator's Manual		Contains Sufficient for <n> Tests

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		Box
121056	QTRACE® Genotyping Plates - FAST	Four ABI FAST MicroAmp® Optical 96-well plates pre-arrayed with INDEL qPCR Assays; individually sealed		Box
121066	QTRACE® Genotyping Plates – Roche 480	Four Roche 480 Optical 96-well plates pre-arrayed with INDEL qPCR Assays; individually sealed		Box
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		Box
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		Box
121221	MultiTRACE™ Genotyping Plate Pack - LC480 v2	Four Roche 480 Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed		Box
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		Box
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		Box
121228	MultiTRACE™ Genotyping Plate Pack – LC480 v3	Four Roche 480 Optical 96-well plates pre-arrayed with INDEL qPCR Assays in multiplex; individually sealed		Box
121139	QTRACE® Extended Panel Genotyping Plate - ABI 0.2mL	Four ABI MicroAmp® Optical 96-well plates pre-arrayed with INDEL qPCR Assays; individually sealed		Box
121045	DigitalTRACE™ QIAcuity Genotyping Plate	Two ABI MicroAmp® Optical 96-well plates pre-arrayed with INDEL dPCR Assays; individually sealed		Box

121056	DigitalTRACE™ EP QIAcuity Genotyping Plate	Two ABI MicroAmp® Optical 96-well plates pre-arrayed with INDEL dPCR Assays; individually sealed		Box

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 $MgCl_2$		Tube
311043	QTRACE® RNaseP Assay	1.1ml buffered solution containing a mix of primers and probe for detecting the RNaseP gene		Tube
	QTRACE® INDEL Assays	375µl buffered solution containing a mix of primers and probe for detecting the variant of interest		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		Tube
	QTRACE® HLA Assays	100µl buffered solution containing a mix of primers and probe for detecting the variant of interest		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		Tube
711295	QTRACE® Universal Positive Control	360µl buffered solution containing synthetic DNA serving as positive control		Tube
711294	DigitalTRACE™ Universal Positive Control	360µl buffered solution containing synthetic DNA serving as positive control		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
211004	QTRACE® INDEL Assay 235		211170	QTRACE® INDEL Assay 884
211005	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		211040	QTRACE® INDEL Assay 916
211313	QTRACE® INDEL Assay 284		211172	QTRACE® INDEL Assay 923
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 H051
211019	QTRACE® INDEL Assay 504		211099	QTRACE® HLA Assay H052
211054	QTRACE® INDEL Assay 519		211100	QTRACE® HLA Assay H053
211020	QTRACE® INDEL Assay 520		211101	QTRACE® HLA Assay H054
211021	QTRACE® INDEL Assay 531		211132	QTRACE® HLA Assay H101
211022	QTRACE® INDEL Assay 548		211133	QTRACE® HLA Assay H102
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

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
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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
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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 Assay H039
811154	DigitalTRACE™ INDEL Assay 472		811094	DigitalTRACE™ HLA Assay H041
811155	DigitalTRACE™ INDEL Assay 482		811095	DigitalTRACE™ HLA Assay H043
811156	DigitalTRACE™ INDEL Assay 493		811096	DigitalTRACE™ HLA Assay H045
811019	DigitalTRACE™ INDEL Assay 504		811098	DigitalTRACE™ HLA Assay H051
811054	DigitalTRACE™ INDEL Assay 519		811099	DigitalTRACE™ HLA Assay H052
811020	DigitalTRACE™ INDEL Assay 520		811100	DigitalTRACE™ HLA Assay H053
811021	DigitalTRACE™ INDEL Assay 531		811101	DigitalTRACE™ HLA Assay H054
811022	DigitalTRACE™ INDEL Assay 548		811133	DigitalTRACE™ HLA Assay H102
811157	DigitalTRACE™ INDEL Assay 555		811134	DigitalTRACE™ HLA Assay H103
811158	DigitalTRACE™ INDEL Assay 567		811279	DigitalTRACE™ HLA Assay H104
811159	DigitalTRACE™ INDEL Assay 574		811280	DigitalTRACE™ HLA Assay H105
811160	DigitalTRACE™ INDEL Assay 585		811281	DigitalTRACE™ HLA Assay H106
811161	DigitalTRACE™ INDEL Assay 597		811282	DigitalTRACE™ HLA Assay H107
811023	DigitalTRACE™ INDEL Assay 601		811283	DigitalTRACE™ HLA Assay H108
811024	DigitalTRACE™ INDEL Assay 615		811284	DigitalTRACE™ HLA Assay H109
811025	DigitalTRACE™ INDEL Assay 626		811290	DigitalTRACE™ HLA Assay H110
811026	DigitalTRACE™ INDEL Assay 634		811291	DigitalTRACE™ HLA Assay H111
811316	DigitalTRACE™ INDEL Assay 647		811292	DigitalTRACE™ HLA Assay H112
811027	DigitalTRACE™ INDEL Assay 650		811293	DigitalTRACE™ HLA Assay H113
811162	DigitalTRACE™ INDEL Assay 663		811296	DigitalTRACE™ HLA Assay H114
811163	DigitalTRACE™ INDEL Assay 670		811297	DigitalTRACE™ HLA Assay H115
811164	DigitalTRACE™ INDEL Assay 678		811298	DigitalTRACE™ HLA Assay H116
811165	DigitalTRACE™ INDEL Assay 681		811299	DigitalTRACE™ HLA Assay H117
811166	DigitalTRACE™ INDEL Assay 694		811300	DigitalTRACE™ HLA Assay H118
811028	DigitalTRACE™ INDEL Assay 706		811319	DigitalTRACE™ HLA Assay 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. Do NOT use heparinized samples, 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

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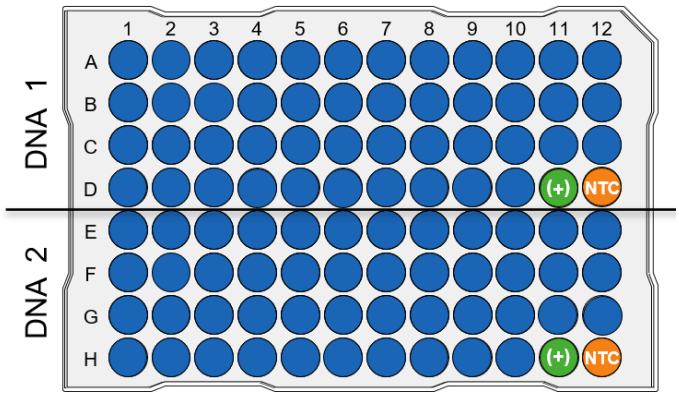
Genotyping Test

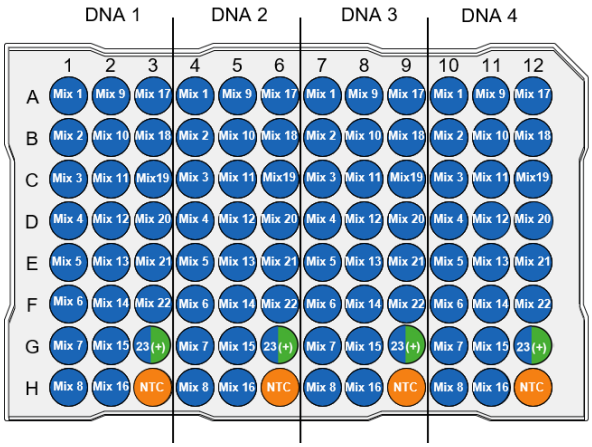
The QTRACE® Analysis System

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.

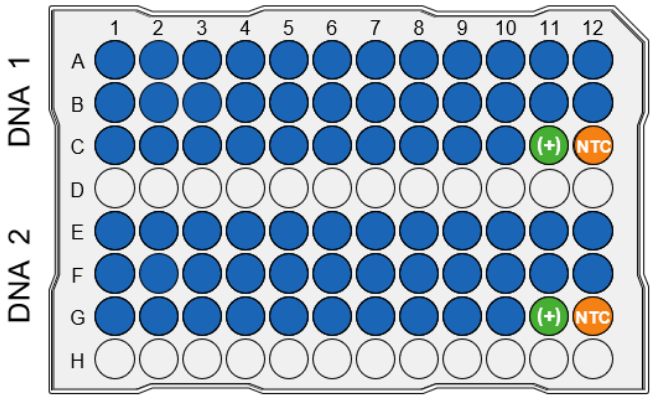
The genotyping plate contains a duplicate set of assays: the 46 quantification assays and the reference (RNaseP) assay that serves as both a positive control and a No Template Control (NTC), pre-dispensed and dried in an optical qPCR plate. The QTRACE® qPCR Master Mix comes supplied with dUTP and UNG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



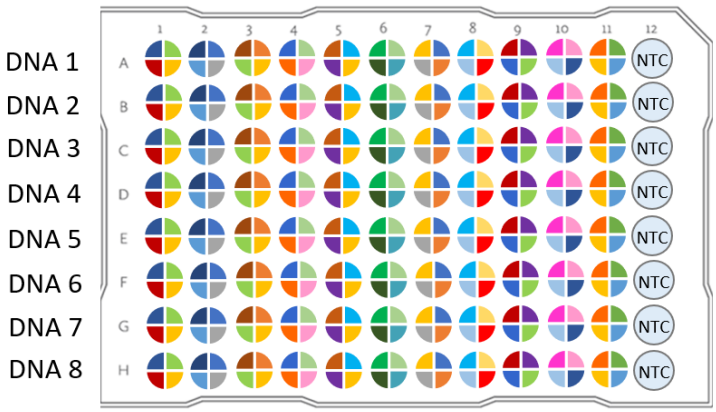
The Extended Panel genotyping plate can be used in rare cases including patients receiving a second transplant or highly similar siblings, where there may be need for additional markers to the standard QTRACE genotyping plate. It contains a duplicate set of 34 quantification assays and the reference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



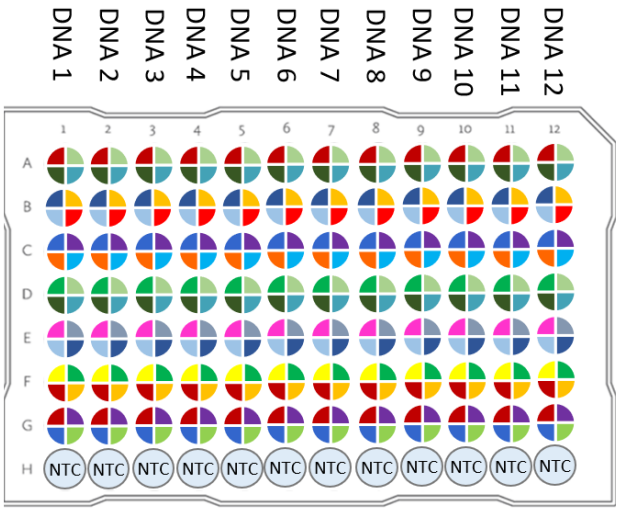
The DigitalTRACE™ Analysis System

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.

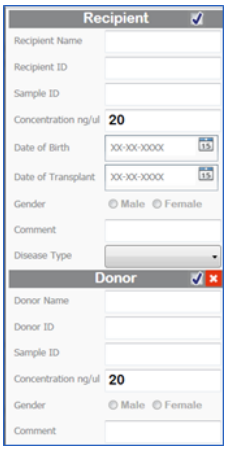
Protocol

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

Press the “Preferences”  button and choose from the available instruments and genotyping plate types.

The QTRACE® Analysis System

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.



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 Preferences for Concentrations).

Once all samples to be comparatively genotyped are entered into the Sample window, press the "Add Typing Samples" 

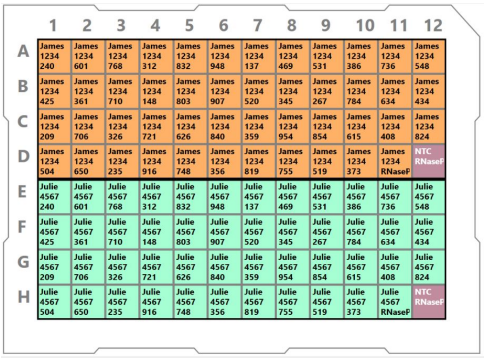
button to add the samples to the plate set up file. You will see the samples now added to the 96 well plate in the middle of the screen.

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:

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):



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 standard genotyping plate:

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 de-ionized 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, de-ionized 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

Sample 1 qPCR Mix	1X	50X
QTRACE® qPCR Master Mix	5.0ul	250.0ul
"recipient ID" DNA	0.8ul	40.0ul
H_2O	19.2ul	960.0ul
	25.0ul	1250.0ul

Sample 2 qPCR Mix	1X	50X
QTRACE® qPCR Master Mix	5.0ul	250.0ul
"Donor ID" DNA	0.1ul	5ul
H_2O	19.9ul	955ul
	25.0ul	1250.0ul

NTC qPCR Mix	1X	3X
QTRACE® qPCR Master Mix	5.0ul	15.0ul
H_2O	20.0ul	60.0ul
	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 µl of the Sample 1 Mix into Wells A1-D6 and in Well D11 of the genotyping plate.

Dispense 25 µl of the Sample 2 Mix into Wells E1-H6 and in Well H11 of the genotyping plate.
Dispense 25 µl 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 multiplexed genotyping plate:

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 de-ionized 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, de-ionized H_2O 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 H_2O and QTRACE® qPCR Master Mix.

Table 2. qPCR Master Mix Composition – MultiTRACE™ plates

Sample 1 qPCR Mix		1X	25X
QTRACE® qPCR Master Mix		5.0ul	125.0ul
"Recipient 1 ID"			
DNA		0.3ul	7.5ul
H_2O		19.7ul	492.5ul
		25.0ul	625.0ul

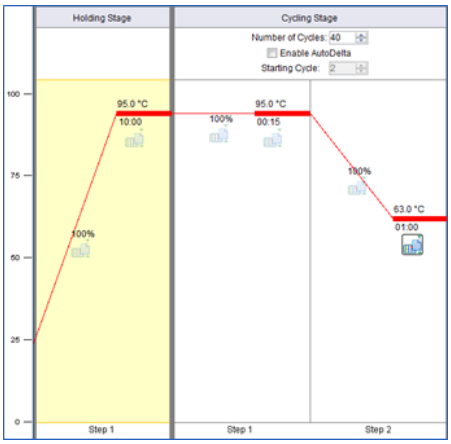
Sample 2 qPCR Mix	1X	25X
QTRACE® qPCR Master Mix	5.0ul	125.0ul
"Donor 1 ID" DNA	0.2ul	5.4ul
H_2O	19.8ul	494.6ul
	25.0ul	625.0ul

Sample 3 qPCR Mix	1X	25X
QTRACE® qPCR Master Mix	5.0ul	125.0ul
"Recipient 2 ID" DNA	0.4ul	8.9ul
H_2O	19.6ul	491.1ul
	25.0ul	625.0ul

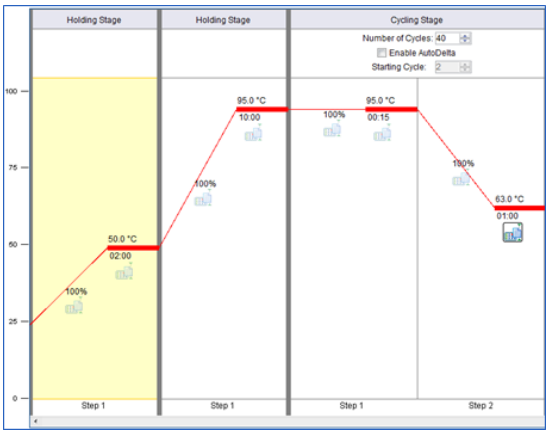
Sample 2 qPCR Mix	1X	25X
QTRACE® qPCR Master Mix	5.0ul	125.0ul
"Donor 2 ID" DNA	0.1ul	2.5ul
H_2O	19.9ul	497.5ul
	25.0ul	625.0ul

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.

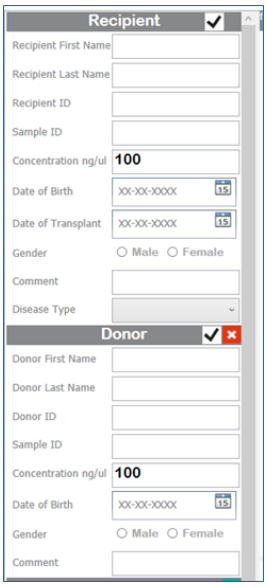


The DigitalTRACE™ Analysis System

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.



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 Preferences 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

"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:

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 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.

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 H_2O.
3. Briefly vortex and centrifuge all tubes before opening.
4. For each sample to be genotyped, lable a tube and a Mix containing sample DNA, de-ionized H_2O and 4x Probe PCR Master Mix as suggested by TRACE Analysis™ Software in Table 1. A No Template Control (NTC) Mix is prepared with de-ionized H_2O 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
H_2O	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
H_2O	8,5 µl	110,5 µl
	12,0 µl	156,0 µl

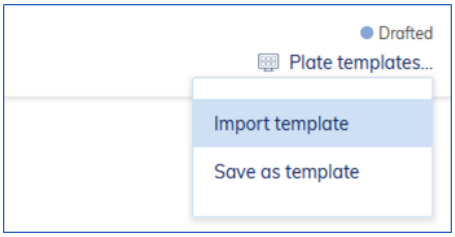
NTC dPCR Mix	1 x	10 x
4x Probe PCR Master Mix	3,0 µl	30,0 µl
H_2O	9,0 µl	90,0 µl
	12,0 µl	120,0 µl

5. **Vortex each tube to thoroughly mix the contents and centrifuge briefly to collect the reaction mix at the bottom of 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 Assay Layout view.
8. **An automated multichannel pipette is recommended in this step to minimize pipetting repetition and increase accuracy.**
9. Visually inspect plate wells from the sides and bottom to confirm consistent volume.
10. 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 Nanoplate with the compatible plate sealer.
14. Load the Nanoplate into the QIAcuity 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 run.

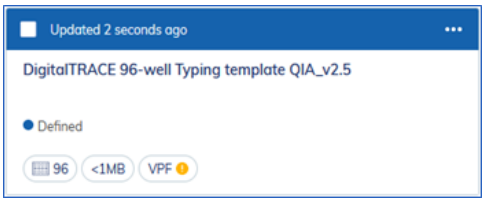
Experiment Setup in QIAcuity Suite Software, v2.5

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

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



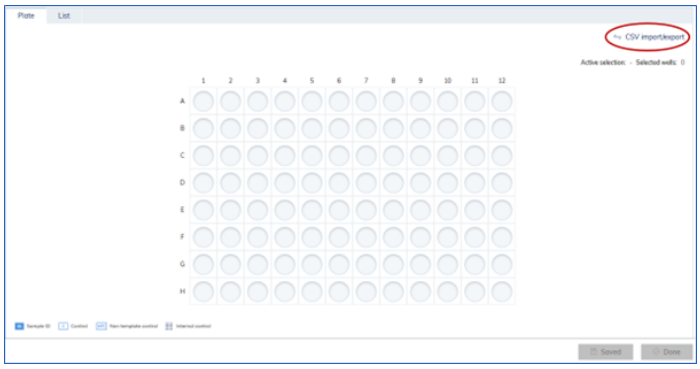
Press "Save changes". The new plate will appear in the main window of the QIAcuity Software Suite:

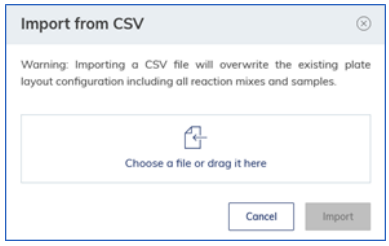


Click on the plate name to open the plate configuration procedure. Type in a new plate name and save the changes.

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

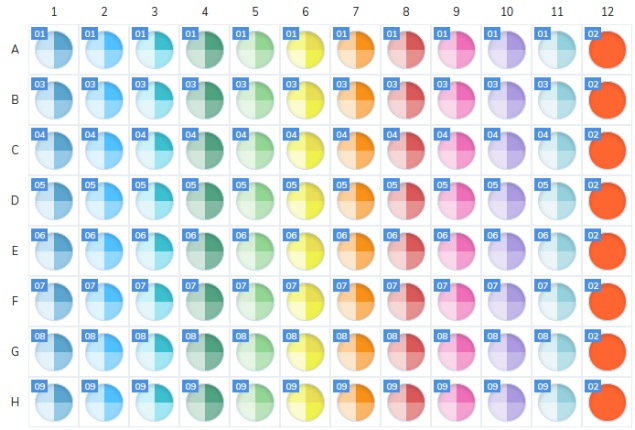
Select "Import from CSV".





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
	60 °C	30 s

Use the following imaging parameters:

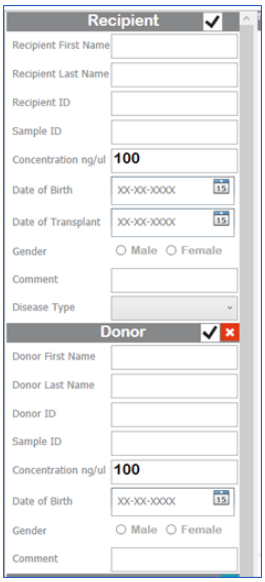
Channel	Exposure duration	Gain
Green	500 ms	6
Yellow	500 ms	6
Orange	Off	Off
Red	300 ms	4
Crimson	400 ms	4

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.



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:

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

1. **Set up all reactions in a pre-PCR lab, under ambient conditions without ice.**
2. Open a MultiTRACE® Genotyping Plate Pack and remove the genotyping plate.
3. Label the genotyping plate with the genotyping test name.
4. Collect the four DNA samples to be screened, as well as 2x ddPCR Supermix Master Mix and de-ionized H_2O. Briefly vortex and centrifuge all tubes before opening.
5. Label five 1.5 ml microcentrifuge tubes:
   a) Sample 1 Mix
   b) 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 H_2O and 2x ddPCR Supermix as suggested by TRACE Analysis™ Software in Table 1. A No Template Control (NTC) Mix is prepared with de-ionized H_2O and 2x ddPCR Supermix.

Table 1. Master Mix Composition

Sample 1 dPCR Mix	1 x	27 x
2x ddPCR Supermix	11,0 µl	297,0 µl
1001 DNA	0,5 µl	13,5 µl
H_2O	10,5 µl	283,5 µl
	22,0 µl	594,0 µl

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
H_2O	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
H_2O	10,5 µl	283,5 µl
	22,0 µl	594,0 µl

Sample 4 dPCR Mix	1 x	27 x
2x ddPCR Supermix	11,0 µl	297,0 µl
1004 DNA	0,5 µl	13,5 µl
H_2O	10,5 µl	283,5 µl
	22,0 µl	594,0 µl

NTC dPCR Mix	1 x	5 x
2x ddPCR Supermix	11,0 µl	55,0 µl
H_2O	11,0 µl	55,0 µl
	22,0 µl	110,0 µl

7. Vortex each tube to thoroughly mix the contents and centrifuge briefly to collect the reaction mix at the bottom of the tube.
8. Remove the adhesive cover from the genotyping plate.
9. Dispense 22 µl of the Sample 1 Mix into Wells A1-G3 by columns of the genotyping plate.
10. Dispense 22 µl of the Sample 2 Mix into Wells A4-G6 by columns of the genotyping plate.
11. Dispense 22 µl of the Sample 3 Mix into Wells A7-G9 by columns of the genotyping plate.
12. Dispense 22 µl of the Sample 4 Mix into Wells A10-G12 by columns of the genotyping plate.
13. Dispense 22 µl of the 5X PCR Master Mix/NTC mixture to wells H3, H6, H9, H12.
14. **A repeat pipettor is recommended to minimize pipetting repetition and increase accuracy.**
15. Refer to the DNA Sample Layout Plate Layout at the end of the protocol.
16. Visually inspect plate wells from the sides and bottom to confirm consistent volume.
17. Seal the plate completely with MicroAmp® Optical Adhesive Film using the MicroAmp® Adhesive Film Applicator.
18. **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.
19. **Remove the Adhesive cover very carefully.**
20. **!!!The following steps from 21 to 26 are only for manual droplet generator users:**
21. Transfer 20 µl of each prepared sample to the sample wells (middle row) of the DG8 cartridge.
22. Add 70 µl of droplet generation oil to each oil well of the DG8 cartridge.
23. Hook the gasket over the cartridge holder using the holes on both sides.
24. Load the cartridge in the QX200 droplet generator.
25. When droplet generation is complete, remove the disposable gasket from the holder and discard it.
26. Pipet 40 µl of the contents of the droplets into a single column of a 96-well PCR plate.
27. Seal the PCR plate with foil plate seals that are compatible with the PX1 PCR plate sealer and the needles in the QX200 droplet reader.
28. Place the plate into the thermal cycler for PCR amplification.
29. Load the plate after amplification into QX200 droplet reader.
30. Import the Sample Setup sheet generated by TRACE Analysis™ Software.
31. Save the file and start the droplet reader.

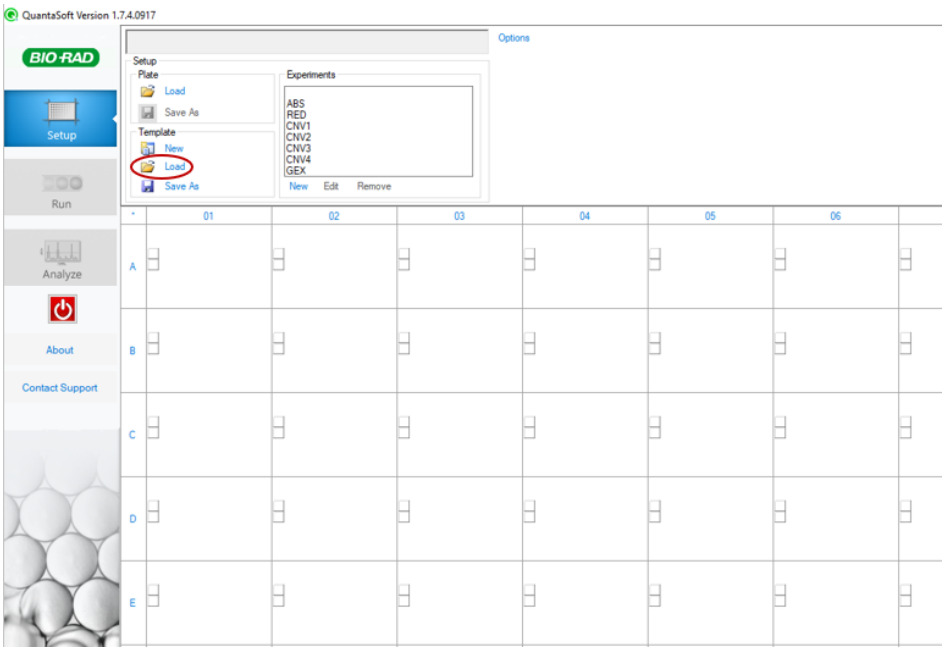
Thermal Cycling Protocol for Biorad

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

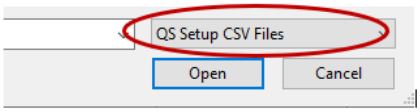
Number of cycles	Temperature	Time	Ramp Rate
1	95 °C	10 min	2 °C/s
40	94 °C	30 s	2 °C/s
	59 °C	60 s	2 °C/s
1	98 °C	10 min	2 °C/s

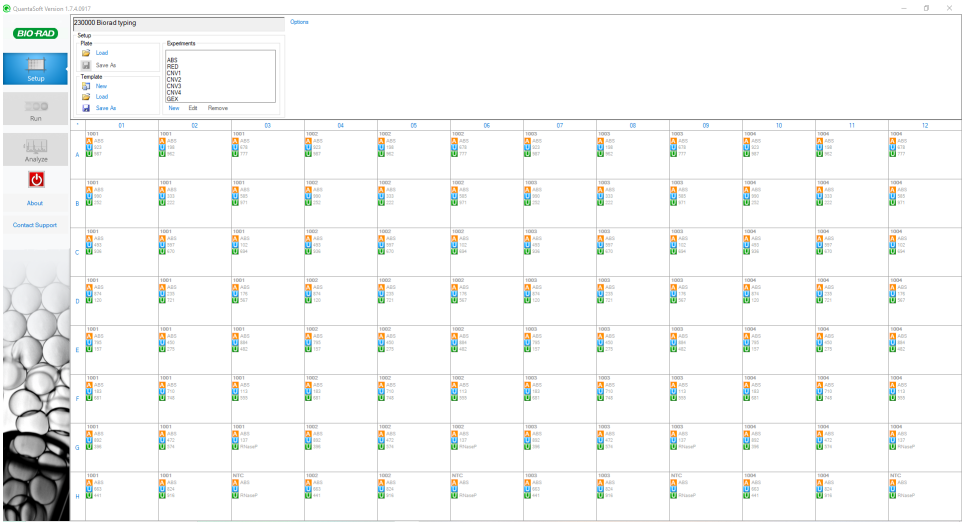
Biorad Droplet Reader Setup in QuantaSoft

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



Select QS Setup CSV Files as file format





Start the droplet reading run.

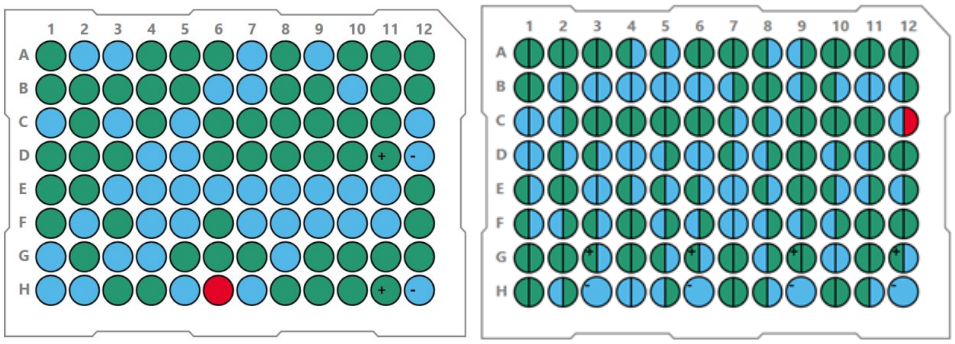
Genotyping Data Analysis and Report

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 colors:



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).

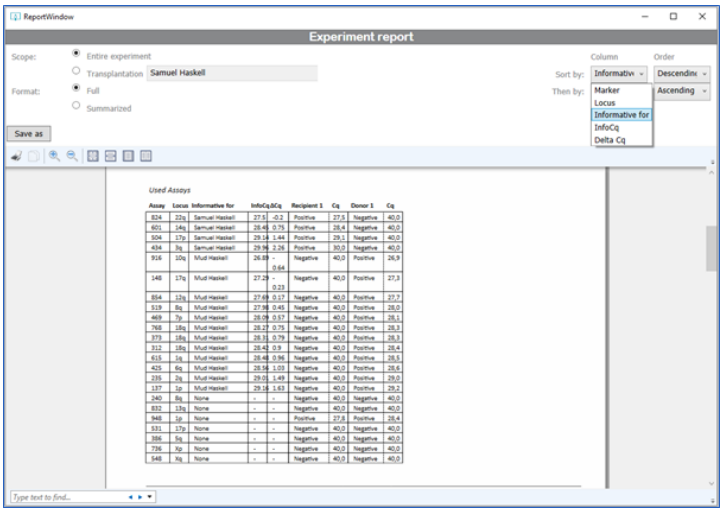
For more information on the Genotyping Algorithm used by TRACE Analysis™ Software, go to the Data Analysis Algorithms Section of this manual.

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.

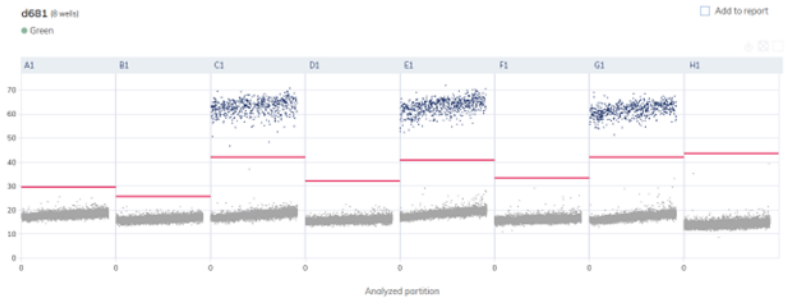
The report generated from a QTRACE® or DigitalTRACE™ Genotyping experiment may be sorted to provide a custom view of the data.



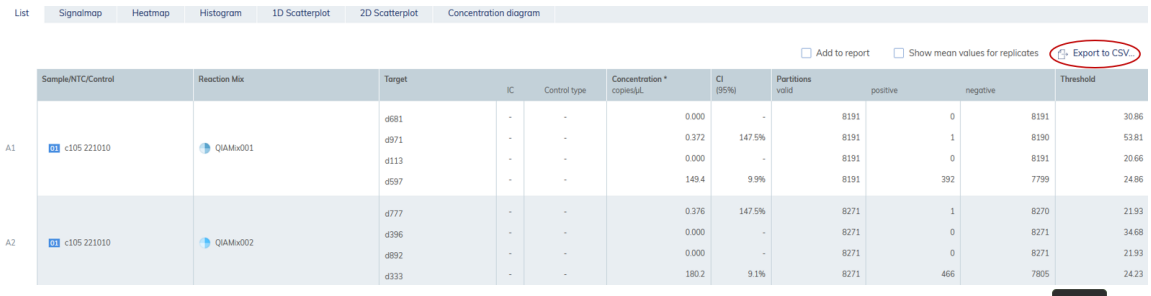
The DigitalTRACE™ Analysis System

Genotyping Data Analysis and Report - QIAcuity

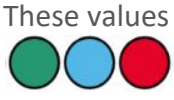
After the QIAcuity run has finished, check in the QIAcuity Software Suite if the automatic thresholds are correct. Adjust the thresholds manually if needed.

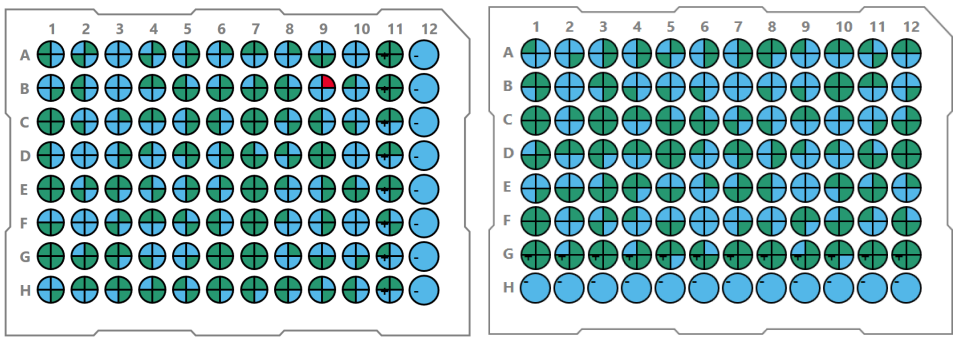


Select all wells on the plate and analyze per target (not per channel), export data by selecting Export to CSV.

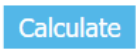


Import dPCR data to TRACE Analysis™ Software by clicking the “Import PCR Data” button. Browse to the location of your exported dPCR data file and select it.
TRACE Analysis™ Software will perform a quality analysis on the data and will present the data in the plate view.
There are three quality scores 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” 

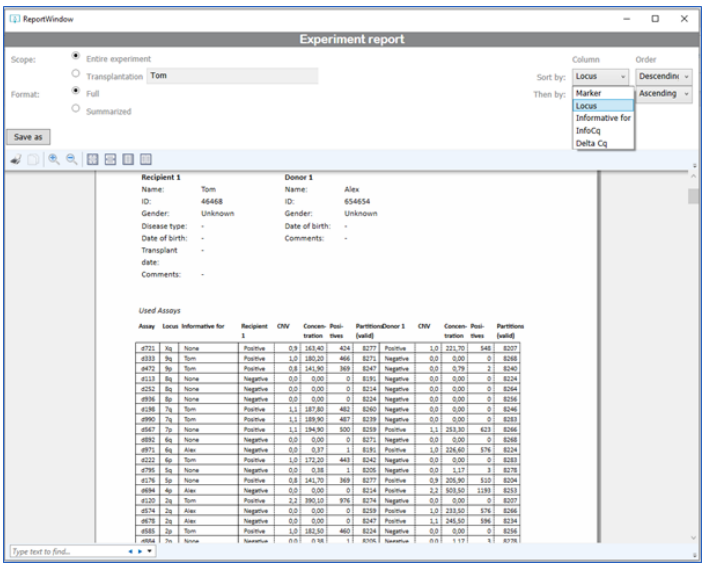
button makes TRACE Analysis™ perform comparative genotyping analysis. It will determine and display markers which are informative for all samples in a group.

Press the "Report" 

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.

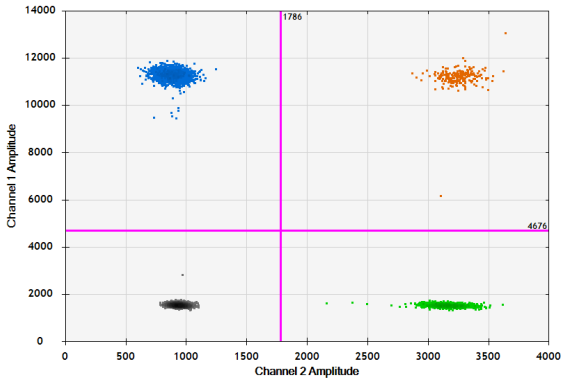
The report generated from a TRACE Analysis™ Genotyping experiment may be sorted to provide a custom view of the data.



To create an anonymized report, go to the Anonymized Reporting Section.

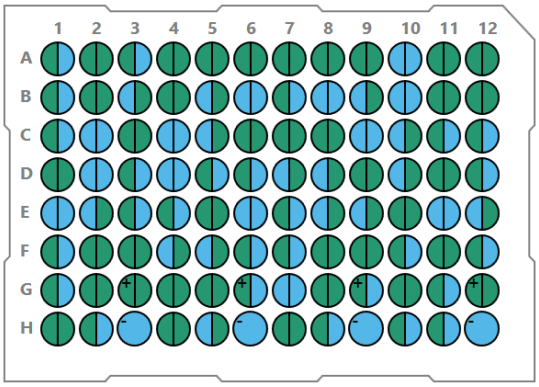
Genotyping Data Analysis and Report - Biorad

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:



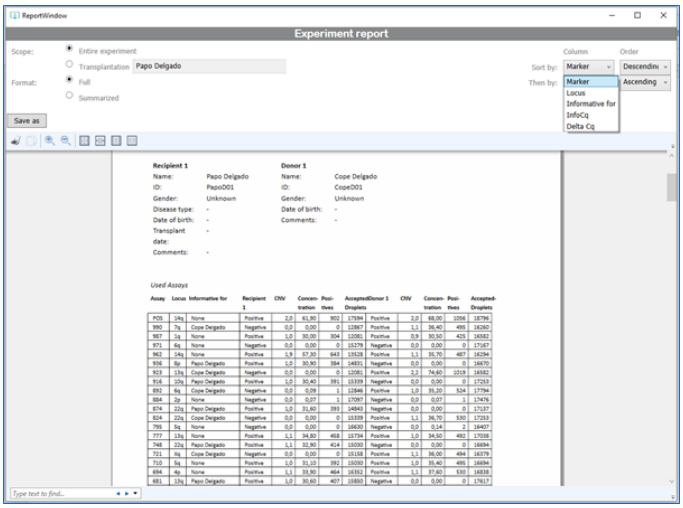
An atypical assay results will exclude the assay from consideration as a potentially informative assay for all samples grouped in the analysis.

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

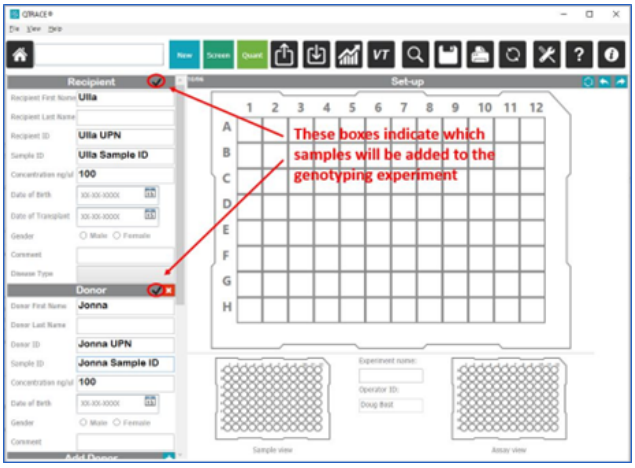
TRACE Analysis™ Software displays each assay which was informative for a single sample in the group being compared, and it also displays the chromosomal location of the informative assays, as well as the positive or negative status of the assays for visual inspection.

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

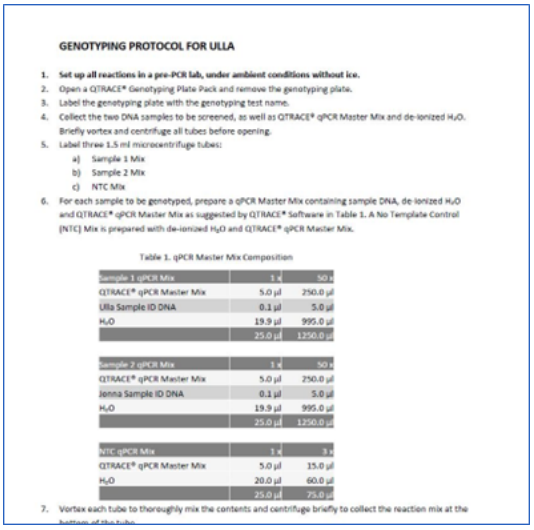


Multiple Donor Analysis Using QTRACE Plates

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.



GENOTYPING PROTOCOL FOR ULLA

1. **Set up all reactions in a pre-PCR lab, under ambient conditions without ice.**
2. Open a QTRACE® Genotyping Plate Pack and remove the genotyping plate.
3. Label the genotyping plate with the genotyping test name.
4. Collect the two DNA samples to be screened, as well as QTRACE® qPCR Master Mix and de-ionized H_2O. Briefly vortex and centrifuge all tubes before opening.
5. Label three 1.5 ml microcentrifuge tubes:
   a) Sample 1 Mix
   b) Sample 2 Mix
   c) NTC Mix
6. For each sample to be genotyped, prepare a qPCR Master Mix containing sample DNA, de-ionized 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

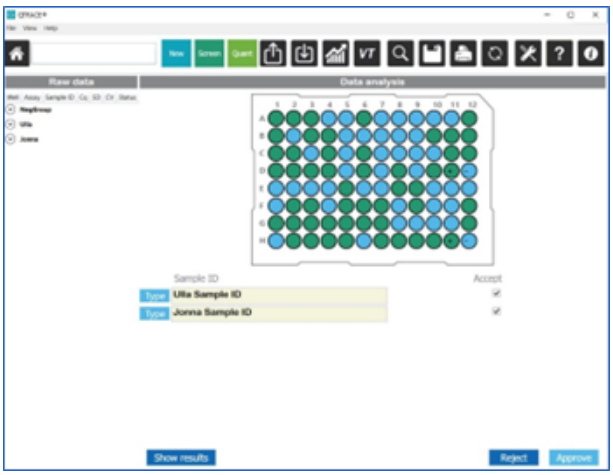
Sample 1 qPCR Mix	1 x	50 x
QTRACE® qPCR Master Mix	5.0 µl	250.0 µl
Ulla Sample ID DNA	0.1 µl	5.0 µl
H_2O	19.9 µl	995.0 µl
	25.0 µl	1250.0 µl

Sample 2 qPCR Mix	1 x	50 x
QTRACE® qPCR Master Mix	5.0 µl	250.0 µl
Jonna Sample ID DNA	0.1 µl	5.0 µl
H_2O	19.9 µl	995.0 µl
	25.0 µl	1250.0 µl

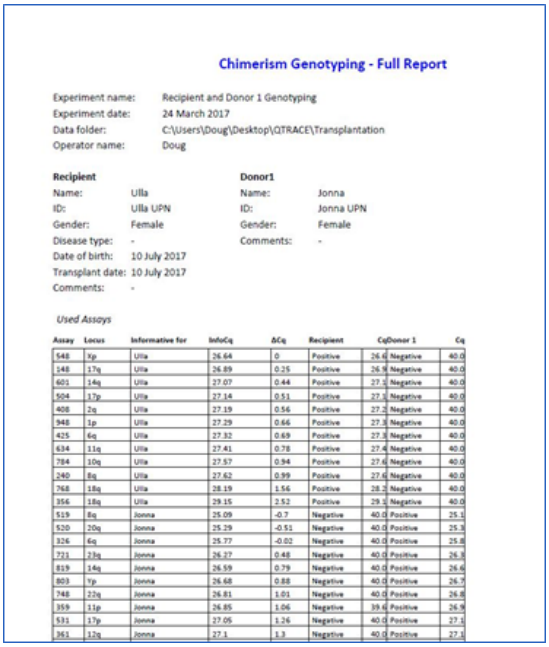
NTC qPCR Mix	1 x	3 x
QTRACE® qPCR Master Mix	5.0 µl	15.0 µl
H_2O	20.0 µl	60.0 µl
	25.0 µl	75.0 µl

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

When the data is imported and approved,



TRACE Analysis™ generates a report for the first pair.



Chimerism Genotyping - Full Report

Experiment name: Recipient and Donor 1 Genotyping
Experiment date: 24 March 2017
Data folder: C:\Users\Doug\Desktop\QTRACE\Transplantation
Operator name: Doug

Recipient		Donor1	
Name:	Ulla	Name:	Jonna
ID:	Ulla UPN	ID:	Jonna UPN
Gender:	Female	Gender:	Female
Disease type:	-	Comments:	-
Date of birth:	10 July 2017		
Transplant date:	10 July 2017		
Comments:	-		

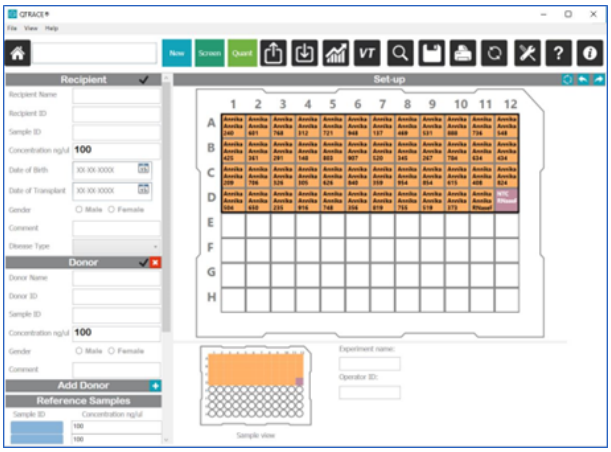
Used Assays

Assay	Locus	Informative for	InfoCq	ΔCq	Recipient	CqDonor 1	Cq
548	Xp	Ulla	26.64	0	Positive	26.6 Negative	40.0
148	17q	Ulla	26.89	0.25	Positive	26.9 Negative	40.0
601	14q	Ulla	27.07	0.44	Positive	27.1 Negative	40.0
504	17p	Ulla	27.14	0.51	Positive	27.1 Negative	40.0
408	2q	Ulla	27.19	0.56	Positive	27.2 Negative	40.0
948	1p	Ulla	27.29	0.66	Positive	27.3 Negative	40.0
425	6q	Ulla	27.32	0.69	Positive	27.3 Negative	40.0
634	11q	Ulla	27.41	0.78	Positive	27.4 Negative	40.0
784	10q	Ulla	27.57	0.94	Positive	27.6 Negative	40.0
240	8q	Ulla	27.62	0.99	Positive	27.6 Negative	40.0
768	18q	Ulla	28.19	1.56	Positive	28.2 Negative	40.0
356	18q	Ulla	29.15	2.52	Positive	29.1 Negative	40.0
519	8q	Jonna	25.09	-0.7	Negative	40.0 Positive	25.1
520	20q	Jonna	25.29	-0.51	Negative	40.0 Positive	25.3
326	6q	Jonna	25.77	-0.02	Negative	40.0 Positive	25.8
721	23q	Jonna	26.27	0.48	Negative	40.0 Positive	26.3
819	14q	Jonna	26.59	0.79	Negative	40.0 Positive	26.6
803	Yp	Jonna	26.68	0.88	Negative	40.0 Positive	26.7
748	22q	Jonna	26.81	1.01	Negative	40.0 Positive	26.8
359	11p	Jonna	26.85	1.06	Negative	39.6 Positive	26.9
531	17p	Jonna	27.05	1.26	Negative	40.0 Positive	27.1
361	12q	Jonna	27.1	1.3	Negative	40.0 Positive	27.1

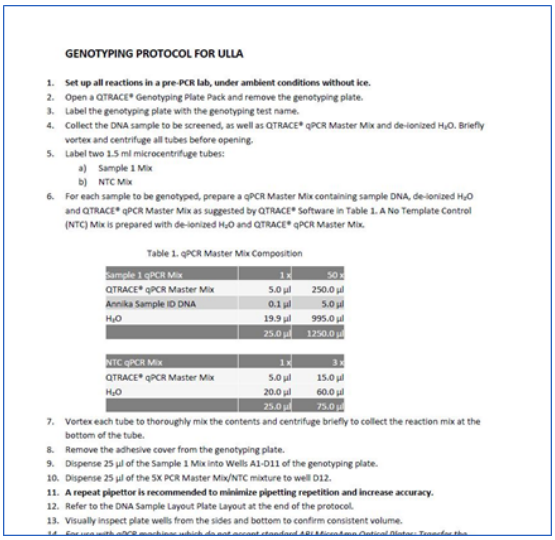
When the second donor is to be analyzed, select the recipient's record in the sample search window next to the home button. The samples which have already been genotyped will appear as they did when initially entered into TRACE Analysis™ Software. Notice that the check boxes by default are not checked,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

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.



GENOTYPING PROTOCOL FOR ULLA

1. **Set up all reactions in a pre-PCR lab, under ambient conditions without ice.**
2. Open a QTRACE® Genotyping Plate Pack and remove the genotyping plate.
3. Label the genotyping plate with the genotyping test name.
4. Collect the DNA sample to be screened, as well as QTRACE® qPCR Master Mix and de-ionized H_2O. Briefly vortex and centrifuge all tubes before opening.
5. Label two 1.5 ml microcentrifuge tubes:
   a) Sample 1 Mix
   b) NTC Mix
6. For each sample to be genotyped, prepare a qPCR Master Mix containing sample DNA, de-ionized 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.

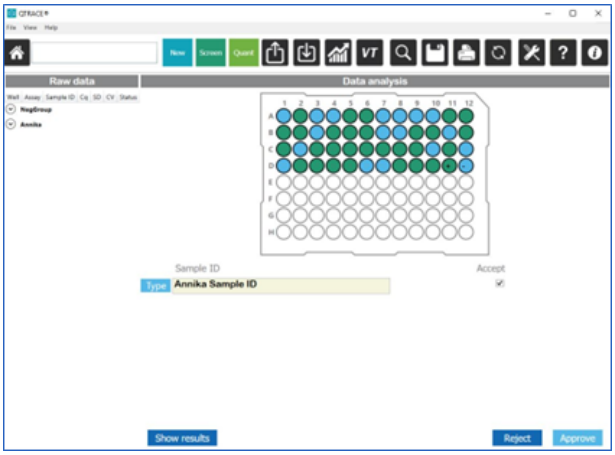
Table 1. qPCR Master Mix Composition

Sample 1 qPCR Mix	1 x	50 x
QTRACE® qPCR Master Mix	5.0 µl	250.0 µl
Annika Sample ID DNA	0.1 µl	5.0 µl
H_2O	19.9 µl	995.0 µl
	25.0 µl	1250.0 µl

NTC qPCR Mix	1 x	3 x
QTRACE® qPCR Master Mix	5.0 µl	15.0 µl
H_2O	20.0 µl	60.0 µl
	25.0 µl	75.0 µl

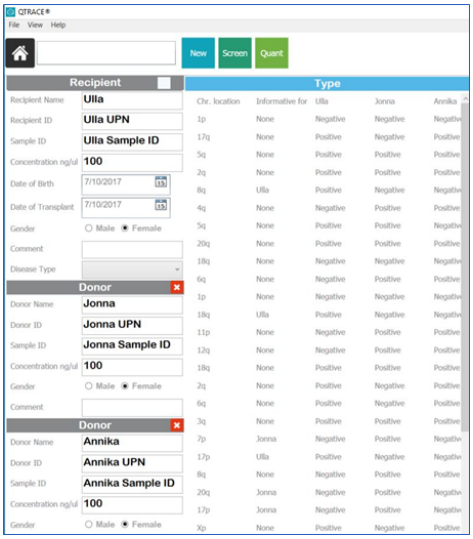
7. Vortex each tube to thoroughly mix the contents and centrifuge briefly to collect the reaction mix at the bottom of the tube.
8. Remove the adhesive cover from the genotyping plate.
9. Dispense 25 µl of the Sample 1 Mix into Wells A1-D11 of the genotyping plate.
10. Dispense 25 µl of the 5X PCR Master Mix/NTC mixture to well D12.
11. **A repeat pipettor is recommended to minimize pipetting repetition and increase accuracy.**
12. Refer to the DNA Sample Layout Plate Layout at the end of the protocol.
13. Visually inspect plate wells from the sides and bottom to confirm consistent volume.

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.

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



Custom Genotyping Panels

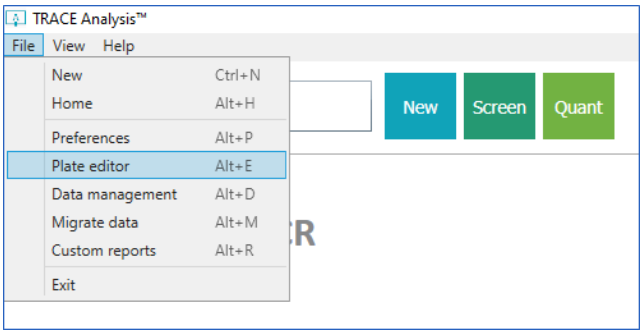
With TRACE Analysis™ Software version 1.08, users of TRACE Analysis™ Software can perform customized genotyping. They can choose which assays they want to use for genotyping experiments. They are able to create, store and use custom genotyping assay panels to perform genotyping using TRACE Analysis™ 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.

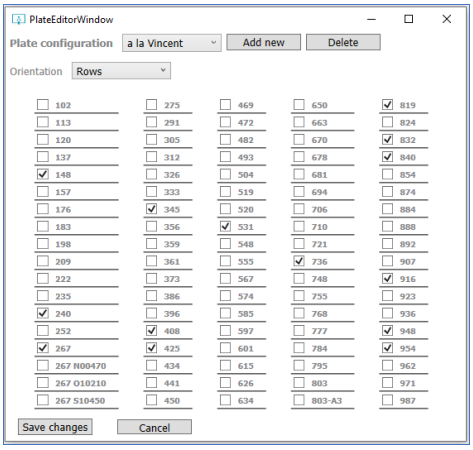
Orientation	Available INDEL Assay Panel Sizes	Plate Throughput
1 Row	10 Assays	8 Samples
2 Rows	22 Assays	4 Samples
3 Rows	34 Assays	2 Samples
4 Rows	46 Assays	2 Samples
1 Column	6 Assays	12 Samples
2 Columns	14 Assays	6 Samples
3 Columns	22 Assays	4 Samples
4 Columns	30 Assays	3 Samples
5 Columns	38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

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

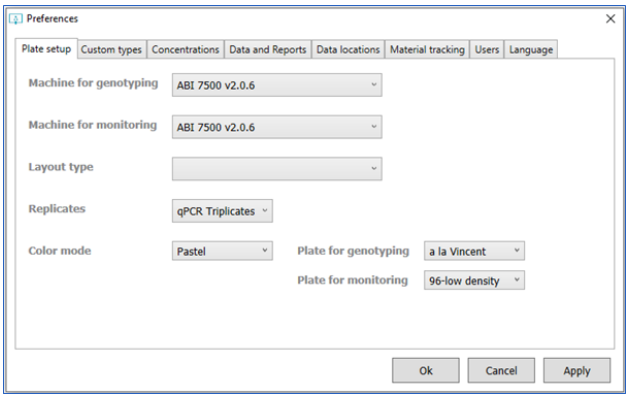


New plate configurations are created by pressing the 'Add new' button. Give the plate a name.

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

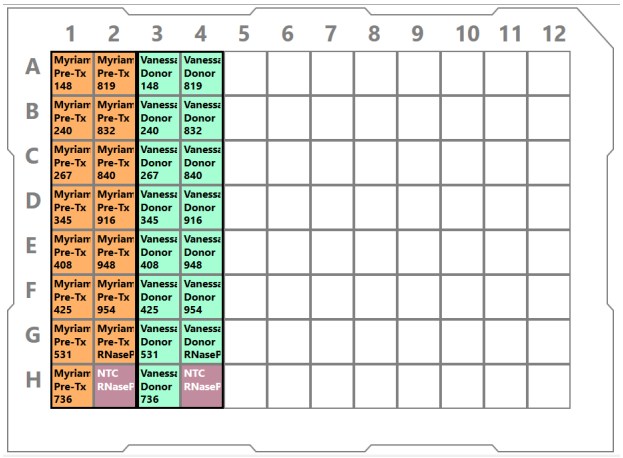


Once a Plate has been saved using the Plate editor, the plate appears as an option in the Plate Configuration menu in the Preferences.

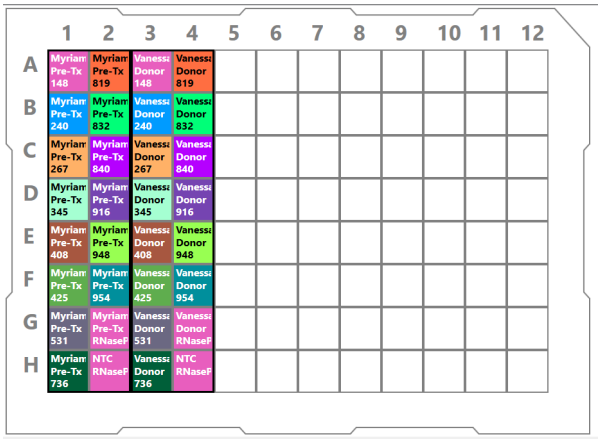


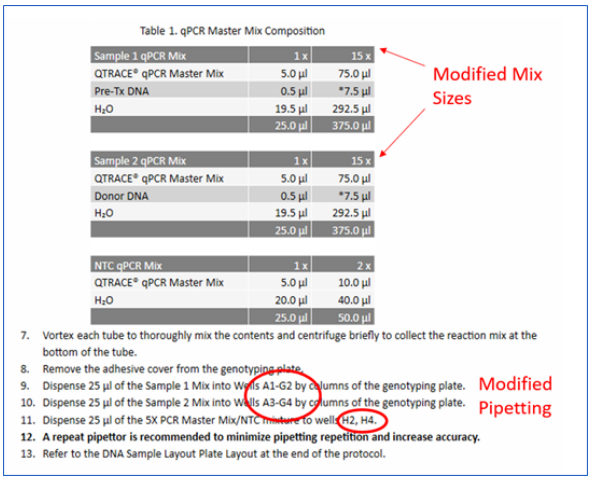
The following is an example of a single donor:patient pair being tested by a 14 member panel with a column configuration:

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

Table 1. qPCR Master Mix Composition

Sample 1 qPCR Mix	1 x	15 x
QTRACE® qPCR Master Mix	5.0 µl	75.0 µl
180425R6-3 DNA	0.5 µl	*7.5 µl
H_2O	19.5 µl	292.5 µl
	25.0 µl	375.0 µl

Sample 2 qPCR Mix	1 x	15 x
QTRACE® qPCR Master Mix	5.0 µl	75.0 µl
180425D6-3 DNA	0.5 µl	*7.5 µl
H_2O	19.5 µl	292.5 µl
	25.0 µl	375.0 µl

Sample 3 qPCR Mix	1 x	23 x
QTRACE® qPCR Master Mix	5.0 µl	115.0 µl
180425R6-4 DNA	0.5 µl	*11.5 µl
H_2O	19.5 µl	448.5 µl
	25.0 µl	575.0 µl

Sample 5 qPCR Mix	1 x	7 x
QTRACE® qPCR Master Mix	5.0 µl	35.0 µl
180425R6-5 DNA	0.5 µl	*3.5 µl
H_2O	19.5 µl	136.5 µl
	25.0 µl	175.0 µl

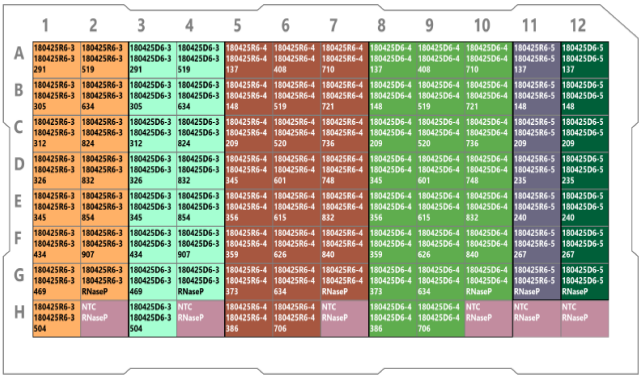
Sample 6 qPCR Mix	1 x	7 x
QTRACE® qPCR Master Mix	5.0 µl	35.0 µl
180425D6-5 DNA	0.5 µl	*3.5 µl
H_2O	19.5 µl	136.5 µl
	25.0 µl	175.0 µl

NTC qPCR Mix	1 x	6 x
QTRACE® qPCR Master Mix	5.0 µl	30.0 µl
H_2O	20.0 µl	120.0 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 µl of the Sample 2 Mix into Wells A3-G4 by columns of the genotyping plate.
11. Dispense 25 µl of the Sample 3 Mix into Wells A5-G7 by columns of the genotyping plate.
12. Dispense 25 µl 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:



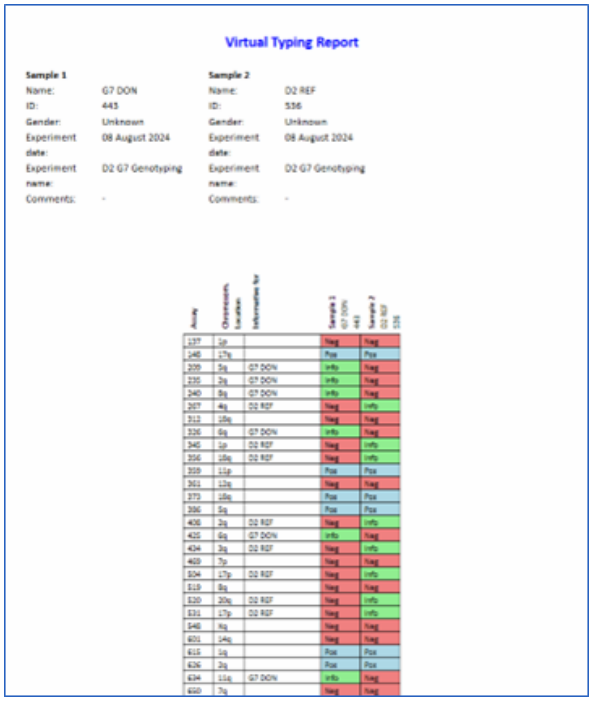
Virtual Typing

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.

Assay	Chromosome location	Informative for ...	443 G7 DON 8889	536 D2 REF 0013
137	1p	-	Negative	Negative
148	17q	-	Positive	Positive
209	5q	G7 DON	Informative	Negative
235	2q	G7 DON	Informative	Negative
240	8q	G7 DON	Informative	Negative
267	4q	D2 REF	Negative	Informative
312	18q	-	Negative	Negative
326	6q	G7 DON	Informative	Negative
345	1p	D2 REF	Negative	Informative
356	18q	D2 REF	Negative	Informative
359	11p	-	Positive	Positive
361	12q	-	Negative	Negative
373	18q	-	Positive	Positive
386	5q	-	Positive	Positive
408	2q	D2 REF	Negative	Informative
425	6q	G7 DON	Informative	Negative
434	3q	D2 REF	Negative	Informative
469	7p	-	Negative	Negative
504	17p	D2 REF	Negative	Informative
519	8q	-	Negative	Negative
520	20q	D2 REF	Negative	Informative
531	17p	D2 REF	Negative	Informative
548	Xq	-	Negative	Negative
601	14q	-	Negative	Negative
615	1q	-	Positive	Positive



Monitoring Test

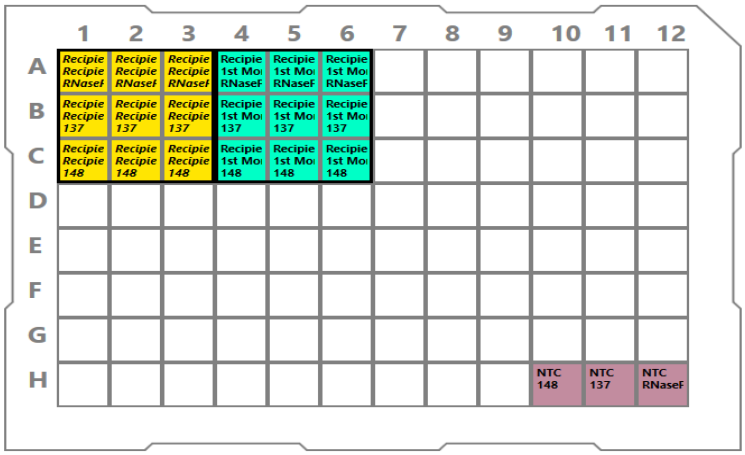
Once recipient specific markers have been found, quantification is performed to monitor engraftment. In the monitoring test, two or more of the informative assays is used to quantify the DNA of interest in an unknown sample relative to a reference sample. Any of the informative assays identified in the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

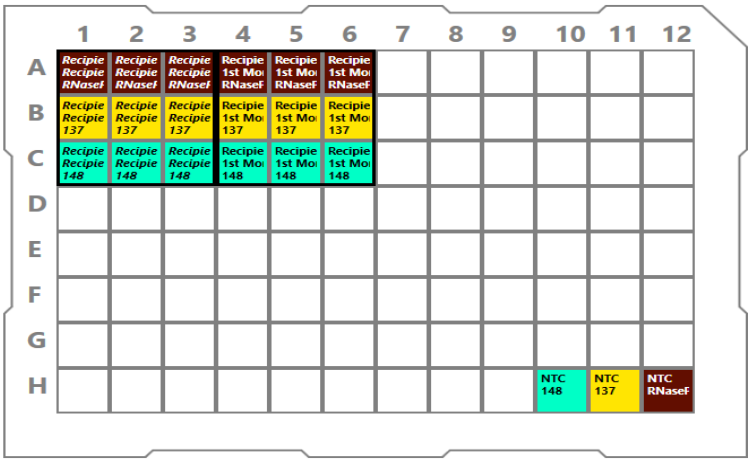
Monitoring Sample View

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.



Monitoring Assay View

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

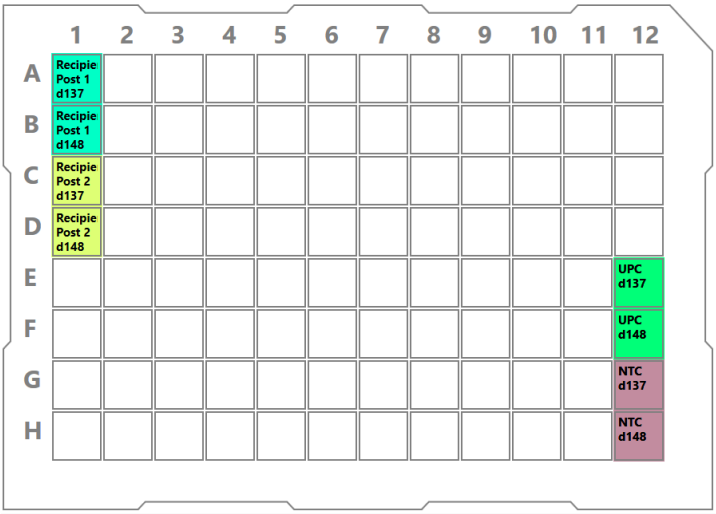


The DigitalTRACE™ Analysis System

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).

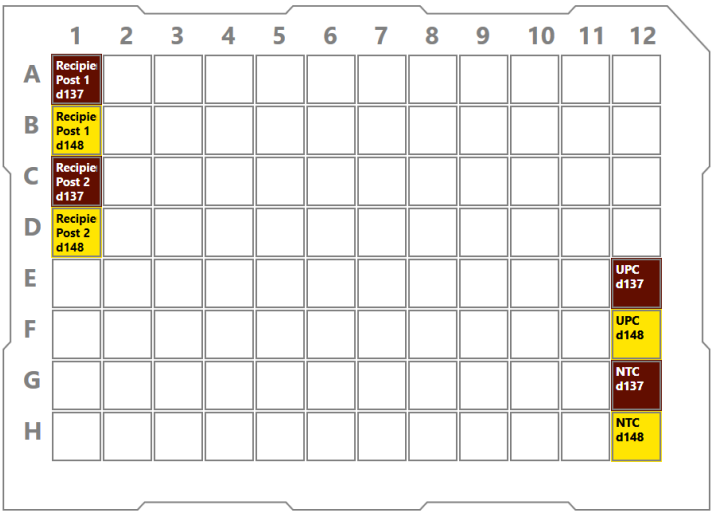
Monitoring Sample View

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 Drag and Drop Plate Layouts 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.

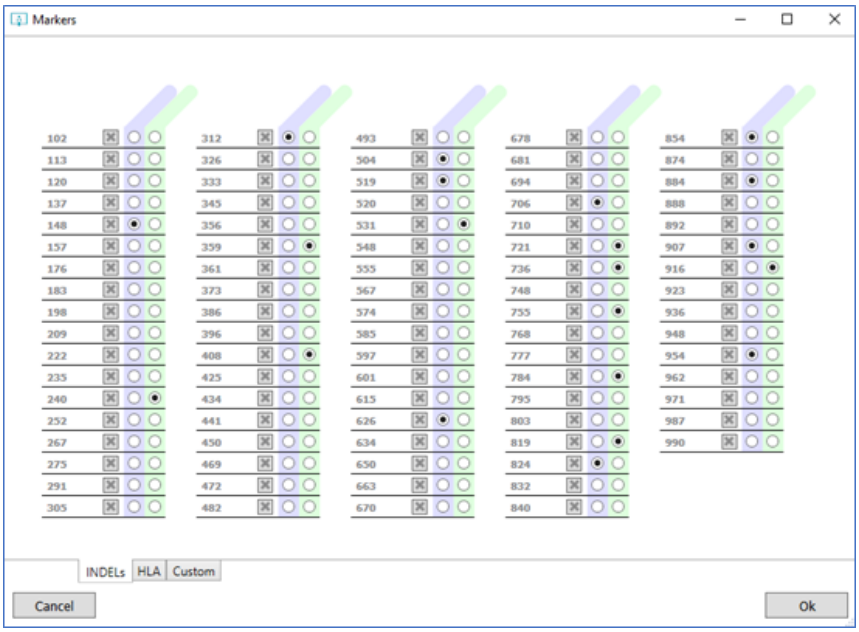
In order to use this feature of TRACE Analysis™:

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.

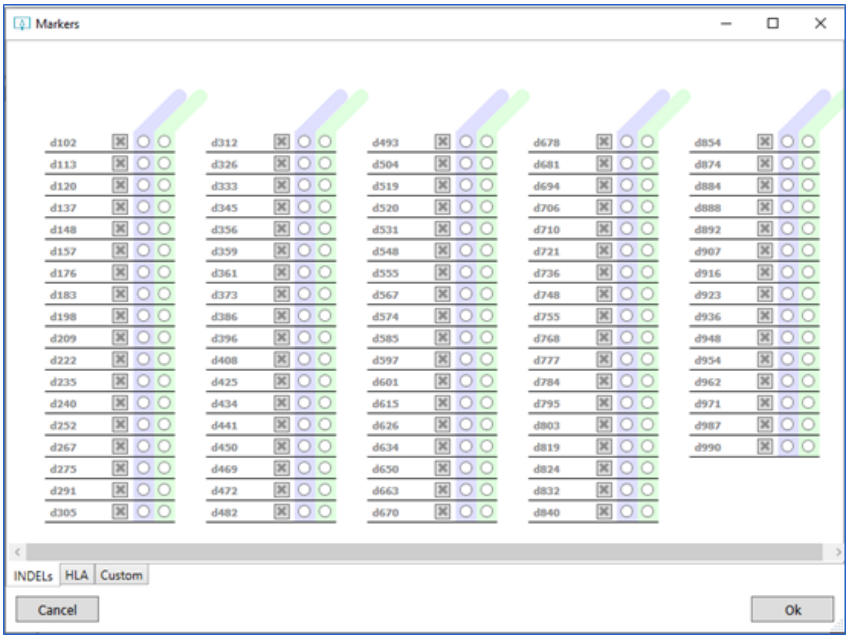
In the Informative Markers section, click the "Add Marker"  button.

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



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.



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

In the Custom tab, custom assays can be added.

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.

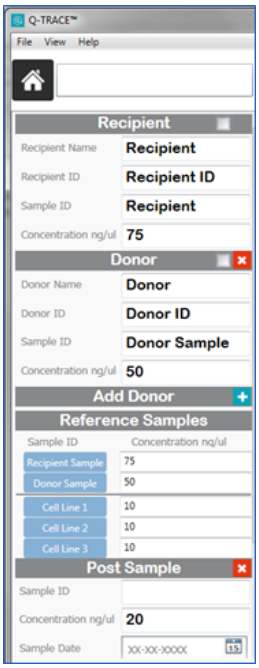
Protocol

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 Assigning Informative Markers).



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.

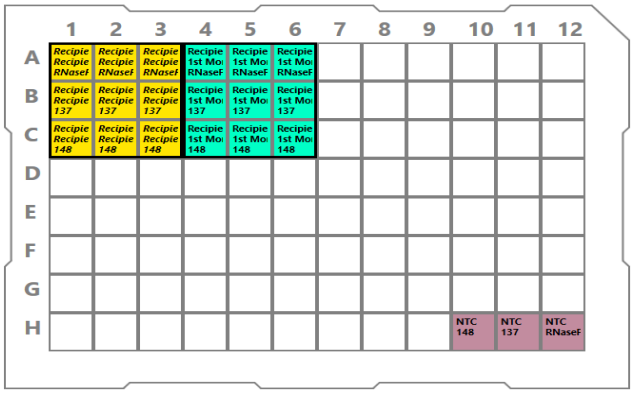
Click on the name(s) of the Assays you wish to use for the experiment.
Once all the information about the sample has been entered, click the “Add Quant Samples” Quant button and the sample will be added to the plate.

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.

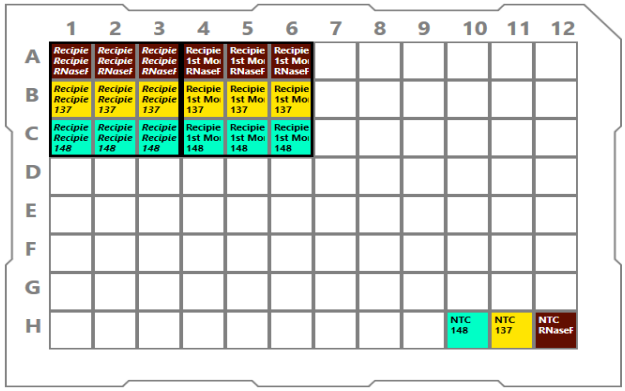
One View (see "Sample Layout view") shows the placement of Pure (pre-transfer) and Mixed Samples (Post-Transfer). Pre samples go in wells A1-A3, B1-B3 and C1-C3. Post samples go in A4-A6, B4-B6 and C4-C6.

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



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 Assigning Informative Markers.

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.

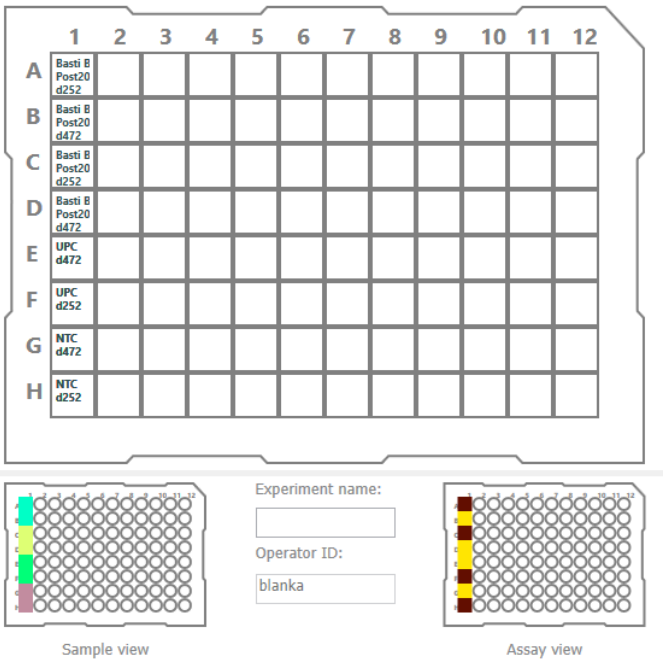
Once all the information about the sample has been entered, click the "Add Quant Samples" 

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 Preferences.
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 up all reactions in a pre-PCR lab, under ambient conditions without ice.**
2. Briefly vortex and centrifuge all tubes before opening.
3. Prepare DNA dilutions as specified in Table 1:

Table 1. DNA dilutions

No.	Name	Sample ID	Concentration	Sample Volume	Water Volume	Total Volume	Amount per Reaction
1	Post Sample	Post_test	26 ng/µl	12,69 µl	48,91 µl	61,60 µl	150 ng
2	Post Sample	Post2_test	41 ng/µl	8,05 µl	53,55 µl	61,60 µl	150 ng

4. Prepare for chosen informative dPCR Assay the following mixture:

Table 2. Master Mix + dPCR Assay mixture

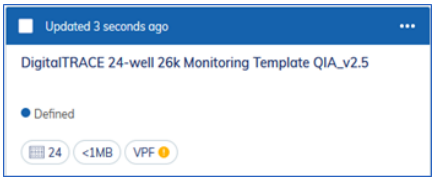
Assay Mix	QIAcuity 4x Master Mix	dPCR 20x assay mix [µl]	Total Volume
d359	46,00 µl	9,20 µl	55,20 µl
d626	46,00 µl	9,20 µl	55,20 µl

5. **IMPORTANT! Vortex at least 5 seconds and spin briefly each prepared Master Mix + dPCR Assay mixture.**
6. Deliver 13,2 µl of QIAcuity Probe Master Mix + dPCR Assay Mix to a 96-well plate as defined in TRACE Analysis™ Software’s Assay Layout view.
7. Add 30,8 µl of each Sample DNA dilution as indicated by TRACE Analysis™ Software’s Layout View. Where NTC and UPC are indicated add 30,8 µl water for NTC and 30,8 µl from Universal Positive Control for UPC.
8. **IMPORTANT! Vortex at least 15 seconds the 96-well plate to mix the contents of each reaction. Centrifuge the 96-well plate briefly using a microcentrifuge.**
9. Transfer 40 µl of each prepared reaction mix into a Nanoplate. Seal the Nanoplate with the compatible plate sealer.
10. Load the Nanoplate into the QIAcuity digital PCR system.
11. Launch the QIAcuity Software Suite.
12. Open the DigitalTRACE template and import the Sample Setup sheet generated by TRACE Analysis™ Software.
13. Save the file and start the run.

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.

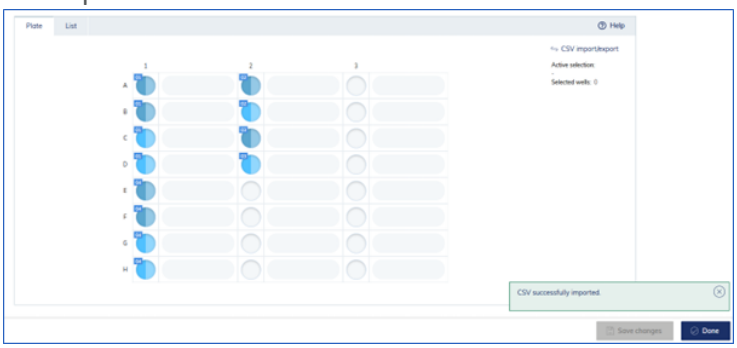
Press Save Plate. The new plate will appear in the main window of the QIAcuity Software Suite.



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".



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:

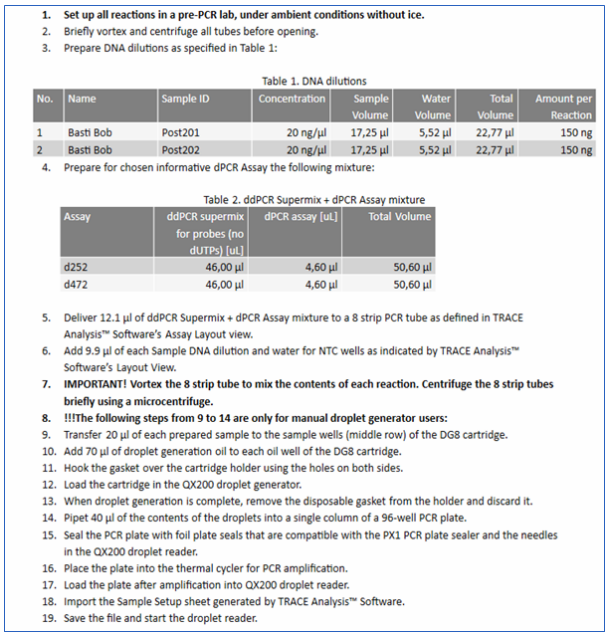
Number of cycles	Temperature	Time
1	95 °C	3 min
40	95 °C	15 s
	60 °C	30 s

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:



1. **Set up all reactions in a pre-PCR lab, under ambient conditions without ice.**
2. Briefly vortex and centrifuge all tubes before opening.
3. Prepare DNA dilutions as specified in Table 1:

Table 1. DNA dilutions

No.	Name	Sample ID	Concentration	Sample Volume	Water Volume	Total Volume	Amount per Reaction
1	Basti Bob	Post201	20 ng/µl	17,25 µl	5,52 µl	22,77 µl	150 ng
2	Basti Bob	Post202	20 ng/µl	17,25 µl	5,52 µl	22,77 µl	150 ng

4. Prepare for chosen informative dPCR Assay the following mixture:

Table 2. ddPCR Supermix + dPCR Assay mixture

Assay	ddPCR supermix for probes (no dUTPs) [uL]	dPCR assay [uL]	Total Volume
d252	46,00 µl	4,60 µl	50,60 µl
d472	46,00 µl	4,60 µl	50,60 µl

5. Deliver 12.1 µl of ddPCR Supermix + dPCR Assay mixture to a 8 strip PCR tube as defined in TRACE Analysis™ Software's Assay Layout view.
6. Add 9.9 µl of each Sample DNA dilution and water for NTC wells as indicated by TRACE Analysis™ Software's Layout View.
7. **IMPORTANT! Vortex the 8 strip tube to mix the contents of each reaction. Centrifuge the 8 strip tubes briefly using a microcentrifuge.**
8. **!!!The following steps from 9 to 14 are only for manual droplet generator users:**
9. Transfer 20 µl of each prepared sample to the sample wells (middle row) of the DG8 cartridge.
10. Add 70 µl of droplet generation oil to each oil well of the DG8 cartridge.
11. Hook the gasket over the cartridge holder using the holes on both sides.
12. Load the cartridge in the QX200 droplet generator.
13. When droplet generation is complete, remove the disposable gasket from the holder and discard it.
14. Pipet 40 µl of the contents of the droplets into a single column of a 96-well PCR plate.
15. Seal the PCR plate with foil plate seals that are compatible with the PX1 PCR plate sealer and the needles in the QX200 droplet reader.
16. Place the plate into the thermal cycler for PCR amplification.
17. Load the plate after amplification into QX200 droplet reader.
18. Import the Sample Setup sheet generated by TRACE Analysis™ Software.
19. Save the file and start the droplet reader.

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
	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

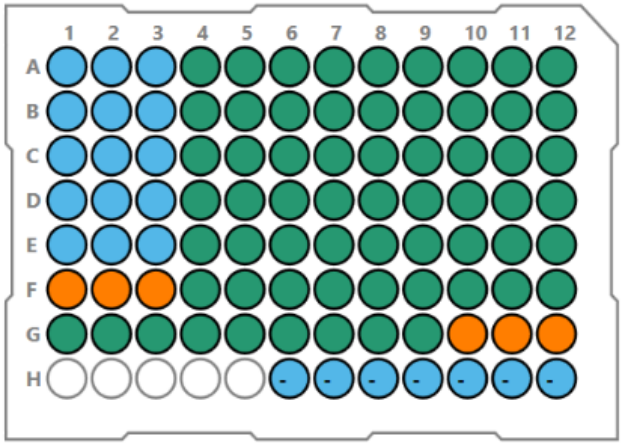
Import qPCR data by clicking the “Import PCR Data” button, and browse to the location of your exported qPCR data file and select it.

The QTRACE® Analysis System

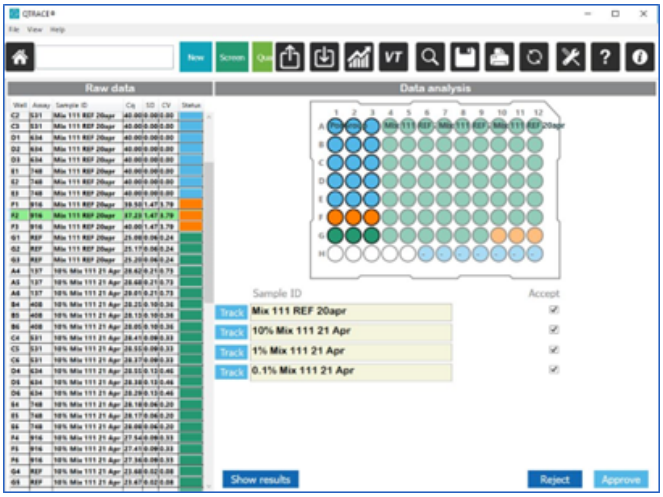
TRACE Analysis™ Software uses a green/amber/red color coding for well highlighting.

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 remain as negative wells.

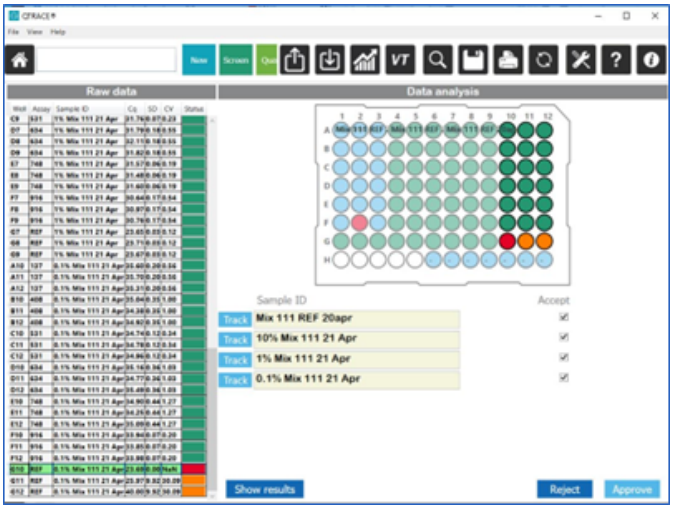
Monitoring plates contain green and light blue colors, indicating true positives or negatives. Amber colored wells highlight replicates which violate user-defined replicate highlighting rules, based on %CV, SD or Cq Range.

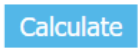


Replicate wells in Monitoring which violate the replicate highlighting preferences set by the user will first be highlighted in Amber.

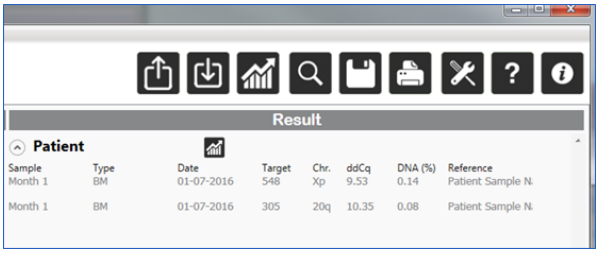


The user can then Accept/Reject individual wells in an attempt to conform to the acceptable variance allowed. Rejected wells turn Red and the remaining wells will either turn green, light blue or remain Amber - if the Rejected well does not cause the remaining replicates to meet acceptable variance.



Once all the data has been inspected, pressing the “Calculate”  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.



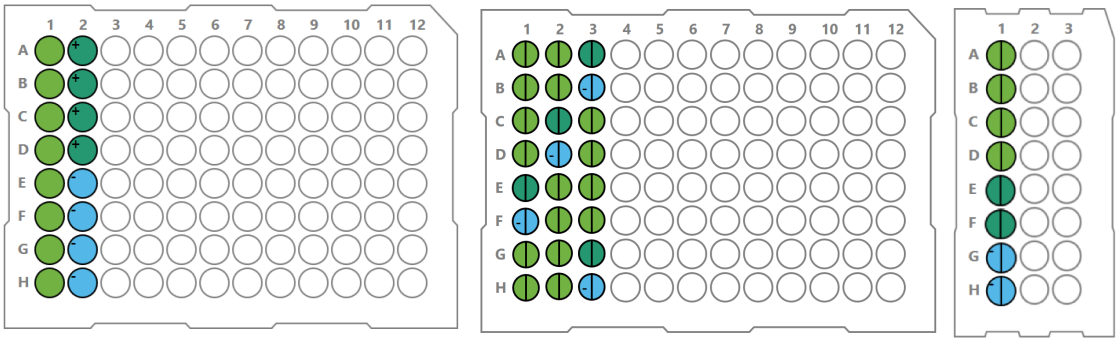
As more data is collected for a particular sample over time, TRACE Analysis™ Software provides a longitudinal view.

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.

The DigitalTRACE™ Analysis System

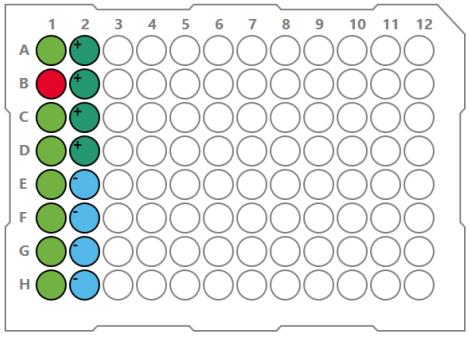
TRACE Analysis™ Software uses a light green/dark green/red color coding for well highlighting.



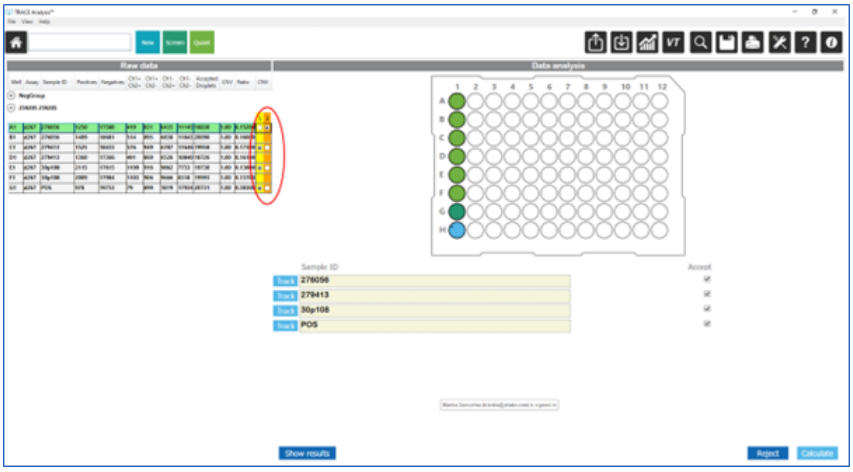
Light green wells represent post sample wells. Dark green wells represent the positive control DNA, Red wells are data which has been rejected.

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.

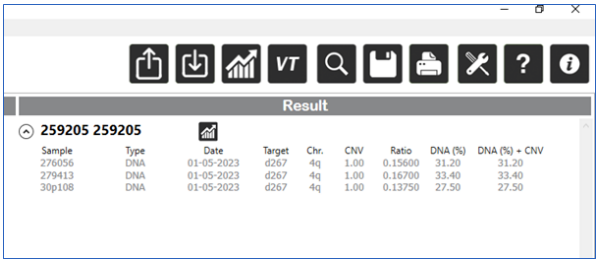


When necessary, for example in case that no CNV information is available from the genotyping, TRACE Analysis™ Software allows the user to change the CNV information for each sample by selecting 1 or 2 in the indicated area under the Rawdata view Show rawdata in order to obtain an accurate quantification result:



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.



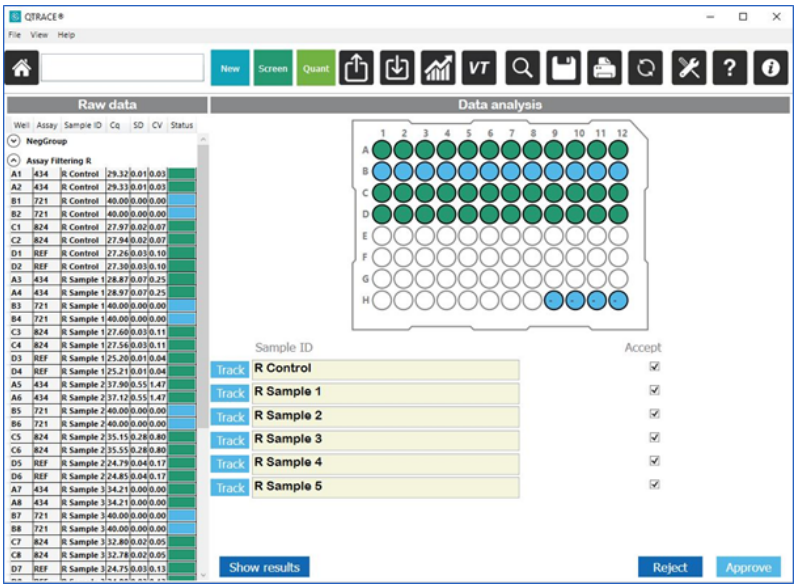
Click on Select assays and then on Report... to generate the Monitoring Report.

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

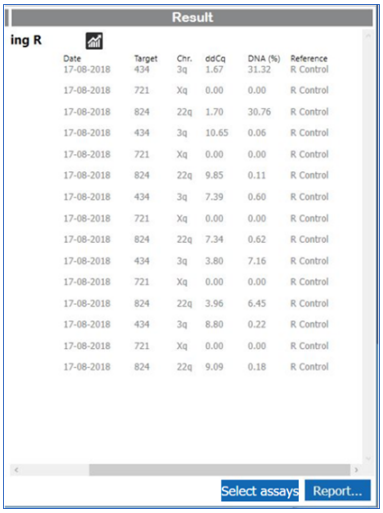
Assay Filtering from Monitoring Reports

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 multi-channel 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:



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.



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.

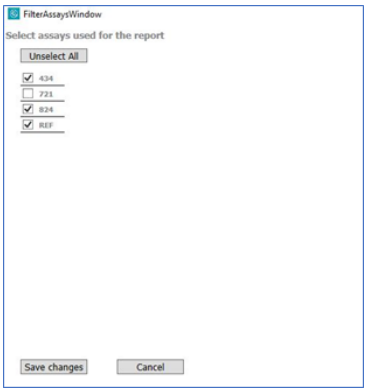
Cell fractions

Sample	Type	Date	Target	Chr.	ΔΔCq	DNA (%)	Reference	Informative for
R Sample 1	BM	17-Aug-2018	434	3q	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	3q	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
					Mean:	**0.06**		
R Sample 3	BM	17-Aug-2018	434	3q	7.39	**0.6**	R Control	R Control
R Sample 3	BM	17-Aug-2018	721	Xq	0	**0**	R Control	R Control
R Sample 3	BM	17-Aug-2018	824	22q	7.34	**0.62**	R Control	R Control
					Mean:	**0.41**		

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

button.

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



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

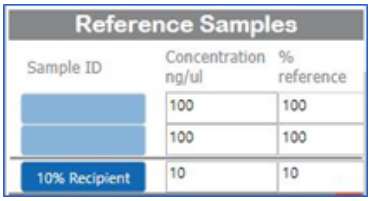
External Reference Functions

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.



Reference Samples		
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

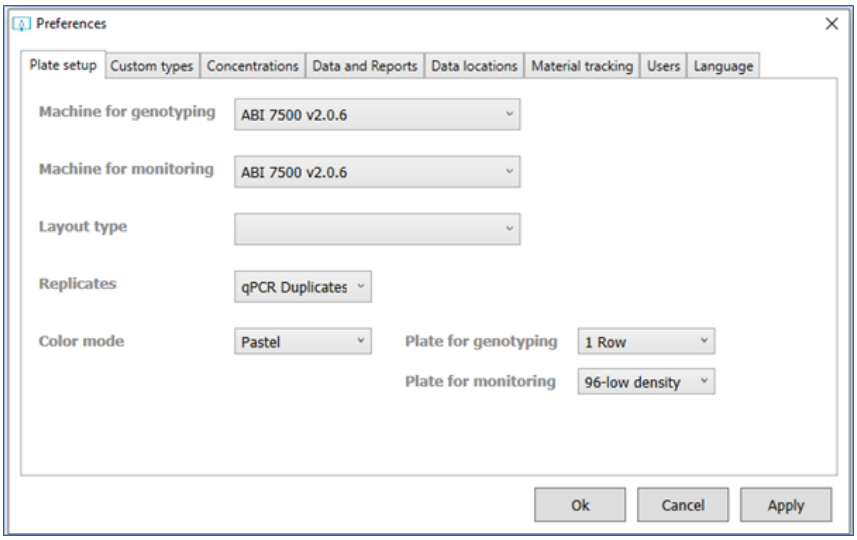
Sample	Type	Date	Target	Chr.	ΔΔCq	DNA (%)	
15	DNA	21-Jan-2020	548	Xq	2.88	**1.36**	
15	DNA	21-Jan-2020	361	12q	2.72	**1.52**	10% Reference Sample
15	DNA	21-Jan-2020	916	10q	2.73	**1.5**	
					Mean:	**1.46**	
15	DNA	21-Jan-2020	916	10q	2.73	**15.04**	
15	DNA	21-Jan-2020	361	12q	2.72	**15.16**	Pure Reference Sample
15	DNA	21-Jan-2020	548	Xq	2.88	**13.6**	
					Mean:	**14.6**	

Simultaneous Genotyping and Monitoring

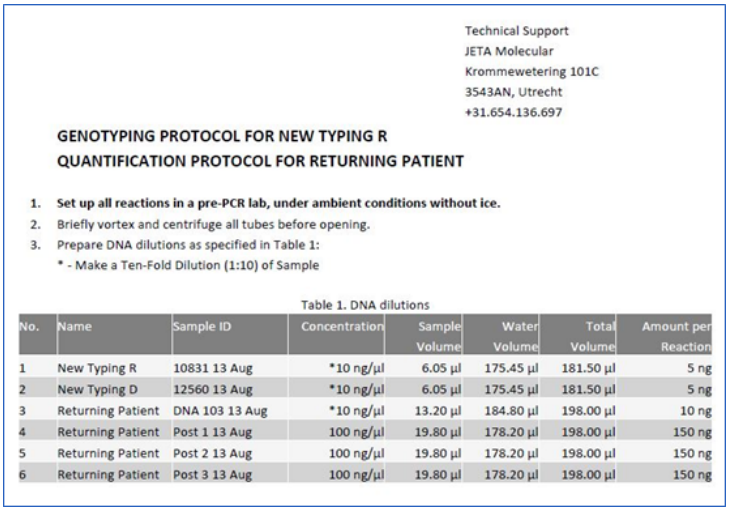
With the use of Custom Genotyping Panels, TRACE Analysis™ Software has the potential to allow concurrent genotyping and monitoring experiments on a single 96-well plate.

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).



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.



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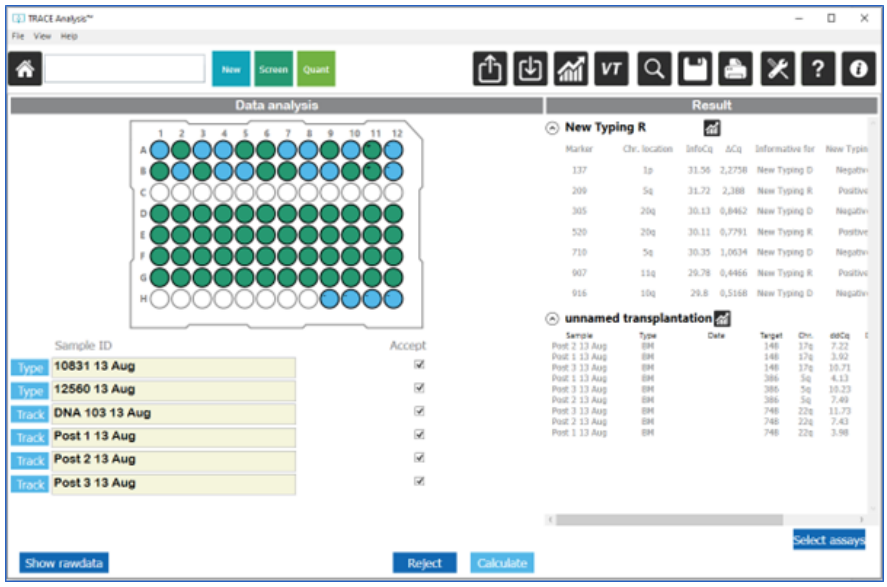
GENOTYPING PROTOCOL FOR NEW TYPING R
QUANTIFICATION PROTOCOL FOR RETURNING PATIENT

1. **Set up all reactions in a pre-PCR lab, under ambient conditions without ice.**
2. Briefly vortex and centrifuge all tubes before opening.
3. Prepare DNA dilutions as specified in Table 1:
   * - Make a Ten-Fold Dilution (1:10) of Sample

Table 1. DNA dilutions

No.	Name	Sample ID	Concentration	Sample Volume	Water Volume	Total Volume	Amount per Reaction
1	New Typing R	10831 13 Aug	*10 ng/µl	6.05 µl	175.45 µl	181.50 µl	5 ng
2	New Typing D	12560 13 Aug	*10 ng/µl	6.05 µl	175.45 µl	181.50 µl	5 ng
3	Returning Patient	DNA 103 13 Aug	*10 ng/µl	13.20 µl	184.80 µl	198.00 µl	10 ng
4	Returning Patient	Post 1 13 Aug	100 ng/µl	19.80 µl	178.20 µl	198.00 µl	150 ng
5	Returning Patient	Post 2 13 Aug	100 ng/µl	19.80 µl	178.20 µl	198.00 µl	150 ng
6	Returning Patient	Post 3 13 Aug	100 ng/µl	19.80 µl	178.20 µl	198.00 µl	150 ng

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™ 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.

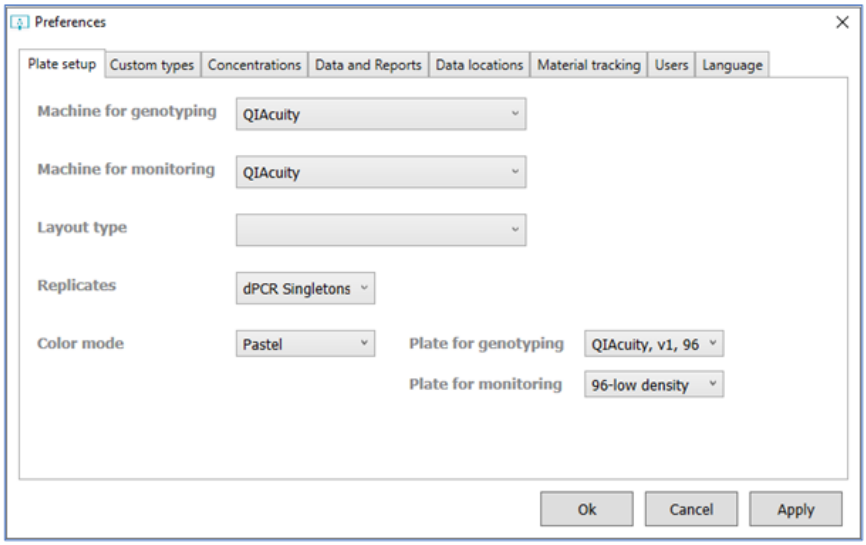
Preferences

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



“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.

“Color mode”:
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.

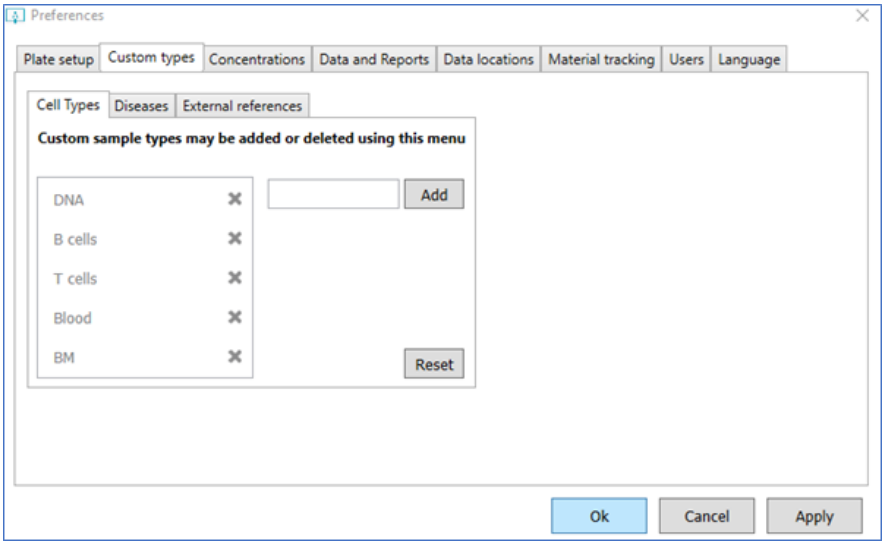
“Plate for genotyping”:
This preference selects the plate chemistry version in use for genotyping. Different plate versions have slightly different assay panel members. If you have created Custom Genotyping Panels, they will appear here.

“Plate for monitoring”:
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.

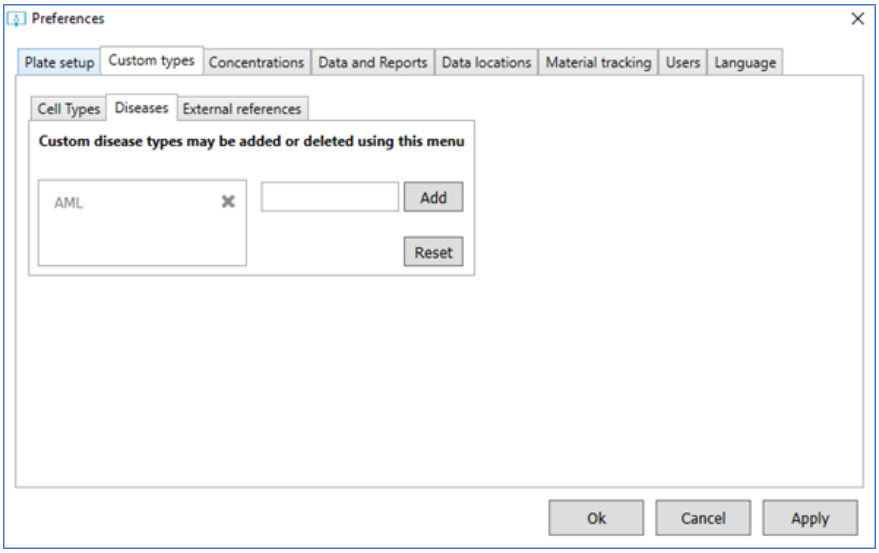
Custom Types Preferences

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.

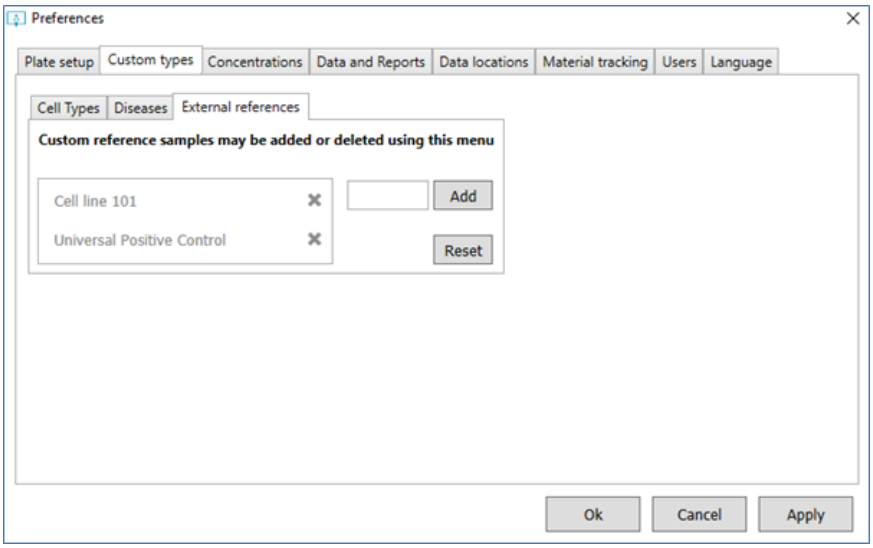
Tab 2: Custom Types Preferences



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™.



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.



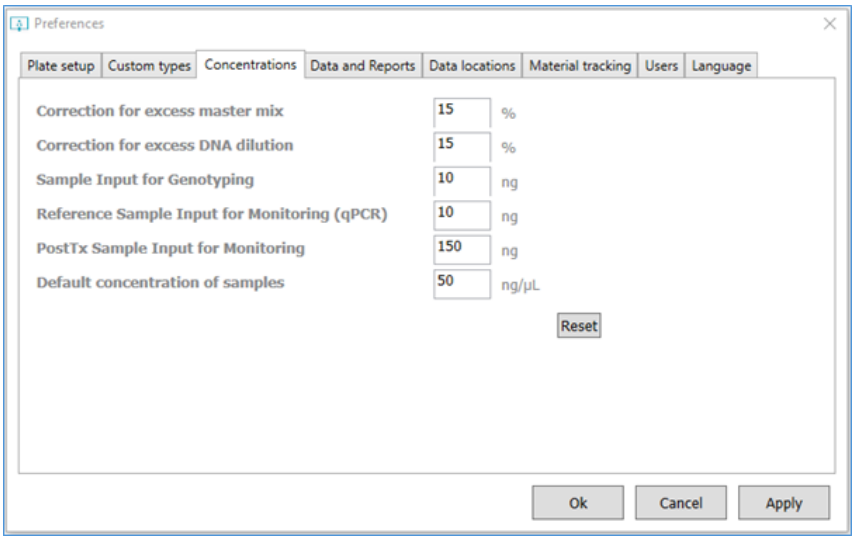
External references tab of the Custom types tab of the Preferences Menu:

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



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

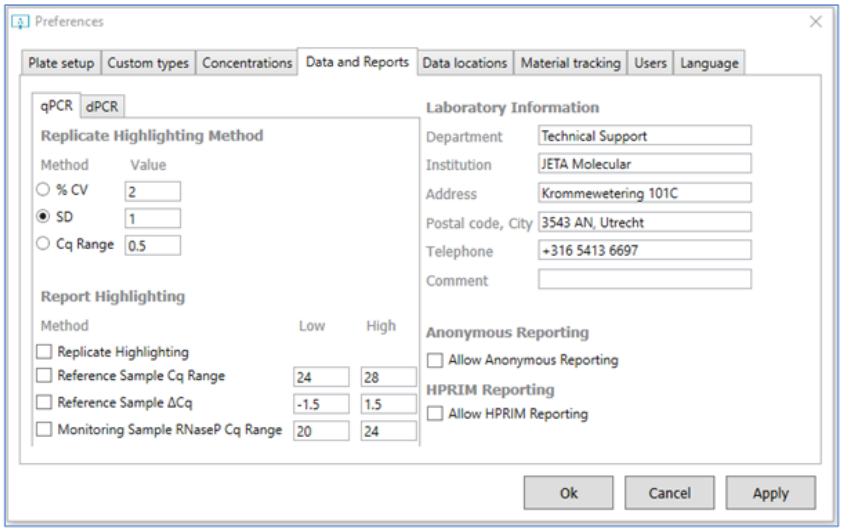
Define the sensitivity to achieve in the experiment by entering the target DNA input per well for the monitoring samples.
While sensitivity may be thought of in terms of cell numbers or percentages or grams of DNA, the software uses nanograms of input to generate an appropriate protocol.

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

qPCR tab:



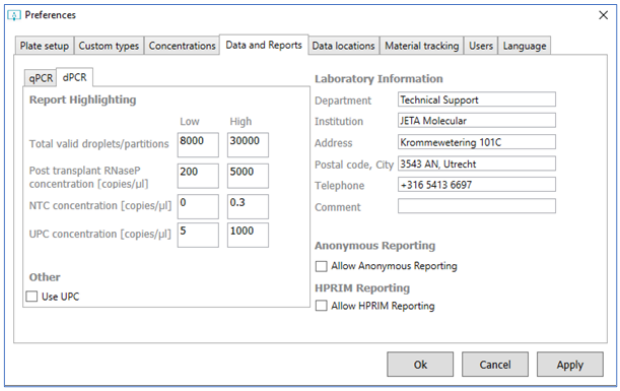
In order to achieve more customized data analysis,TRACE Analysis™ has a tab called Data and Reports in the Preferences section of the software.
There is a Replicate Highlighting Section and a Report Highlighting Section.

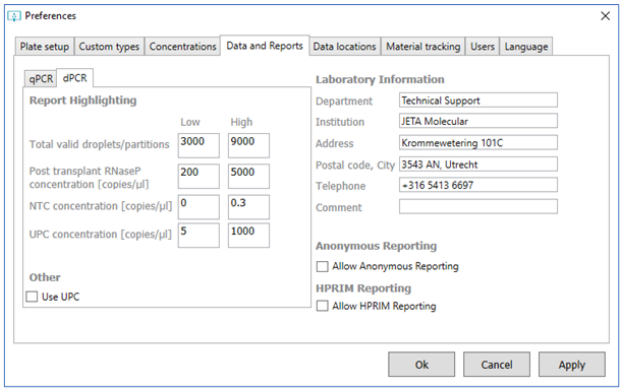
In Replicate Highlighting, users can select between three methods to highlight variance in replicate samples. Replicates displaying variance in %CV, SD or Cq range may be user defined, based on the lab's practices. The chosen method is applied to all replicates in monitoring, whether it is the reference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





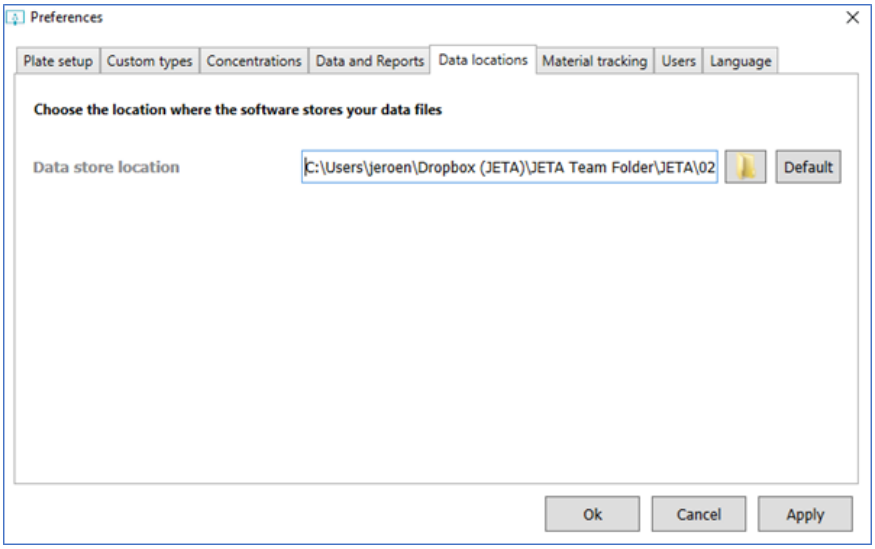
The number of valid droplets/partitions, post transplant RNase P concentration, UPC and NTC concentration can be defined here. If the actual droplet/partition number or RNaseP, NTC or UPC concentration falls outside of the defined range, data will be highlighted in the report.

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



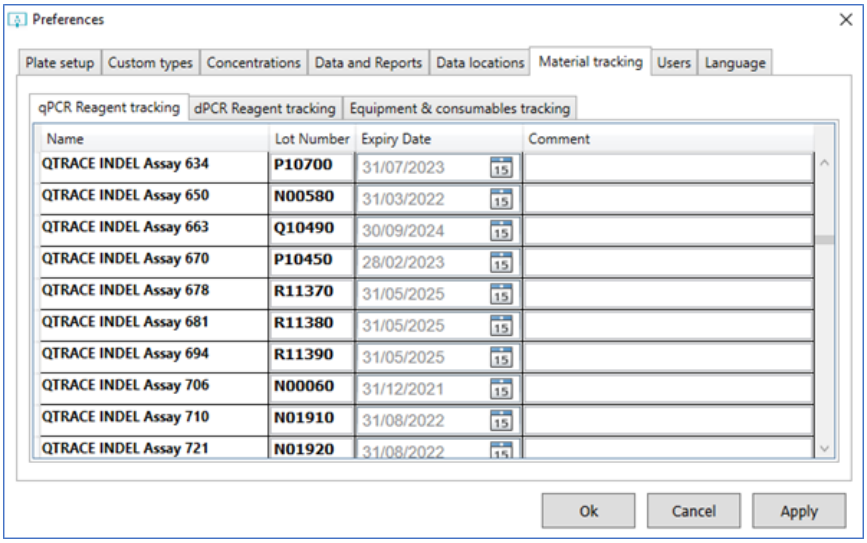
Data locations tab of the Preferences Menu:
Define where TRACE Analysis™ Software stores the data it generates.
For labs using multiple copies of TRACE Analysis™ Software, this location is likely best set as a shared location on a server. When all local copies of TRACE Analysis™ Software point to the 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.

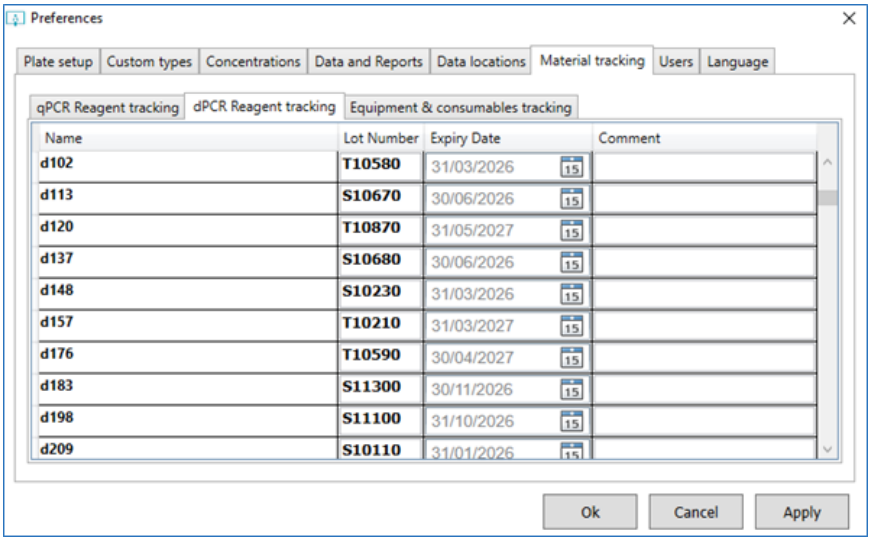
qPCR Reagent tracking tab

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



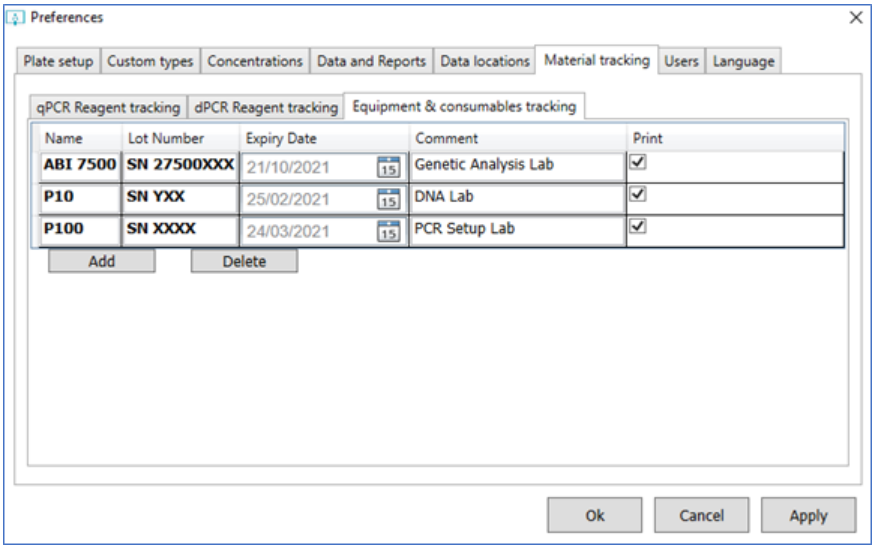
dPCR Reagent tracking tab

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



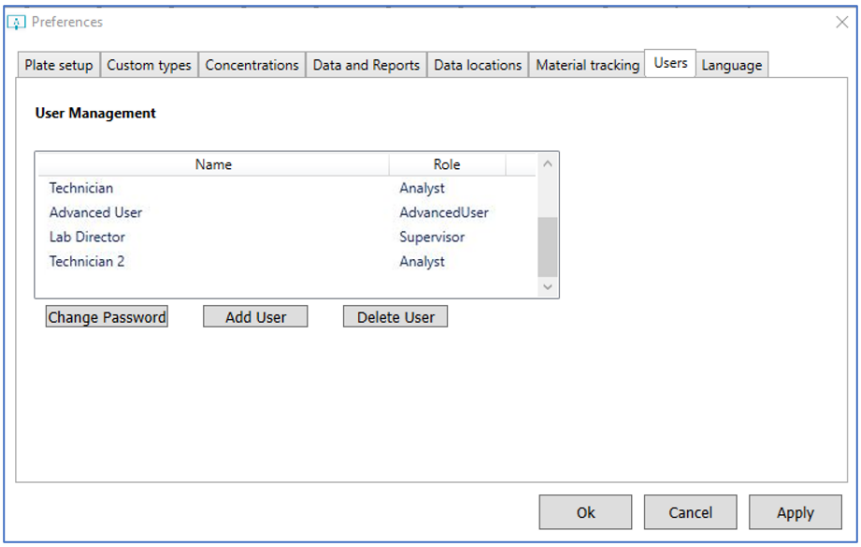
Equipment & consumables tracking tab

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



Users Preferences

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



Language Preferences

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

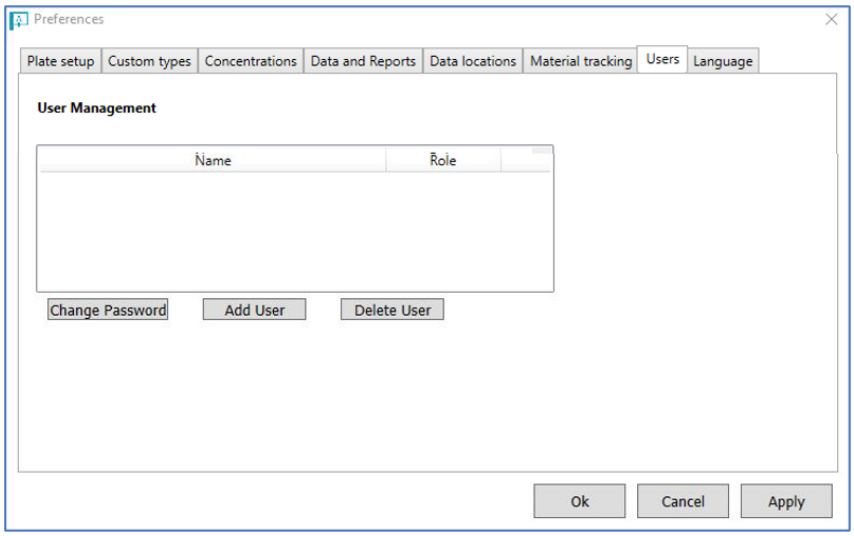
Software Access And User Profile Management

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

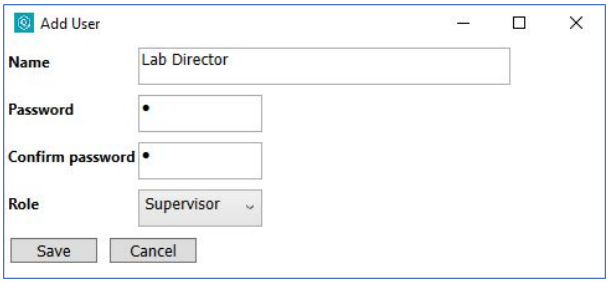
When TRACE Analysis™ launches for the fist time, User Profiles need to be established.

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

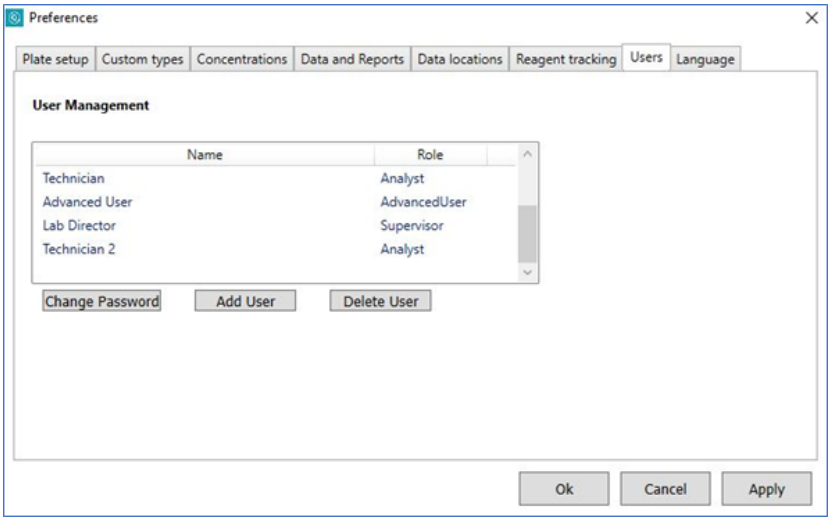
In the Preferences, under the new User tab, there is an option for creating New Users.



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



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



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**

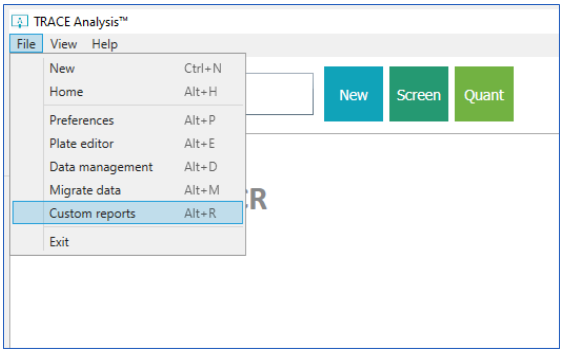
File Management

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.

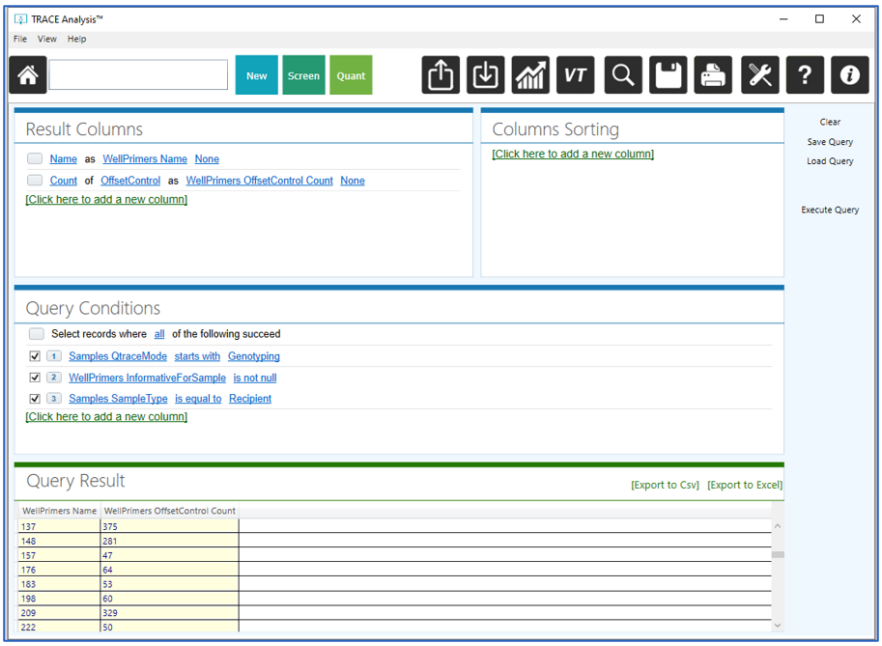
Data storage and retrieval in TRACE Analysis™ Software version 2.0 is enhanced through the introduction of a relational database data storage format. Previous versions of software relied on generation of .xml files which were stored in various folders, making the information captured in the software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



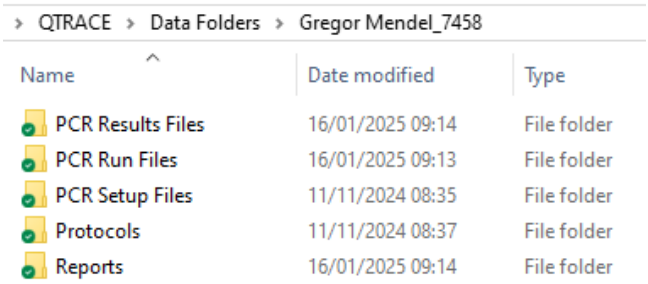
For the logging of errors, files are created in your temporary directory (C:\Users\Your Profile\AppData\Local\Temp), named QTRACE.log(n).

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.

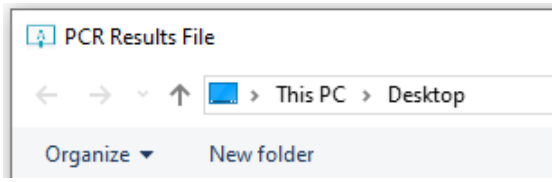
Experimental Files Saved in TRACE Analysis™ Software

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

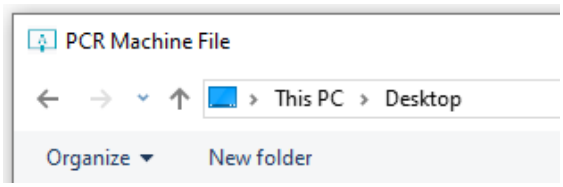


Within each of these folders are all the files used to track the recipient. When PCR data is imported into TRACE Analysis™, the user also has the ability to identify the location of the original PCR machine file.

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™.



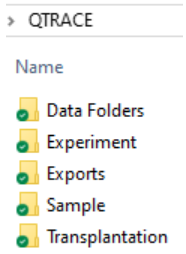
After selecting the results file, TRACE Analysis™ prompts the user to identify the location of the data collection file from the PCR machine. The imported file is automatically saved to the PCR Runs File folder.



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

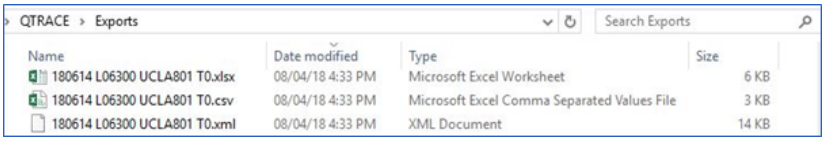
and put it into the Run Files folder for each patient in the same experiment. With the reports that are generated from each experiment, they are differentially saved – only the report for the specific patient goes into that patients file, while the other patient sample has a separate report, and it is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



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.



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	-	Negative	40	Negative	40
9	706	16p	None	-	Positive	30.3095	Positive	30.2039
10	755	11q	None	-	Negative	40	Negative	40
11	NTC	14q	None	-	Negative	40	Negative	40
12	POS	14q	None	-	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

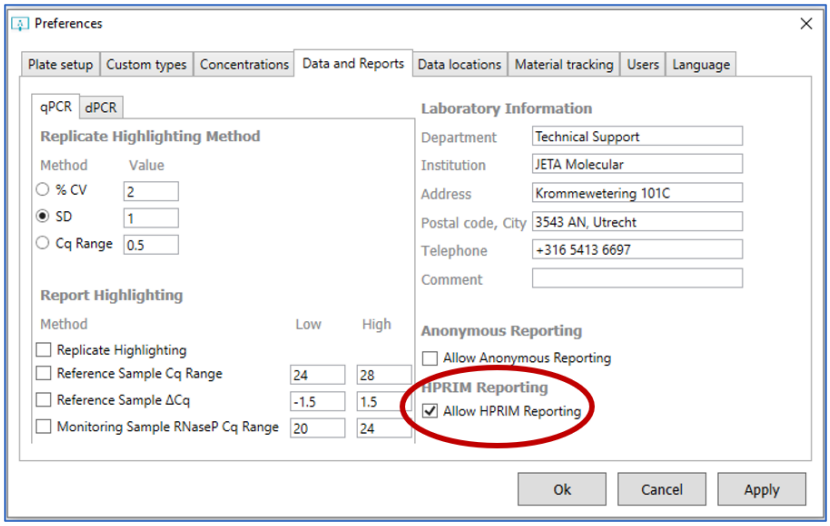
assay combination are exported, in addition to the Mean % DNA for all assays tested on a single sample.

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	Reference	
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'

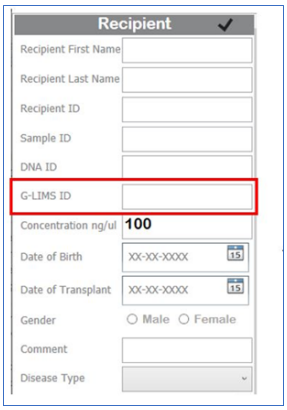


b) Créer un nouveau couple donneur- receveur

Les nouveaux champs sont encadrés en rouge.

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 .

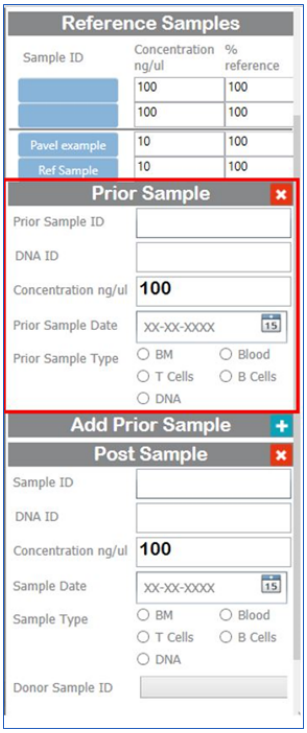
c) Renseigner le nouveau champ '**Q-LIMS ID**'. Il apparait dans le bloc 'Recipient' après avoir sélectionné l'échantillon de référence pour le test de quantification.



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.

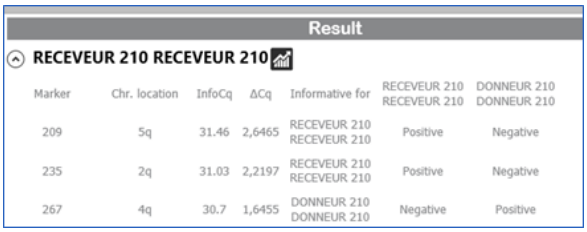


e) Valider les résultats

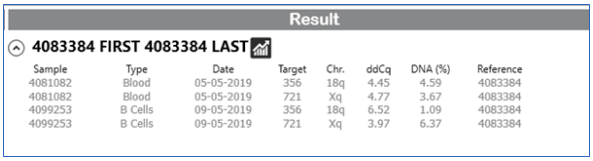
- Après avoir importé les résultats de qPCR de votre patient et après avoir vérifié les données de votre plaque dans la fenêtre « Data analysis », cliquer sur le bouton 'Calculate' Calculate.

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

Exemple: résultat de génotypage



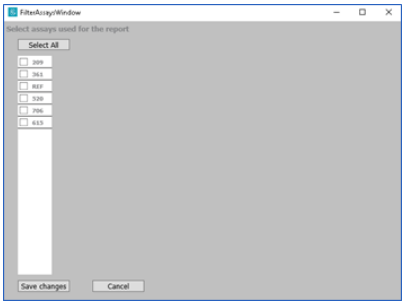
Exemple: résultat de quantification



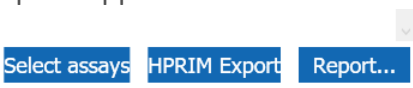
- Cliquer ensuite sur le bouton ‘Select assays’ 

. 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.

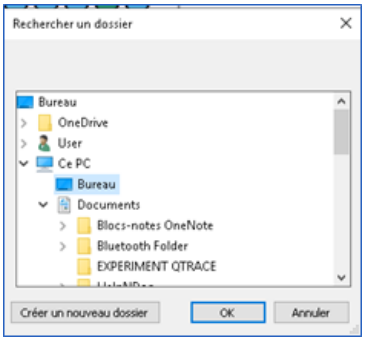


- Cliquer ensuite sur ‘Save changes’ pour valider vos résultats. Les boutons ‘HPRIM Export’ et ‘Report’ apparaissent alors.



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:



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.

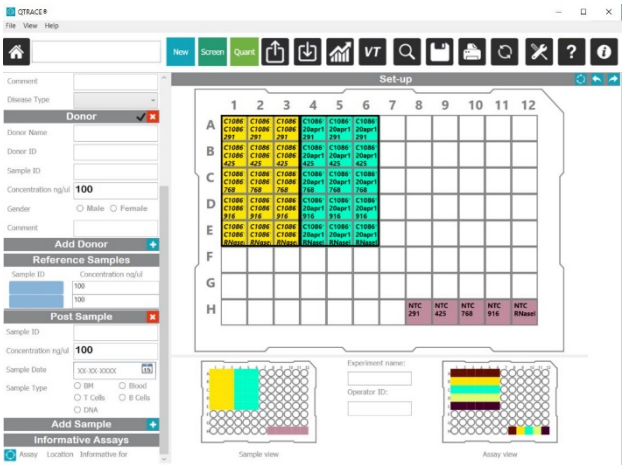
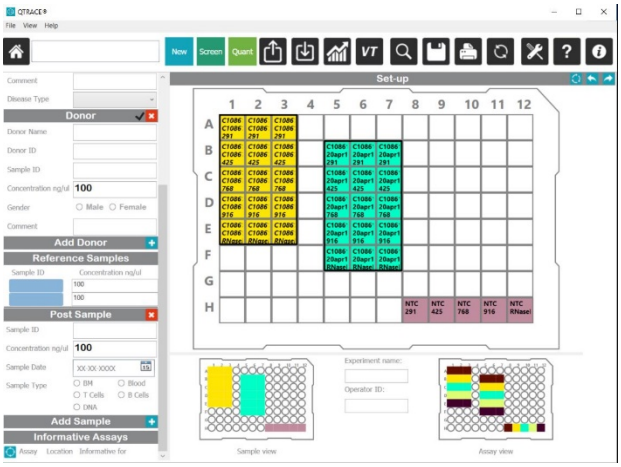
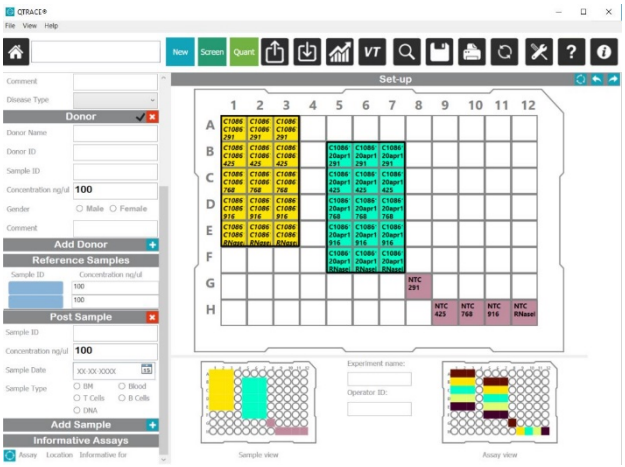
Reset
Reset the experiment plate completely.

Undo
Reset the last placement onto the experimental plate.

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

Drag and Drop Plate Layouts

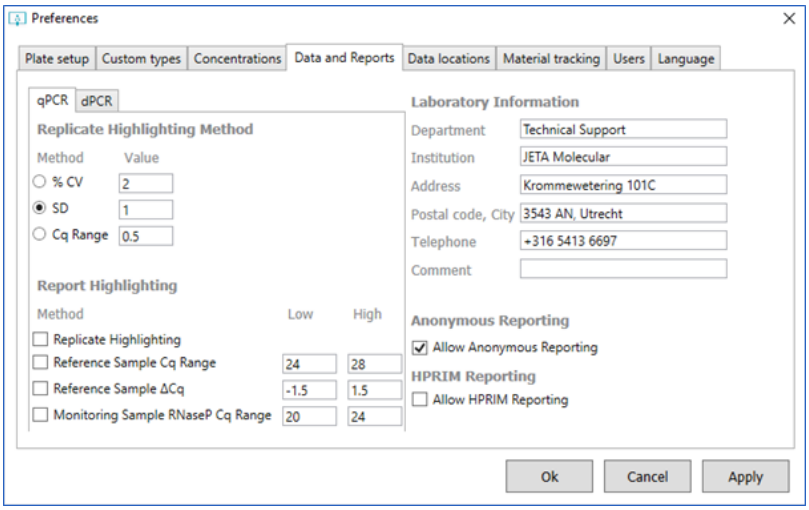
TRACE Analysis™, Version 1.5 introduced movable wells for customized plate layout capability. Once monitoring samples are placed onto the plate, the user has the ability to move a well from one location to another location. Whole sample groups can be moved by left-clicking and holding the mouse button. 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.

In a monitoring experiment, once the Quant button is pressed, samples and assays are added to the plate.	
Entire Sample Blocks can be moved by holding the left-click mouse button while touching a well in the layout	
Individual Sample Blocks can be moved by holding the right-click mouse button while touching a well in the layout	

Anonymized Reporting

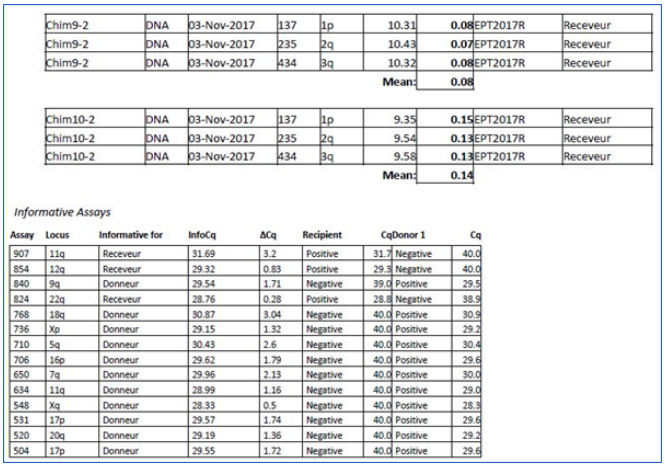
The ability to generate anonymized reports from TRACE Analysis™ Software is an option in the Preferences.

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.



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.



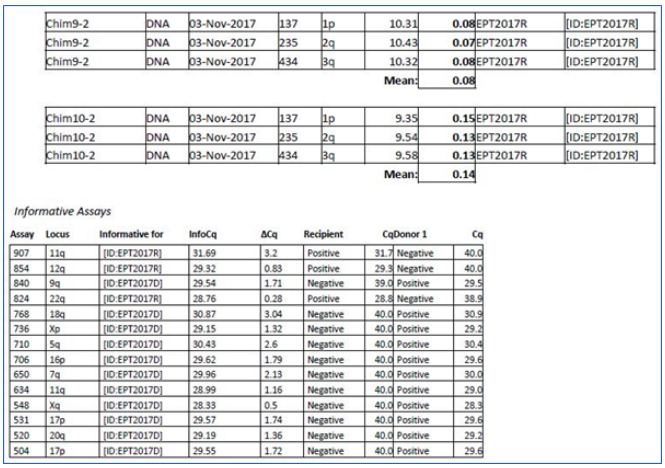
Chim9-2	DNA	03-Nov-2017	137	1p	10.31	**0.08**	EPT2017R	Receveur
Chim9-2	DNA	03-Nov-2017	235	2q	10.43	**0.07**	EPT2017R	Receveur
Chim9-2	DNA	03-Nov-2017	434	3q	10.32	**0.08**	EPT2017R	Receveur
					Mean:	**0.08**		

Chim10-2	DNA	03-Nov-2017	137	1p	9.35	**0.15**	EPT2017R	Receveur
Chim10-2	DNA	03-Nov-2017	235	2q	9.54	**0.13**	EPT2017R	Receveur
Chim10-2	DNA	03-Nov-2017	434	3q	9.58	**0.13**	EPT2017R	Receveur
					Mean:	**0.14**		

Informative Assays

Assay	Locus	Informative for	InfoCq	ΔCq	Recipient	CqDonor 1		Cq
907	11q	Receveur	31.69	3.2	Positive	31.7	Negative	40.0
854	12q	Receveur	29.32	0.83	Positive	29.3	Negative	40.0
840	9q	Donneur	29.54	1.71	Negative	39.0	Positive	29.5
824	22q	Receveur	28.76	0.28	Positive	28.8	Negative	38.9
768	18q	Donneur	30.87	3.04	Negative	40.0	Positive	30.9
736	Xp	Donneur	29.15	1.32	Negative	40.0	Positive	29.2
710	5q	Donneur	30.43	2.6	Negative	40.0	Positive	30.4
706	16p	Donneur	29.62	1.79	Negative	40.0	Positive	29.6
650	7q	Donneur	29.96	2.13	Negative	40.0	Positive	30.0
634	11q	Donneur	28.99	1.16	Negative	40.0	Positive	29.0
548	Xq	Donneur	28.33	0.5	Negative	40.0	Positive	28.3
531	17p	Donneur	29.57	1.74	Negative	40.0	Positive	29.6
520	20q	Donneur	29.19	1.36	Negative	40.0	Positive	29.2
504	17p	Donneur	29.55	1.72	Negative	40.0	Positive	29.6

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	DNA	03-Nov-2017	137	1p	10.31	**0.08**	EPT2017R [ID:EPT2017R]
Chim9-2	DNA	03-Nov-2017	235	2q	10.43	**0.07**	EPT2017R [ID:EPT2017R]
Chim9-2	DNA	03-Nov-2017	434	3q	10.32	**0.08**	EPT2017R [ID:EPT2017R]
					Mean:	**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	**0.13**	EPT2017R [ID:EPT2017R]
Chim10-2	DNA	03-Nov-2017	434	3q	9.58	**0.13**	EPT2017R [ID:EPT2017R]
					Mean:	**0.14**	

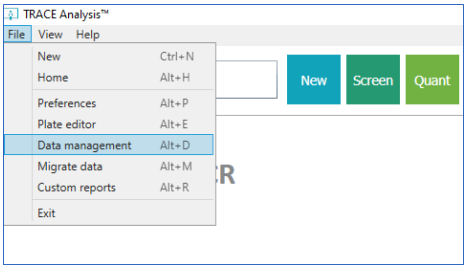
Informative Assays

Assay	Locus	Informative for	InfoCq	ΔCq	Recipient	CqDonor 1	Cq
907	11q	[ID:EPT2017R]	31.69	3.2	Positive	31.7 Negative	40.0
854	12q	[ID:EPT2017R]	29.32	0.83	Positive	29.3 Negative	40.0
840	9q	[ID:EPT2017D]	29.54	1.71	Negative	39.0 Positive	29.5
824	22q	[ID:EPT2017R]	28.76	0.28	Positive	28.8 Negative	38.9
768	18q	[ID:EPT2017D]	30.87	3.04	Negative	40.0 Positive	30.9
736	Xp	[ID:EPT2017D]	29.15	1.32	Negative	40.0 Positive	29.2
710	5q	[ID:EPT2017D]	30.43	2.6	Negative	40.0 Positive	30.4
706	16p	[ID:EPT2017D]	29.62	1.79	Negative	40.0 Positive	29.6
650	7q	[ID:EPT2017D]	29.96	2.13	Negative	40.0 Positive	30.0
634	11q	[ID:EPT2017D]	28.99	1.16	Negative	40.0 Positive	29.0
548	Xq	[ID:EPT2017D]	28.33	0.5	Negative	40.0 Positive	28.3
531	17p	[ID:EPT2017D]	29.57	1.74	Negative	40.0 Positive	29.6
520	20q	[ID:EPT2017D]	29.19	1.36	Negative	40.0 Positive	29.2
504	17p	[ID:EPT2017D]	29.55	1.72	Negative	40.0 Positive	29.6

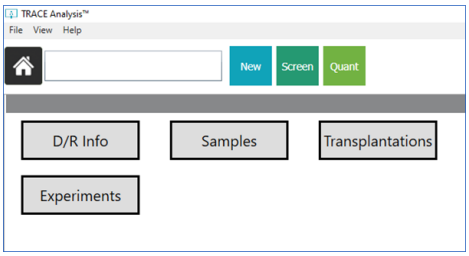
Modification of Existing Data

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

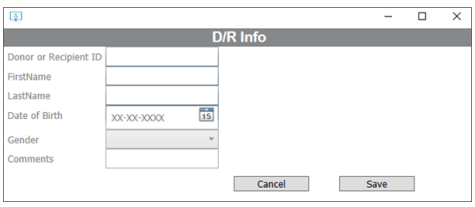
To make changes to existing files, select the Data Management item from the File Menu



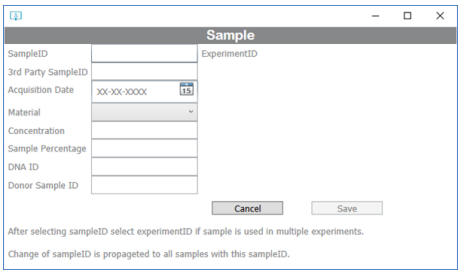
Selecting from the four different buttons allows users to edit some information related to these topics.



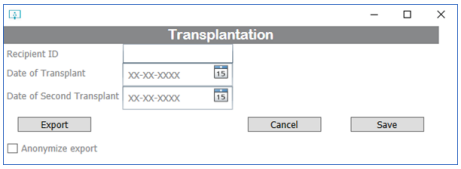
The 'D/R Info' button allows editing of Donor and Recipient information.



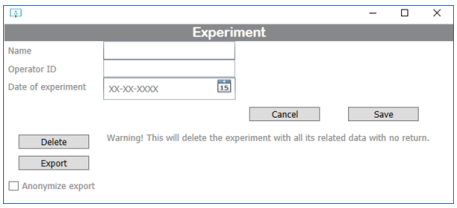
Sample Information may be edited by pushing the 'Samples' button.



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



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



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 ΔCq (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 ΔCq 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 ΔCq (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 ΔCq 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 ΔΔCq 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	ΔCq	ΔΔCq	$2^{(-\Delta\Delta Cq)}$	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 ΔΔCq. 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-(ΔΔCq). 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 ΔΔCq, 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, ΔΔCq, 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 Monitoring Data Analysis and Report 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.

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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. ΔRn (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)

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