

ABA iPLEX[®] Pro Chimeric ID Panel Protocol

This document gives the assay protocol for the Assays by Agena (ABA) iPLEX Pro Chimeric ID Panel (Chimeric ID). The Chimeric ID Panel accurately determines the percent contribution of donor and recipient DNA in samples taken following an allogeneic bone marrow transplant. The BME Chimerism report allows users to designate pure patient and recipient samples. These samples are used as references to determine informative loci and automatically report the composition of subsequent samples.

For complete information on equipment and reagents required, isolating and quantitating DNA, and setting up the experiment in the software, see the *iPLEX Pro Reagents User Guide*. For detailed instructions on desalting and dispensing analyte and acquiring data, see the applicable instrument user guide. All user guides are available on AgenaCx.com.

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Required Agena Reagents and Software

The Chimeric ID Panel may be run in 96 or 384-well format. The following items (Table 1) are required. Upon receipt, store the items as described in Table 1.

Table 1. Required Agena Reagents for the Chimeric ID Panel

Materials Provided	Number of Reactions Sufficient For	Shipping Conditions	Storage Temperature	Storage Location (see Table 3)
Chimeric ID Panel <ul style="list-style-type: none"> Chimeric ID PCR Primer Mixes (1-8) Chimeric ID Extend Primer Mixes (1-8) 	960	Dry Ice	-10 to -25°C	Lab Area 2
PCR Reagent Set <ul style="list-style-type: none"> MgCl₂ 10X PCR Buffer dNTP Mix PCR Enzyme 	Large: 3840 Medium: 960 Small: 192	Dry Ice	-10 to -25°C	Lab Area 2
iPLEX Pro Reagent Set <ul style="list-style-type: none"> 3-Pt Calibrant iPLEX Termination Mix iPLEX Buffer Plus (10X) iPLEX Pro Enzyme SAP Buffer SAP Enzyme 	Large: 3840 Medium: 960 Small: 192	Dry Ice	-10 to -25°C	Lab Area 2
SpectroCHIP Arrays	10x384: 3840 10x96: 960 2x384: 768 2x96: 192	Ambient Temperature	Room Temperature	Lab Area 3
Clean Resin (28g or 40g)	varies	Ambient Temperature	Room Temperature	Lab Area 3 (paraffin-cover Clean Resin)

See *MassARRAY System: Recommended Lab Equipment and Set-up*, available on AgenaCx.com (Product Support > Applications & Systems > MassARRAY System), for more detailed information on recommended lab set up and recommended vendors.

Table 2. Required Software

Software	Source
MassARRAY Typer v4.1.83 or higher	Agena Bioscience (download from AgenaCx.com: Product Support > Software > Typer)
BME Chimerism Report v1.0.14 or higher	Agena Bioscience (download from AgenaCx.com: Product Support > Applications & Systems > Sample Integrity and Composition)
'R' Environment, including RODBC, doBy, gap, and RSQLite packages	http://cran.r-project.org See MassARRAY Typer Release Notes on AgenaCx.com (Product Support > Software > Typer) for instructions for downloading the R software and installing the necessary packages.
MassARRAY Analyzer software <ul style="list-style-type: none"> • RT Workstation v3.7 or higher • Chip Prep Control v1.6 or higher (if using MassARRAY System with Chip prep control) 	Agena Bioscience (part of MassARRAY Analyzer and MassARRAY System with Chip prep module)

Assay Protocol

Table 3. Lab Area Activities

Lab Area	Activities
1	Isolation and dilution of DNA and preparation of the DNA sample plate.
2	Pre-PCR preparation, including preparation of the PCR reaction plate, preparation of PCR cocktails, and addition of PCR cocktails to the reaction plate. Preparation of the SAP and extension cocktails.
3	Thermocycling the reaction plate after addition of PCR cocktails; addition of the SAP cocktail to the reaction plate and thermocycling; addition of the extension reaction cocktails to the reaction plate and thermocycling; desalting; nanodispensing; and data acquisition.

A. Preparing Sample DNA Plate

IMPORTANT

Perform this procedure in **Lab Area 1**. See *MassARRAY System: Recommended Lab Equipment and Set-up*, available on AgenaCx.com (Product Support > Applications & Systems > MassARRAY System), for more detailed information on recommended DNA extraction and quantification methods and kits.

The Chimeric ID Panel accepts 10-240 ng of input DNA per well.

1. Dispense 2 μL (5-120 ng/ μL) of DNA to each well of a new reaction plate.
2. Visually inspect the individual wells from the bottom of the plate to confirm uniform and adequate DNA sample is present in every well before continuing.
3. Seal the plate and centrifuge at 2,000 x g for one minute.

STOPPING POINT

Purified genomic DNA can be stored at 4°C for up to 24 hours. To store DNA longer than 24 hours, it is recommend to at store at -20°C. Avoid repeated freezing and thawing of DNA.

B. PCR Amplification

IMPORTANT

Prepare the PCR cocktails and add to the reaction plate in **Lab Area 2**. Thermocycle the PCR reaction plate in **Lab Area 3**. Make sure all reagents are thawed completely at room temperature and enzymes are kept on ice. Make sure reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely at room temperature and spun down.

1. Prepare the PCR master mix in a 1.5 mL tube placed on ice by adding reagents in the order in which they are listed in Table 4. Do NOT add PCR primers, as they are added separately. Prepare more cocktail than the number of PCR reactions to be performed. Either prepare for one extra reaction or use a percentage extra to ensure sufficient overage is present to overcome typical pipetting variation.

Table 4: PCR Reaction

Reagent	Per Well (µL)	Final Concentration
HPLC-grade water	0.8	n/a
10X PCR Buffer	0.5	1X
MgCl ₂	0.4	2 mM
dNTP Mix	0.1	500 µM
PCR Enzyme	0.2	0.2 U/ µL
PCR Master Mix Final Volume	2.0	
Chimeric ID PCR Primer (add separately to each multiplex)	1.0	
PCR Cocktails Final Volume	3.0	
DNA, 5-120 ng/µL	2.0	
PCR Reaction Final Volume	5.0	

- Vortex the tube for 3 seconds and briefly centrifuge.
- Label eight new 0.5 mL tubes 1-8.
- Prepare each individual PCR cocktail by adding the appropriate amount of PCR master mix and specific PCR primer to the appropriate tube (PCR primer 1 to tube 1, PCR primer 2 to tube 2, etc.).
- Vortex the tubes for 3 seconds and briefly centrifuge.
- Dispense 3 µL PCR cocktail into each sample well of the sample plate, for a final PCR reaction volume of 5 µL.
- Seal the PCR reaction plate, pulse vortex 5 times, then centrifuge at 3200 x g for 5 seconds.
- Visually inspect the individual wells from the bottom of the PCR reaction plate to confirm uniform and adequate cocktail solution is present in every well before continuing.
- Thermocycle the PCR reaction plate using the following conditions:

95°C	2 minutes	1 cycle
95°C	30 seconds	45 cycles
60°C*	30 seconds	
72°C	1 minute	
72°C	5 minutes	1 cycle
10°C		Hold

*Note difference from standard iPLEX Pro thermocycling temperature.

STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

C. SAP Reaction

IMPORTANT

Prepare the SAP cocktail in **Lab Area 2**. Add the SAP cocktail to the reaction plate and thermocycle the plate in **Lab Area 3**. Make sure all reagents are thawed completely at room temperature and enzymes are kept on ice. Make sure all reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely at room temperature and spun down.

1. Prepare the SAP cocktail in a 1.5 mL tube on ice as shown in Table 5. Prepare more cocktail than the number of SAP reactions to be performed. Either prepare for one extra reaction or use a percentage extra to ensure sufficient coverage is present to overcome typical pipetting variation.

Table 5: SAP Cocktail

Reagent	Per Well (µL)	Final Concentration
HPLC-grade water	1.53	n/a
10X SAP Buffer	0.17	0.24X
SAP Enzyme	0.30	0.073 U/µL
Final Volume SAP Cocktail	2.00	

2. Vortex the tube containing SAP cocktail for 3 seconds and briefly centrifuge.
3. Centrifuge the reaction plate at 3200 x g for 5 seconds.
4. Dispense 2 µL of SAP cocktail into each sample well of the PCR reaction plate.
5. Seal the reaction plate, pulse vortex 5 times, then centrifuge at 3200 x g for 5 seconds.
6. Visually inspect the individual wells from the bottom of the reaction plate to confirm uniform and adequate solution is present in every well before continuing.
7. Thermocycle the reaction plates using the following conditions:

37°C	40 minutes
85°C	5 minutes
10°C	Hold

STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

D. iPLEX Pro Extension Reaction

IMPORTANT

Prepare the extension reaction cocktails in **Lab Area 2**. Add the extension reaction cocktails to the reaction plate and thermocycle the plate in **Lab Area 3**. Make sure all reagents are thawed completely at room temperature and enzymes are kept on ice. Make sure all reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely at room temperature and spun down.

1. Prepare the extension master mix in a 1.5 mL tube on ice, as shown in Table 6. Do NOT add extend primers, as they are added separately.
Prepare more master mix than the number of extension reactions to be performed. Either prepare for one extra reaction per extension cocktail or use a percentage extra to ensure sufficient overage is present to overcome typical pipetting variation.

Table 6. Extension Cocktails

Reagent	Per Well (µL)	Final concentration
HPLC-grade water	0.62	n/a
iPLEX Buffer Plus	0.20	0.222X
iPLEX Termination Mix	0.20	0.222X
iPLEX Pro Enzyme	0.04	0.142 U/ µL
Extension Master Mix Final Volume	1.06	
Chimeric ID Extend Primers (added separately)	0.94	
Extension Cocktails Final Volume	2.00	

2. Vortex the tube for 3 seconds and briefly centrifuge.
3. Label eight new 0.5 mL tubes 1-8.
4. Prepare each individual extension cocktail by adding the appropriate amount of extension master mix and specific extend primer to the appropriate tube (extend primer 1 to tube 1, extend primer 2 to tube 2, etc.).
5. Vortex the tubes for 3 seconds and briefly centrifuge.
6. Centrifuge the reaction plate at 3200 x g for 5 seconds.
7. Dispense 2 µL of extension reaction cocktail into the appropriate wells of the reaction plate.
8. Seal the reaction plate, pulse vortex 5 time, then centrifuge at 3200 x g for 5 seconds.
9. Visually inspect the individual wells from the bottom of the reaction plates to confirm uniform and adequate solution is present in every well before continuing.

10. Thermocycle the reaction plate using the following conditions.

95°C	30 seconds		
95°C	5 seconds		
52°C	5 seconds	5 cycles	40 cycles
80°C	5 seconds		
72°C	3 minutes		
10°C	Hold		

STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

E. Water Addition

1. Add HPLC-grade water to each well of the reaction plate using a 12-channel multipipettor.
 - a. For 96-well plates, add 41 µL.
 - b. For 384-well plates, add 16 µL.
2. Seal the plate and centrifuge at 3200 x g for 1 minute.

STOPPING POINT

If not proceeding directly to processing the plate on the MassARRAY System, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing more than 24 hours). Do not store for more than 2 weeks.

Analyte Desalting, Dispensing, and Data Acquisition

Follow the instructions in the appropriate instrument user guide to desalt and dispense analyte onto SpectroCHIP® Arrays and to acquire data with the MassARRAY® Analyzer. Use settings for **iPLEX Pro genotyping**.

Generating Reports

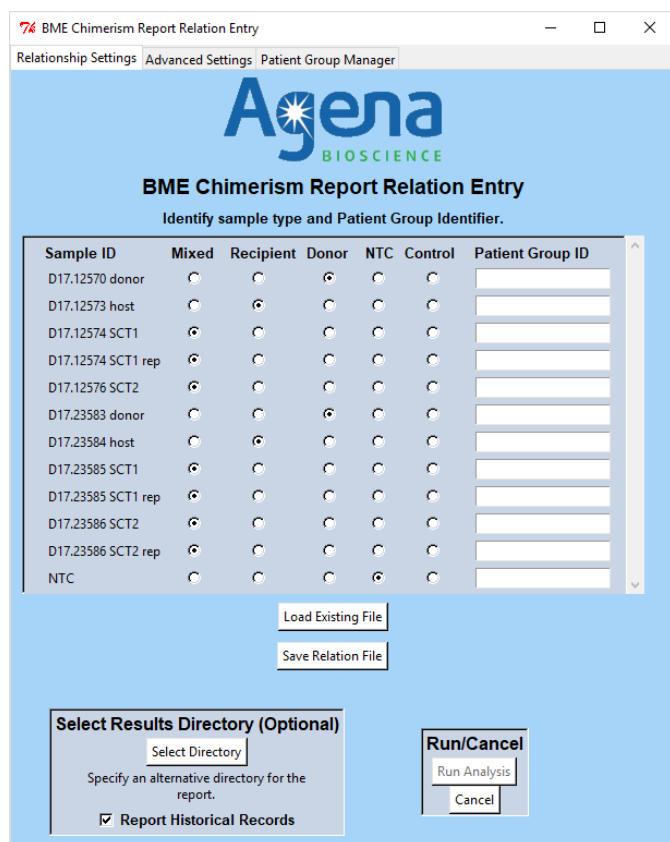
A. Overview

After samples are run on the MassARRAY System and analyzed by the BME Chimerism Report software, they are automatically added to the BME Chimerism database. Once pure samples from a recipient or a donor have been run once, they do not need to be run again, as the data is already in the database and can be used for future comparisons. You may also remove a particular donor from the BME Chimerism database in order to exclude them from future analysis. Recipients and donors are linked by a Patient Group ID, which is unique to the recipient (for example, the recipient’s hospital ID number). Each Patient Group ID may be associated with only 1 recipient, and up to 5 donors.

After you load the plates to be analyzed in Typer Analyzer and start the report run, the BME Chimerism Report Relation Entry window (Figure 1) will appear, populated with the Sample IDs of the samples on the loaded plates. For each sample, you will enter (or upload via a prepared file) the Patient Group ID and specify whether the sample is pure recipient, pure donor, mixed, an NTC, or control sample.

NOTE: To have the sample type automatically populate the Report Relation Entry window along with sample IDs when you load the plate in Typer Analyzer, include “donor”, “recipient”, “ntc”, or “control” in the sample name.

Figure 1. BME Chimerism Report Relation Entry Window

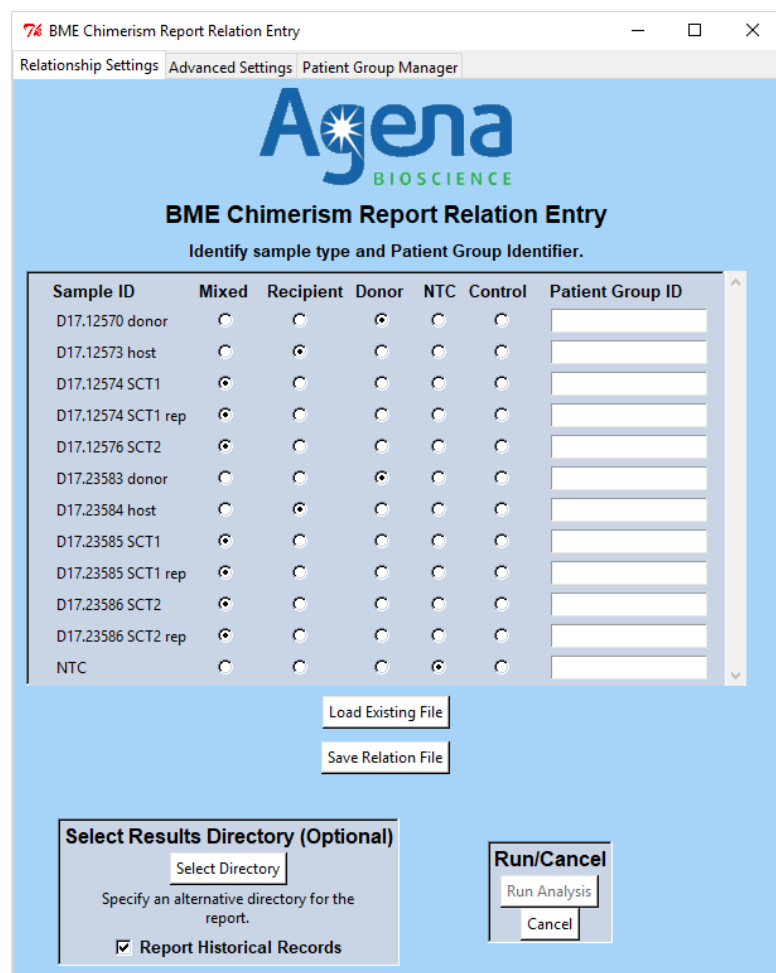


B. Generating the BME Chimerism Report

- 1) Open MassARRAY TyperAnalyzer and in the Project Explorer pane double click on the SpectroCHIP Arrays of interest. The SpectroCHIP Arrays will be added to the Chip List.
- 2) Load the SpectroCHIP Arrays by checking the box next to the SpectroCHIP Array names in the Chip List.
- 3) Once the SpectroCHIP Arrays are loaded, select **File > Reports > BME Chimerism Report-v1** in the MassARRAY TyperAnalyzer menu bar.

The BME Chimerism Report Relation Entry window will appear. This window has 3 tabs: Relationship Settings, Advanced Settings, and Patient Group Manager.

In the Relationship Settings Tab:



Sample ID	Mixed	Recipient	Donor	NTC	Control	Patient Group ID
D17.12570 donor	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.12573 host	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.12574 SCT1	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.12574 SCT1 rep	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.12576 SCT2	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.23583 donor	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.23584 host	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.23585 SCT1	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.23585 SCT1 rep	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.23586 SCT2	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
D17.23586 SCT2 rep	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
NTC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	

4) Enter the sample type and Patient Group ID. You may enter the information manually or load an existing file.

To enter information manually:

- a. Enter the Patient Group ID for each sample. For NTC or Control samples, leave this field blank.
- b. For each sample, select the sample type (Mixed, Recipient, Donor, NTC, or Control).
- c. Click **Save Relation File**. The information you entered will be saved in the *Known-Trio_file.csv* in the Details folder in the report results folder in the Typer/bin/Reports/BMEChimerism folder.

To load an existing file:

- a. Create a CSV file with SampleID in column A, Type in column B (Mixed, Recipient, Donor, NTC, or Control), and PatientID in column C (for NTC or Control samples leave this field blank).

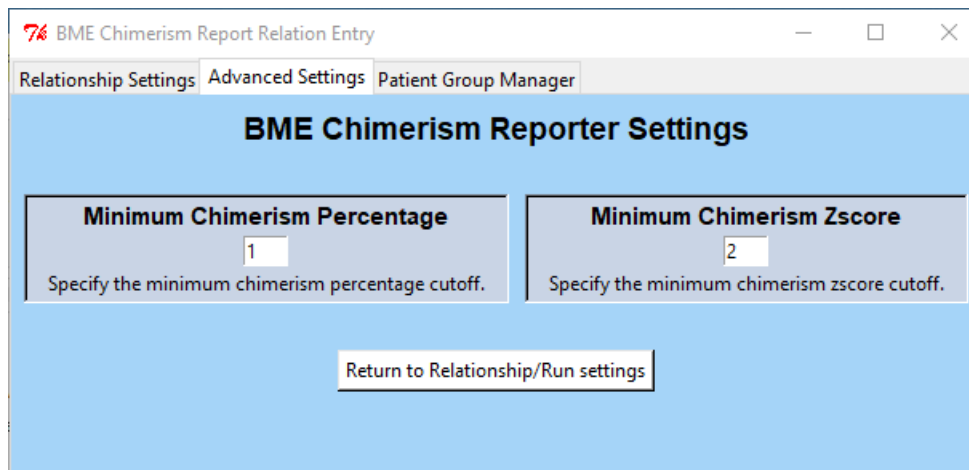
	A	B	C
1	SampleID	Type	PatientID
2	05_M3_F1_F2_rep1	mixed	334
3	05_M3_F1_F2_rep10	mixed	334
4	05_M3_F1_F2_rep2	mixed	334
5	05_M3_F1_F2_rep3	mixed	334
6	05_M3_F1_F2_rep4	mixed	334
7	05_M3_F1_F2_rep5	mixed	334
8	10_M3_F1_F2_rep4	mixed	334
9	10_M3_F1_F2_rep9	mixed	334
0	100_F1	donor	334
1	100_F1_rep2	ntc	
2	100_F2	donor	334
3	100_F2_rep2	ntc	
4	100_M3	recipient	334
5	100_M3_rep2	ntc	
6	15_M3_F1_F2_rep1	mixed	334
7	15_M3_F1_F2_rep10	mixed	334
8	15_M3_F1_F2_rep2	mixed	334
9	15_M3_F1_F2_rep3	mixed	334

- b. Click on the **Load Existing File** button, navigate to the file you would like to use, and click **Open**. Data from your file will populate the Relationship Settings tab.
- c. Click **Save Relation File**.

- 5) If you want to save the results report in a location different than the default location (Typer/bin/Reports/BMEChimerism), click on the **Select Directory** button and specify the new location.
- 6) Select the **Report Historical Records** box if you would like the report to also use previously run samples that contain the same Patient Group IDs as your current run.

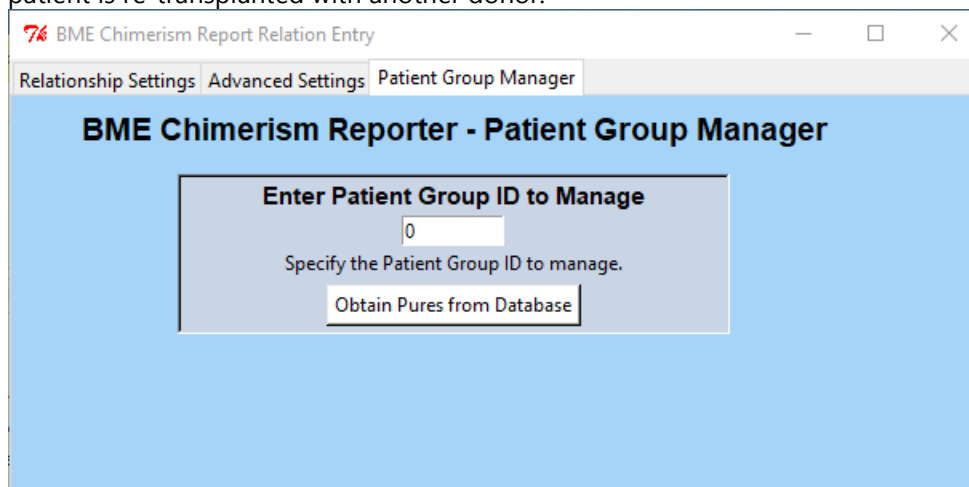
In the Advanced Settings Tab:

- 7) Optional step: This tab shows the default analysis settings: by default, samples with a chimerism percentage above 1% AND a z-score higher than 2 will be labeled as *Chimerism Detected* in the results report. You may change these settings if desired.

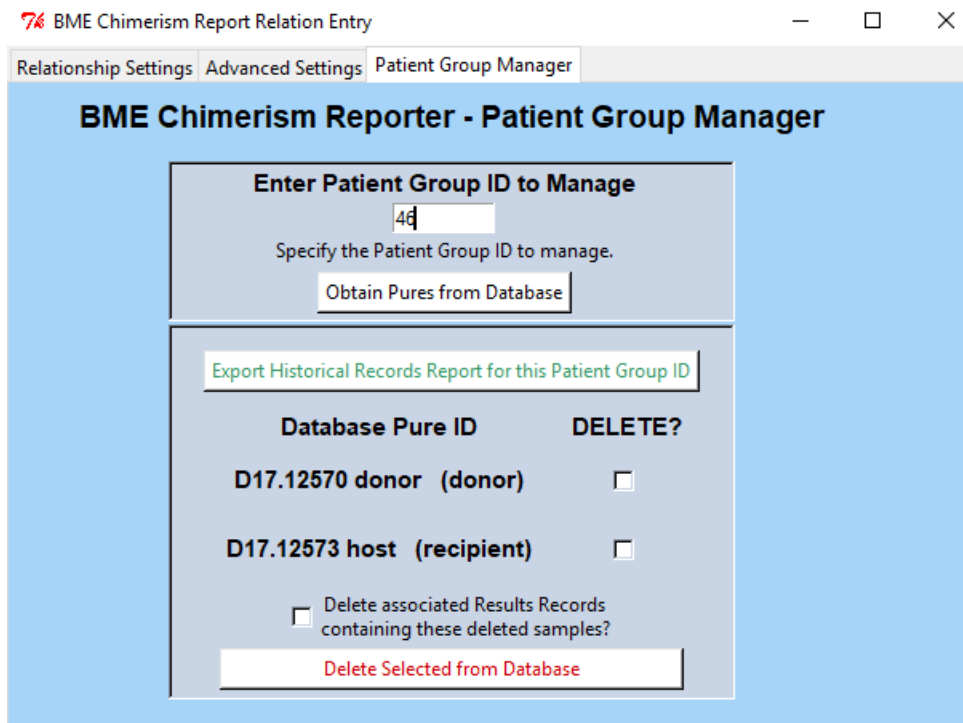


In the Patient Group Manager Tab:

- 8) Optional step: You may delete a pure sample from the analysis if, for example, there was an annotation error or if the patient is re-transplanted with another donor.



- a. Enter the Patient Group ID.
- b. Click on **Obtain Pures from Database**.
A list of all the pure donor and pure recipient samples associated with that Patient Group ID will be listed.

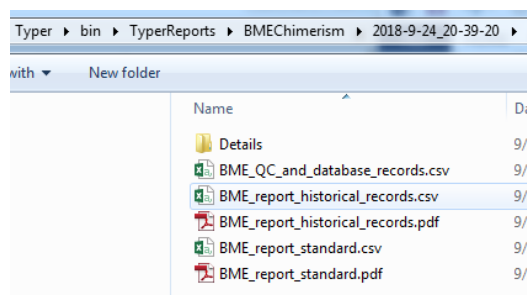


- c. Check the box next to the samples you want to delete from the BME Chimerism Database.. This will exclude them from being used in any future analysis.
 - d. If you would like to not just delete the checked samples from the database, but also remove all existing comparison records that include the checked samples, check the box at the bottom of the window (**Delete associated Results Records containing these deleted samples?**) Note that this only removes the data from the BME Chimerism database. The data is still in the Typer database and can be run again with the BME Chimerism Report software; this will then repopulate the BME Chimerism database with these samples.
 - e. Click **Delete Selected from Database**.
- 9) Optional step: Note that this tab can also be used at any time to export all the results in the BME Chimerism database for a given patient.
- a. Enter the Patient Group ID.
 - b. Click on **Obtain Pures from Database**.
 - c. Click on **Export Historical Records Report for this Patient Group ID**.
The PDF of the historical report will automatically open, and will be stored in the Typer/bin/TyperReports/BMEChimerism/PGI_Historical_Records folder in a folder labeled with the Patient Group ID.

In the Relationship Settings Tab:

10) Click **Run Analysis**.

When the report is complete, the BME Standard Report will automatically open in both PDF and CSV formats. All reports will be placed in a date-and time-stamped folder in the Typer/bin/TyperReports/BMEChimerism folder, unless a different location was specified (step 5 above).



C. Reading the BME Chimerism Report

Details Folder

This folder contains a number of files pertaining to generation of the report, including the baseline file and the analysis settings file.

BME Standard Report

This report is available in both PDF and CSV formats, and gives the results for each sample:

- DATE: Note that this is the date the sample was run on the MassARRAY Analyzer 4, not the date the report was generated.
- RESULT: *Chimerism Detected*, *No Chimerism Detected*, or *No Call*. See Table 7 for call criteria.
- Z SCORES:
 - Z Score: The average z-score of all the “informative assays” (assays where the donor and recipient differ).
 - Z Score HMzg: The average z-score of all the informative assays where the comparison is homozygous (e.g., donor is GG and recipient is AA). This field will be blank if it is a multi-donor comparison.
 - Z Score Htzg: The average z-score of all the informative assays where the comparison is heterozygous (e.g., donor is GA and recipient is AA). This field will be blank if it is a multi-donor comparison.
- DONOR and RECIPIENT %: The percent that each donor and the recipient contribute to the sample is reported.
- WARNINGS: When the chimerism % is above the discordance % threshold (default 5%) and below the z-score cutoff (default 2) the following message will appear: Insufficient Z score to call Chimerism.

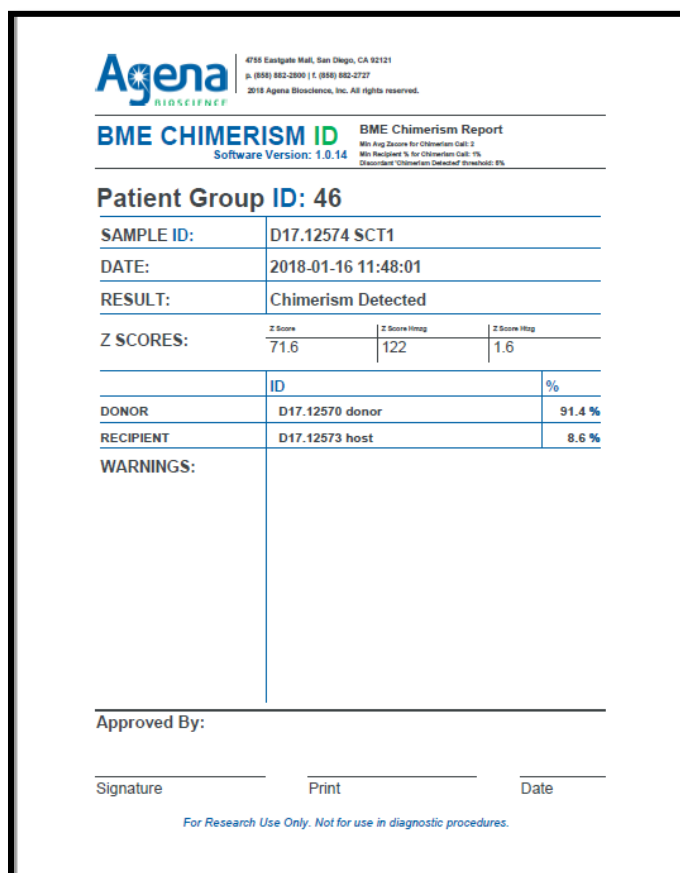
Table 7. Chimerism Call Criteria

Parameter				
Chimerism %	< CHI%	CHI% < X < DSC%	≥ DSC%	≥ CHI%
Z-score	-	< ZSM	< ZSM	≥ ZSM
Call	No Chimerism Detected	No Chimerism Detected	No Call	Chimerism Detected

Table 8. Parameter Description

Parameter	Default	Description	Variable Name in Settings File*
Chimerism % (CHI)	1%	Threshold for a sample to qualify for a potential call of Chimerism Detected.	callPercent
Z-score min (ZSM)	2	Z-score threshold for chimerism confirmation.	zscorecallMin
Discordance (DSC)	5%	Threshold for discordance check of the z-score.	upperChimerismDiscordance

*Default settings file at Typer/bin/Reports/BMEChimerism



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BME CHIMERISM ID BME Chimerism Report
 Software Version: 1.0.14
Min Avg Zscore for Chimerism Call: 2
 Min Recipient % for Chimerism Call: 5%
 Discordant Chimerism Detected Threshold: 5%

Patient Group ID: 46

SAMPLE ID: D17.12574 SCT1
 DATE: 2018-01-16 11:48:01
 RESULT: Chimerism Detected

Z SCORES:	Z Score	Z Score (log)	Z Score (log)
	71.6	122	1.6

	ID	%
DONOR	D17.12570 donor	91.4 %
RECIPIENT	D17.12573 host	8.6 %

WARNINGS:


Approved By: _____

Signature _____ Print _____ Date _____

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

BME Historical Records Report

This report is generated if the **Report Historical Records** box in the Relationship Settings tab of the BME Chimerism Report Relation Entry window is checked, and is available in both PDF and CSV formats. It shows, for each patient (e.g., each Patient Group ID), every comparison in the BME Chimerism database ever made for that Patient Group ID.



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BME CHIMERISM ID Historical records of Patient Group ID
Software Version: 1.0.14 Min Avg Z-score for Chimerism Call: 2
Min Recipient % for Chimerism Call: 1% Discard/Ret "Chimerism Detected" Threshold: 0%

Patient Group ID: 46

SAMPLE	DATE: 2018-01-16 11:48:01	DONOR ID	%	
ID: D17.12574 SCT1		D17.12570 donor	91.4 %	
Result:		RECIPIENT ID	%	
Chimerism Detected		D17.12573 host	8.6 %	
WARNINGS		Z Score	Z Score Wtavg	Z Score Wtq
		71.8	122	1.6

SAMPLE	DATE: 2018-01-16 11:48:02	DONOR ID	%	
ID: D17.12576 SCT2		D17.12570 donor	85.4 %	
Result:		RECIPIENT ID	%	
Chimerism Detected		D17.12573 host	13.6 %	
WARNINGS		Z Score	Z Score Wtavg	Z Score Wtq
		89	150.8	2.3

SAMPLE	DATE: 2018-01-16 11:48:04	DONOR ID	%	
ID: D17.12574 SCT1 rep		D17.12570 donor	91.2 %	
Result:		RECIPIENT ID	%	
Chimerism Detected		D17.12573 host	8.8 %	
WARNINGS		Z Score	Z Score Wtavg	Z Score Wtq
		59.2	91.6	1.3

Approved By:

Signature _____ Print _____ Date _____

Page 1 of 1 for Patient Group ID: 46
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BME QC and Database Records

This report, available in CSV format, contains information on QC and which samples were added to the BME Chimerism database:

- Sample/Control QC FAIL Records: A list of samples that failed QC and the reason.
- Pure Samples Not added to Database: A list of any pure samples that failed QC and were not added to the database.
- Status of Pure Samples Being Entered into Database: A list of every pure sample in the current run and whether it already existed in the database or is new and was added to the database.

- Mixed Samples Missing Required Pure Samples For Analysis: A list of any mixed samples that did not have an associated pure sample on the current plate or in the database. Such samples cannot be analyzed.
- MixedSample-Donor comparisons with Not Enough Informative Assays For Analysis: Informative assays are assays where the donor and recipient differ. Any comparisons with an insufficient number of these assays will be listed here.
- Status of Result Records Being Added to Database: Lists each comparison and whether it already exists in the database, or is new and was added to the database.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	BME Chimerism Reporter software version					1.0.14								
2	Database Entry Records and Pure Sample Information													
3														
4													
5	Sample/Control QC FAIL Records													
6														
7	Mixed Sample 'D17.23585 SCT1 rep' FAILED QC (not enough assays passing QC with 76.09%)													
8	#####													
9														
10														
11													
12	Pure Samples Not added to Database													
13														
14	#####													
15														
16														
17													
18	Status of Pure Samples Being Entered into Database													
19														
20	NEW - Pure sample 'D17.12570 donor' for Patient Group '46' added to database as 'donor'													
21	NEW - Pure sample 'D17.12573 host' for Patient Group '46' added to database as 'recipient'													
22	NEW - Pure sample 'D17.23583 donor' for Patient Group '47' added to database as 'donor'													
23	NEW - Pure sample 'D17.23584 host' for Patient Group '47' added to database as 'recipient'													
24	#####													
25														
26														
27													
28	Mixed Samples Missing Required Pure Samples For Analysis													
29														
30														
31														
32	#####													
33														
34														
35													
36	MixedSample-Donor comparisons with Not Enough Informative Assays For Analysis													
37														
38														
39														
40	#####													
41														
42														
43													
44	Status of Result Records Being Added to Database													
45														
46	NEW - Record for mixed sample D17.12574 SCT1 and recipient sample D17.12573 host + donors D17.12570 donor added to database													
47	NEW - Record for mixed sample D17.12574 SCT1 rep and recipient sample D17.12573 host + donors D17.12570 donor added to database													
48	NEW - Record for mixed sample D17.12576 SCT2 and recipient sample D17.12573 host + donors D17.12570 donor added to database													
49	NEW - Record for mixed sample D17.23585 SCT1 and recipient sample D17.23584 host + donors D17.23583 donor added to database													
50	NEW - Record for mixed sample D17.23586 SCT2 and recipient sample D17.23584 host + donors D17.23583 donor added to database													
51	NEW - Record for mixed sample D17.23586 SCT2 rep and recipient sample D17.23584 host + donors D17.23583 donor added to database													

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The MassARRAY System, the BME Chimerism software, and the iPLEX Pro Chimeric ID Panel are For Research Use Only. Not for use in diagnostic procedures.

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