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1. Description

Components **1×24-well ChimerXplain PCR Plate:** lyophilized Reaction Mix 1 (ChimerXact Primer 1–4 to amplify D10S2325, D12S391, P450CYP19, and D2S1360, respectively), Reaction Mix 2 (ChimerXact Primer 5–9 to amplify D9S1118, MYCL1, D7S1517, D11S554, and D8S1132, respectively), and Reaction Mix 3 (ChimerXact Primer 10 to amplify SE33).

1×0.5 mL PCR Resuspension Buffer (10×)

1× Positive Control DNA:

100 ng lyophilized genomic DNA of WT47 cells [European Collection of Cell Cultures (ECACC): ECACC No. 88052063].

Capacity For 8 tests.

Product format 24-well plate containing lyophilized Reaction Mix 1, Reaction Mix 2, and Reaction Mix 3 in 8×3 wells (1 test per row).

Storage The ChimerXplain PCR Plate is best stored at –20 °C, and may be stored at 2–8 °C for up to 4 weeks protected from light in the original aluminium pouch.

Store the Resuspension Buffer at 2–8 °C.

Store the dissolved Positive Control DNA at –80 °C.

The expiration date is indicated on the label.

1.1 Principle of the ChimerXplain Kit

The ChimerXplain Kit allows the identification of appropriate markers to distinguish donor and recipient cells. Three different multiplex PCR reactions (ChimerXplain Kit, Reaction Mix 1–3) are performed to determine the length of ten highly polymorphic STR loci using both donor and recipient DNA. One PCR primer of each

locus is labeled with different fluorescent dyes (FL, Joe, or TMR). After amplification of the STR loci, PCR products are separated by capillary electrophoresis. By comparing the STR sizes for every allele between donor and recipient, one or more informative STRs that differ in length can be selected (refer to figure 1).

For a precise and reliable chimerism quantification, it is recommended to amplify the selected STRs in singleplex PCR reactions using the ChimerXact Kits after the initial identification of the discriminatory loci with the ChimerXplain Kit. For each STR that is assayed using the ChimerXplain Kit, singleplex PCR reactions (ChimerXact Kits 1–10) are available for further chimerism analysis.

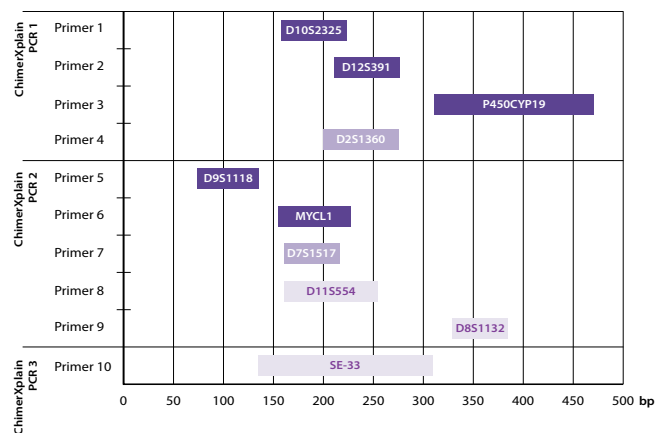


Figure 1: Overview on size range of products. Dark purple: labeled with FL; purple: labeled with Joe; light purple with purple font: labeled with TMR.

2. Short protocol

The amounts and conditions given are based on extensive optimization and validation using an Applied Biosystem 310 Genetic analyzer. Parameters and DNA amounts might need to be adapted for other capillary sequencer models.

2.1 Genomic DNA isolation

1. Isolate genomic DNA by any protocol that yields high-purity DNA.
2. Dilute sample DNA and positive control DNA (WT47 cells) in 10 mM Tris/HCl, pH 8 to a final concentration of 0.8 µg/µL.

2.2 Set up PCR experiment

1. For resuspension solution 1 (RS1) combine 4.8 ng genomic DNA and 5.5 µL 10× PCR Resuspension Buffer and adjust with water to a final volume of 55 µL.
2. For resuspension solution 2 (RS2) combine 0.8 ng genomic DNA and 2.5 µL 10× PCR Resuspension Buffer and adjust with water to a final volume of 25 µL.

Column 1 (ChimerXact primer 1–4)	Column 2 (ChimerXact primer 5–9)	Column 3 (ChimerXact primer 10)
Reaction Mix 1	Reaction Mix 2	Reaction Mix 3

Scheme of the ChimerXplain PCR Plate.

▲ **Note:** For correct orientation position plate with label away from you.

- Dissolve lyophilized Mix 1 and 3 each with 25 µL RS1 containing 2.2 ng genomic DNA.
- Dissolve lyophilized Mix 2 using 25 µL RS2 containing 0.8 ng genomic DNA.
- Run PCR using the following program:

Cycling conditions

LID=105 °C

T=95 °C	11 minutes		
T=96 °C	1 minute		
T=94 °C	30 seconds		} 9 cycles
T=60 °C	30 seconds	R (RAMP) = 1 °C/second	
T=70 °C	45 seconds	R (RAMP) = 1 °C/second	} 21 cycles
T=90 °C	30 seconds		
T=60 °C	30 seconds	R (RAMP) = 1 °C/second	} 21 cycles
T=70 °C	45 seconds	R (RAMP) = 1 °C/second	
T=60 °C	30 minutes		
Hold 4 °C			

The thermal cycler profile is optimized for Eppendorf and MJ Research cyclers.

2.3 Capillary electrophoresis

- Sample preparation:
Electrophoreses mastermix: Mix 1.5 µL size standard and 23.5 µL Hi-Di™ formamide per sample. Add 1 µL of ChimerXplain PCR product to one 25 µL mastermix aliquot. Denature samples at 95 °C for 3 minutes and snap cool.
- Prepare a sample sheet:
Prepare a GeneScan™ sample sheet for 4 dyes. Type sample name, mark size standard column, and enter sample information for all samples to be analyzed.
- Prepare a GeneScan injection list:
Set up a new injection list and select the appropriate sample sheet. For all samples select
“GS STR POP4 (1 mL) A”
“inj. Secs” to 5
“inj. kV” to 15.0
“Run kV” to 15.0
“run °C” to 60
“Run Time” to 30
matrix file
Optionally activate the autoanalysis checkbox.
▲ **Note:** The injection parameters might have to be adapted depending on the signal intensities. Lowering the injection time or voltage will decrease the peak signals.
Load the denatured samples onto the sample tray, close the doors, and start electrophoresis.

2.4 Data analysis

- Set up analysis parameters:
Define the analysis parameters for peak detection. When employing the GeneMapper® software use the microsatellite sizing-only application.
- Set up a size standard:
Assign sizes to the respective peaks of a representative run containing the size marker.
- Set up a project for sample analysis:
Define samples to be analyzed. Choose appropriate analysis parameters, a size standard, and a matrix for the analysis. Perform data analysis.

2.5 Select STR peaks

Display the electropherograms and the corresponding tables of all samples. Select the peak(s) for each STR locus according to the dye and the size ranges (refer to figure 1 or table in 3. Appendix).

A template table for the comparison of donor and recipient allele lengths can be found in the appendix.

2.6 Select informative STR markers

- Exclude all STRs with identical lengths.
- Prefer an allelic pattern with the two, e.g., donor peaks surrounding the two recipient peaks (DRRD or RDDR) or alternating configurations like DRDR or RDRD.
- Alternatively, choose two homozygous loci.
- In addition, heterozygous STRs differing in only one allele can be used.
- Finally, a STR heterozygous in one case and homozygous in the other case can be used.

The complete and detailed user manual for the ChimerXplain Kit and the ChimerXact Kits can be downloaded here: www.miltenyibiotec.com/chimerism-analysis or ordered free of charge using the order number 140-002-982.

3. Appendix: Template for comparison of donor and recipient allele length

ChimerXplain PCR 1:

No.	Locus ID	Chromosome	Dye	Code	Length range	Recipient allele 1	Recipient allele 2	Donor allele 1	Donor allele 2
1	D10S2325	10	FL	B	163–213				
2	D12S391	12	FL	B	213–269				
3	P450CYP19	15	FL	B	314–464				
4	D2S1360	2	Joe	G	200–273				

ChimerXplain PCR 2:

No.	Locus ID	Chromosome	Dye	Code	Length range	Recipient allele 1	Recipient allele 2	Donor allele 1	Donor allele 2
5	D9S1118	9	FL	B	80–128				
6	MYCL1	1	FL	B	156–225				
7	D7S1517	7	Joe	G	164–212				
8	D11S554	11	TMR	Y	166–253				
9	D8S1132	8	TMR	Y	330–379				

ChimerXplain PCR 3:

No.	Locus ID	Chromosome	Dye	Code	Length range	Recipient allele 1	Recipient allele 2	Donor allele 1	Donor allele 2
10	SE-33	6	TMR	Y	138–305				

All protocols and data sheets are available at www.miltenyibiotec.com.

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