EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.012_GenePrint® 10 Cell Line Authentication

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented: 1 October 2015

Updated:	Ashima Amin	1 October 2015
	Name	**Date**

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

The GenePrint® 10 System is used for the identification and authentication of human cell lines and for research use. The system allows co-amplification and three-color fluorescent detection of ten loci (nine STR loci and Amelogenin), including THO1, D21S11, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, and TPOX. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92×10^9.

The GenePrint® 10 System is compatible with the ABI PRISM® 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xl Genetic Analyzers. The GenePrint® 10 System contains all materials necessary to amplify STR regions of human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the GenePrint® 10 5X Master Mix. An internal lane standard (ILS) and allelic ladder are provided for sizing and genotyping of amplified fragments, and the 2800M Control DNA is supplied as a positive control.

The ILS is added to every sample after amplification and used within each capillary electrophoresis run to determine the size of each amplified product. The allelic ladder consists of the most common alleles at a particular locus and is used as a standard to positively identify each allele. The 2800M Control DNA has a known genotype and can be used to verify genotyping accuracy.

Specimen Types: DNA

Pre-amplification Components Box (VWR 76201-514) or (Promega B9510)

- 250 µl GenePrint® 10 5X Master Mix
- 250 µl GenePrint® 10 5X Primer Pair Mix
- 1.25 ml Water, Amplification Grade
- 25 µl 2800M Control DNA, 10ng/µl

Post-amplification Components Box

- 50 µl GenePrint® 10 Allelic Ladder Mix
- 150 µl Internal Lane Standard 600

Materials to be supplied by the User:

- GeneAmp PCR 9700 thermo cycler with a silver or gold-plated silver sample block (Applied Biosystems)
- Microcentrifuge
- MicroAmp optical 96-well reaction plates (Applied Biosystems)

Storage Conditions:

For long-term storage, store all components except the 2800M Control DNA at –20°C.

The GenePrint® 10 5X Primer Pair Mix, GenePrint® 10 Allelic LadderMix and Internal Lane Standard 600 (ILS 600) are light-sensitive and must be stored in the dark.

Pre-amplification and post-amplification reagents should be stored and used separately with different pipettes, tube racks, etc.

Amplification Steps:

1. Thaw the GenePrint® 10 5X Master Mix and GenePrint® 10 5X Primer Pair. Mix completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 5X Primer Pair Mix or the 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom on the tube.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Increase the number of reactions by 10-15% to compensate for pipetting error. Use an amplification prep sheet for all GenePrint® 10 samples. Template can be found in EIGC012.Geneprint Amplification.

3. Add the final volume of each reagent listed in Table 1 to a sterile tube. Add amplification grade water to the tube first, then add GenePrint® 10 5X Master Mix and GenePrint® 10 5X Primer Pair Mix. The temple DNA will be added at step 5.

Table 1. PCR Amplification Mix for Amplification of Extracted DNA.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 25.0µl	×		=	
GenePrint® 10 5X Master Mix	5.0µl	×		=	
GenePrint® 10 5X Primer Pair Mix	5.0µl	×		=	
template DNA (10ng)[1,2]	up to 15µl				
total reaction volume	25µl				

4. Vortex the PCR amplification mix for 5–10 seconds, then pipet PCR amplification mix into each reaction well of the MicroAmp plate.

   Note: Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

5. Add template DNA (10ng) for each sample to the respective well containing PCR amplification mix.

6. For the positive amplification control, vortex the tube of 2800M Control DNA, then add 1.0µl of 2800M Control DNA to a reaction well containing PCR amplification mix.

7. For the negative amplification control, pipet amplification grade water into a reaction well containing PCR amplification mix.

8. Seal the plate. Vortex gently. Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

9. Place the MicroAmp plate in the 9700-thermal cycler.

10. Select and run the recommended protocol. Be sure that Max mode is selected as the ramp speed. The preferred protocol for use with the GeneAmpR PCR System 9700 thermal cycler is provided below with the estimated total cycling time is 1.5 hours.

    Note: Program can be found saved at

    Thermocycler ABI 9700-6
    User : mmw
    Method: geneprint10

Thermal Cycling Protocol[1]
96°C for 1 minute, then:
94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds for 30 cycles, then:
60°C for 10 minutes
4°C soak

11. After completion of the thermal cycling protocol, proceed with fragment analysis or store amplified samples at –20°C in a light-protected box.

    Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

Instrument Setup and Sample Preparation

Materials to be supplied by the User:

- 95°C dry heating block, water bath or thermal cycler
- Plate block in -20, water bath, or ice.
- centrifuge compatible with 96-well plates
- MicroAmpR optical 96-well plate and septa, or equivalent
- Hi-Di™ formamide (Fisher 4311320)
- 3130xl Genetic Analyzer with POP-7® Polymer (Fisher 4363785)
- Buffer (10x) with EDTA (Fisher 402824)

1. Thaw the Internal Lane Standard 600.
   Note: Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining and mixing the Internal Lane Standard 600 and Hi-Di™ formamide as follows:
   [(0.5µl ILS 600) × (# samples)] + [(9.5µl Hi-Di™ formamide) × (# samples)]

Size Standard	0.5 µl per sample
Hi-Di Formamide	9.5 µl per sample
	10.0 µl per sample master mix

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks.

   -If the peak heights are too low, we recommend altering the formamide/internal lane standard mix to contain 1.0µl of ILS 600 and 9.0µl of Hi-Di™ formamide.

   -If the peak heights are too high, we recommend altering the loading cocktail to contain 0.25µl of ILS 600 and 9.75µl of Hi-Di™ formamide.

3. Vortex for 10–15 seconds to mix.

4. Add 10µl of formamide/internal lane standard mix into each well.

5. Add 1µl of amplified sample (or 1µl of GenePrint®10 Allelic Ladder Mix). Cover wells with septa.

6. Centrifuge plate briefly to remove air bubbles from the wells.

7. Denature samples at 95°C for 3 minutes, then immediately chill on cold plate block, crushed ice or in an ice-water bath for at least 3 minutes.
   Note: Denature samples just prior to loading the instrument.

8. Create Sample Sheet and upload. Template can be found in EIGC.012_GenePrint Sample Sheet. Be to enter Project and order in the 2 highlighted fields. Enter samples in the correct wells and delete wells not being used. No special characters besides dashes, underscores, or periods allowed. Keep sample names below 10 characters. Save at txt file.

9. Change buffer (1x) and water in reservoir chambers. Change the buffer is the waste reservoir. This should be done each day the machine is being run.

10. Make sure there is enough polymer for your samples. Each run of 16 uses about 25 µl of polymer.

11. Assemble plate holder and put on machine. Make sure the notch in the assembly matches the notch on the instrument. Take care not to push on the machine too hard when placing assembly on machine.

12. Link sample sheet to plate.

13. Select the green arrow in software to start the run.

14. Log in reagent lot numbers for all in Allele file for client.