

35.1 Purpose

To amplify DNA samples using the GlobalFiler (GF) PCR Amplification Kit (Applied Biosystems).

35.2 Responsibility

DNA Unit Personnel.

35.3 GlobalFiler Background

35.3.1 The GlobalFiler PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay for the amplification of human genomic DNA. The kit amplifies and fluorescently labels in a single PCR amplification reaction 21 autosomal STR loci: D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA, SE33, TH01, TPOX, vWA; 1 Y-STR locus: DYS391; 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel); and Amelogenin.

35.3.2 These procedures generally follow those outlined in the Applied Biosystems GlobalFiler PCR Amplification Kit User Guide. Please refer to the user guide for additional details and troubleshooting.

35.4 GlobalFiler Amplification

Note: Amplification setup must occur in an amplification hood.

35.4.1 The total reaction volume is 25µl; 10µl of Reaction Mix plus optimally ~0.3ng of purified human DNA (volume of DNA + dH₂O = 15µl) as determined by qPCR for evidentiary samples. It may also be used for casework knowns that are extracted instead of GlobalFiler Express. The kit supplied positive control (DNA Control 007) concentration is stated to be 0.1 ng/µl by the manufacturer, 3 µl neat would suffice for the target DNA template. If there is any reproducible discrepancy with the positive control, the tubes may be quanted and more or less of the control can be re-amplified if necessary.

Note: Mixture and degraded samples may require more DNA to be amplified, routinely ~0.5 ng. Higher amounts may be amplified based on these reasons. The target DNA template amount is a generalization; analysts should use their training and experience along with validation data to amp the appropriate amount of genomic DNA.

- 35.4.2 To obtain the targeted amount of DNA; dilutions with dH₂O may have to be made. This step is documented on DNA QR-22c and maintained in the batch file.
- 35.4.3 The Reaction mix is prepared per manufacturer's instructions (Applied Biosystems GlobalFiler PCR Amplification Kit User Guide): Make enough reaction mix for the number of reactions needed plus additional reactions to compensate for pipetting volume variations. (QR-323)
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|---------------------------------------|----------------|
| Master Mix | 7.5µl/reaction |
| Primer Set | 2.5µl/reaction |
| Aliquot 10µl of Reaction Mix per tube | |
| DNA + dH ₂ O | 15µl/reaction |
| Total volume | 25µl/reaction |
- 35.4.4 An aliquot of the reaction mix (10µl) is dispensed into 0.2ml amplification tubes, strip tubes, or into 0.2ml 96 well plates along with the appropriate volume of DNA + dH₂O. Multiple quantities of DNA from one sample may be amplified when Taq inhibitors are present in the sample or when the sample DNA is significantly degraded.
- 35.4.4.1 See SOP 1 regarding the standard controls that are processed with evidentiary samples and see DNA SOP-20 regarding sample concentration.
- 35.4.5 The samples are amplified in a Veriti 96-Well Thermal Cycler using the 100% ramping rate, a VeritiPro using the 9700-simulation mode, or a BioRad C-1000 Thermal Cycler using the ramp speed of 2.1°C/sec. The amplification parameters are 95°C for 1 min, followed by 29 cycles of the following (94°C for 10 sec, 59°C for 90 sec), then 60°C for 10 min, and finally, 4°C.
- 35.4.6 For detection of the amplification products, see SOP-38 GlobalFiler™, GlobalFiler™ Express, and Yfiler™ Plus Detection.
- 35.4.7 See DNA SOP-36 for full details on amplifying DNA from single source samples (database and known samples) using the GlobalFiler Express PCR Amplification Kit (Applied Biosystems). See DNA SOP-19 for full details on amplifying DNA from single source samples (database and known samples) extracted from the EZ2 or the EZ2 Normalization method using the GlobalFiler PCR Amplification Kit with 29 cycles (see above for paramaters).
- Also referenced is: GlobalFiler PCR Amplification Kit User Guide