AMPLIFICATION USING THE MINIFILER SYSTEM		
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Amplification using the Minifiler System

I. General Information for AmpFℓSTR® MiniFiler™ PCR Amplification

The MiniFiler™ PCR Amplification Kit from Applied Biosystems is a miniature STR (miniSTR) test that utilizes reduced size primers to target Amelogenin and eight of the larger STR loci amplified with Identifiler® (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA). The MiniFiler™ amplification results in amplicons that are significantly shorter in length than those produced with Identifiler® (see **Figure 1**). MiniFiler™ can be used in conjunction with Identifiler® to recover the larger loci that typically drop-out due to sample degradation. It can also be used for samples that may be inhibited and show no amplification with Identifiler®.

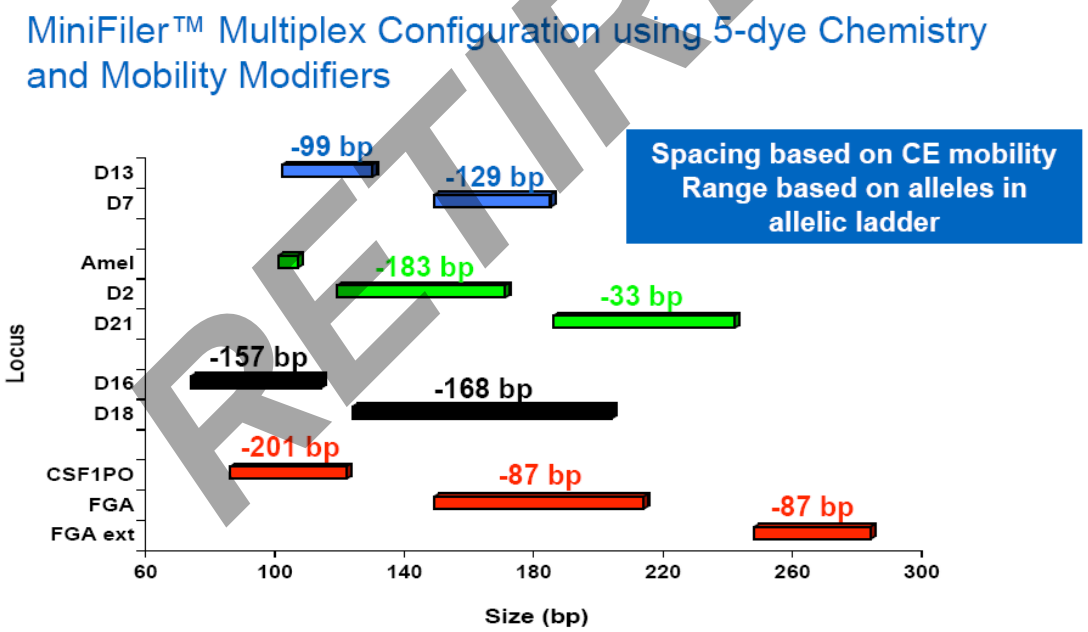

Figure 1. Amplicon size reduction of MiniFiler™ compared to the same STR loci in Identifiler®. Image from Applied Biosystems's "MiniFiler™ Kit Multiplex Configuration," 2006. http://marketing.appliedbiosystems.com/images/Product_Microsites/Minifiler1106/pdf/MplexConfig.pdf

The target DNA concentration for amplification using the MiniFiler™ system is 500 pg. The minimum DNA concentration required for amplification in this system is 100 pg (minimum quantitiation value of 10 pg/µL). If a sample is found to contain less than 10 pg/µL of DNA, then the sample should not be amplified in MiniFiler™. It can be re-extracted, reported as containing insufficient DNA, concentrated using a Microcon DNA Fast Flow or possibly submitted for High Sensitivity testing (see **Table 1**).

TABLE 1: For MiniFiler™

Minimum Desired Template	100 pg
Template Volume for Amp	10 µL
Minimum Sample Concentration in 200 µL	10.0 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning* to 50 µL	2.5 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning** to 20 µL	1.0 pg/µL

* Sample concentration **prior** to processing with a Microcon DNA Fast Flow and elution to 50 µL

** Sample concentration **prior** to processing with a Microcon DNA Fast Flow and elution to 20 µL

Since MiniFiler™ has a template amplification volume of 10 µL, the extraction negative **must have a quantitation value of < 0.2 pg/µL**. Thus, if the extraction negative is > 0.2 pg/µL, it should be re-quantitated. If it fails again, the sample set must be re-extracted prior to amplification (see **Table 2**).

TABLE 2:

Amplification System	Sensitivity of Amplification	Extraction Negative Control Threshold
MiniFiler™	10 pg	0.20 pg/µL in 10 µL

II. Generation of Amplification Sets

Amp sets are generated by supervisors following review of quantification results. Furthermore, samples may be submitted for amplification through sample request documentation.

III. PCR Amplification – Sample Preparation

1. Samples amplified with MiniFiler™ reagents should be prepared with irradiated TE^{-4}.

Prepare dilutions for each sample, if necessary, according to **Table 3**.

TABLE 3: Dilutions

Dilution	Amount of DNA Template (□L)	Amount of UltraPure TE^{-4} (□L)
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18
0.05	2.5	47.5
0.04	4 or (2)	96 or (48)
0.02	2 or (1)	98 or (49)
0.01	2	198
0.008	4 or (2)	496 or (248)

The target DNA template amount for MiniFiler™ is 500 pg.

To calculate the amount of template DNA and diluent to add, the following formulas are used:

$$\text{Amt of DNA } (\mu L) = \frac{\text{Target Amount (pg)}}{(\text{Sample concentration, pg}/\mu L)(\text{Dilution factor})}$$

The amount of diluent to add to the reaction = 10 µL – amt of DNA (µL)

For samples with quantitation values ≤50 pg/µL but ≥10 pg/µL, aliquot 10 µL extract.

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B. Positive Control

For MiniFiler™, DO NOT make a dilution of the 100 pg/µL AmpF*l*STR Control DNA 007. Instead, combine 5 µL of the Control DNA with 5 µL of irradiated TE^{-4}. This yields a total volume of 10 µL with 500 pg in the amplification.

C. Amplification Negative Control

10 µL of irradiated TE^{-4} will serve as an Amplification Negative Control.

D. Master Mix Preparation

1. Retrieve the MiniFiler™ Primer Set and MiniFiler™ Master Mix from the refrigerator and store in a Nalgene cooler on the bench. **Record the lot numbers of the reagents.**

2. Vortex or pipette the reagents up and down several times to thoroughly mix the reagents. After vortexing, centrifuge reagents at full speed briefly to ensure that no sample is trapped in the cap.

3. Consult the amplification documentation for the exact amount of MiniFiler™ Primer Set and Master Mix to add. The amount of reagents for one amplification reaction is listed in **Table 4**.

TABLE 4: MiniFiler™ PCR amplification reagents for one sample

Reagent	Per reaction
MiniFiler™ Primer Set	5.0 µL
MiniFiler™ Master Mix	10.0 µL
Reaction Mix Total:	15.0 µL
DNA	10.0 µL

E. Reagent and Sample Aliquot

1. Vortex master mix to thoroughly mix. After vortexing, briefly tap or centrifuge the master mix tube to ensure that no reagent is trapped in the cap.

2. Add 15 µL of the MiniFiler™ reaction mix to each of the stratalinked PCR tubes that will be utilized, changing pipette tips and remixing reaction mix as needed.

   NOTE: Use a new sterile filter pipette tip for each sample addition. Open only one tube at a time for sample addition.

3. Arrange samples in a rack in precisely the positions they appear on the sheet.

4. **Witness step.** Ensure that your samples are properly positioned.

5. Prior to adding sample or control, pipette each sample or control up and down several times to thoroughly mix. The final aqueous volume in the PCR reaction mix tubes will be 25 µL. After addition of the DNA, cap each sample before proceeding to the next tube.

6. After all samples have been added, take the rack to the amplified DNA area for Thermal Cycling.

IV. Thermal Cycling

1. Turn on the ABI 9700 Thermal Cycler. (See manufacturer's instructions).

2. Choose the following files in order to amplify in MiniFiler™:

MiniFiler
User: casewk File: mini

PCR Conditions for the Perkin Elmer GeneAmp PCR System 9700

9700 **MiniFiler** User: casewk File: mini	The mini file is as follows: Soak at 95°C for 11 minutes 30 Cycles: : Denature at 94°C for 20 seconds : Anneal at 59°C for 2 minutes : Extend at 72°C for 1 minute 45 minute incubation at 60°C. Storage soak indefinitely at 4°C

3. 9700 Instructions

a. Place the tubes in the tray in the heat block **(do not add mineral oil)**, slide the heated lid over the tubes, and fasten the lid by pulling the handle forward. Make sure you use a tray that has a 9700 label.

b. Start the run by performing the following steps:

c. The main menu options are RUN CREATE EDIT UTIL USER. To select an option, press the F key (F1…F5) directly under that menu option.

d. Verify that user is set to "casewk." If it is not, select the USER option (F5) to display the "Select User Name" screen.

e. Use the circular arrow pad to highlight "casewk." Select the ACCEPT option (F1).

f. Select the RUN option (F1).

g. Use the circular arrow pad to highlight the desired STR system. Select the START option (F1). The "Select Method Options" screen will appear.

h. Verify that the reaction volume is set to **25**µL for MiniFiler™ and the ramp speed is set to **9600 (very important)**.

i. If all is correct, select the START option (F1).

j. The run will start when the heated cover reaches 103°C. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed, hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

k. Upon completion of the amplification, remove samples and press the STOP button repeatedly until the "End of Run" screen is displayed. Select the EXIT option (F5). Wipe any condensation from the heat block with a Lint free wipe and pull the lid closed to prevent dust from collecting on the heat block. Turn the instrument off.

NOTE: **Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach in the Post-Amp area.**