Amplification using the Yfiler™ System

1 General Information for Amplification

1.1 The Yfiler™ Amplification System from Life Technologies targets sixteen (16) locations on the Y chromosome. The system includes loci with tri-, tetra-, penta- and hexa-nucleotide repeats and utilizes five dyes (6-FAM™, VIC®, NED™ and PET® for samples and LIZ® for the GeneScan™ 500 size standard).

LOCUS	REPEAT	Dye Label
DYS456	tetra-nucleotide	6-FAM™ (blue)
DYS389I	tetra-nucleotide	
DYS390	tetra-nucleotide	
DYS389II	tetra-nucleotide	
DYS458	tetra-nucleotide	VIC® (green)
DYS19	tetra-nucleotide	
DYS385a/b	tetra-nucleotide	
DYS393	tetra-nucleotide	NED™ (yellow)
DYS391	tetra-nucleotide	
DYS439	tetra-nucleotide	
DYS635	tetra-nucleotide	
DYS392	tri-nucleotide	
Y GATA H4	tetra-nucleotide	PET® (red)
DYS437	tetra-nucleotide	
DYS438	penta-nucleotide	
DYS448	hexa-nucleotide	

1.2 The target DNA concentration for amplification using the Yfiler™ system is 500 pg male DNA. The minimum DNA concentration required for amplification in this system is 100 pg male DNA (minimum quantitation value of 10 pg/ul male DNA). If a sample is found to contain less than 10.0 pg/µL male DNA, then the sample should not be amplified in Yfiler™. It can be re-extracted, reported as containing insufficient male DNA, or concentrated using a Micro-concentrator. (see Table 1)

TABLE 1: For Yfiler™

Minimum Desired Template of male DNA	100.00 pg
Template volume for amp	10 µL
Minimum Sample Concentration of male DNA in 200 µL	10.0 pg/µL
Minimum Sample Concentration of male DNA in 200 µL prior to Microconning* to 50 µL	2.5 pg/µL
Minimum Sample Concentration of male DNA 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

2 Generation of Amplification Test Batches

2.1 Refer to the manuals within the Test Batches folder of the LIMS Process Manual for specific procedures relating to LIMS test batch creation and other procedures. Any case file documentation developed outside of the LIMS system should be scanned to a PDF document and attached to the appropriate electronic case record.

2.2 Amplification test batches are generated following review of quantification results. Furthermore, samples may be submitted for amplification via the "add test" function in LIMS.

3 PCR Amplification – Sample Preparation

3.1 Samples amplified with Yfiler™ reagents should be prepared with UltraPure water.

3.2 Prepare dilutions for each sample, if necessary, according to Table 2.

TABLE 2: Dilutions

Dilution	Amount of DNA Template (uL)	Amount of ULTRAPURE Water (uL)
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)

3.3 The target DNA template amount for Yfiler™ is 500 pg male DNA.

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

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

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

3.3.2 Create the male positive control by making a 0.5 dilution of Control DNA 007:

- Label tube MPC
- Aliquot 5µL of UltraPure water into tube MPC
- Aliquot 5µL of Control DNA 007 into tube MPC

3.3.3 Create the female negative control by making a 0.01 dilution of Control DNA 9947A:

- Label tube **FNC**
- Aliquot **198µL** of **UltraPure water** into tube **FNC**
- Aliquot **2µL** of **Control DNA 9947A** into tube **FNC**

4 Amplification Negative Control

4.1 UltraPure water will serve as an amplification negative control.

5 Master Mix Preparation

5.1 Retrieve Yfiler™ primers and Yfiler™ reaction mix from the refrigerator. Retrieve ABI Taq Gold from the freezer. Reagents may be stored in a Nalgene cooler on bench. **Record the lot numbers of the reagents.**

5.2 Vortex or pipette the reagents up and down several times to thoroughly mix the reagents. **Do not vortex Taq Gold** as it may degrade the enzyme.

5.3 After vortexing, centrifuge reagents briefly at full speed to ensure that no sample is trapped in the cap.

5.4 Consult the amplification documentation for the exact amount of Yfiler™ primers, reaction mix and ABI Taq Gold to add. The amount of reagents for one amplification reaction is listed in Table 3.

Table 3 - Yfiler™ PCR amplification reagents for one sample

Reagent	Per reaction
Yfiler™ PCR Reaction Mix	9.2 µL
Yfiler™ Primer Set	5.0 µL
AmpliTaq Gold DNA Polymerase (5U/µL)	0.8 uL
Mastermix total in each sample:	15 µL
DNA	10 µL

6 Reagent Aliquot

6.1 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.

6.2 Add **15 µL** of the Yfiler™ master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.

7 Witnessing Step

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

7.2 Have a witness confirm the order of input and output samples:

7.2.1 Input samples – From the main test batch screen, ensure that the extract tube label and entire LIMS input sample ID match for each sample. For Zygem samples in 0.2ml tubes, the witness will only need to confirm the tube label on the side of the tube.

7.2.2 Output samples – Go to the "Load Plate" screen in LIMS and ensure that the amp tube labels correspond to the order on the plate. The entire amp tube label must be read for each sample.

8 Sample and Control Aliquot

8.1 Use a new sterile filter pipette tip for each DNA sample or control addition. Open only one tube at a time for sample addition.

8.2 Prior to adding each sample or control to the master mix, pipette each up and down several times to thoroughly mix. Add the appropriate amount of DNA extract and diluant to each amp tube. The final aqueous volume in the PCR reaction mix tubes will be 25µL. After addition of the DNA and diluant (as needed), cap each sample before proceeding to the next tube.

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

9 Thermal Cycling

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

PCR Conditions for the Perkin Elmer GeneAmp PCR System 9700

9700 Yfiler™ user: casewk file: yfiler	The Yfiler™ file is as follows: Initial Incubation Step: Hold 95°C for 11 minutes Cycle (30 cycles) Denature at 94°C for 1 minute Anneal at 61°C for 1 minute Extend at 72°C for 1 minute Final Extension: Hold 60°C for 80 minutes Final Hold: Hold 4°C ∞.

10 Instructions for the 9700

10.1 Place the tubes in the tray in the heat block, slide the heated lid over the tubes, and fasten the lid by pulling the handle forward.

10.2 Start the run by performing the following steps:

10.3 Note: 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.

10.4 Verify that user is set to “casewk.”

10.4.1 If it is not, select the USER option (F5) to display the “Select User Name” screen.

10.4.2 Use the circular arrow pad to highlight “casewk.” Select the ACCEPT option (F1).

10.5 Select the RUN option (F1).

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

10.7 Verify that the reaction volume is set to **25**µL for Yfiler™.

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

10.9 Update usage log.

10.10 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.

10.11 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.

10.11.1 NOTE: The 4°C storage soak step is not meant to store samples for an extended period. Samples should be removed from the instrument and placed in the 4°C refrigerator at the earliest convenience.

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

10.13 After completion of the thermal cycling protocol, store amplified product at 4°C and proceed with fragment analysis.

11 Complete the LIMS Test Batch

11.1 Fill out the Performed By tab for the Test Batch Review.

11.2 Select all output samples and click Review to perform the test batch approval.

11.3 Schedule the samples to the appropriate STR test batch and create the test batch.

11.4 A batch reviewer will then complete the Test Batch Tech Review.