IDENTIFILER™ SAMPLE PREPARATION FOR AMPLIFICATION		
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Identifiler Sample Preparation for Amplification

GENERAL INFORMATION

The Identifiler Kit is a PCR Amplification Kit manufactured, sold, and trademarked by Applied Biosystems (ABI).

1. The target DNA template amount for Identifiler™ 28 cycles is 500 pg.
   The target DNA template amount for Identifiler™ 31 cycles is 100 pg.

   To calculate the amount of template DNA and UltraPure water (diluent) to add, the following formulas are used. The sample concentration is the quantitation value:

$$\text{DNA extract added } (\mu L) = \frac{\text{Target DNA Template Amount (pg)}}{(\text{sample concentration, pg}/\mu L)(\text{dilution factor})}$$

The volume of diluent to add (µL) = Volume of sample aliquot (µL) – amount of DNA extract (µL)

Generation of Amplification Sheets and Preparing DNA aliquots for amplification

1. Follow applicable procedures for preparation of an amplification test batch in the LIMS.

   To determine the appropriate system for amplification of samples, refer to Table 1

TABLE 1: PCR amplification input based on Quant values

Quant value at 1:10 dilution pg/µL	**Quant value neat pg/µL**	**Amplification Sheet**	**Dilution**
High Yield DNA extraction ≥ 0.4 pg/µL	≥ 4.0* to 20 pg/µL	Amplify with ID for 31 cycles*	Neat = 1
High Yield DNA /HSC extraction ≥ 2.0 pg/µL	≥ 20 pg/µL	Amplify with ID for 28 cycles	As appropriate
HSC extraction ≥ 0.7 pg/µL	≥ 7.5 pg/µL	Microcon and amplify with ID 28	As appropriate

*Samples providing less than 20 pg per amplification can only be amplified with the permission of a supervisor.

Samples with concentrations between or equal to 20 pg/µL and 100 pg/µL (less than or equal to 500 pg amplified) may be automatically amplified in duplicate; see the concordant analysis policy (section 1).

Identifiler – Sample and Amplification Set-up

1. For each sample to be amplified, label a new tube. Add DNA and UltraPure water as specified by the amplification documentation. (Samples amplified with Identifiler reagents should be prepared with UltraPure water).
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	38
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)

a) Centrifuge samples at full speed briefly.
b) Label tubes appropriately for dilutions. Add the correct amount of UltraPure water as specified by the amplification documentation and Table 1.
c) Pipette sample up and down several times to thoroughly mix sample.
d) Set the sample aside until you are ready to aliquot it for amplification.

Samples and Controls

a. **For an Identifiler™ 28 cycle amplification, make a 0.5 (1/2) dilution of the ABI Positive (A9947) control at 100 pg/ µL (5 µL in 5 µL of water).**

This yields 50 pg/µL of which 5 µL or 250 pg will be used.

b. **For an Identifiler™ 31 cycle amplification, make a 0.2 (1/5) dilution of the ABI Positive (A9947) control at 100 pg/µL (4 µL in 16µL of water).**

This yields 20 pg/µL of which 5 µL or 100 pg will be used.

3. 5 µL of UltraPure water will serve as an amplification negative control.

4. Arrange samples in precisely the positions they appear on the sheet.

5. Have a witness confirm the order of input and output samples:
   i. **Input samples-**from the main test batch screen, insure that the extract tube label and entire LIMS input sample ID match for each sample.

ii. **Output samples-**Go to the "Load Plate" screen in LIMS and ensure that the amp tube labels correspond to the order on the plate.

Master Mix Preparation

1. Retrieve **Identifiler™** primers and reaction mix from the refrigerator and Taq Gold from the freezer. Store in a Nalgene cooler, if desired. Record the lot numbers of the reagents.

2. Vortex or pipette the reagents up and down several times. Centrifuge reagents at full speed briefly. **Do not vortex TAQ GOLD**.

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

TABLE 3: Identifiler™ PCR amplification reagents for one sample

Reagent	Per reaction
Primer mix	2.5 µL
Reaction mix	5 µL
AmpliTaq Gold DNA Polymerase (5U/µL)	0.5 µL
Mastermix total:	8 µL
DNA	5 µL

Reagent and Sample Aliquot

1. Vortex master mix. After vortexing, briefly centrifuge or tap master mix tube on bench.

2. Add **8 µL** of the Identifiler™ master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.

3. Prior to immediately adding each 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 13µL. After addition of the DNA, cap each sample before proceeding to the next tube.

4. After all samples have been added, return DNA extracts to storage and take the rack to the amplified DNA area for Thermal Cycling (continue to section C).

An alternative method for amplification is to use a 96-well plate.

1. Positive Control

   If only half a plate of samples are amplified, only one PE is necessary, however, to encompass all of the injections required for a full plate of samples, amplify two or more PEs (10 µL in 10µL of water).

2. Sealing the Plate
   a. If using a PCR plate, place a super pierce strong seal on top of the plate, and place the plate in the plate adapter on the ABgene heat sealer.
   b. Push the heat sealer on top of the plate for 2 seconds.
   c. Rotate the plate and reseal for 2 additional seconds.
   d. Label the plate with "A" for amplification and the date and time. (A011104.1300)

Thermal Cycling – all amplification systems

1. Turn on the ABI 9700 Thermal Cycler.

2. Choose the following files in order to amplify each system:

Identifiler 28	Identifiler 31
user: **hisens** or **casewk** file: **id28**	user: **hisens** or **casewk** file: **id31**

3. The following tables list the conditions that should be included in each file. If the files are not correct, bring this to the attention of the Quality Assurance Team and a supervisor.

Identifiler PCR Conditions for the Applied Biosystems GeneAmp PCR System 9700

9700 **Identifiler 28 or 31** user: **hisens** or **casewk** file: **id28** or **id31**	The Identifiler file is as follows: Soak at 95°C for 11 minutes : Denature at 94°C for 1 minute 28 or 31 Cycles : Anneal at 59°C for 2 minutes : Extend at 72°C for 1 minute 60 minute incubation at 60°C. Storage soak indefinitely at 4°C

9700 Instructions

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. Make sure you use a tray that has a 9700 label.

2. Start the run by performing the following steps:

3. 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.

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

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

6. Select the RUN option (F1).

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

8. Verify that the reaction volume is set to **13µL** for **Identifiler**. The ramp speed is set to **9600**.

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

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.

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

    After the amplification process, the samples are ready to be loaded on the fluorescent instruments. They may be stored in the appropriate refrigerator at 2-8°C for a period of up to 6 months.

    NOTE:

    Turn instruments off ONLY when the Main Menu is displayed, otherwise there will be a "Power Failure" message the next time the instrument is turned on. If this happens, it will prompt you to review the run history. Unless you have reason to believe that there was indeed a power failure, this is not necessary. Otherwise, press the STOP button repeatedly until the Main Menu appears.

    In case of an actual power failure, the 9700 thermal cycler will automatically resume the run if the power outage did not last more than 18 hours. The history file contains the information at which stage of the cycling process the instrument stopped. Consult the Quality Assurance Team on how to proceed.

Amplification Troubleshooting

PROBLEM: No or only weak signal from both the positive control and the test samples

Possible Cause	Recommended Action
Mistake during the amplification set up such as not adding one of the components or not starting the thermal cycler	Prepare new samples and repeat amplification step
Thermal cycler defect or wrong program used	Check instrument, notify QA team, prepare new samples and repeat amplification step

PROBLEM: Positive control fails but sample signal level is fine

Possible Cause	Recommended Action
Mistake during the amplification set up such as not adding enough of the positive control DNA	Prepare new samples and repeat amplification step
Positive control lot degraded	Notify QA team to investigate lot number, prepare new samples and repeat amplification step with a new lot of positive control

PROBLEM: Presence of unexpected or additional peaks in the positive control

Possible Cause	Recommended Action
Contamination by other samples, contaminated reagents	Notify QA team to investigate the amplification reagents, prepare new samples and repeat amplification step
Non-specific priming	Notify QA team to check thermal cycler for correct annealing settings, prepare new samples and repeat amplification step

PROBLEM: Strong signal from the positive controls, but no or below threshold signal from DNA test sample

Possible Cause	Recommended Action
The amount of DNA was insufficient or the DNA is severely degraded	Amplify a larger aliquot of the DNA extract Concentrate the extracted DNA using a Microcon device as described in the Microcon procedure. Re-extract the sample using a larger area of the stain or more biological fluid to ensure sufficient high molecular DNA is present
Test sample contains PCR inhibitor (e.g. heme compounds, certain dyes)	Amplify a smaller aliquot of the DNA extract to dilute potential Taq Gold polymerase inhibitors Purify the extracted DNA using a Microcon device as described in the Microcon procedure. Re-extract the sample using a smaller area of the stain to dilute potential Taq Gold polymerase inhibitors Re-extract the samples using the organic extraction procedure

The decision on which of the above approaches is the most promising should be made after consultation with a supervisor.