Amplification Setup

1. Purpose

This document describes the procedure to manually set up an amplification plate using the Globalfiler and Investigator 24plex amplification kits on the Proflex PCR System.

2. Summary

Amplification reagents from commercially purchased kits are added to DNA samples to generate DNA profiles through PCR. The Proflex PCR system is used for amplification.

3. Procedure

Documentation

1. Open a DOC-307 Case Chronicle document and save a copy to the Batches in Progress folder on the Shared Drive as the Batch ID with the following naming format: YYMMDDAnalyst Initials (Example: 230215SW)
    a. If previous processing was performed for this batch, then open the previously saved document.
2. Click on the Amp tab of the saved Case Chronicle.
3. Enter the following information into the header of the document:
    a. Batch ID
    b. Performed by
    c. Date of amplification
    d. Reagent Lot Numbers
4. Ensure the correct kit is selected from the drop-down list.
5. Add the number of Samples to the Sample field of the header.
    a. This information is used by the DOC-307 Case Chronicle to calculate the volume of master mix components and should include the total number of samples, reagent blanks, positive control, and amplification negative control. An additional 15% is automatically included for overage to account for pipetting error.
6. Enter the Positive Control and Negative Control into field 1A and 1B, respectively.
7. Enter the sample identification numbers for all samples and reagent blanks that will be amplified in the batch layout in the subsequent fields.
    a. The Leave at least one empty space for the Allelic Ladder on the Load Plate every 3 columns.

Manual Amplification Plate Setup

1. Prepare a positive control sample for the kit in use prior to amplification set-up using the volumes below:

Kit	Positive Control Name	Positive Control Volume	Water/TE Volume
Globalfiler	007	9µL	6µL
Investigator 24Plex	9948	5µL	10µL
Yfiler Plus	007	3 µL	57µL

a. The positive control must be prepared fresh daily.

2. Prepare the reaction reagents by thawing completely, then vortex 3 to 5 seconds and centrifuge briefly before opening.
3. Create a master mix for each Sample, Reagent blank, including overage, and mix thoroughly by vortexing.
    a. Refer to the header of the Amp tab of DOC-307 Case Chronicle for exact volumes suitable for the number of samples.

Kit	Reaction Mix Volume	Primer Mix Volume
Globalfiler	7.5µL	2.5µL
Investigator 24Plex	7.5µL	2.5µL
Yfiler Plus	10.0µL	5.0µL

4. Pipette master mix into the applicable wells according to the volumes specific for the amplification kit listed below.

Kit	Total Master Mix Volume
Globalfiler	10.0µL
Investigator 24Plex	10.0µL
Yfiler Plus	15.0µL

5. Pipette sample or a control into the applicable wells according to the requirements of the amplification kit listed below.
    a. The negative control must be the same water that was used for any associated sample dilutions.

Kit	Sample Volume
Globalfiler	15.0µL
Investigator 24Plex	15.0µL
Yfiler Plus	10.0µL

6. Seal the reaction plate or tube strip with strip caps.
7. Centrifuge to remove any bubbles.

Proflex PCR System Run

1. Place plate or strip tubes on the heating block.
2. Close the cover.
3. Touch **Open Method**.
4. Touch the name of the appropriate amplification kit.
5. Touch **Verify Block**.
6. The Run Parameters screen is displayed:

7. Verify the name of the run method that you have selected.
8. Touch **Start Run**.
    a. The cover is heated to the required temperature, then the run begins.
    b. While the run is in progress, the Home screen is displayed.
    c. When the run is complete, the Status Dial/s on the Home screen displays Done and Remove Samples.
9. Remove the samples and store appropriately.
10. Touch **Done** once you have removed the samples.

Benchmark PCR Run

1. Place PCR strips or plate on the sample block.
2. Close the lid and turn the lid knob clockwise until it clicks.
3. Touch **All Program**.
4. Select desired kit program and then touch **Run**.
5. Check the sample volume and lid temperature in the Run Settings window.
6. Touch **Ok** to begin the run.
7. Remove the samples and storage appropriately once the run is complete.

4. References

GlobalFiler™ PCR Amplification Kit USER GUIDE
Investigator® 24plex QS Handbook
Yfiler™ Plus PCR Amplification Kit User Guide
ProFlex™ PCR System USER GUIDE

5. Definitions

N/A