Use of the VersaPlex™ 27PY System to Amplify Extracted DNA

Instructions for Use of Product DC7020.

Promega

Quick Protocol

Protocol for Amplification and Analysis of Extracted DNA

This document is a quick protocol for experienced users to amplify extracted DNA in a 25µl reaction volume. Prior to using a VersaPlex™ 27PY System for the first time, thaw all pre-amplification and post-amplification components. Store reagents at 2–10°C, where components are stable for 6 months. Do not refreeze.

Before You Begin

Determine the concentration of genomic DNA for your samples. Note that different DNA quantification methods can yield different quantification values. We strongly recommend that you perform experiments to determine the optimal DNA template amount based on your DNA quantification method.

Optional: Record the DNA template amount as optimized in your laboratory.

PCR Setup

1. Centrifuge pre-amplification component tubes briefly, then vortex for 15 seconds before each use. Do not centrifuge after vortexing.
2. Determine the number of reactions including positive and negative controls. Add 1 or 2 reactions to this number.
3. Prepare the PCR amplification mix by combining the components as shown below.

PCR Amplification Mix Component	Volume per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 25µl	×		=	
VersaPlex™ 27PY 5X Master Mix	5.0µl	×		=	
VersaPlex™ 27PY 5X Primer Pair Mix	5.0µl	×		=	
Template DNA (1.0ng)	up to 15µl				
Total volume	**25µl**				

4. Vortex the PCR amplification mix for 5–10 seconds, and then add PCR amplification mix to each reaction well.
5. Add the template DNA.
6. For the positive amplification control, vortex the 2800M Control DNA, and then dilute an aliquot to 1.0ng in the desired template DNA volume. Add 1.0ng of diluted DNA to a reaction well containing PCR amplification mix.
7. For the negative amplification control, pipet Water, Amplification Grade, or TE–4 buffer instead of template DNA into a reaction well containing PCR amplification mix.
8. Seal or cap the plate. Optional: Briefly centrifuge the plate.

Notes:

Protocol for Amplification and Analysis of Extracted DNA (continued)

PCR

The following protocol was developed for use with the ProFlex® PCR System with default ramp mode (no emulation), the GeneAmp® PCR System 9700 with a silver or gold-plated silver sample block with Max Mode as the ramp speed and the Veriti® 96-Well Thermal Cycler with a 100% ramping rate.

1. Program the thermal cycler with the following conditions. Refer to the technical manual for more information. When amplifying extracted DNA, we recommend using 29 cycles. Optimize the cycle number as required.

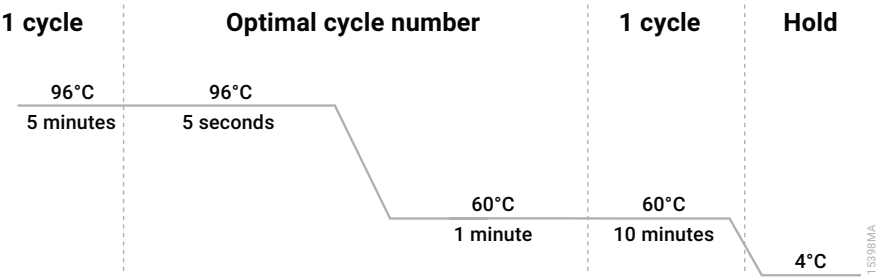

Figure 1. The thermal cycling protocol for the ProFlex® PCR System, GeneAmp® PCR System 9700 and Veriti® 96-Well Thermal Cycler.

2. Proceed with fragment analysis, or store amplified samples at –20°C protected from light.

Notes:

Instrument Setup and Sample Preparation

A passing spectral calibration must be generated using the VersaPlex™ 6C Matrix Standard (Cat.# DG4960) prior to sample analysis. See the *VersaPlex™ 6C Matrix Standard Technical Manual* #TMD056 for more information.

Instrument Setup

1. For the Applied Biosystems® 3500 or 3500xL Genetic Analyzer, set the oven temperature to 60°C, and then select "Start Pre-Heat". When the Oven Temperature and Detection Cell Temperature turn green, you may proceed with the first injection.
2. Use the following parameters when setting up the instrument. Refer to the instrument user's manual for additional details.

Genetic Analyzer	Run Module	Dye Set	Injection Parameters[1]	Run Parameters
Applied Biosystems® 3500	HID36_POP4	Promega J6	1.2kV; 15 seconds	13kV; 1,500 seconds
Applied Biosystems® 3500xL	HID36_POP4	Promega J6	1.2kV; 24 seconds	13kV; 1,500 seconds

[1]Injection time may be modified to increase or decrease the observed peak heights.

Optional: Record the injection conditions as optimized in your laboratory.

Notes:

Instrument Setup and Sample Preparation (continued)

Sample Preparation

Prepare samples for capillary electrophoresis immediately before loading.

1. Centrifuge post-amplification component tubes briefly, and then vortex for 15 seconds before each use. Do not centrifuge after vortexing.
2. Calculate the number of samples including the number of allelic ladders per run. Add 1 or 2 samples to this number.
3. Prepare a loading cocktail by combining and mixing the WEN ILS 500 and Hi-Di™ formamide. You may need to optimize the volume of WEN ILS 500.

Component	Volume per Sample	×	Number of Samples	=	Final Volume
WEN ILS 500	0.5µl	×		=	
Hi-Di™ formamide	9.5µl	×		=	
Total volume	**10µl**				

Optional: Record the volume of WEN ILS 500 per sample as optimized in your laboratory.

4. Vortex the loading cocktail for 10–15 seconds, and pipet 10µl of formamide/ILS mix into each well.
5. Add 1µl of amplified sample (or 1µl of VersaPlex™ 27PY Allelic Ladder Mix). Cover wells with appropriate septa, and centrifuge plate briefly.
6. Denature samples at 95°C for 3 minutes, and then immediately chill on crushed ice or freezer block or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.
7. Place the plate on the instrument, and start the capillary electrophoresis run.

Notes:

Data Analysis

The panels, bins and stutter text files needed for data analysis using GeneMapper® ID-X software, version 1.2 or higher, are available for download at: **https://www.promega.com/resources/software-firmware/versaplex-27py-genemapper-id-x-software-panels-and-bin-sets/**

Additional protocol information is in Technical Manual #TMD055, available online at: **www.promega.com**

