

Use of the PowerPlex® 18D System to Amplify Extracted DNA

INSTRUCTIONS FOR USE OF PRODUCTS DC1802 AND DC1808.

Protocol for Amplification and Analysis of Extracted DNA

This document is a quick protocol for experienced users to amplify extracted DNA. Quick protocols also are available for amplifying DNA from storage card punches and swabs. For complete protocol information and troubleshooting tips see the *PowerPlex® 18D System Technical Manual #TMD031*, which is available online at: www.promega.com/protocols/

Before You Begin

The locus-to-locus balance for the PowerPlex® 18D System is optimized for direct-amplification methods. Amplification of extracted DNA may result in locus-to-locus imbalance. Determine the concentration of genomic DNA for your samples. Note that different quantitation methods yield different quantitation values. We strongly recommend that you perform experiments to determine the optimal DNA template amount based on your DNA quantitation method.

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

PCR Setup

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

Component	Volume per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 25.0µl	×		=	
PowerPlex® D 5X Master Mix	5.0µl	×		=	
PowerPlex® 18D 5X Primer Pair Mix	5.0µl	×		=	
Template DNA (1ng)	up to 15µl				
Total volume	25µl				

5. Vortex the PCR amplification mix for 5–10 seconds, then add PCR amplification mix to each reaction well.
6. Add the template DNA.
7. For the positive amplification control, vortex the 2800M Control DNA, then dilute an aliquot to 1ng in the desired template DNA volume. Add 1ng of diluted DNA to a reaction well containing PCR amplification mix.
8. For the negative amplification control, pipet Water, Amplification Grade, or TE⁻⁴ buffer instead of template DNA into a reaction tube containing PCR amplification mix.
9. Seal the plate. Briefly centrifuge the plate if desired.

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Thermal Cycling

The PowerPlex® 18D System is designed for use with the GeneAmp® PCR System 9700 with 9600 mode as the ramp speed.

1. Program the thermal cycler with the following conditions. Refer to the technical manual for more information.

For extracted DNA we recommend using 30 cycles. Optimize the cycle number as required.

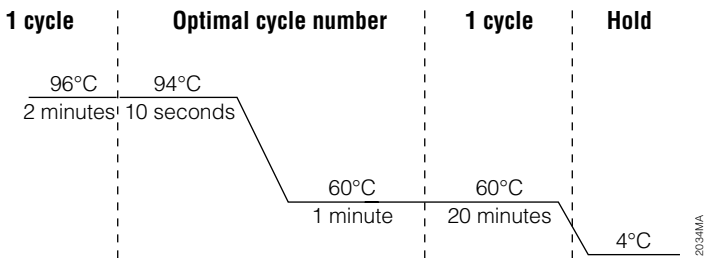


Figure 1. The thermal cycling protocol for the GeneAmp® PCR System 9700 thermal cycler.

Optional: Record the cycle number as optimized in your laboratory.


2. Proceed with the analysis, or store amplified samples at -20°C in a light-protected box until ready to analyze.

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Instrument Setup and Sample Preparation

 A passing spectral calibration must be generated using the PowerPlex® 5C Matrix Standards (Cat.# DG4850) prior to sample analysis. See the *PowerPlex® 5C Matrix Standard Technical Manual #TMD049* for more information.

Instrument Setup

1. For the Applied Biosystems® 3500 or 3500xL Genetic Analyzer, we recommend preheating the oven at 60°C for at least 30 minutes prior to 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 Time
Applied Biosystems® 3500	HID36_POP4	Promega G5	1.2kV, 15 seconds	1,210–1,500 seconds
Applied Biosystems® 3500xL	HID36_POP4	Promega G5	1.2kV, 24 seconds	1,210–1,500 seconds
Applied Biosystems® 3130 and 3130xL	HIDFragmentAnalysis36_POP4	G5 ²	3kV, 5 seconds	1,500 seconds
ABI PRISM® 3100 and 3100-Avant	HIDFragmentAnalysis36_POP4	G5 ²	3kV, 5 seconds	1,500 seconds

¹Injection time may be modified (2–24 seconds) to increase or decrease the observed peak heights.

²Confirm that the active dye set is the file generated for the PowerPlex® 5-dye chemistry.

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

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Instrument Setup and Sample Preparation (continued)

Sample Preparation

Prepare samples for capillary electrophoresis immediately before loading.

1. Thaw the post-amplification components. Centrifuge the WEN Internal Lane Standard 500 (WEN ILS 500) briefly, 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 reactions to this number.
3. Prepare a loading cocktail by combining and mixing the WEN ILS 500 and Hi-Di™ formamide.

Component	Volume per Sample	×	Number of Samples	=	Final Volume
WEN ILS 500	0.5µl ¹	×		=	
Hi-Di™ formamide	9.5µl	×		=	

¹The volume of internal lane standard used in the loading cocktail can be adjusted to change the intensity of the size standard peaks based on laboratory preferences.

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/internal lane standard mix into each well.
5. Add 1µl of amplified sample (or 1µl of PowerPlex® 18D Allelic Ladder Mix) to each well. Cover wells with appropriate septa, and centrifuge plate briefly.
6. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.
7. Place the plate assembly on the autosampler.
8. Start the capillary electrophoresis run.

Additional Notes:

Data Analysis

The panels, bins and stutter text files needed for data analysis using GeneMapper® ID software, version 3.2, and GeneMapper® ID-X software, version 1.2 or higher, are available for download at:

www.promega.com/resources/software-firmware/genemapper-id-software-panels-and-bin-sets/

For complete protocol information see the *PowerPlex® 18D System Technical Manual* #TMD031, available online at:

www.promega.com/protocols/

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