Use of the PowerPlex® 18D System to Amplify DNA from Storage Card Punches

INSTRUCTIONS FOR USE OF PRODUCTS DC1802 AND DC1808.

Protocol for Amplification and Analysis of DNA from Storage Card Punches

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

Before You Begin

The PowerPlex® 18D System is compatible with the following sample types:

FTA® Sample Types	NonFTA Sample Types
Buccal cells collected on FTA® cards with Whatman EasiCollect™ or Fitzco Sampact™ devices	Buccal samples on Bode Buccal DNA Collector™ devices
Buccal cells collected with sterile swabs transferred to FTA® or Indicating FTA® cards	Blood and buccal samples on nonFTA card punches (e.g., S&S 903)
Liquid blood (from collection or storage Vacutainer® tubes or finger sticks) spotted onto FTA® cards	

No preprocessing steps are required for FTA® card punches. For nonFTA cards, the punches must be preprocessed as described below. For complete protocol information see the *PunchSolution™ Kit Technical Manual* #TMD038, which is available online at: **www.promega.com/protocols/**

1. Add 10µl of PunchSolution™ Reagent (Cat.# DC9271) to one 1.2mm punch placed in a well of the 96-well reaction plate.

   Note: Do not cover the plate or place the plate in a thermal cycler with a closed, heated lid.
2. Incubate plate at 70°C for 30 minutes or until wells are dry.

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	15µl	×		=	
PowerPlex® D 5X Master Mix	5.0µl	×		=	
PowerPlex® 18D 5X Primer Pair Mix	5.0µl	×		=	
Total volume	**25µl**				

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5. Vortex the PCR amplification mix for 5–10 seconds.
6. For FTA® storage cards, add one or two 1.2mm punches from a card containing a buccal sample or one 1.2mm punch from a card containing whole blood to the appropriate wells of the reaction plate. For nonFTA card punches, add the PCR amplification mix to the PunchSolution™ Reagent-treated punches.

   Note: It also is acceptable to add the FTA® card punch first, then add the PCR amplification mix.
7. For the positive amplification control, vortex the tube of 2800M Control DNA, dilute to 5ng/µl and add 1µl (5ng) to a reaction well containing 25µl of PCR amplification mix. Do not add a punch to this control reaction.
8. Reserve a well containing PCR amplification mix as a negative amplification control.
9. Seal the plate, and briefly centrifuge the plate.

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 DNA from punches, we recommend using 27 cycles. Optimize the cycle number as required.

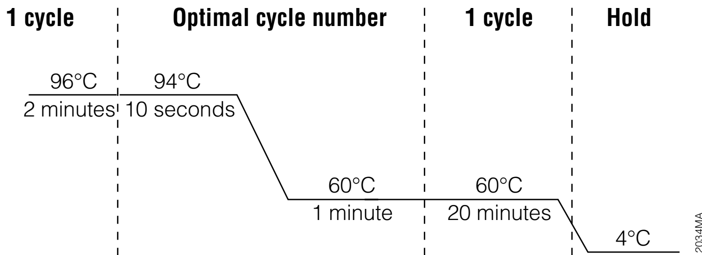

Figure 1. The thermal cycling protocol for the GeneAmp® PCR System 9700 thermal cycler.

   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.

Additional Notes:

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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 3130*xl*	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

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

[1]The 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.

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 Technical Manual* #TMD031, available online at:
www.promega.com/protocols/

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