Use of the PowerPlex® Fusion System to Amplify DNA from Swabs

INSTRUCTIONS FOR USE OF PRODUCTS DC2402 AND DC2408.

Quick PROTOCOL

Protocol for Amplification and Analysis of DNA from Swabs

This document is a quick protocol for experienced users to amplify DNA from swabs. Quick protocols are also available for amplifying extracted DNA and DNA from storage card punches. For complete protocol information and troubleshooting tips see the *PowerPlex® Fusion System Technical Manual* #TMD039, which is available online at: **www.promega.com/protocols/**

Preprocessing Swabs

For complete protocol information see the *SwabSolution™ Kit Technical Manual* #TMD037, which is available online at: **www.promega.com/protocols/**

1. Place buccal swab head in a 1.5ml tube.
2. Add 1ml of SwabSolution™ Reagent (Cat.# DC8271) to each buccal swab head.
3. Place tube in a heat block, and incubate at 70°C for 30 minutes.
   Note: Buccal swab extracts can be stored at 4°C for up to 6 months.

PCR Setup

1. Thaw all pre-amplification components just prior to use.
2. Vortex the components thoroughly for 15 seconds. Centrifuge tube 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	13µl	×		=	
PowerPlex® Fusion 5X Master Mix	5.0µl	×		=	
PowerPlex® Fusion 5X Primer Pair Mix	5.0µl	×		=	
Total volume	23µl				

5. Vortex the PCR amplification mix for 5–10 seconds.
6. Add 2µl of swab extract to 23µl of PCR amplification mix.
7. For the positive amplification control, add 2µl of 2800M Control DNA (diluted to 5ng/µl) to a reaction well containing 23µl of PCR amplification mix. Do not add SwabSolution™ Reagent to this control reaction.
8. Seal the plate. Briefly centrifuge the plate if desired.

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Printed in USA. Revised 6/16.
Part #9FB148

Use of the PowerPlex® Fusion System to Amplify DNA from Swabs

INSTRUCTIONS FOR USE OF PRODUCTS DC2402 AND DC2408.

Quick PROTOCOL

PCR

The PowerPlex® Fusion System is validated for use with the GeneAmp® PCR System 9700 with a silver or gold-plated silver sample block with Max mode as the ramp speed.

1. Program the thermal cycler with the following conditions. Refer to the technical manual for more information. For swab extracts we recommend using 27 cycles. Optimize the cycle number as required.

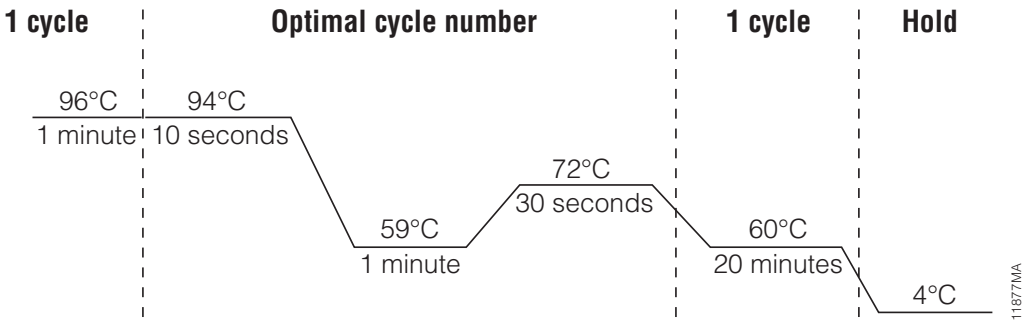

Figure 1. The thermal cycling protocol for the GeneAmp® PCR System 9700 thermal cycler.

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:

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Promega

Printed in USA. Revised 6/16.
Part #9FB148

Use of the PowerPlex® Fusion System to Amplify DNA from Swabs

INSTRUCTIONS FOR USE OF PRODUCTS DC2402 AND DC2408.

Quick PROTOCOL

Instrument Setup and Sample Preparation

A passing spectral calibration must be generated using the PowerPlex® 5C Matrix Standard (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 seconds
Applied Biosystems® 3500xL	HID36_POP4	Promega G5	1.2kV, 24 seconds	1,210 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.

Additional Notes:

ORDERING / TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Promega

Printed in USA. Revised 6/16.
Part #9FB148

Use of the PowerPlex® Fusion System to Amplify DNA from Swabs

INSTRUCTIONS FOR USE OF PRODUCTS DC2402 AND DC2408.

Quick PROTOCOL

Instrument Setup and Sample Preparation (continued)

Sample Preparation

Prepare samples for capillary electrophoresis immediately before loading.

1. 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. You may need to optimize the volume of WEN ILS 500.

Component	Volume Per Reaction	×	Number of Samples	=	Final Volume
WEN ILS 500	0.5µl[1]	×		=	
Hi-Di™ formamide	9.5µl	×		=	

[1]The volume of WEN ILS 500 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® Fusion Allelic Ladder Mix). 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® Fusion System Technical Manual* #TMD039, available online at: **www.promega.com/protocols/**

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Printed in USA. Revised 6/16
Part #9FB148