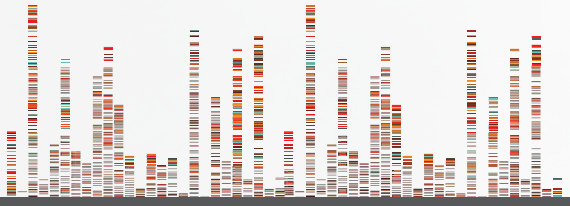

info@lmrc.ir

IG_17 Plex® DRT PCR Amplification Kit

Quick Reference Protocol

LegaloMed

Protocol for blood or buccal cells on Treated & Non-Treated paper substrates

All components of the IG_17 Plex DRT PCR Amplification Kit should be stored at —30 to —15 °C. Avoid repeated thawing and freezing. The primer mix and allelic ladder must be stored protected from light. DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. Under these conditions, the components are stable until the expiration date indicated on the box.

Further information

For more information, refer to the IG_17Plex DRT PCR Amplification Kit User Guide

Notes before starting

- Setup all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Before opening the tubes, thaw PCR components, vortex (EXCEPT IG_DRT PCR Additive), and then centrifuge briefly to collect the contents at the bottom of the tubes.
- Use disposable tips with hydrophobic filters to minimize cross-contamination risks.

Table1: The IG_17Plex DRT PCR Amplification Kit is compatible with the following types of single-source samples for direct PCR amplification:

Using Chemically-Treated paper substrates	Using Untreated paper substrates
Liquid blood (from collection or storage Vacutainer® tubes or finger sticks) spotted Onto FTA® or Nucleic® or IG_Gene® cards	Buccal samples on Bode Buccal DNA Collector™ devices
Buccal cells collected on FTA® or Nucleic® cards or Whatman EasiCollect™ or Fitzco Sampact™ devices	Blood and buccal samples on non FTA cards (e.g., S&S 903)
Buccal cells collected with swabs transferred onto FTA® or Nucleic cards or Indicating FTA® cards	

Procedure

1. Collect one (for Blood samples) and two (for Buccal cells samples) 1.2 mm punch from the center of the storage card into a 0.2 ml PCR grade plate or 0.2 ml PCR grade microtube.

   Note 1: For buccal cells collected using Indicating FTA Cards, take the punch from a white area.This color indicates successful sample Transfer

   Note 2: Do not add water to the wells on the reaction plate before adding the punches. If you observe static issues with the paper discs, you can prepare and dispense the 12-pL reaction mix into the wells of the reaction plate before adding the punches. Alternatively, dispense 4 µL IG_DRT PCR Additive into each sample before adding the punches.

   Note 3: Make the punch as close as possible to the center of the sample to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.

   Note 4: If you are using a Bode Buccal DNA Collector™, makea 1.2 mm punch as close as possible to the tip of the DNA collector to ensure optimum peak intensity.

2. Determine the number of reactions including positive and negative controls. Add 1 or 2 reactions to this number to provide excess volume for the loss that occurs during reagent transfers.
3. Prepare the PCR amplification mix by combining the components as shown below (Table 2).

Table 2. PCR amplification mix setup

Components	Volume per Reaction		
	Blood or Buccal cells on Treated & Untreated Papers	Positive Control	Negative Control
IG_17Plex DRT Master Mix	6 µl	6 µl	6 µl
IG_17Plex DRT Primer Mlx	2 µl	2 µl	2 µl
IG_DRT PCR Additive	4 µl	-	4 µl
Control DNA U266*	-	1-4 µl	-
Distilled H2O	-	0-3 µl	-
Total Volume	12 µl		

* Amounts of Control DNA may have to be adapted after setting optimal PCR cyclenumber in your laboratory if signals are too low or too high.

Note 1: Do not add any template DNA, blank disc or water to the negative control PCR tube.

Note 2: The volumes of positive control are suggested amounts and can be adjusted if peak heights are too high for your optimize number.

Note 3: IMPORTANT! This kit is optimized for a 12-µL PCR volume to overcome the PCR inhibition that is epected when amplifying unpulfiled samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

4. Vortex the reaction mix for 2 seconds. centrifuge briefly and then dispense 12 µl to each 0.2 ml microtube or reaction plate
5. Close the microtube or seal the MicroAmp™ Optical 96-Well Reaction Plate.
6. Briefly centrifuge reactions to ensure discs are fully submerged.

Perform PCR

1. Program the thermal cycler according to the manufacturer's instructions, using the conditions shown in figure 1:

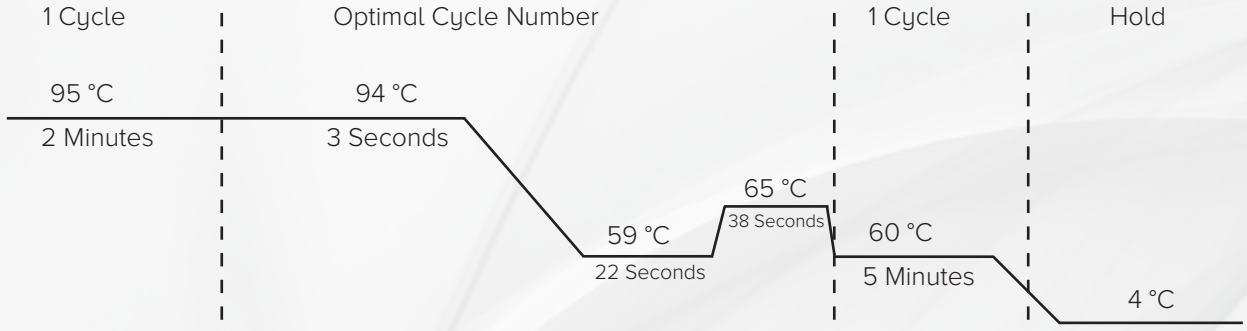

Figure 1: Standard cycling conditions

* Based on our studies the optimum PCR cycle number is 28, 29 or 30 cycles for blood samples on treated & untreated papers, and is 29, 30 cycles for buccal samples on treated papers.

IMPOTANT! You Should perform a single initial sensitivity experiment to determine the appropriate cycle number use the kit

2. Load the plate into the thermal cycler, then start the run.
3. When the cycling protocol is completed, immediately perform Capillary Electrophoresis or store the amplified DNA at —25 °C to —15 °C.

Perform Capillary Electrophoresis

1. Pipet the required volumes of components into an appropriately sized microtube:

Reagent	Volume per Reaction
Gene Scale_500 IGO Size Standard	0.5 µL
Hi-DI™ Formamlde	10 µL

Note 1: Include volume for additional samples to provide excess volume for the loss that occurs during reagent transfers.

Note 2: IMPORTANT! The volume of size standard indicated in the table is suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Vortex the microtube, then briefly centrifuge.
3. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate or appropriate microtube, add:

- 10 µL of the formamide/size standard mixture
- 1 µL of PCR product or Allelic Ladder

Note: For blank wells, add 10 µL of Hi-Di Formamide.

4. Close the microtube (or seal the Reaction Plate), then briefly vortex and centrifuge.
5. Heat the microtube or the reaction plate at 95 °C for 3 minutes.
6. Immediately place the microtube or the reaction plate on ice for 3 minutes.
7. Place the sample tray on the autosampler, then start the electrophoresis run.

Note 1: IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between runs.

Note 2: Perform internal validation studies to verify the required allelic ladder injection frequency, to ensure accurate genotyping of all samples in your laboratory environment.

Revision history

Revision	Date	Description
B	Oct 2022	Revised and Changed PCR Components & Protocol for treated & untreated papers
A	January 2022	Baseline for probable future revisions.

For up-to-date licensing information and product-specific disclaimers, see the respective LegaloMed kit handbook or user manual