PACE-IR™ Quick Reference Guide

Introduction

3CR Bioscience's PACE-IR™ Genotyping Master Mix is a PCR-based, allele-specific chemistry for the analysis of Single Nucleotide Polymorphisms (SNPs) and insertion/deletions (indels). It is designed for enhanced performance with samples containing PCR inhibitors. For full details of the PACE-IR™ genotyping chemistry, please refer to the PACE-IR™ Genotyping Master Mix User Guide at www.3crbio.com.

Included in the kit

PACE-IR™ Genotyping Master Mix (2x concentration).

Not included in the kit

- PCR plate and optically clear seal
- Template DNA
- PCR-grade water
- Assay mix (see User Guide).

Storage

-20˚C for long periods, 4˚C for periods of up to four weeks. Defrost thoroughly and gently mix before use. Avoid multiple freeze / thaw cycles.

Safety warnings and precautions

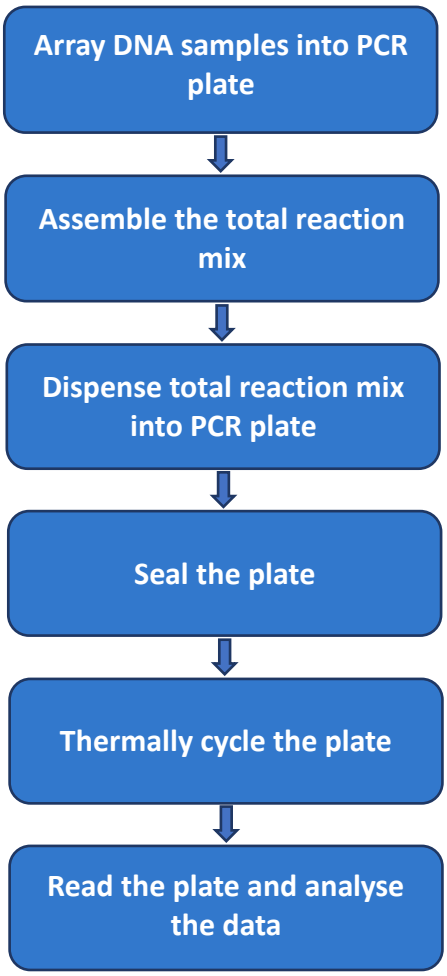
This product should only be handled by trained laboratory personnel. It is advisable to wear suitable PPE when using the product. In case of contact with skin or eyes, wash immediately with water.

Using PACE-IR™ Genotyping Master Mix

1. Array DNA samples into PCR plate.
   A small number of no-template controls (NTCs) should be included on each plate. Positive controls (samples of known genotype) can also be included if desired. Once arrayed, the DNA can be dried down or used in hydrated form.

2. Assemble the PACE-IR™ total reaction mix.
   Table 1 details the reagent volumes required for preparing the total reaction mix for different plate types. Prepare sufficient total reaction mix for the number of samples multiplied by the chosen reaction volume required, plus an extra 5%.

PACE-IR™ genotyping process



	Hydrated DNA method (µL per well)			Dried DNA method (µL per well)			
	96-well plate	384-well plate	384-well Array tape	96-well plate	384-well plate	1536-well plate	384-well Array tape
2x PACE-IR GMM	5.0	2.5	0.8	5.0	2.5	0.5	0.4
PACE Assay mix	0.138	0.069	0.022	0.138	0.069	0.014	0.011
Water	N/A	N/A	N/A	5	2.5	0.5	0.4
DNA	5.0	2.5	0.8	N/A	N/A	N/A	N/A
TOTAL	**10.0**	**5.0**	**1.6**	**10**	**5.0**	**1.0**	**0.8**

***Table 1*. Reagent volumes for assembly of total reaction mix. Total volumes indicated are recommended for the associated PCR plate type.**

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3. Dispense total reaction mix into the PCR plate.
   Add the required amount of total reaction mix to each DNA sample in the reaction plate in accordance with *Table 1*.

4. Seal the PCR plate.
   Seal the plate with an optically clear seal. The plate should then be centrifuged briefly to locate all liquid at the bottom of the wells.

5. Thermally cycle the plate.
   PACE-IR™ Genotyping Master Mix can be used with any standard Peltier-based or water bath thermal cycler. Run the thermal cycling protocol detailed in *Table 2*.

Step	Description	Temperature	Time	Nº. Cycles
1	Enzyme activation	94˚C	15 min	1
2	Template denaturation	94˚C	20 secs	10
	Annealing and extension	65-57˚C	60 secs (drop 0.8˚C per cycle)	
3	Denaturation	94˚C	20 secs	30
	Annealing and extension	57˚C	60 secs	

***Table 2*. Thermal cycling conditions for PACE-IR™ genotyping reactions.**

Template denaturing	94˚C	20 secs	3
Annealing and extension	57˚C	60 secs	

***Table 3*. Thermal cycling conditions for recycling PACE-IR™ genotyping reactions.**

6. Read the plate and analyse the data.
   After thermal cycling, read fluorescence in a FRET-capable plate reader. To analyse the data, import it into a genotype cluster analysis software package. Display the data in a cluster and analyse the genotyping clusters as shown in *Figure 1*.

   If sufficiently defined genotype clusters are not obtained after the initial thermal cycling protocol, the plate should be cycled for an additional three cycles using the conditions detailed in *Table 3* and read/analysed again. The additional cycling can be repeated until tight and well separated clusters are observed, though this is rarely required.

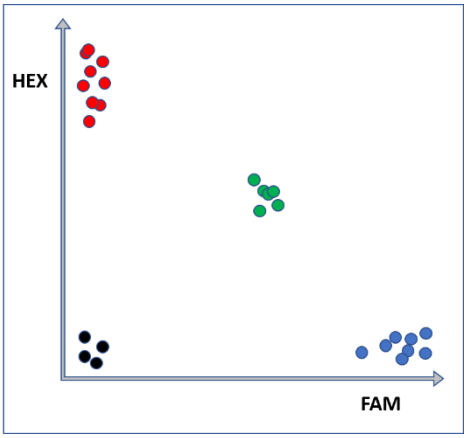

***Figure 1* Example cluster plot for PACE-IR™ genotyping data. The red and blue clusters are homozygous for the alleles reported with HEX and FAM, respectively, whilst the green cluster represents individuals that are heterozygous for the polymorphism.**

Ordering information

Please visit www.3crbio.com

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