ProbeSure-IR™ Quick Reference Guide

Introduction

3CR Bioscience's ProbeSure-IR™ Genotyping Master Mix is designed for use in PCR genotyping applications with hydrolysis probe-based assays. It is specifically formulated for enhanced performance with samples containing PCR inhibitors, for use in endpoint fluorescent detection of Single Nucleotide Polymorphisms (SNPs) and insertion / deletions (indels). For full details of ProbeSure-IR Genotyping Master Mix, please refer to the ProbeSure-IR™ Genotyping Master Mix User Guide at www.3crbio.com.

Included in the kit

ProbeSure-IR™ Genotyping Master Mix (2x concentration)

Not included in the kit

- PCR plate and optically clear seal
- Template DNA
- PCR-grade water
- Primers and probes (or both combined into an assay mix).

Storage

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

Safety warnings and precautions

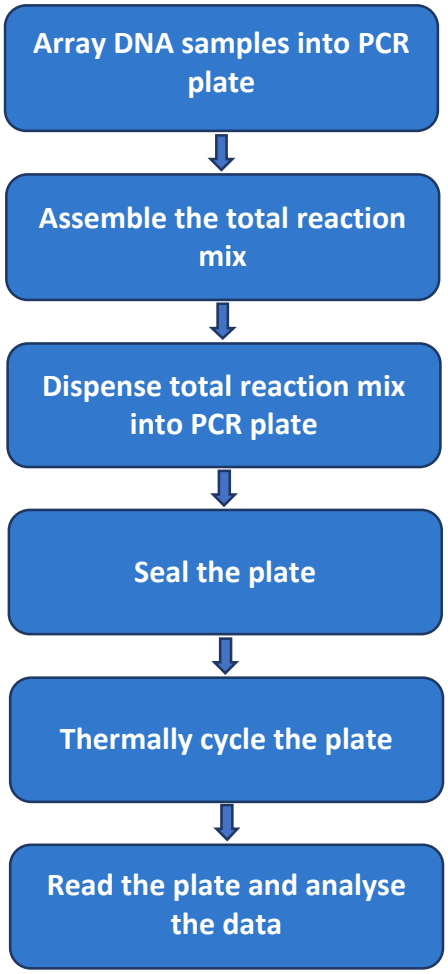
This product should only be handled by trained laboratory personnel. It is advisable to wear suitable PPE when using the product. In case of contact with skin or eyes, wash immediately with water.

Using ProbeSure-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 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%.

ProbeSure-IR™ genotyping process flow



	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 ProbeSure-IR GMM	5.0	2.5	0.8	5.0	2.5	0.5	0.4
Assay mix	variable						
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**	**10.0**	**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.**

ProbeSure-IR™ Quick Reference Guide

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.
   ProbeSure-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	N°. Cycles
1	Enzyme activation	95˚C	10-15 min	1
2	Template denaturation	95˚C	10-15 secs	35-45
	Annealing and extension	57-65˚C	60 secs	

***Table 2*. Thermal cycling conditions for ProbeSure-IR™.**

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*.

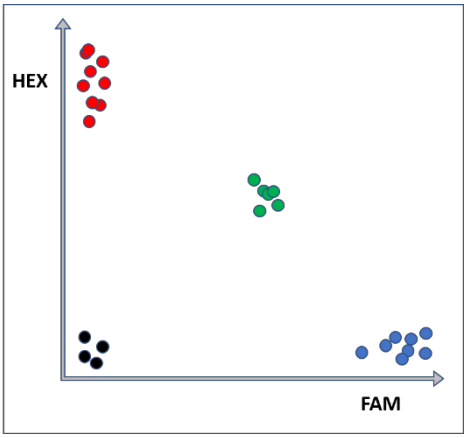


***Figure 1*. Example cluster plot for ProbeSure-IR™ genotyping data. The red and blue clusters are homozygous for the alleles reported with HEX and FAM, respectively, whilst the green cluster represents individuals that are heterozygous for the polymorphism.**

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.

Description	Temperature	Time	N°. Cycles
Template denaturing	95˚C	10-15 secs	3
Annealing and extension	57-65˚C	60 secs	

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

Ordering information

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