BHQ Probe Master Mix quick start guide

For research use only. Not for use in diagnostic procedures.

This document provides an overview of the steps for performing endpoint genotyping using BHQ® Probe Master Mix. For more details on any of the steps, please refer to the full user manual, that can be accessed at: www.lgcgroup.com/BHQMasterMix.

It is assumed that:

1. A suitable probe-based assay has been ordered for your experiment.
2. You will need DNA template and water in addition to your probe-based assay.
3. DNA is at the appropriate concentration for the genome size of your organism and has been dispensed into reaction plates or Array Tape®.

Step	Procedure	Details
1	**Prepare a primer-probe mix (if applicable)**	• Please note: To perform endpoint genotyping using primers and BHQ or BHQplus® Probes, two primers and two probes are required. Each probe should be labelled with a unique fluorophore and BHQ quencher for the detection of each allele. • If you are using a pre-combined primer-probe mix, such as ValuMix™ Assays for SNP genotyping, proceed to Step 2 (assuming mix has been hydrated). • Hydrate primers and probes to desired stock concentration e.g. 100 µM. • Prepare a primer-probe mix containing the two primers and the two probes e.g. a 20x mix. • If using BHQ Probes and primers, LGC suggests a final reaction concentration of 900 nM for each primer and 200 nM for each probe.
2	**Prepare a genotyping mix**	• Combine the primer-probe mix prepared in Step 1 with 2x BHQ Probe Master Mix. • Prepare a sufficient volume for the planned experiment.
3	**Dispense genotyping mix**	• Dispense the prepared genotyping mix across the DNA samples. • Ensure that the final concentration of BHQ Probe Master Mix is 1x. If dried down DNA is used, include water in the genotyping mix (in Step 2) to adjust the final concentration of BHQ Probe Master Mix.
4	**Perform the thermal cycle**	• Essential: 95 ºC for 15 min is required to activate the enzyme. • PCR protocol can be programmed as per experimental requirements.
5	**Read fluorescence of completed reactions**	• Completed reactions should be read on an appropriate FRET-capable plate reader. • Please refer to the information supplied with your probe(s) to determine the appropriate read settings for your completed reactions. • The passive reference dye ROX (if included) is read at the following wavelengths: Excitation 575 nm, Emission 610 nm.
6	**Plot data in Cartesian plot**	• Endpoint genotyping data should be plotted in a cluster plot format. • Please refer to the information supplied with your probe(s) to determine which fluorophore corresponds to which allele.

www.lgcgroup.com/genomics • genomics@lgcgroup.com

@LGCGenomics LGC.Genomics lgc-genomics

Science for a safer world