BHQplex CoPrimers: ultimate flexibility for multiplex PCR

Multiplex PCR with built-in flexibility for assay interchangeability

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

BHQplex CoPrimers: maximise multiplex PCR

BHQplex™ CoPrimers™ enable multiplex PCR, whilst maintaining workflow flexibility. Their unique design allows assay interchangeability, overcoming a common shortfall of traditional multiplex PCR technologies. The added flexibility of interchangeability in turn supports a much broader range of applications including gene expression, adventitious presence (AP) testing, copy number variation, and SNP differentiation. Now you can think beyond the panel-based workflows for SNP genotyping, common in marker-assisted selection (MAS) breeding programs. Maximise multiplex PCR with BHQplex CoPrimers.

BHQplex CoPrimers enables assay interchangeability without cross-reactivity, for flexible multiplexing

The unique oligonucleotide structure of BHQplex CoPrimers probes reduces the formation of primer dimers and enhances binding specificity to the target.

This structure enables interchangeability of assays from a pre-designed functional set without introducing cross reactivity.

BHQplex reduces data turn-around times

BHQplex CoPrimers generates twice as much data per well in dual-plex format, so projects require less time to generate the same amount of data.

BHQplex CoPrimers drives laboratory efficiencies, reducing costs

Multiplex PCR has shown to reduce cost per SNP call by 30-40% compared to single-plex PCR due to master mix, plastics, and labour savings. Multiplex PCR increases laboratory capacity without additional headcount or capital equipment.

Wide range of fluorescent dyes to fit most PCR detection instruments on the market

The BHQplex CoPrimers chemistry can be used on the same liquid handling instruments as other hydrolysis and non-hydrolysis probe PCR chemistries.

Figure 1 outlines the BHQplex CoPrimers mechanism of action.

BHQplex CoPrimers genotyping system

The fluorescently-labelled BHQplex CoPrimers consists of a short “Primer” sequence and a “Capture” sequence, connected by a flexible linker to enable cooperative binding of the fragments to the target of interest (Fig. 1).

The non-extendable flexible linker connecting the Primer and the Capture sequence prevents the polymerase from extending through the Capture sequence, retaining the primer specificity in each round of amplification.

Due to its abbreviated length, the Primer sequence allows for a design of highly selective primers capable of detecting allelic changes as short as a single nucleotide.

Non-specific amplicons that do not have a complementary region to the Capture sequence, such as primer dimers, are not propagated. Because this process is repeated during each PCR cycle, it results in an exponential reduction of nonspecific amplification.

When the variant is placed at the optimal position near the 3’ end of the Priming sequence, BHQplex CoPrimers amplify the matched target preferentially, while the amplification of the mismatched target is significantly suppressed.

BHQplex CoPrimers contain an internal “Black Hole Quencher®” (BHQ®) dye, which greatly reduces the fluorescence emitted when in proximity to the reporter dye. On extension of the Priming fragment, the fluorescent dye is cleaved, releasing the fluorophore, and generating a fluorescent signal.

Fig. 1a: Ingredients

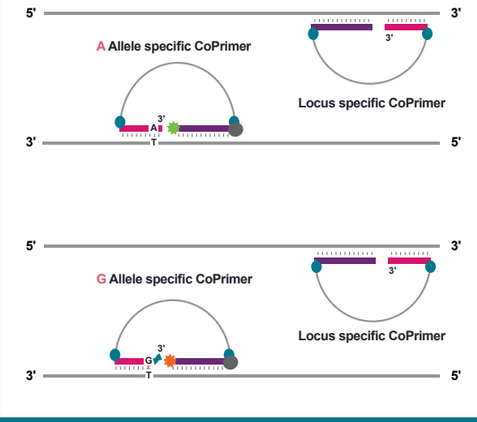

Fig. 1b: The reaction

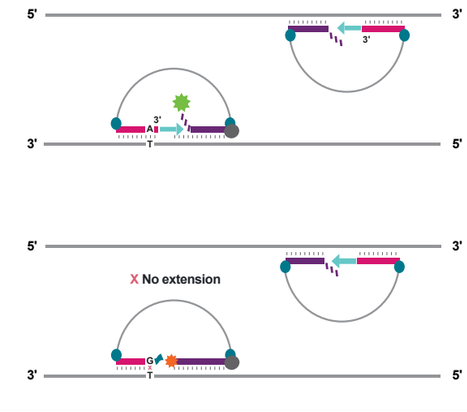

Fig. 1: Alelle-specific BHQplex CoPrimers (in this case, interrogating a [G/A] nucleotide change) are designed to hybridise over the variant with the interrogating nucleotide being positioned at the penultimate base of the Priming sequence.

Fig. 1a: [A]-BHQplex CoPrimers hybridises to the bottom strand of the [A] template, forming a perfect match. Subsequently, the DNA polymerase initiates from the priming sequence, and the green fluorophore on the capture fragment is cleaved.

Fig. 1b: [G]-BHQplex CoPrimers hybridises to the bottom strand of the [A] template, but fails to amplify due to the lack of 3’ end complementarity.

Legend

- A/G Allele-specific priming agents (forward primer)
- Locus-specific priming segment (reverse primer)
- Capture segments (forward and reverse primers)
- Flexible linker
- Fluorophores
- Quencher
- Primer extension

How do I get my BHQplex CoPrimers?

Our proprietary software enables us to design BHQplex CoPrimers for multiplex PCR that increase your assay specificity and allow interchangeability.

Email us at the address below to learn how your lab can reduce costs and improve efficiency to achieve more than you thought possible.

genomics@lgcgroup.com

BHQplex CoPrimers: do more with less.

Integrated tools. Accelerated science.

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