Guide

KASP assay design – anchoring

For submission of sequence information

BIOSEARCH™
TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Guide

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1. Introduction

Anchoring is a special approach to KASP™ assay design. It enables the KASP assay to differentiate between two highly similar sequences. Assay primers are designed to make use of a single unique base (or bases) that is only present in the sequence of interest and is not found in any similar sequences that may occur in the genome. Once identified, the unique base(s) can be used to "anchor" the assay to the region of interest.

2. How anchoring works

A typical KASP assay utilises three primers; two allele specific forward primers and a reverse common primer. The two allele specific primers differ only at the 3' end with each primer being specific to one of the different alleles to be genotyped. It is the allele specific primers that result in the generation of the fluorescent signal that is measured upon completion of the reaction.

In a standard (non-anchored) assay the common reverse primer is needed only to drive the PCR. Anchoring makes additional use of the reverse primer. Anchoring requires that the flanking sequence around the target single nucleotide polymorphism (SNP) or insertion or deletion (indel) must contain one or more bases that are unique to the sequence of interest. The unique base(s) must be within 70 bp of the SNP or indel. These bases can be used as an "anchoring point" to make the assay specific to only the region of interest. In anchored assays the reverse common primer is designed with the unique base(s) at the 3' end. This will mean that the anchored assay will preferentially amplify the region that contains the anchoring point.

Anchoring the assay via the reverse common primer is similar to the function of the allele specific forward primers whereby the primer with the correct complementary base at the 3' end will preferentially anneal and extend. The 3' end of the primers (both the allele-specific and anchored commons) are important in introducing the specificity of the reaction because the Taq polymerase complex will initiate extension from the 3' end of primers. Any mismatching or non-complementary sequence at the 3' end of the primer will cause the PCR to stall.

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3. When anchoring assays can help

Anchoring allows KASP assays to genotype SNPs or indels in DNA samples that are known to contain multiple DNA sequences that are highly similar to the sequence surrounding the polymorphism of interest.

Without anchoring the presence of highly similar sequences would have a negative effect on the specificity of the reaction and could result in the assay failing to correctly report all genotypes and / or a lack of separation between the genotyping clusters.

The two most common scenarios in which anchored KASP assays are beneficial are:

- Homology – anchoring of KASP assays is recommended when the target sequence for the KASP assay is not unique within the genome, and sequences that are highly similar to the target sequence are present at one or more additional locations in the same genome.

- Polyploid genomes – anchoring of KASP assays is of benefit when genotyping allopolyploids i.e. organisms with multiple genomes that are derived from two or more ancestral species. Anchoring can generally be used in cases when the target sequence is not unique within the multiple genomes as a result of orthologous sequences that are present in one or more of the multiple genomes.

4. How to submit sequences for anchoring

Please submit sequence information for your target SNPs or indels using the appropriate LGC, Biosearch Technologies' SNP submission form. When entering the flanking sequences, any base(s) that you would like to use as an anchoring point should be surrounded by "<" and ">" (figure 1).

SNP ID	Sequence
Example01	ATAACTACTTTTAAAGGCACATTATTCAACCTCACT**\<G\>**TTTCATCCTC**[A/T]** CATAATGACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG

Figure 1. Example of a sequence entered into Biosearch Technologies' SNP submission form. The target SNP (highlighted in **pink**) must be surrounded by "[" and "]", the anchor point (highlighted in **teal**) must be surrounded by "<" and ">" .

For details of how to format your sequence submission please refer to section 2 of the KASP assay design guide.

For best results the anchoring point should be as close as possible to the target polymorphism. Please do not submit sequences with anchoring points that are >70 bp from the target polymorphism. These would be too far away from the polymorphism to design a robust KASP assay and will therefore be disregarded by Biosearch Technologies' assay design software.

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5. Examples

Example 1 – Homology

If the sequence surrounding the target polymorphism is affected by homology then anchoring can be used to make the KASP assay specific to the target sequence. This is shown in the following example (figure 2).

Anchor

Example

SNP@Chr6:6638363	AACATGGCGTCCGGGGGTAAGATAAGACC**K**AACCGTAA**G**TACCCACTGCAGCCAAGAGTT
Chr3:54828053	TGGCGTCCGGGGGTA**T**GATAAGACCTAACCGTAA**A**TACC**G**ACTGCAGCCAAGAGTT
Chr6:3395586	CATGGCGTCC**T**GGGGTAAGATAAGACCTAACCGTAA**A**TACC**G**ACTGCA
Chr14:14221507	GGTA**T**GATAAGACCTAACCGTAA**A**TACCCACTGCAGC**CAA**GCAAT
Chr11:2891379	CCGGGGGTA**T**GATAAGACCTAACCGTAA**A**TACC**G**ACTGCA
Chr7: 67545571	GGGGGTAAGATAAG**G**CCTAACCGTAA**A**TAC

Figure 2. An example alignment showing the target SNP with flanking sequence and homologous sequences. **The target SNP is coloured pink, the anchor point is coloured teal** and **bases which do not match the target sequence are coloured orange**.

In the alignment shown above (figure 2) the very top sequence is the target SNP with its flanking sequence. The lower sequences represent homology located elsewhere in the genome. The unique base in the target sequence that can be used as an anchoring point is shown.

To submit this sequence to Biosearch Technologies for anchored KASP assay design it should be entered into Biosearch Technologies' SNP submission form as shown below (figure 3).

SNP ID	Sequence
Example02	AAACATGGCGTCCGGGGGTAAGATAAGACC[T/G]AACCGTAA<G>TACCCACTGC AGCCAAGAGTT

Figure 3. The target SNP sequence from figure 2 as it should be entered into Biosearch Technologies' SNP submission form with both the [SNP] and the <anchor point> correctly identified in the sequence.

Example 2 – Polyploid genome

When genotyping organisms with polyploid genomes, anchoring can be used to make the KASP assay specific to a single target genome. This is shown in the following example (figure 4).

D genome anchor

Example

Chr4D	CCGTTAGGGTCGCGTCCT**C**ACTCAGCGG**S**GGTCCAAATTGGAGTCGGTTTGATCGCAAGG
Chr4A	CCGTTAGGGTCGCGTCCTAACTCAGCGGGGGTCCAAATTGGAGTCGGTTTGATCGCAAGG
Chr4B	CCGTTAGGGTCGCGTCCTAACTCAGCGG**S**GGTC**T**AAATTGGAGTCGGTTTGATCGCAAGG

Figure 4. An example alignment from a polyploidy organism showing the three (A,B and D) genomes. A SNP present in the top and the bottom sequence is shown by the IUPAC ambiguity code "S".

B genome anchor

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This example (figure 4) shows three genomes (A, B and D). There is SNP present in both the D and the B genomes. In this example anchoring can be used to design a KASP assay to target either the D or the B genome.

To submit this sequence to Biosearch Technologies for anchored KASP assay design it should be entered into Biosearch Technologies' SNP submission form. Please note that each anchor is only specific to a single genome and, if both the possible genome specific assays are required, the sequences must be submitted on separate lines as shown below (figure 5).

SNP ID	Sequence
D-genomeSNP	CCGTTAGGGTCGCGTCCT<C>ACTCAGCGG[C/G]GGTCCAAATTGGAGTCGGTT TGATCGCAAGG
B-genomeSNP	CCGTTAGGGTCGCGTCCTAACTCAGCGG[C/G]GGTC<T>AAATTGGAGTCGGTT TGATCGCAAGG

Figure 5. The SNP sequences from figure 4 as they should be entered into Biosearch Technologies' SNP submission form with both the [SNP] and the <anchor points> correctly identified in the sequences.

Example 3 – Multiple anchor points

If there are multiple possible anchoring sites available in the sequence flanking the target polymorphism then they can all be included in the sequence(s) submitted to Biosearch Technologies. This is shown in the following example (figure 6).

	Anchor, Anchor, Anchor
Example	
SNP@Chr9:5358261	AAGCTGCT**TAGG**TTTAGTAGGCGGTAGACG**W**TGCCAGCAAAGGT**A**GAGATC**G**ATAGAATT
Chr2:17765942	AAGCTGCT**AGAT**TTTT**T**GTAGGCGGTAGACGATGCCAGCAAAGGT**G**GAGATC**C**ATAGAATT
Chr5:20581366	AAGCTGCT**AGAT**TTTAGTAGA**C**GGTAGACGATGCCAGCAAAGGT**G**GAGATC**C**ATA**C**AATT

Figure 6. An example alignment showing the target SNP with flanking sequence and homologous sequences. This example includes multiple anchor points.

When submitting sequences with multiple anchor points all of the possible anchors should be surrounded by "<" and ">". If the anchor point consists of several consecutive bases then "<" should be added before the first base and ">" should be added after the final base so that the entire anchor point is surrounded by "<" and ">". Biosearch Technologies' assay design software will use the best anchor from all those identified in the submitted sequence.

To submit the sequence as shown in figure 6 to Biosearch Technologies for anchored KASP assay design it should be entered into Biosearch Technologies' SNP submission form as shown below.

SNP ID	Sequence
Example03	AAGCTGCT<TAGG>TTTAGTAGGCGGTAGACG[A/T]TGCCAGCAAAGGT<A>GAG ATC<G>ATAGAATT

Figure 7. The SNP sequence from figure 6 as it should be entered into Biosearch Technologies' SNP submission form with the [SNP] and the multiple <anchor points> correctly identified in the sequence.

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6. Useful links

[Biosearch Technologies Genotyping services SNP submission template](#)
Use this form for submission of sequence information for genotyping service projects.

[Biosearch Technologies KOD and KBD SNP submission template](#)
Use this form for submission of sequence information for KASP assays for use in your own laboratory.

[KASP assay design guide](#)
Guide explaining how to submit DNA sequence information to Biosearch Technologies.

[Request a free KASP trial kit](#)
Trial kit to try KASP genotyping in your own lab.

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