

# Guide

## KASP assay design

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For submission of sequence information

# Guide

KASP assay design

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# Guide

## KASP assay design

This document explains how sequence information should be submitted for KASP™ assay design. The different polymorphisms that can be detected by KASP are outlined, with examples of how the sequence information should be submitted (using the appropriate sequence submission form). Submitted sequences should be formatted as outlined in section 2 to ensure that your order is processed efficiently.

### 1. KASP chemistry: assay design capabilities

KASP assays can be used for the detection of the following polymorphism types:

- a. Single nucleotide polymorphisms (SNPs)
- b. Insertions or deletions (indels)
- c. Multi-nucleotide variants (MNVs)
- d. Haplotypes

Please note that for all assay design requests LGC Biosearch Technologies™ require a minimum of 50 bp of sequence on each side of the polymorphism.

Examples of the sequence format that should be used for each type of polymorphism are illustrated below.

#### a. Single nucleotide polymorphisms

KASP assays can be designed to detect bi-allelic single nucleotide polymorphisms (SNPs) within any organism. The SNP of interest should be submitted within [square brackets] and can either be formatted as [allele1/allele2] or [IUPAC code].

Example SNP ID	Sequence
SNP_alleles	GCTCTCTGTGTCTGATCCAAGAGGCGAGGCCAGTTTCATTTGAGCATTAA [A/G] TGTC AAGTTCTGCACGCTATCATCATCAGGGCCGAGGCTTCTCTTTGTT
SNP_IUPAC	GCTCTCTGTGTCTGATCCAAGAGGCGAGGCCAGTTTCATTTGAGCATTAA [R] TGTC AAGTTCTGCACGCTATCATCATCAGGGCCGAGGCTTCTCTTTGTT
rs12913832	GCTCTCTGTGTCTGATCCAAGAGGCGAGGCCAGTTTCATTTGAGCATTAA [A/G] TGTC AAGTTCTGCACGCTATCATCATCAGGGCCGAGGCTTCTCTTTGTT

Figure 1. Examples of how to format sequence for KASP assay design for a SNP. Alleles are coloured teal and pink in the first example, and the appropriate IUPAC code is coloured orange in the second example.

When submitting SNPs that are identified with RefSNP (rs) numbers the sequence is optional. It is advisable to submit the sequence in addition to the rs number to ensure that Biosearch Technologies are using exactly the sequence that you require.

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### b. Insertions or deletions

KASP assays can be designed for the detection of insertions or deletions (indels) of any size. One allele-specific primer will be designed to incorporate the indel sequence and the second allele-specific primer will be designed to the same target sequence excluding the indel. The common reverse primer will be designed to sequence present in all samples.

Example SNP ID	Sequence
1bp_indel	ATTGTCAATAATATGTGGAGGTCGTGTTGGTACAACATGATAGCATAAAG [ <b>A</b> / ] GTGATGATTAGTTTCCGTAATGCGTCCGCTGGGCTGATGGGCCTTCTGGA
1bp_indel_alt	ATTGTCAATAATATGTGGAGGTCGTGTTGGTACAACATGATAGCATAAAG [ <b>A</b> / - ] GTGA TGATTAGTTTCCGTAATGCGTCCGCTGGGCTGATGGGCCTTCTGGA
15bp_indel	ATTGTCAATAATATGTGGAGGTCGTGTTGGTACAACATGATAGCATAAAG [ <b>AGATCCATACATTTA</b> / ] GTGATGATTAGTTTCCGTAATGCGTCCGCTGGGCTGATGGGCCTTCTGGA
15bp_indel_alt	ATTGTCAATAATATGTGGAGGTCGTGTTGGTACAACATGATAGCATAAAG [ <b>AGATCCATACATTTA</b> / - ] GTGATGATTAGTTTCCGTAATGCGTCCGCTGGGCTGATGG GCCTTCTGGA

Figure 2. Examples of how to format sequence for KASP assay design for indels. The 'insertion' allele is shown in teal in all examples. The 'deletion' allele is omitted and may optionally be represented by a dash as shown in the second and fourth examples (pink dash).

Detection of large indels is also possible using KASP. There is no upper size limit for the indel, aside from the requirement for the sequence to all be present on the same strand of DNA. To submit an indel that is larger than 100 bp you should provide 50 bp of sequence at the start and end of the indel region within the square bracket and add an N in the centre to represent the remainder of the indel sequence.

Example SNP ID	Sequence
Large_indel	ATTGTCAATAATATGTGGAGGTCGTGTTGGTACAACATGATAGCATAAAG [ <b>AGATCCATACATTTAAGTCGGTCAAATTATGGATCCTTCAAGATCTTAAGNCCTTAA</b> <b>GGTAACTTGACTTAGTGCATAGGTGGAAGGTTTATAGTAGCT</b> / ] GTGATGATTAGTTT CCGTAATGCGTCCGCTGGGCTGATGGGCCTTCTGGA

Figure 3. Example of how to format sequence for KASP assay design for a large indel. Within the square brackets, 50 bp at the start and end of the indel should be specified (shown in teal) with an N in the centre (shown in purple) to represent the remainder of the sequence. The 'deletion' allele is omitted in this example.

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### c. Multi-nucleotide variants

Detection of multi-nucleotide variants (MNV) is possible using KASP. Sequence information for MNVs should be submitted using the [allele1/allele2] format.

Example SNP ID	Sequence
MNV	TGGGCAGCATTAGTAGAAGAAAGTACAAGACCGTGTGTAGAGGATACTCT [GATATACTTGAG/CAGTCC]AGCAGATAGCGTTGGATAGGCGACAGGATTATTGGAG CGCCGTCGAGAAC

Figure 4. Example of how to format sequence for KASP assay design for a MNV. Alleles are coloured teal and pink in this example.

If either or both of the MNV alleles are longer than 100 bp then you should submit 50 bp of sequence at the start and end of the allele with an N in the centre to represent the remainder of the MNV allele sequence.

Example SNP ID	Sequence
Large_MNV	TGGGCAGCATTAGTAGAAGAAAGTACAAGACCGTGTGTAGAGGATACTCT [GATATACTTGAGGTGTCAGTTGATGCTTGATATACTTGACGTTCTCTACANTGTGCCA GCTACGCCGATGGGCGGTCCACACGGAATTCAGAGGTGGGATT /CAGTCCGCAGCTAGGGGGGTGATGGTTCGCAAGAAAATATACGGTCTATGNATGTAAC TTTTATCATAAAGGTAGACACCAGTTATTGTTTCGGGTCGTAGA]AGCAGATAGCGTTGG ATAGGCGACAGGATTATTGGAGCGCCGTCGAGAAC

Figure 5. Example of how to format sequence for KASP assay design for a large ( $\geq 100$  bp) MNV. Alleles are coloured teal and pink in this example. The N representing the remainder of the MNV allele sequence is coloured purple.

### d. Haplotypes

KASP assays can be designed to detect haplotypes of any size. Sequence information for haplotypes should be submitted using the [allele1/allele2] format. The [square brackets] should surround all SNPs that are part of the haplotype. Any nearby SNPs that are not considered to be part of the haplotype should be outside of the square brackets and should be identified using the appropriate IUPAC ambiguity code.

Example SNP ID	Sequence
Haplotype	CAAACACCAAACCTGGTGTAGTCGTGGTTTACAACACGGGAGTTCAAACCTG [TATCCGAATGACGAATGTTACGTCCTTAAAC /CATCCGAATCACGAATGTTACGTCCTTAAAG]CATCATGAAATGAGTTTAGTTTGGG TGGCTCGTAAGTAGACATAAGGCAC

Figure 6. Example of how to format sequence for KASP assay design for haplotypes. Alleles are coloured teal and pink in this example.

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### 2. Formatting guidelines for sequence submission

Sequence information for assay design should be submitted using the appropriate SNP submission template (see Section 4).

#### SNP ID

Use this field to provide a unique identifier for the assay (i.e. an assay name).

- Can be up to 20 characters in length
- Use alphanumeric and underscore characters only e.g. ABCD1234\_ab
- Do not begin the name with a hyphen (-)

#### 2.1 Sequence information

##### Sequence orientation

Enter the sequence in the 5' – 3' orientation.

##### Upper case and lower case bases

Enter the sequence information using upper case characters (ATCG). If particular regions of the surrounding sequence are less certain, use lower case characters to identify the uncertain bases (atcg). A combination of both upper case and lower case characters is acceptable.

##### Identify the target SNP using square brackets

The SNP of interest (target site) must be identified by surrounding it with square brackets. Use the appropriate IUPAC code (e.g. [R]) or a forward slash (/) character to denote the two possible alleles (e.g. [A/G]). The IUPAC codes for ambiguous nucleic acids are detailed in table 1 for your reference.

Symbol	Translation
R	A or G
Y	C or T
M	A or C
K	G or T
S	C or G
W	A or T
N	A or C or G or T

Table 1. IUPAC codes for ambiguous specification of nucleic acids.

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### Identify the target indel using square brackets

The location of the indel of interest (target site) must be identified by surrounding it with square brackets.

See figures 2, 3 and 4 for examples of how to format sequence for indels of varying sizes.

### Surrounding SNPs and indels

If there are additional known SNPs in the sequence surrounding the SNP of interest, these must be identified using IUPAC codes. These surrounding SNPs must not be surrounded by square brackets. Do **not** mark surrounding SNPs as Ns.

If there are known indels in the sequence surrounding the SNP of interest, these must be identified as Ns.

These surrounding indels must not be surrounded by square brackets.

### Avoiding bases

If there are particular regions of sequence that should be completely avoided during assay design, replace these bases with an N.

### Acceptable sequence submission formats

Acceptable sequence submission formats	Example
[First allele/second allele]	ATAACTACTTTTTAAAGGCACATTATTCAACCTCACTGTGCATTTTCATCCTC [A/T] TGCATA ATGACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG
IUPAC code	ATAACTACTTTTTAAAGGCACATTATTCAACCTCACTGTGCATTTTCATCCTC [W] TGCATAA TGACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG
Rs Number	rs123456* *NOTE: It is advisable to submit the sequence in addition to the rs number to ensure that Biosearch Technologies are using exactly the sequence that you require.
indels	ATAACTACTTTTTAAAGGCACATTATTCAACCTCACTGTGCATTTTCATCCTC [/T] TGCATAAT GACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG

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### Unacceptable sequence submission formats

Unacceptable sequence submission formats	Example
Multiple IUPAC codes without marking the target site in square brackets	ATAACTACTTTTAAAGGCACATTATSCAAACCTCAYYTGCATTTTCATCCTCWTGCATAAT GACAGTTRTTCTCTCCCAAGTCTCCWWCTGGCACAGTTAAAG
Insufficient surrounding sequence (<50 bases either side)	CACTGTGCATTTTCATCCTC [A/T] TGCATAATGACAGTTATTCTCT
Surrounding SNPs marked with square brackets	ATAACTACTTTT [T/G] AAAGGCACATTATT [C/G] AACCTCACTGTGCATTTTCATCCTC [A/T] TGCATAATGACAGTTATTCTCTCCA [A/C] GTCTCCTTCTGGGACAGTTAAAG

### Marking special design requirements

Marking special requirements	Example	Design
Surrounding polymorphisms – mark these with IUPAC codes (without square brackets)	ATAACTACTTTTAAAAGGCACATTATTSAACCTCACTGTGCATTTTCATCCTC [A/T] TGCATAATGACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG	Primer design software will take surrounding SNPs into account during primer design.
Avoiding bases – designate as Ns	ATAACTACTTTTAAAGGCACAMTATTCAACCTCACTGTG CATTTNATCCNN [A/T] TGCATAATGACAGTTATTCTCTCC AAGTCTCCTTCTGG	Primer design software will avoid the bases designated as Ns in assay primers.
Suspicious bases – user lower case characters	ATAACTACTTTTAAAGGCACATTATTCAACCTCActgtgcat ttcatcctC [A/T] TGCATAATGACAGTTATTCTCTCCCAAGTCT CCTTCTGG	Bases in lower case characters will be avoided by primer design software.

### 3. Homologous sequences

When designing KASP assays, homologous sequences should be avoided. If this is not possible, anchoring may be used to increase the specificity of the assay. For more details on anchoring, please view our [guide to anchoring](#).



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### 4. Submitting sequence information to Biosearch Technologies

Sequence information for KASP assay design must be submitted to Biosearch Technologies using the appropriate SNP submission template (Excel format).

#### Genotyping service projects

Please [download](#) the sequence submission form for genotyping service projects.

#### Assays to be run in your own laboratory

Please [download](#) the sequence submission form for KASP assays to run in your own laboratory.

To run KASP genotyping in your own laboratory you will require both KASP Assay Mix and KASP Master Mix. Biosearch Technologies manufacture KASP Master Mix with three different levels of ROX; these are the Low, Standard, and High ROX formulations. These formulations only differ in the level of ROX that they contain and are otherwise identical. To determine the appropriate version of KASP Master Mix for your qPCR instrument or plate reader, please visit [our website](#).



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