



KASP assay design

Guide to submission of sequence information

This document is intended as a guide to 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 SNP submission form). Submitted sequences should be formatted as outlined in Section 2, to ensure that your order is processed efficiently.

1. KASP chemistry: product capabilities

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

Single nucleotide polymorphisms

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

```
CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTATTAAGTCGAGC[C/G]
AGCTGCTAGACGTCGCAGTCGACACAGCTAGCCTAGGACAAAGTCTCGTG

CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTGATTAAGTCGAGC[S]
AGCTGCTAGACGTCGCAGTCGACACAGCTAGCCTAGGACAAAGTCTCGTG
```

Figure 1. Examples of how to format sequence for KASP assay design for a SNP

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 standard sequence. The common reverse primer will be designed to sequence present in all samples.

```
CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTGGCTAGCTGATTAA[A/]
AGCTGCTAGACGTCGCAGTCGACACTGACGTCCTAGGACAAAGTCTCGTG

CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTGGCTAGCTGATTAA[A/-]
AGCTGCTAGACGTCGCAGTCGACACTGACGTCCTAGGACAAAGTCTCGTG
```

Figure 2. Examples of how to format sequence for KASP assay design for a 1 bp indel.

```
CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTGGCTAGCTGATTAA[ATAGACGTCGATCGT/]
AGCTGCTAGACGTCGCAGTCGACACTGACGTCCTAGGACAAAGTCTCGTG
CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTGGCTAGCTGATTAA[ATAGACGTCGATCGT/-]
AGCTGCTAGACGTCGCAGTCGACACTGACGTCCTAGGACAAAGTCTCGTG
```

Figure 3. Example of how to format sequence for KASP assay design for a 15 bp indel.

Large indels

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 sequence for a large indel, 50 bp of sequence at the start and end of the indel region should be included within the square bracket with an N in the centre to represent the remainder of the indel sequence.

```
CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTGGCTAGCTGATTAA[TGCTAGACGTCGCAGT
CGACTGCTAGACGTCGCAGTCGACACGTCGCAGTNTGCTAGACGTCGCAGTCGACTGCTAGACGT
CGCAGTGACACGTCGCAGT/]AGCTGCTAGAGCTGCTAGACGTCGCAGTCGACACTGACGTCCTAG
GACAAAGTCTCGTG
```

Figure 4. 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 with an N in the centre to represent the remainder of the sequence.

2. Formatting guidelines for sequence submission

Sequence information for assay design should be submitted using the appropriate SNP submission template (see Section 3). Please note that we require 50 bases of sequence on each side of the submitted polymorphism.

Assay name field

Use this field to provide a unique identifier for the assay.

- 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 (-)

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) should be identified by surrounding it with square brackets. Use the appropriate IUPAC code (e.g. [R], [S]) or a forward slash (/) character to denote the two possible alleles (e.g. [A/G]).

Identify the target indel using square brackets

The location of the indel of interest (target site) should 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 known SNPs in the sequence surrounding the SNP of interest, these should be identified using IUPAC codes. These surrounding SNPs should 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 should be identified as Ns. These surrounding indels should 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]	ATAACTACTTTTAAAGGCACATTATTCAACCTCACTGTGCATTTTCATCCTC[A/T]TGCATA ATGACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG
IUPAC code	ATAACTACTTTTAAAGGCACATTATTCAACCTCACTGTGCATTTTCATCCTC[W]TGCATAA TGACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG
Rs Number	Rs123456* *N.B. It is advisable to submit the sequence in addition to the Rs number to ensure that LGC Genomics are using exactly the sequence that you require.
Indels	ATAACTACTTTTAAAGGCACATTATTCAACCTCACTGTGCATTTTCATCCTC[/T]TGCATAAT GACAGTTATTCTCTCCCAAGTCTCCTTCTGGGACAGTTAAAG

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] TGCATAATGACAGTTATTCTCTCCCA[A/C]GTCTCCTTCTGGGACAGTTAAAG

Marking special design requirements

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

3. Submitting sequence information to LGC

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

Genotyping service projects

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

Assays to be run in your own laboratory

Please note that LGC manufacture KASP Master mix with varying levels of ROX. KASP Master mix is available in 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 instrument, please visit www.lgcgroup.com/mastermixcheck

1. KASP assays to run on qPCR instruments or FRET-capable plate readers
Please [download](#) the SNP submission form for KASP assays to run in your own laboratory.
2. KASP assays to run on the Fluidigm system
Please [download](#) the SNP submission form for Fluidigm assays.

For any queries about this guide please contact:

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