CRISPR/Cas9-based genome editing and validation screening

KAPA HiFi ensures high-fidelity amplification of targeting materials and screening libraries

CRISPR/Cas9 is a naturally occurring adaptive immune system in prokaryotes that has been adopted by biologists and optimized for highly specific, versatile, powerful genome editing.

The CRISPR/Cas9 system enables fast, precisely targeted genome cleavage followed by replacement of the original DNA with user-defined sequences. Some examples of the vast array of applications for CRISPR/Cas9 editing include:

- replacement of defective genes;
- enhancement or alteration of gene function; and
- targeted gene interruption or removal of target sequences.

Genome editing is only effective when the sequences of the guide RNAs and repair templates are correct.

- The sequence of the single-guide RNA (gRNA) is essential to accurately guide the Cas9 nuclease to the desired site.
- In experiments using a repair template, errors in the template will be incorporated into the genome.

Preparation of targeting materials with KAPA HiFi increases the accuracy of guide RNAs and repair templates, thus:

- improving the efficiency of CRISPR/Cas9 targeting;
- reducing off-target effects; and
- increasing accuracy of replacement sequences.

CRISPR is an acronym for Clustered Regularly Interspaced Short Palindromic Repeats. In the original bacterial systems, the guide RNAs recognized invading viral sequences that needed to be destroyed. By replacing these viral sequences with custom sequences, scientists can now direct the Cas9 nuclease to their targets of choice.

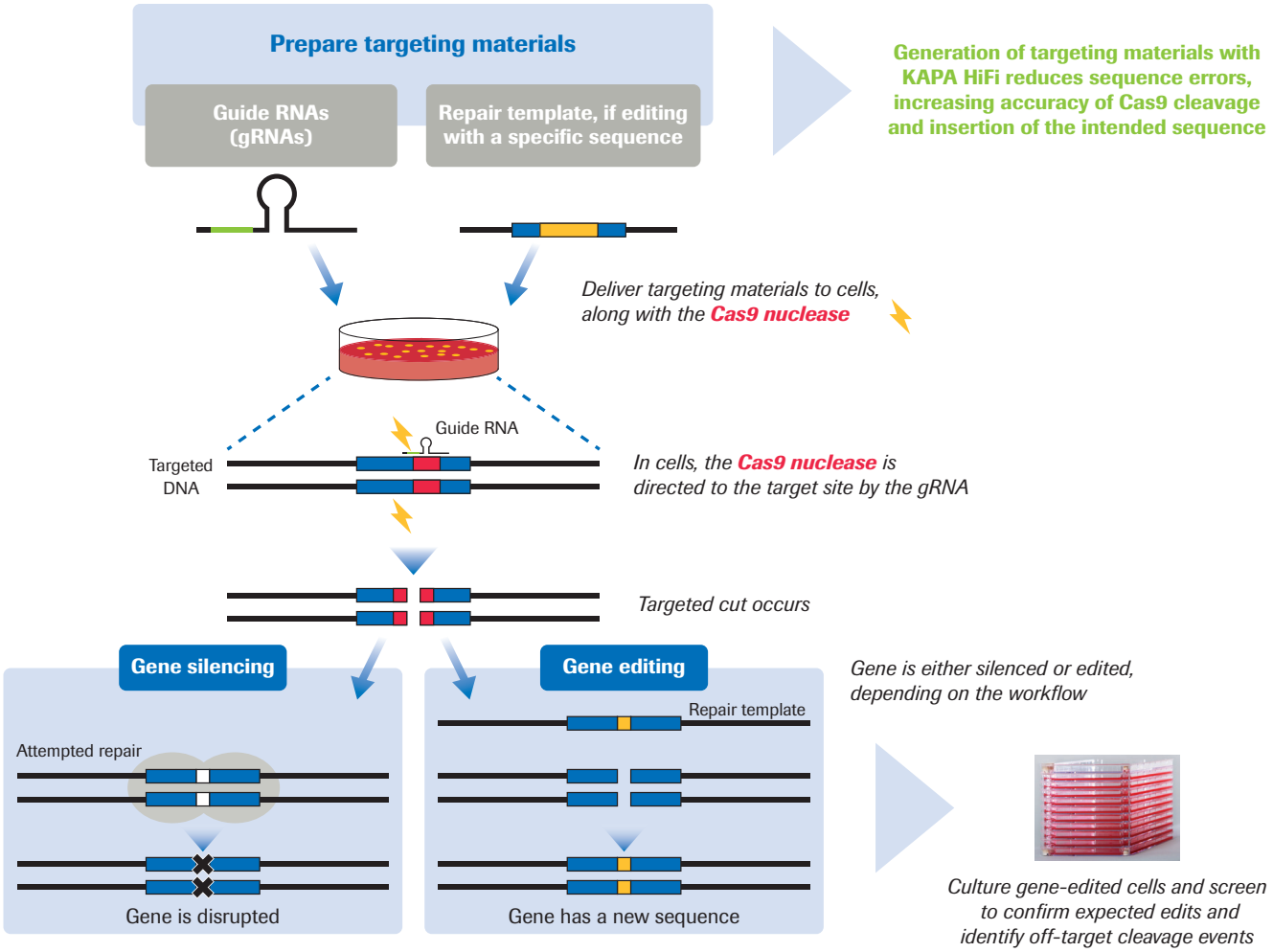


Figure 1. A high-level overview of the CRISPR/Cas9 workflow. Adapted from http://sitn.hms.harvard.edu/flash/2014/crispr-a-game-changing-genetic-engineering-technique.

Screening of CRISPR/Cas9 clones verifies accuracy of genome editing. Following isolation of clones, PCR-amplification of target sites creates the input material for NGS library preparation.

The low error rate and high processivity of KAPA HiFi ensures sensitive, accurate detection of target sites even across challenging genome regions, such as those with GC- or AT-rich content.

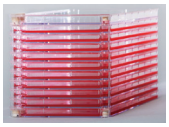
Researchers using CRISPR/Cas9 may also be interested in CIRCLE-seq, a method developed to screen for off-target Cas9 cleavage events. KAPA HiFi enhances the precision of CIRCLE-seq, ensuring that detected mutations are not amplification errors.

Consult our dedicated Support & Applications Scientists for guidance in using KAPA HiFi in CRISPR/Cas9 genome editing, validation screens, and CIRCLE-seq screening for off-target effects.

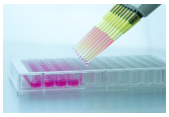
Contact us: **support.seqls@roche.com**

Additional resource:

Targeted CRISPR indel screening using an automated KAPA HyperPrep workflow. Roche Application Note 2018. Contact your Rep for the AppNote.



Clones of CRISPR/Cas9 edited cells



Generate amplicons with KAPA HiFi

Prepare sequencing libraries with KAPA HyperPrep

Library quantification with KAPA Library Quantification Kits

Sequencing

Figure 2. Overview of validation screen following CRISPR/Cas9 gene editing.

Ordering information

Code	Description	Pack size
KK2101	KAPA HiFi PCR Kit	100 U
KK2102	KAPA HiFi PCR Kit	250 U
KK2501	KAPA HiFi HotStart PCR Kit	100 U
KK2502	KAPA HiFi HotStart PCR Kit	250 U
KK2601	KAPA HiFi HotStart ReadyMix Kit	1.25 mL
KK2602	KAPA HiFi HotStart ReadyMix Kit	6.25 mL
KK2800	KAPA HiFi HotStart Uracil+ Kit	10 rxn
KK2801	KAPA HiFi HotStart Uracil+ Kit	50 rxn
KK2802	KAPA HiFi HotStart Uracil+ Kit	250 rxn

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For more information about other Roche products for this workflow, ask your Roche Sequencing representative or visit:
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