CIRCLE-seq for CRISPR/Cas9 Off-target Screening

KAPA HiFi minimizes amplification bias in CIRCLE-SEQ

CRISPR/Cas9 gene editing can result in off-target cleavage events, impacting the validity of final results. Cleavage of unintended targets can impair cell function, lead to cell death, or produce misleading conclusions. Thus, it is important to be able to screen for these off-target mutations.

There are several methods to screen for off-target Cas9 cleavage, each with advantages and disadvantages:

- **Digenome-seq:** An *in vitro* assay with a simple workflow, but lacking enrichment for cleavage sites
- **GUIDE-seq:** An assay that enriches for cleavage sites, but includes labor-intensive cell-based steps
- **CIRCLE-seq:** A sensitive *in vitro* assay that targets Cas9 cleavage sites, reducing sequencing costs (Figures 1 and 2)

CIRCLE-seq was developed to overcome the challenges associated with off-target screening. This method provides greater sensitivity and reproducibility than other workflows. CIRCLE-seq is also highly scalable, as it avoids the many limitations associated with culturing living cells.

KAPA HiFi enhances the precision of CIRCLE-seq, ensuring that detected mutations are not amplification errors.

- Error rates are 50 – 100 times lower than wild-type *Taq*
- Proofreading is enhanced by efficient 3' to 5' exonuclease activity
- Amplification bias is extremely low, ensuring uniform sequence coverage

CIRCLE-seq is an acronym for Circularization for *in vitro* reporting of cleavage effects by sequencing.
It is a rapid, accessible, scalable *in vitro* assay for sensitive detection of off-target CRISPR/Cas9 cleavage.

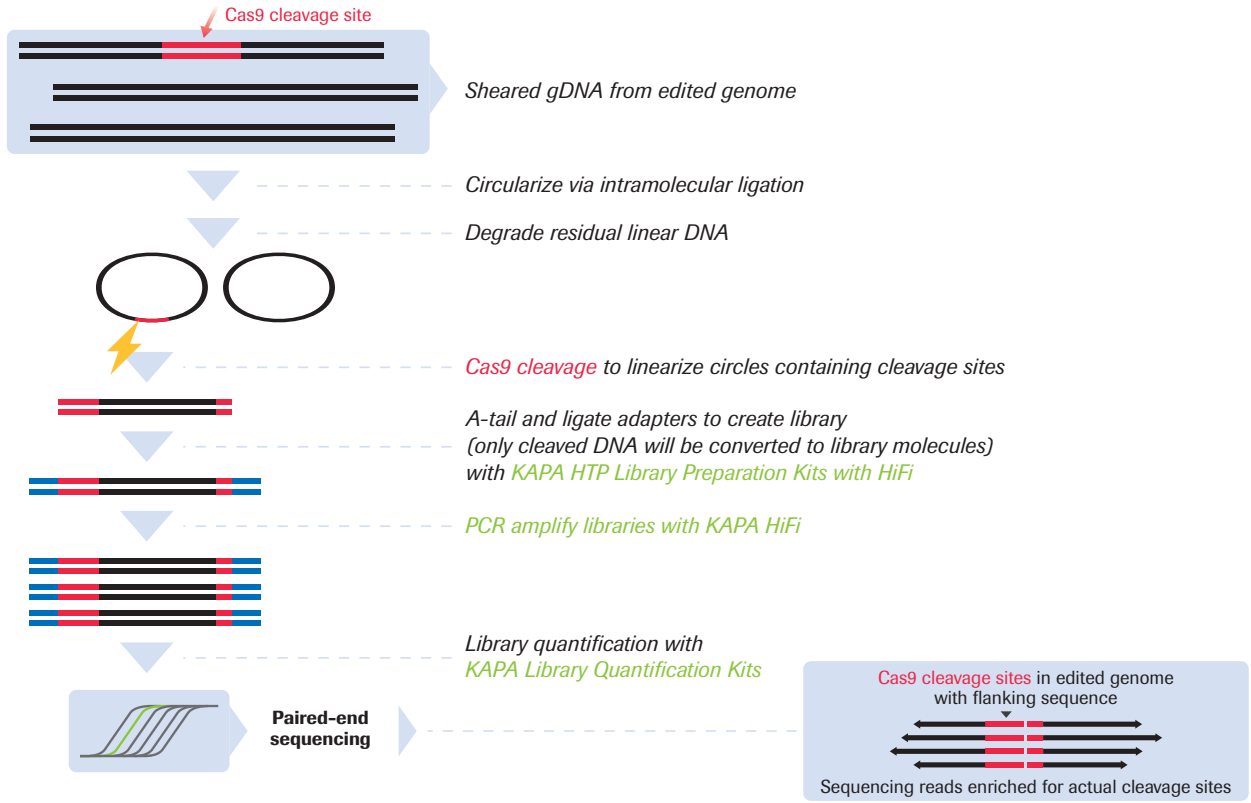


Figure 1. An overview of the CIRCLE-seq workflow. Genomic DNA from the edited genome is randomly sheared to ~300 bp and then, through a series of steps employing the KAPA HTP Library Prep Kit, is converted into sequencing libraries enriched for Cas9 cleavage sites. Briefly, intramolecular ligation circularizes the sheared DNA, and unwanted linear DNA is degraded. Circular DNA is then treated with the Cas9–sgRNA complex and cleaved; DNA lacking Cas9 sites remains circular. The linear DNA molecules are then A-tailed and ligated to adapters, then amplified with KAPA HiFi. Final libraries are then sequenced via paired-end high-throughput sequencing to identify Cas9 cleavage sites. Figure adapted from Tsai, et al., 2017. For complete methods and figures, see Tsai, et al 2017.

The specificity and sensitivity of CIRCLE-seq enables greater detection of off-target Cas9 cleavage sites while reducing sequencing costs.

- Detects more off-target events than either Digenome-seq or GUIDE-seq
- Eliminates the need for laborious cell culture required for Guide-seq
- Greatly reduces background reads compared to Digenome-seq

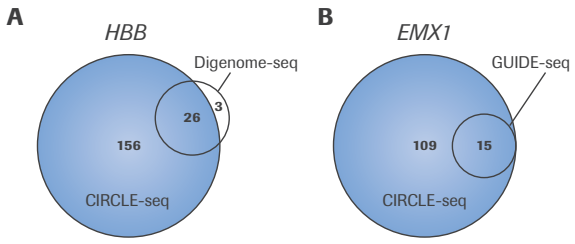


Figure 2. CIRCLE-seq identifies more off-target cleavage events than GUIDE-seq or Digenome-seq. A) CIRCLE-seq detected more off-target sites than Digenome-seq with 10-fold fewer sequencing reads following gRNA targeting of the beta globin gene. Digenome-seq detected 29 off-target sites; CIRCLE-seq detected 26 of 29 of those off-target sites, as well as 156 additional sites. **B)** CIRCLE-Seq identified a larger number of off-target sites than GUIDE-seq following CRISPR/Cas9 editing with a gRNA targeting the EMX1 gene. Adapted from Tsai et al. 2017, Supplementary Figures 6 and 8.

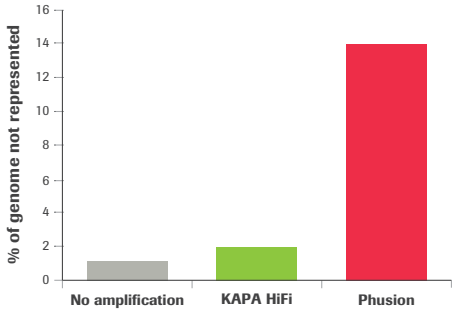


Figure 3. KAPA HiFi amplifies challenging regions such as GC- or AT-rich DNA, ensuring uniform library coverage. This figure demonstrates that fewer regions of challenging genomes (*P. falciparum* is shown here) are missed when sequencing libraries are prepared with KAPA HiFi, compared to other polymerases.

Ordering information

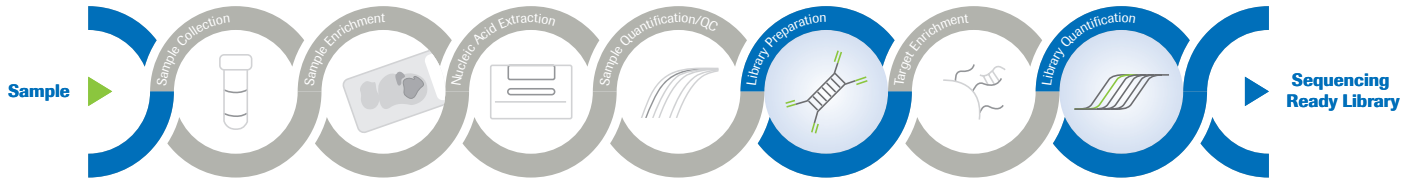
Code	Description	Pack size
KK2101	KAPA HiFi PCR Kit	100 U
KK2102	KAPA HiFi PCR Kit	250 U
KK2501	KAPA HiFi HotStart PCR Kit	100 U
KK2502	KAPA HiFi HotStart PCR Kit	250 U
KK2601	KAPA HiFi HotStart ReadyMix Kit	1.25 mL
KK2602	KAPA HiFi HotStart ReadyMix Kit	6.25 mL
KK2800	KAPA HiFi HotStart Uracil+ Kit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

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

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

Featured publication

CIRCLE-seq: a highly sensitive *in vitro* screen for genome-wide CRISPR–Cas9 nuclease off-targets.
Tsai et al. *Nature Methods*, 2017 vol 14: pages 607 – 614



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MC-US-03360 SS301004 A456 2/19