Single-cell transcriptomics

KAPA HiFi reliably amplifies low-abundance transcripts for NGS

Roche

Single-cell RNA-seq enables the interrogation of gene expression within individual cells.
These methods enrich cell-specific information, offering the potential to answer important questions about rare disease-associated cells—such as circulating tumor cells—or about distinct cell types in healthy tissues.

Despite this great potential, successful single-cell transcriptomics faces several challenges, including:

- the very small amounts of RNA typically present in individual cells, usually in the low picogram range; and
- the potential loss of molecular complexity during preparation of sequencing libraries, especially the loss of low-abundance transcripts.

Smart-seq2 is a powerful method developed by Picelli *et al.* to preserve the complexity of single-cell RNA while streamlining library preparation.
In this workflow, cDNAs are first synthesized with SMART technology (Figure 1, inset). This method leverages the robust, high-fidelity KAPA HiFi HotStart polymerase for sensitive, accurate, full-length transcript coverage, and yields cDNA that is ready for input into NGS library preparation workflows.

KAPA HiFi also drives the success of many library preparation workflows, resulting in high-complexity libraries that yield excellent mapping and alignment metrics over a broad range of input.

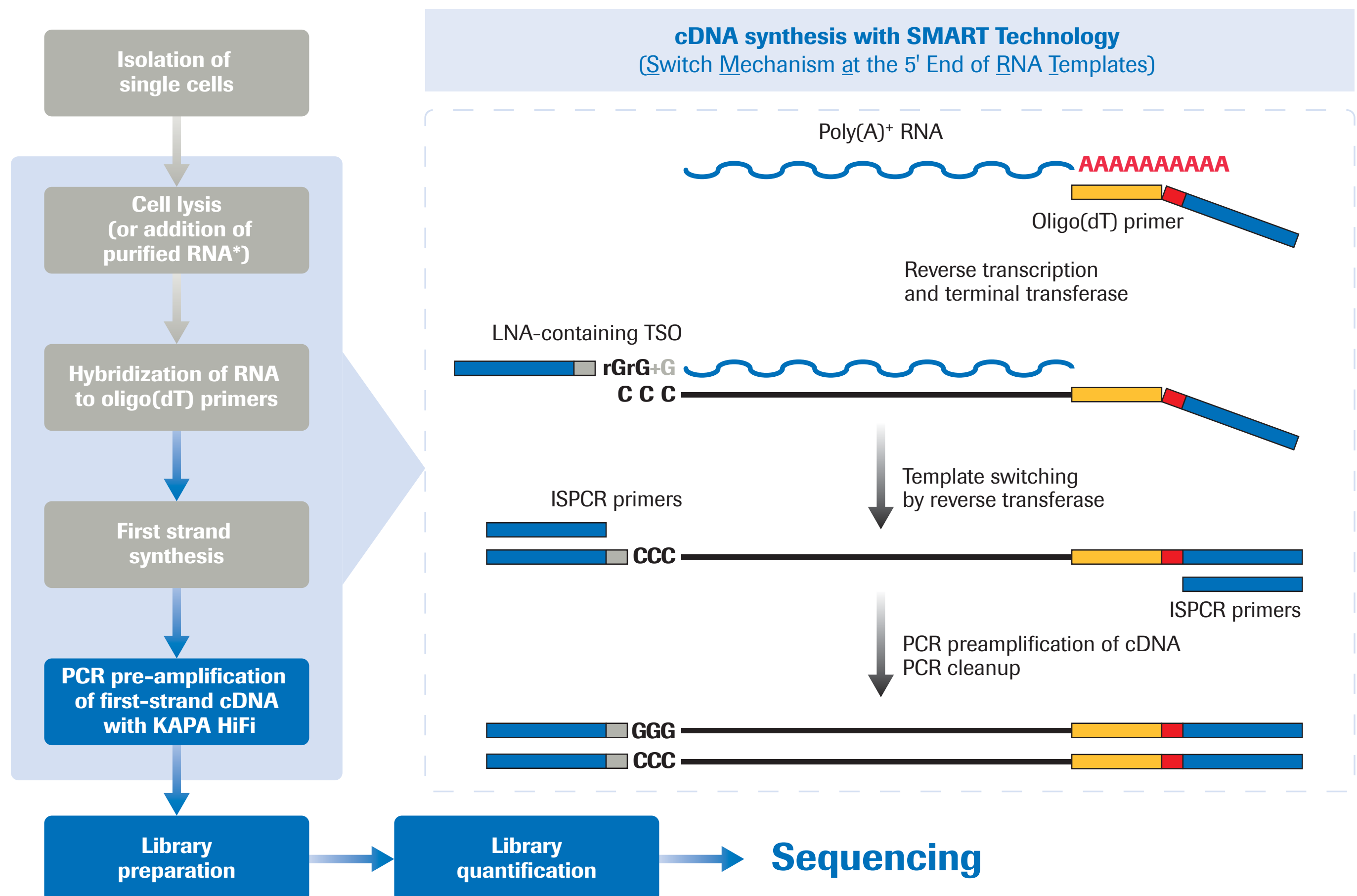

Figure 1. Streamlined single-cell RNA-seq workflow, from single cell to sequencing-ready library. The steps of SMART cDNA synthesis are summarized in the inset above; for the full workflow see Picelli et al. (2014). Full-length RNA-seq from single cells using Smart-seq2. Following SMART cDNA synthesis with HiFi, the cDNA serves as input into library preparation. For optimal sequencing efficiency, final libraries are then quantified with the KAPA Library Quantification Kit prior to pooling. Abbreviations used in Figure 1: LNA=locked nucleic acid; TSO=template-switching oligonucleotide; ISPCR primers=the primers used for amplification following reverse transcription. *Details provided in Picelli et al. (2014).

Data on file.
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Smart-seq2 outperforms the SMARTer kit in a comparison carried out by Picelli et al., (2013). Amplification steps in Smart-seq2 employ KAPA HiFi HotStart ReadyMix, while the SMARTer kit uses Advantage 2 polymerase (Takara). Smart-seq2 with KAPA HiFi yielded:

- longer, more full-length cDNAs;
- better detection of transcripts with high GC content; and
- libraries that more closely resemble a no preamplification control, demonstrating lower levels of bias.

Use of KAPA HiFi in the Smart-seq2 workflow enables automation of SMART cDNA synthesis. The previous Smart-seq protocol was not readily automatable due to necessary small-volume bead-based cleanup steps; integration of KAPA HiFi into the SMART-seq2 protocol eliminated the need for these steps (see Picelli et al., 2013).

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

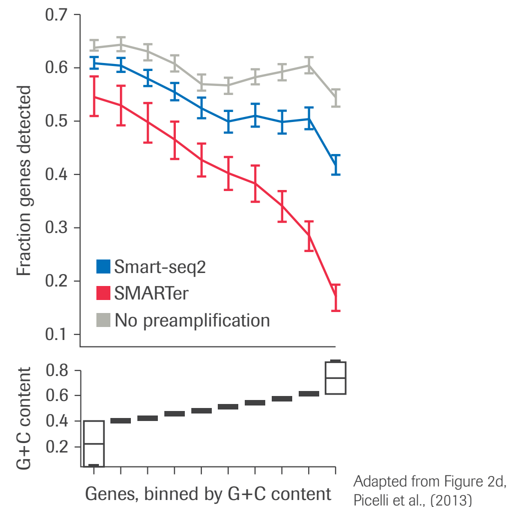
Featured publications

Full-length RNA-seq from single cells using Smart-seq2. Picelli, et al. *Nature Protocols*, 2014 vol 9: pages 171 – 181

Smart-seq2 for sensitive full-length transcriptome profiling in single cells. Picelli, et al. *Nature Methods* 2013 vol. 10, no. 11: pages 1096 – 1098

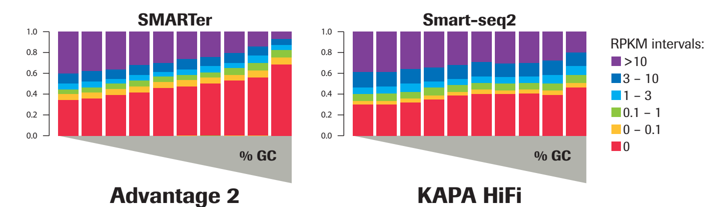
Full-Length mRNA-Seq from single cell levels of RNA and individual circulating tumor cells. Ramskold, et al. *Nat Biotechnol.* 2012 vol. 30, no. 8: 777 – 782

A



Adapted from Figure 2d, Picelli et al., (2013)

B

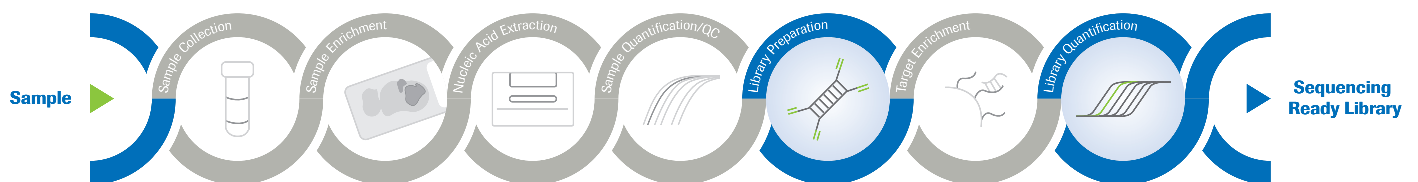


Adapted from Supplementary Figure 6, Picelli et al. (2013)

Figure 2. Smart-seq2 with KAPA HiFi provides more sensitive full-length transcriptome profiling than the SMARTer workflow. A) Mean fraction of genes detected (RPKM>1) per bin, sorted by GC content. mRNA-seq data was included as a no-preamplification control. Error bars denote SEM ($n \geq 4$), and the lower panel shows the GC range per bin. **B)** The fraction of genes with no reads (red) or at increasing expression level intervals, binned by the GC content of the genes. The polymerase used for preamplification is indicated. For complete figures and captions, see Picelli et al., 2013.

Consult our dedicated Support & Applications scientists for guidance in using KAPA HiFi for your single-cell ultra-low-input RNA sequencing experiments!

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



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