Oxford NANOPORE Technologies
APPLICATIONS

A method for high-output, full-length isoform sequencing and accurate poly(A) tail measurement

A new high-throughput, full-length cDNA nanopore sequencing kit that captures full transcript information in individual reads, including start and poly(A) sites, UTRs, and poly(A) tail lengths

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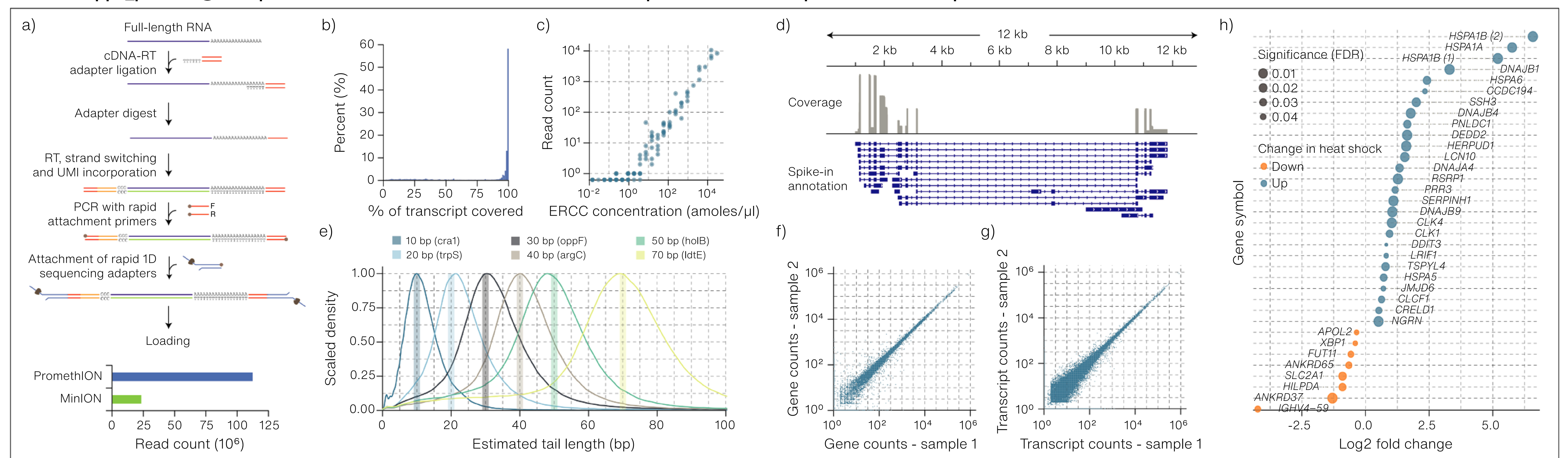

Fig. 1 PCR-cDNA Sequencing Kit a) protocol and raw-read output b-d) spike-in length, quantification, coverage e) poly(A) tail length f-g) gene and transcript counts h) differential gene expression

Oxford Nanopore's latest cDNA sequencing kit captures full transcript sequences in individual reads, including transcript 5' and 3' ends and the length of the poly(A) tail

We have developed a novel method of preparing and sequencing cDNA using the new PCR-cDNA Sequencing Kit (SQK-PCS111). This method incorporates a unique molecular identifier (UMI), and reverse transcription is initiated from a ligated adapter to avoid priming from poly(A) stretches within the transcripts. To demonstrate the method, we grew human GM24385 cells under normal or heat-shocked conditions at 42°C for 1 hour in multiple replicates. We spiked extracted total RNA with SIRV-Mix 3 quantitative panel (Lexogen) and sequenced on single PromethION™ and MinION™ Flow Cells per sample, typically generating ~100 million and 20 million reads, respectively (Fig. 1a). SIRV Mix 3 panel transcripts demonstrate a high proportion of reads that cover the transcripts fully from 5' to 3' (Fig. 1b), accurate quantification compared with known concentrations (Pearson's R = 0.96, Fig. 1c), and clean and full-transcript-spanning coverage profiles (Fig. 1d). Using engineered E. coli transcripts with known 3' poly(A) tail lengths (10-70 nt) and *tailfindr* software, we illustrate the kit's ability to accurately measure poly(A) tail lengths at biological length ranges (Fig. 1e). Gene counts are highly correlated between replicates (>0.98 Pearson on log-transformed counts, Fig. 1f) as are transcripts counts (>0.93, Fig. 1g). Using 40 million raw reads as a starting point per sample, we identified tens of genes as differentially expressed after heat shock (*DESeq2*, p-adj <0.05), including several known heat-shock genes (Fig. 1h).

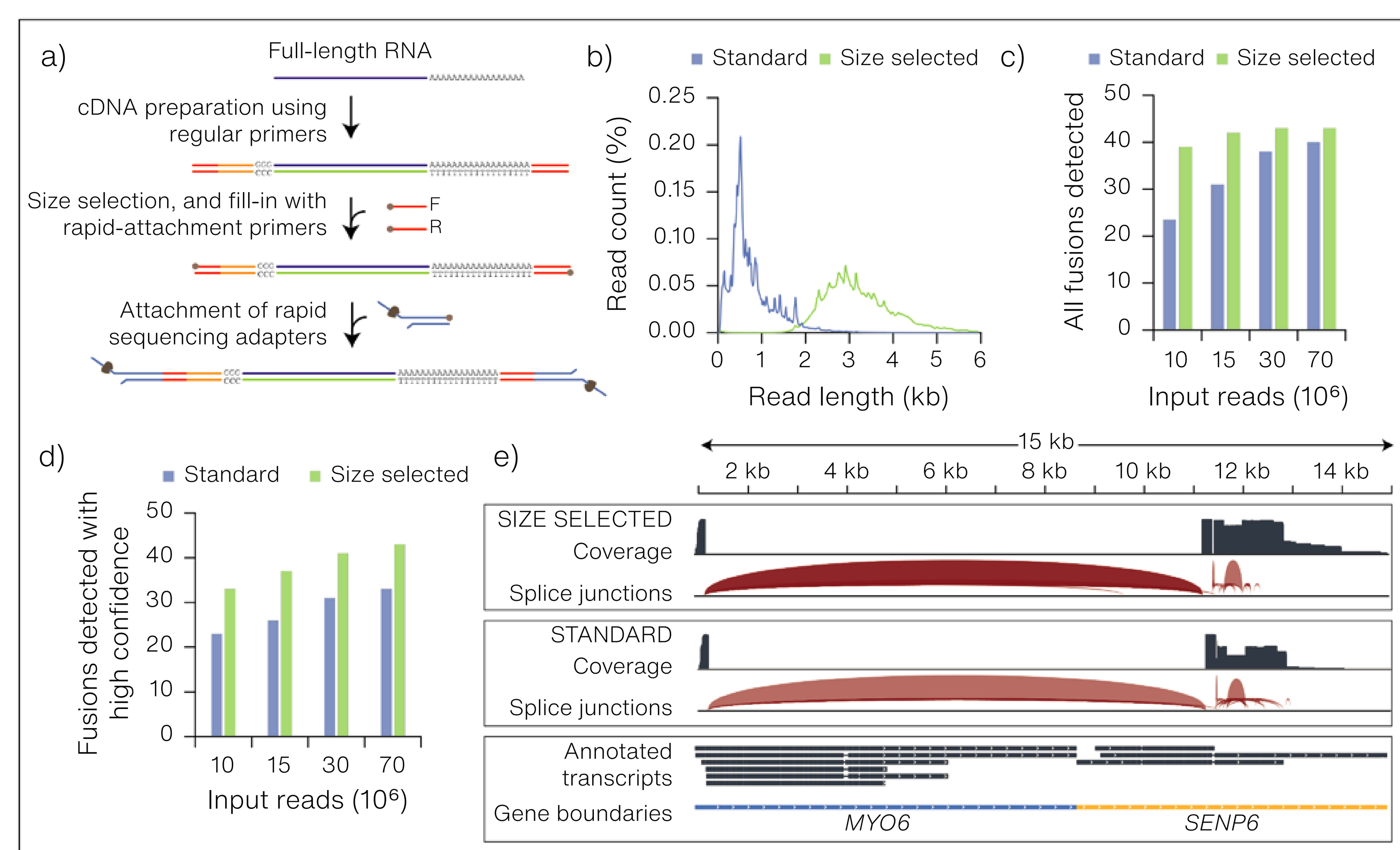

Fig. 2 Size selection a) workflow b) read lengths c) and d) fusions detected e) *MYO6/SENP6*

Full isoform-level characterisation of very long fusion transcripts by size selection

We performed a modified SQK-PCS111 cDNA protocol to select for long cDNA molecules, using total RNA extracted from a MCF-7 human breast adenocarcinoma cell line (Figs. 2a and b). Detection of known fusion transcripts were evaluated using *JAFFAL* (Figs. 2c and d). With size selection, only 10-15 million reads were required to detect the same number of fusions as ~70 million standard reads. Combining standard and size-selected data increases the total number of fusions detected. Oxford Nanopore cDNA reads cover fusion transcripts from their 5' to 3' end, allowing isoform-level analysis of fusion products. Shown here is the cancer-associated fusion *MY06/SENP6* (Fig. 2e).

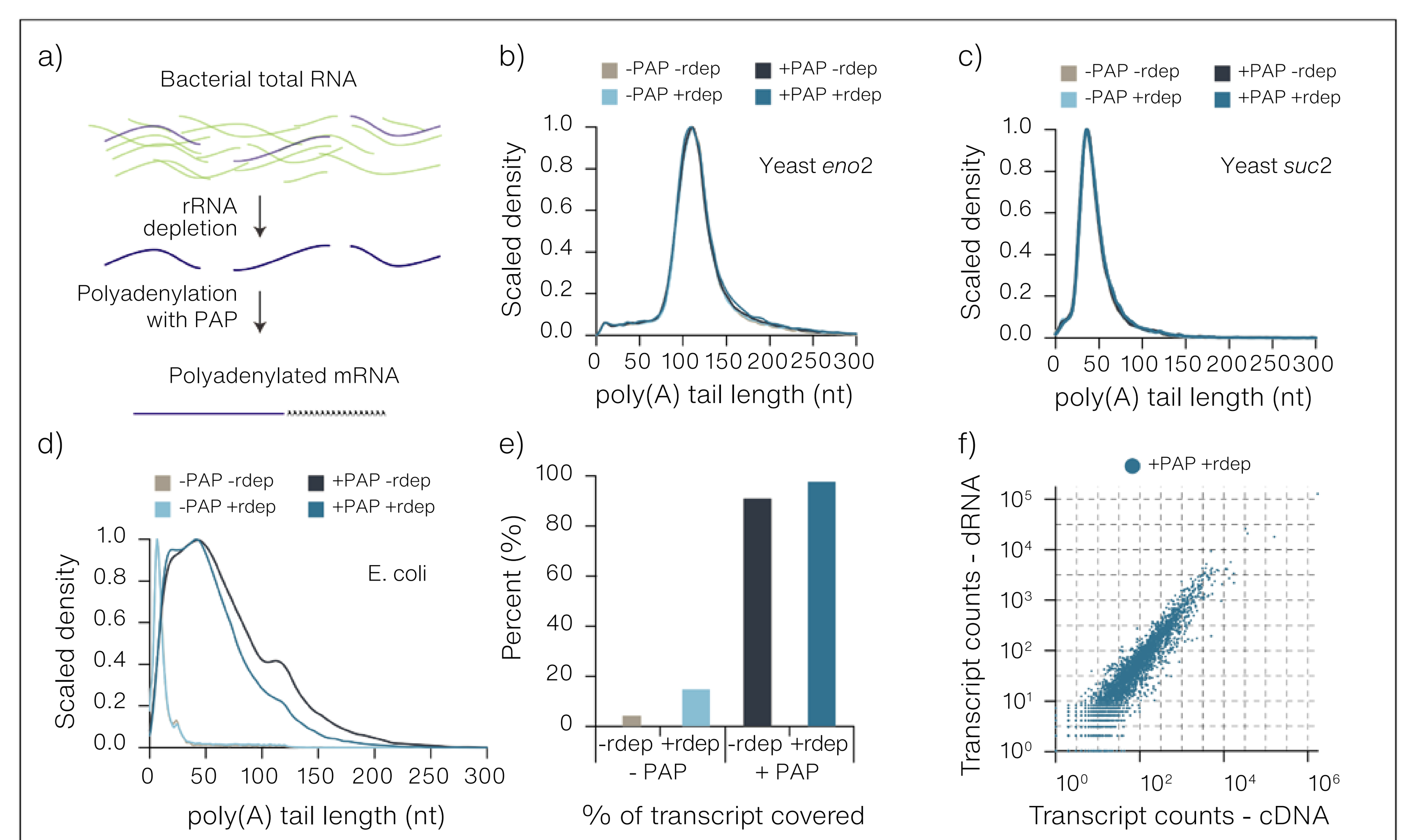

Fig. 3 Poly(A) tailing a) protocol, b-d) tail-length e) E. coli read fraction and f) gene counts

Enzymatic polyadenylation enables sequencing of non-poly(A) transcriptomes

Bacterial mRNA can be prepared for sequencing by depleting the predominant rRNA, before polyadenylating the remaining mRNA by incubation with E. coli poly(A) polymerase (PAP, Fig. 3a). Two yeast IVT transcripts with known tail lengths of 100 nt (Fig. 3b) and 30 nt (Fig. 3c) were spiked into extracted RNA and the libraries sequenced using the PCS111 Kit. The resulting reads mapping to the yeast transcripts had the expected tail length (Figs. 3b and c). The PAP treatment added a poly(A) tail of about 50 nt to the 3' end of E. coli transcripts (Fig. 3d), which enabled their sequencing (Fig. 3e). The same method is compatible with Oxford Nanopore's direct RNA Sequencing Kit, to quantify E. coli gene-expression levels (Fig. 3f).

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