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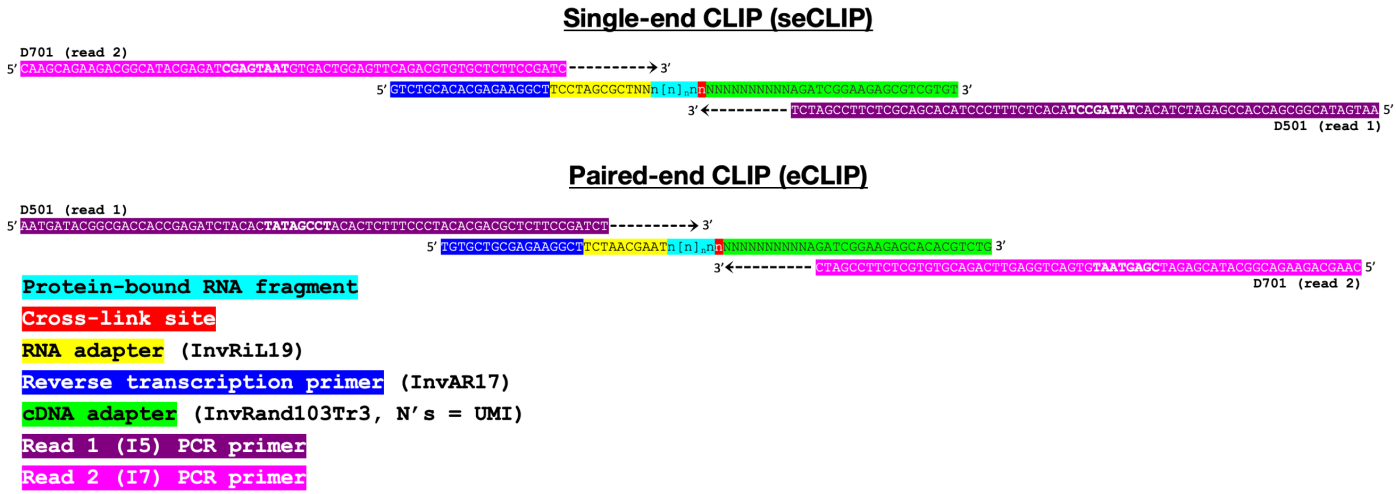
Supplementary information

Transcriptome-wide identification of RNA-binding protein binding sites using seCLIP-seq

In the format provided by the authors and unedited

Supplementary Figure 1

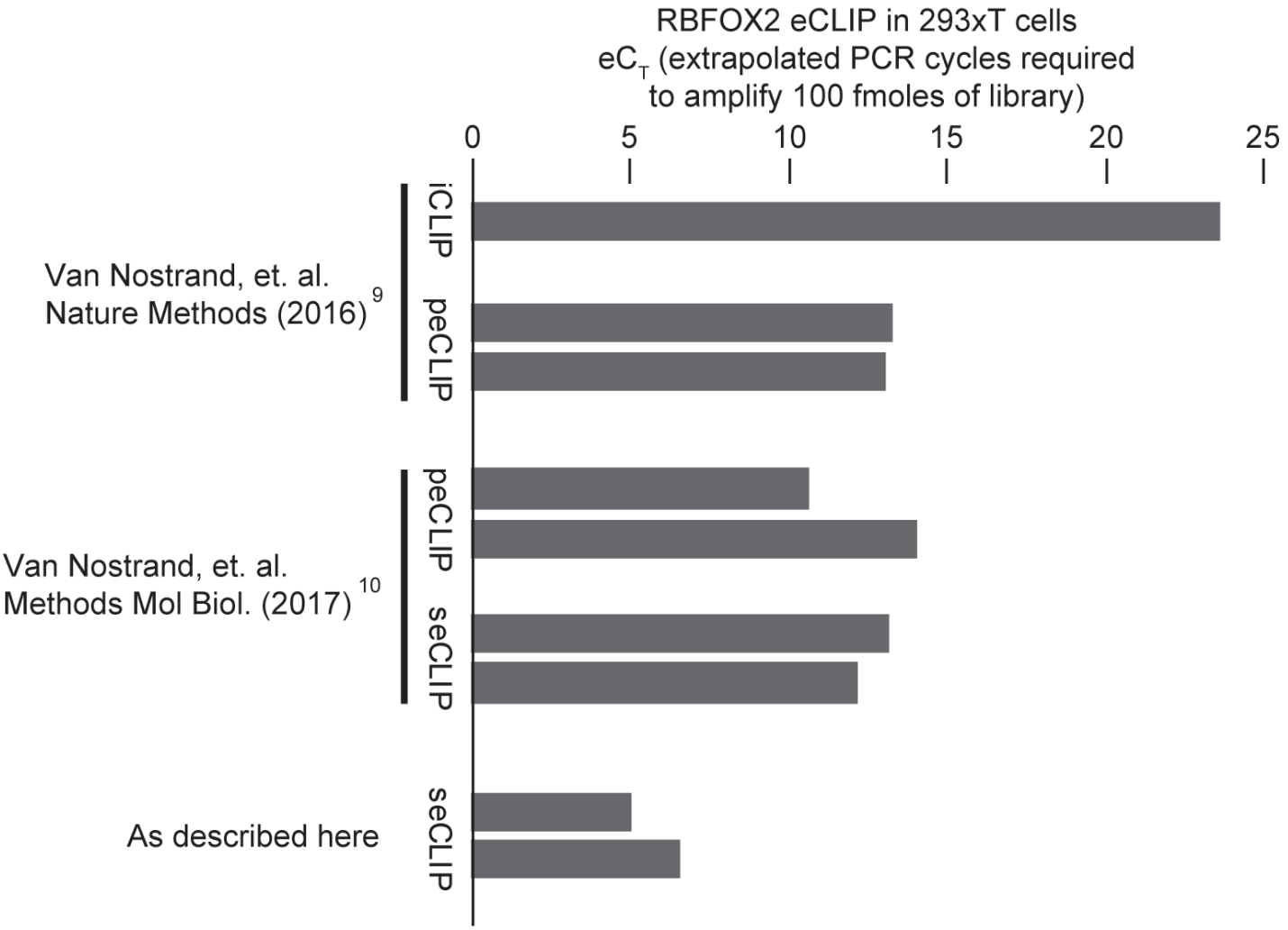


Supplementary Figure 1

Schematic of sequencing read structures in seCLIP and eCLIP methods.

In seCLIP, modification of the adapter strategy places the unique molecular identifier (UMI) and cross-link site at the beginning of the first sequencing read, thus necessitating single-end sequencing to obtain this information. Specifically, the RT primer and cDNA adapter sequences have been modified such that their respective complementarities to the D50x and D70x index PCR primers have been switched. Conversely, eCLIP requires paired-end sequencing to reliably capture this information as it is at the beginning of read 2. Nucleotides in bold indicate bases used for identifying and demultiplexing Illumina sequencing adapters, with D501 and D701 shown as examples. Refer to "Illumina Adapter Sequences" documentation for information regarding other Illumina adapters.

Supplementary Figure 2



Supplementary Figure 2

Representative comparison of RBFOX2 library amplification in seCLIP, peCLIP, and iCLIP methods.

RBFOX2 single-end CLIP, as described here, of 20M 293xT cells requires approximately 7 fewer PCR cycles than experiments using previously published seCLIP and peCLIP methods and approximately 18 fewer PCR cycles than experiments using the iCLIP method to reach the same molar amount (100 fmoles) of sequencing library. Extrapolated C_T (eC_T) is calculated in the same way as the a-eC_T metric mentioned herein but assumes an idealized 2-fold amplification per PCR cycle rather than the experimentally derived 1.84-fold amplification per cycle used in the a-eC_T metric calculation.