

Supplemental information for

Tn5 transposase and tagmentation procedures for massively scaled sequencing projects

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Supplemental Items:

Supplemental Methods: Complete sequence of Tn5 expression vector

Supplemental Fig 1. Plasmid map of pTXB1-Tn5

Supplemental Fig 2. Sequencing library characteristics

Supplemental Fig 3. Tagmentation sequence bias

Supplemental Fig 4. Library characteristics from ultra low DNA input amounts

Supplemental Fig 5. Library complexity at variable input amounts

Supplemental Table 1: Detailed list of reaction conditions in Figure 3

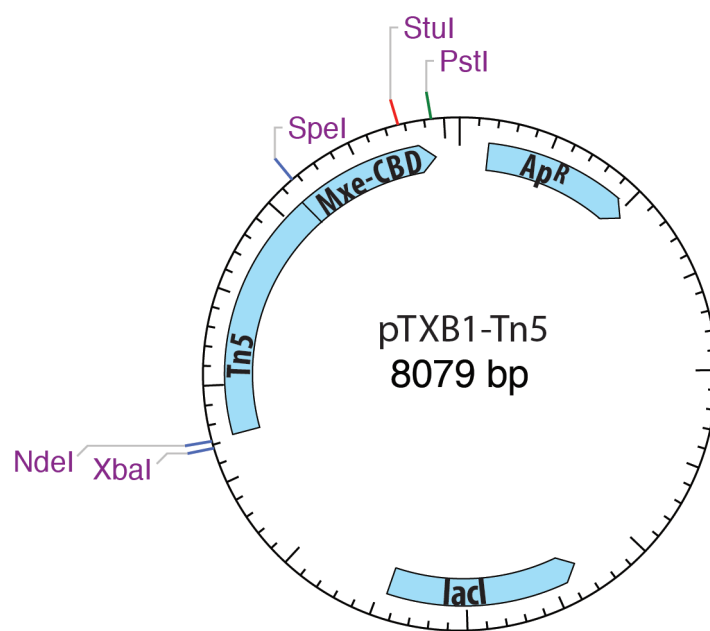
Supplemental Methods

Complete sequence of vector used for Tn5 production (see Supplemental Figure 1 for a Plasmid map).

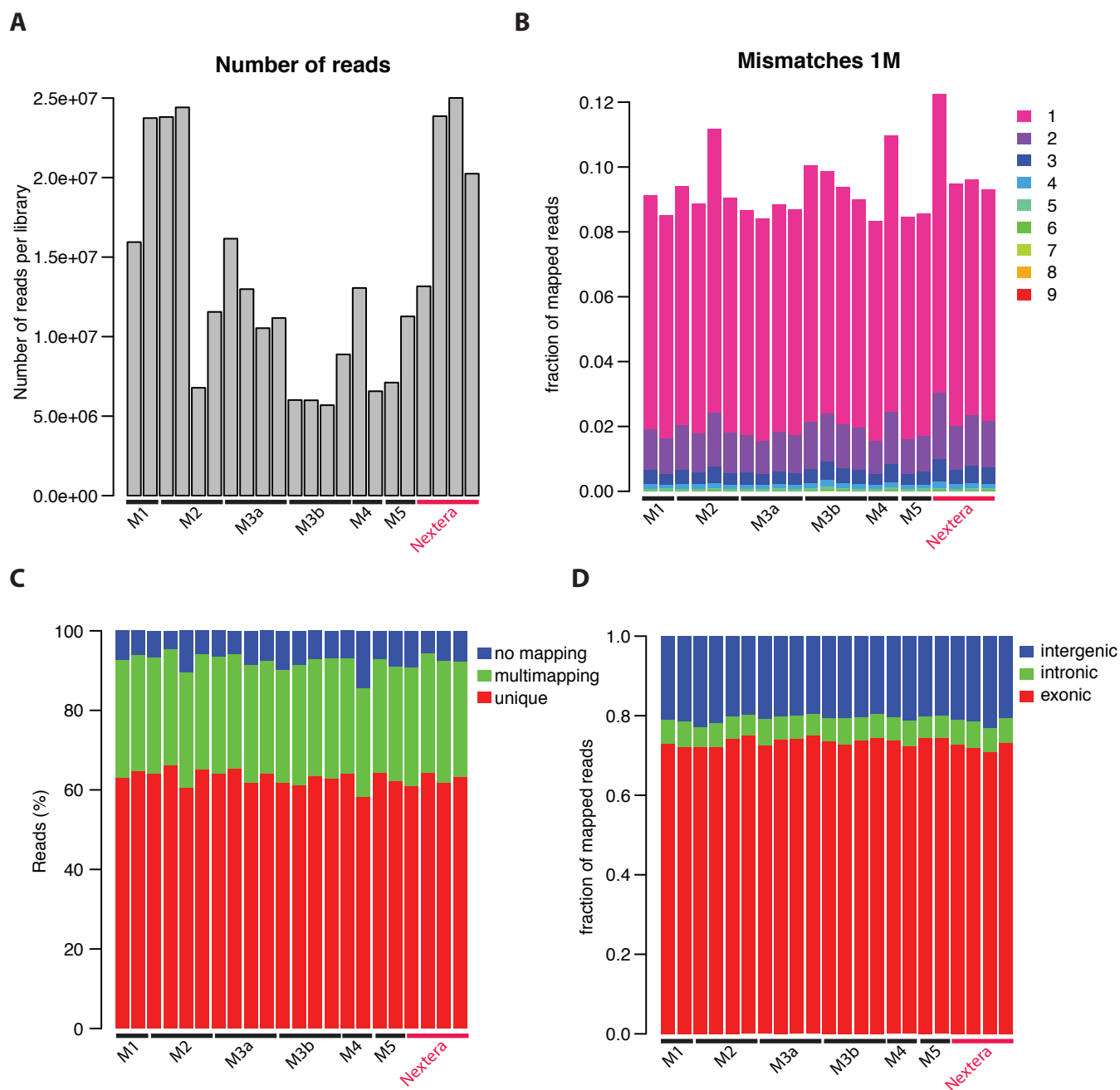
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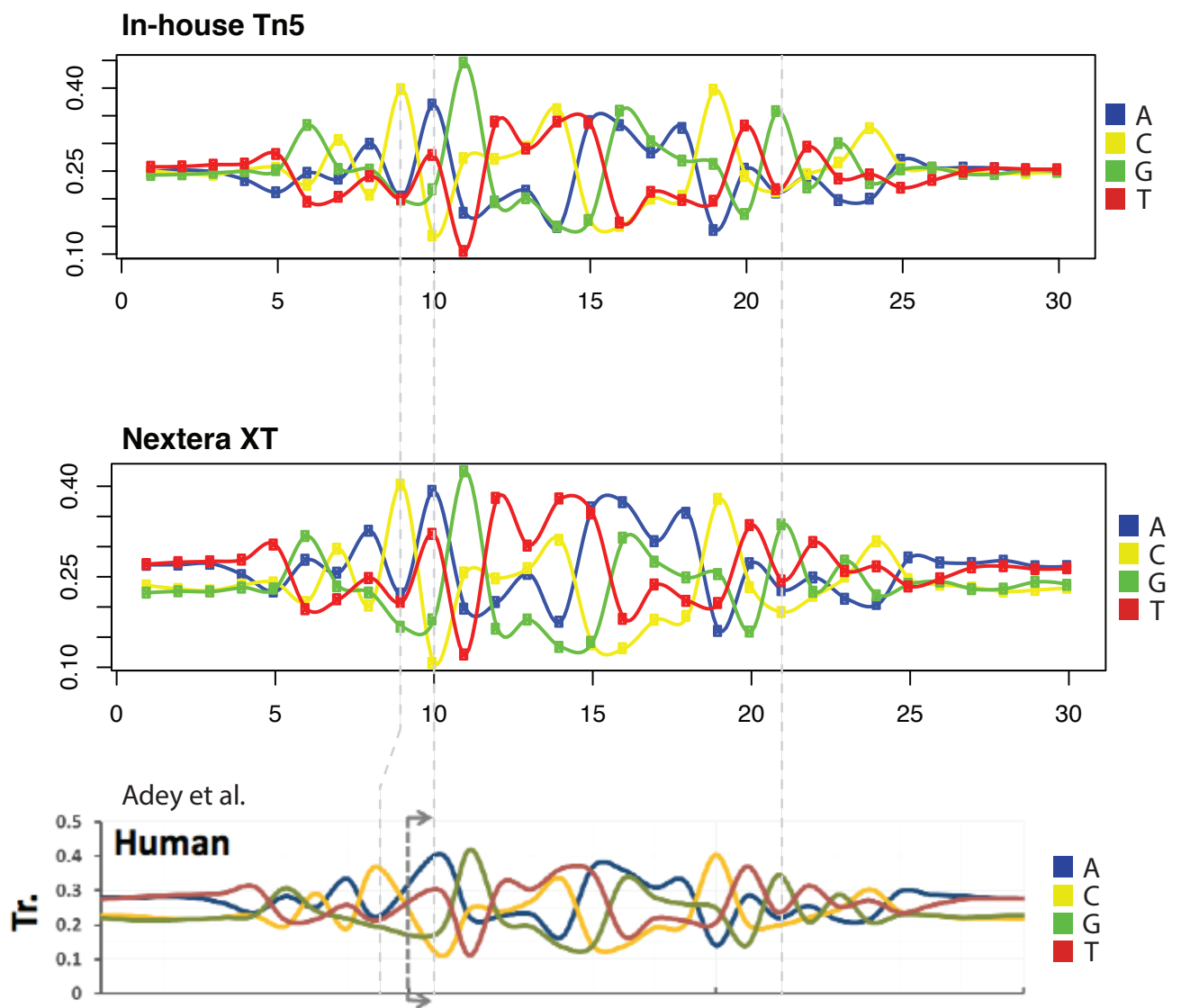


Supplemental Figure 1. Map of the pTXB1-Tn5 plasmid.



Supplemental Figure 2. Mapping statistics of RNA-seq libraries.

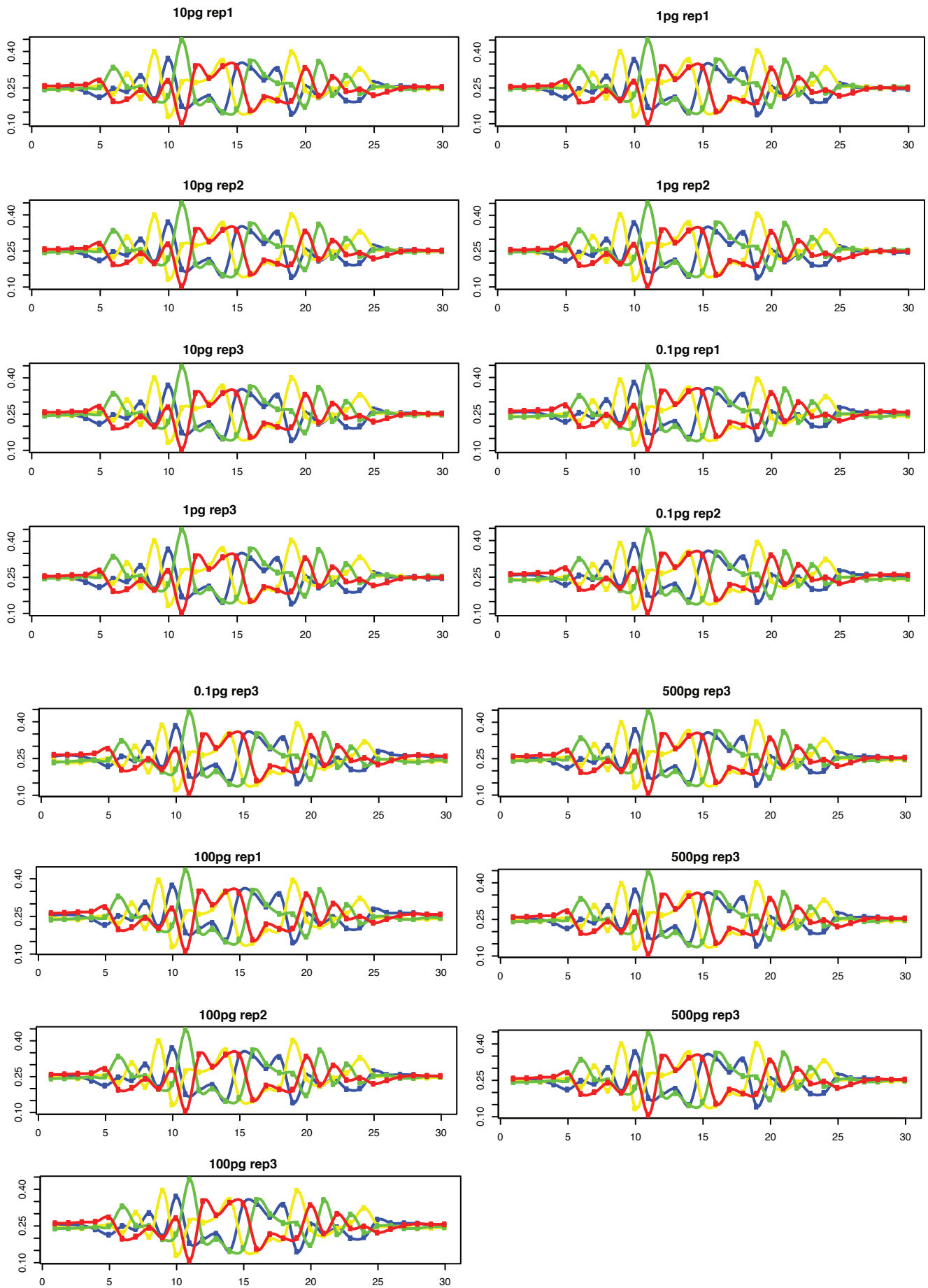
Libraries were generated with in-house or commercial Tn5 and reaction buffers as detailed in Supplemental Table 1. **(A)** Read depth per library. **(B)** Mismatch frequencies in libraries. **(C)** Percent reads mapping either uniquely to genome and transcriptome, to multiple locations (multimapping) or without any identifiable origin (no mapping). **(D)** Fraction of reads mapping to exonic, intronic or intergenic regions.



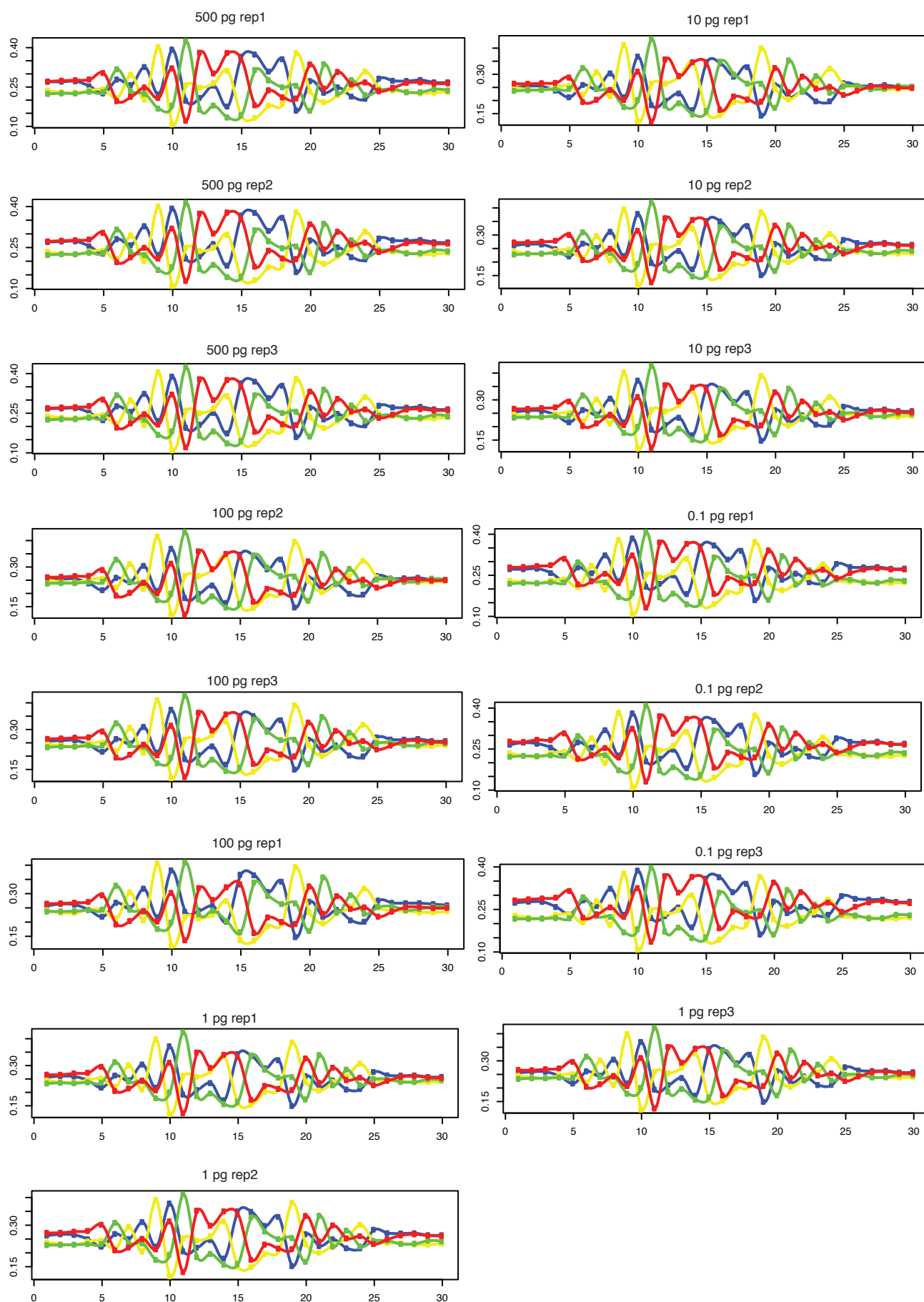
Supplemental Figure 3. Tagmentation sequence bias

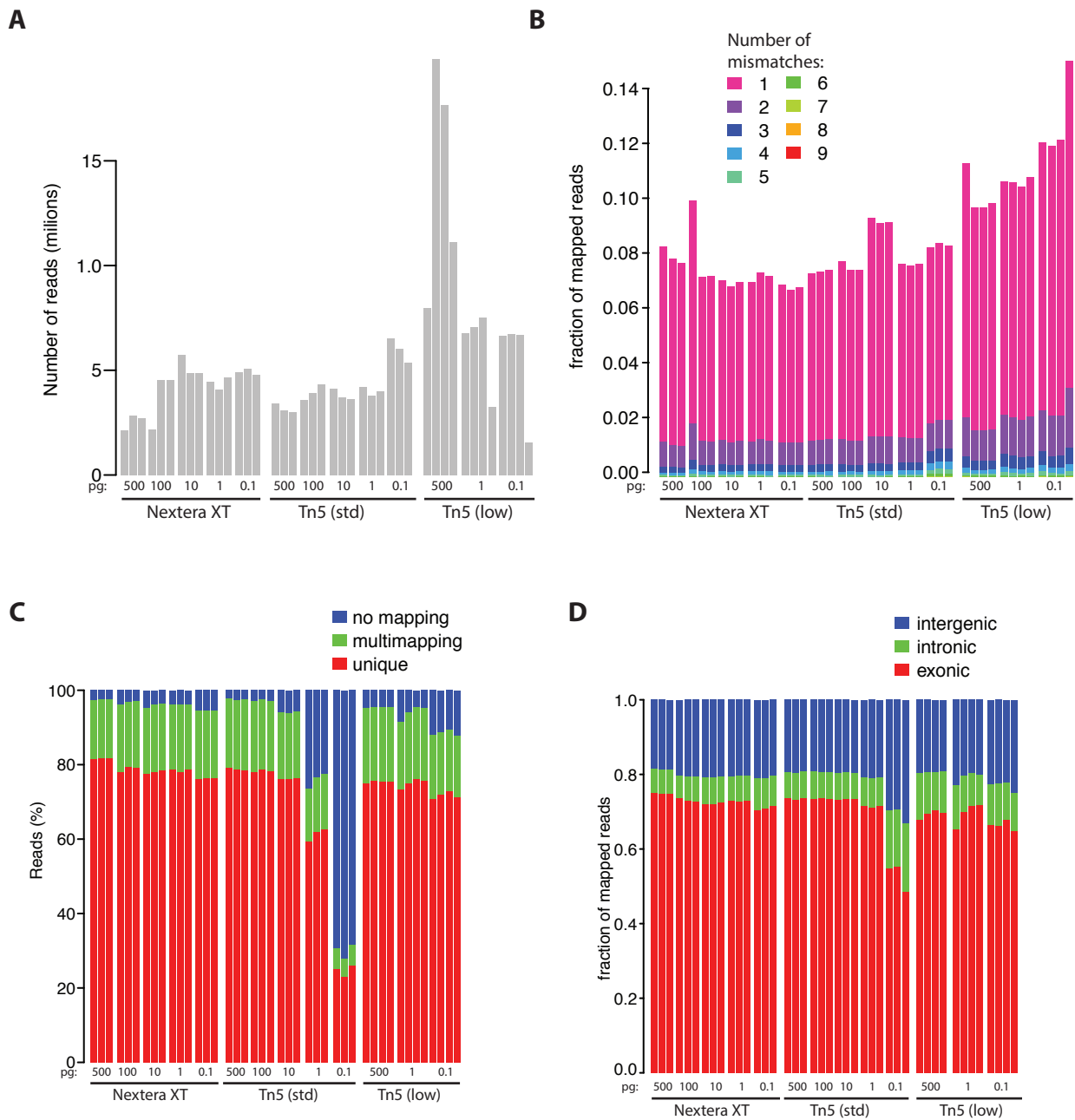
Analyses of sequencing libraries generated with in-house Tn5 or Nextera XT demonstrate a bias in tagmentation sites very similar to that already described in Adey et al. 2010. The following two pages show bias estimated from libraries generated from different starting amounts.

In-house Tn5 libraries



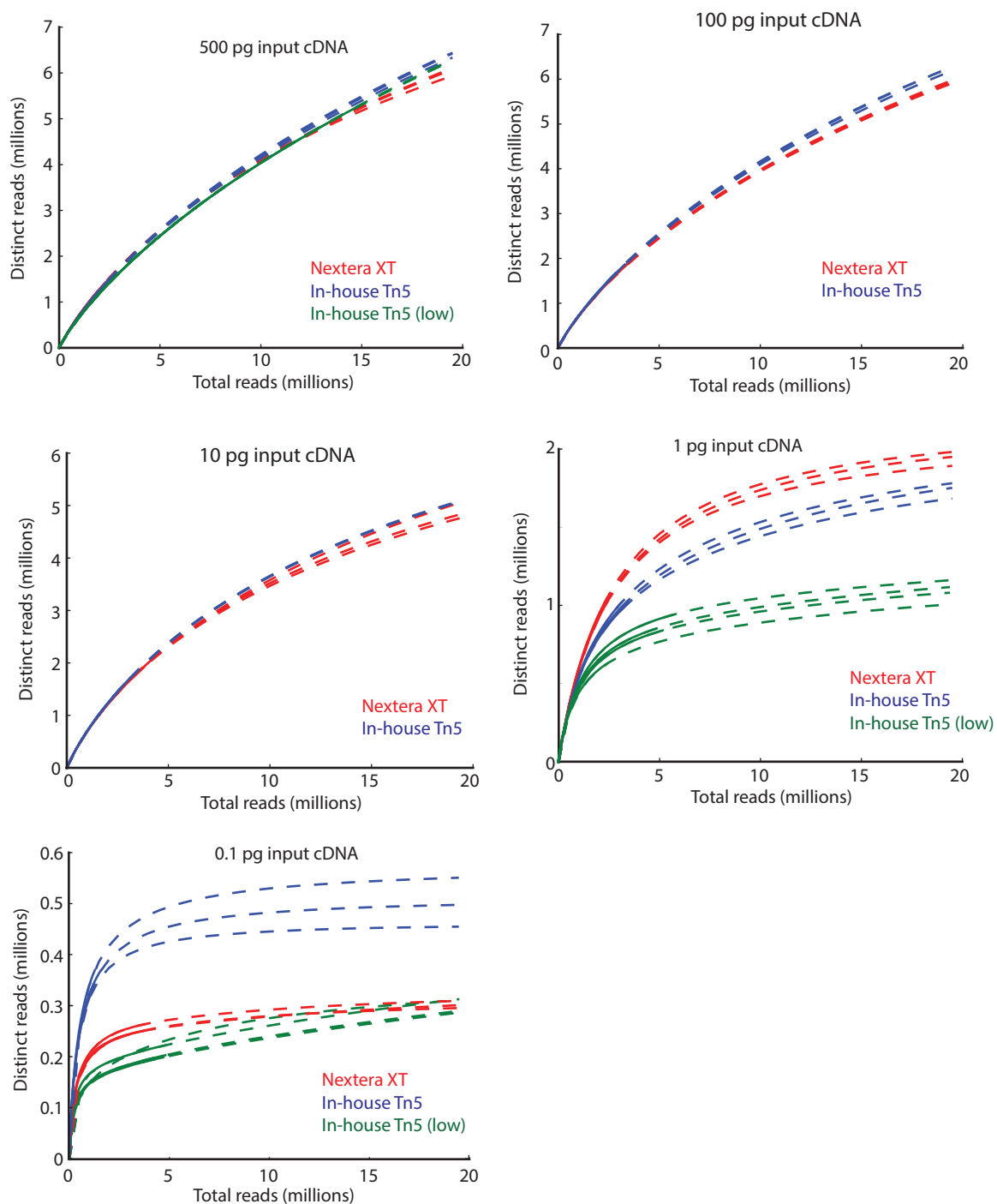
Nextera XT libraries





Supplemental Figure 4. Characteristics of libraries generated from ultra-low cDNA input.

Libraries were generated with Nextera XT, in-house Tn5 and buffers using standard (std) or only a 1% of the amounts of Tn5 (low). Starting amounts in picogram cDNA is indicated below each graph. **(A)** Read depth per library. **(B)** Mismatch frequencies in libraries. **(C)** Percent reads mapping either uniquely to genome and transcriptome, to multiple locations (multimapping) or without any identifiable origin (no mapping). **(D)** Fraction of reads mapping to exonic, intronic or intergenic regions.



Supplemental Figure 5. Library complexity.

Library complexities were estimated using preseq (Daley et al. Nature Methods 2013). Solid lines represent subsampling of sequenced data and dashed lines extrapolations of the complexities in libraries if they were to be sequenced deeper.

Supplemental Table 1. Detailed list of reaction conditions compared in Fig 3.

Name	Tagmentation reaction volume (μl)	Tn5 transposase	Tagmentation buffer (5X)	Purification after tagmentation	Enrichment PCR	Figure
P1 (Standard Nextera®)	50	Nextera®-provided	Nextera® kit	Columns (DNA Clean & Concentrator)	Nextera® kit	Fig 3A,B
Modified 1	20	Nextera®-provided	Nextera® kit	5 μl NT buffer	Nextera® XT kit	Fig 3A,B
Modified 2	20	Nextera®-provided	Nextera® kit	5 μl NT buffer	KAPA HiFi DNA Polymerase	Fig 3A,B
Modified 3a	20	Nextera®-provided	Nextera® kit	0.01% SDS	KAPA HiFi DNA Polymerase	Fig 3A,B,C,D
Modified 4	20	Nextera®-provided	Nextera® kit	0.001% SDS	KAPA HiFi DNA Polymerase	Fig 3A,B
Modified 5	20	Nextera®-provided	Nextera® kit	ddH ₂ O	KAPA HiFi DNA Polymerase	Fig 3A,B
Modified 3b	20	In-house Tn5	50 mM TAPS-NaOH pH 8.5, 25 mM MgCl ₂ , 50% DMF	0.01% SDS	KAPA HiFi DNA Polymerase	Fig 3C,D