Supplemental material for

Sequencing Illumina libraries at high accuracy on the ONT MinION using R2C2

by

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Contents:

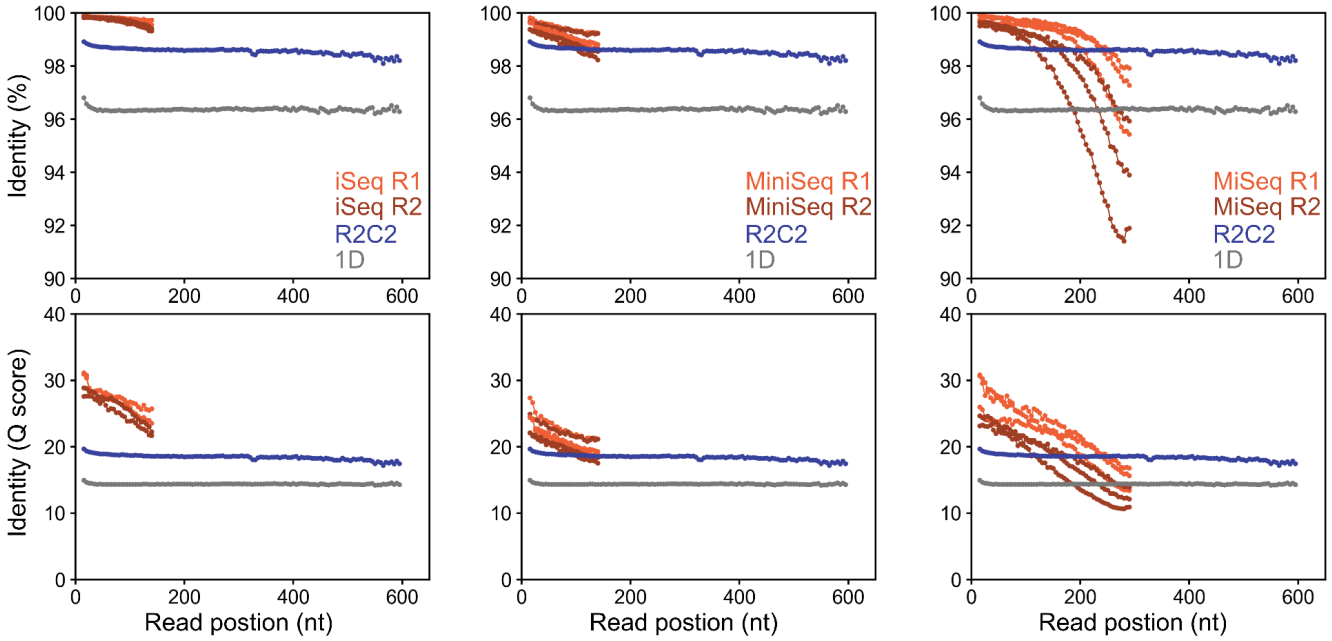

Fig. S1: Read position dependent accuracy of benchtop Illumina sequencers and ONT sequencers. Publicly available iSeq (left), MiniSeq(center), and MiSeq (right) reads of genomic E.coli DNA were processed to evaluate read accuracy. This accuracy, is shown for 3 separate sequencing runs for each read position as percent (top) or log converted Q score (bottom). In each case, Illumina benchtop sequencer accuracy is compared to R2C2 and 1D ONT data generated by us for this study as shown in figure 2B.

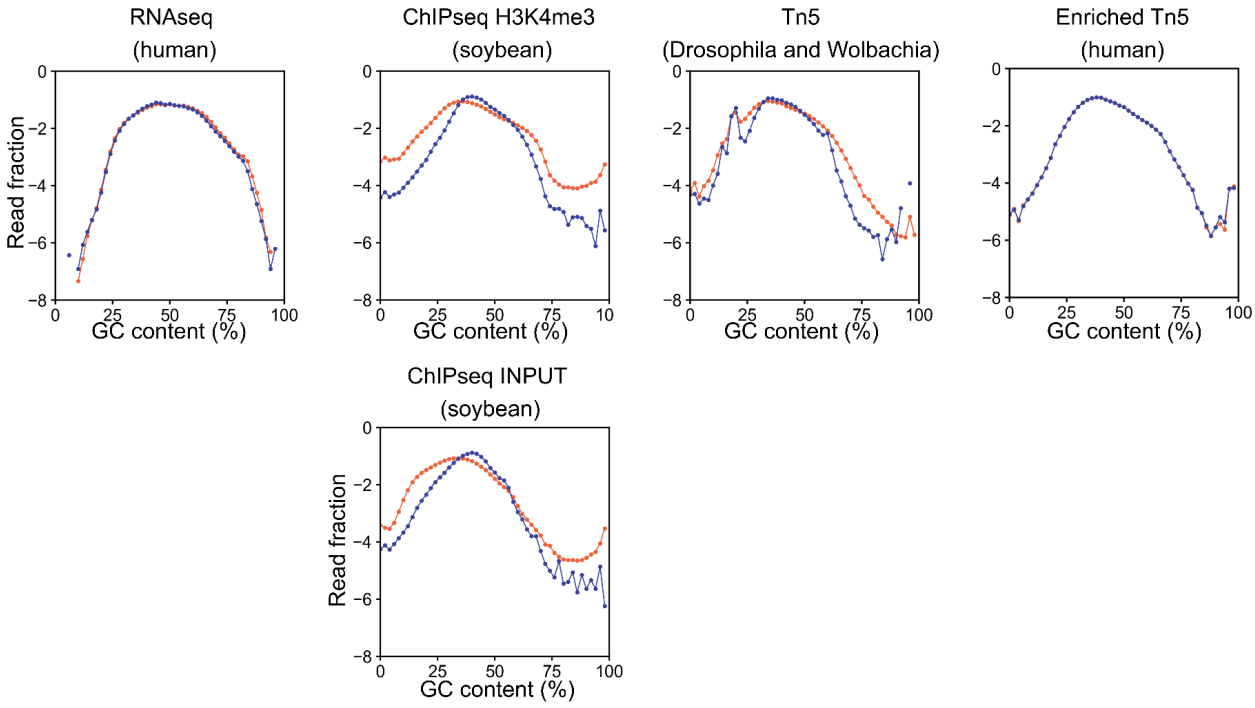

Fig. S2: GC-content of Illumina and R2C2 reads sampling from the same library. Log_{10} converted read fractions of reads with different GC content is shown for all experiments performed for this study. For the ChIP-seq study, read fractions for both libraries in the analyzed pool are shown (H3K4me3 and INPUT). Illumina reads are shown in orange and R2C2 reads are shown in blue.

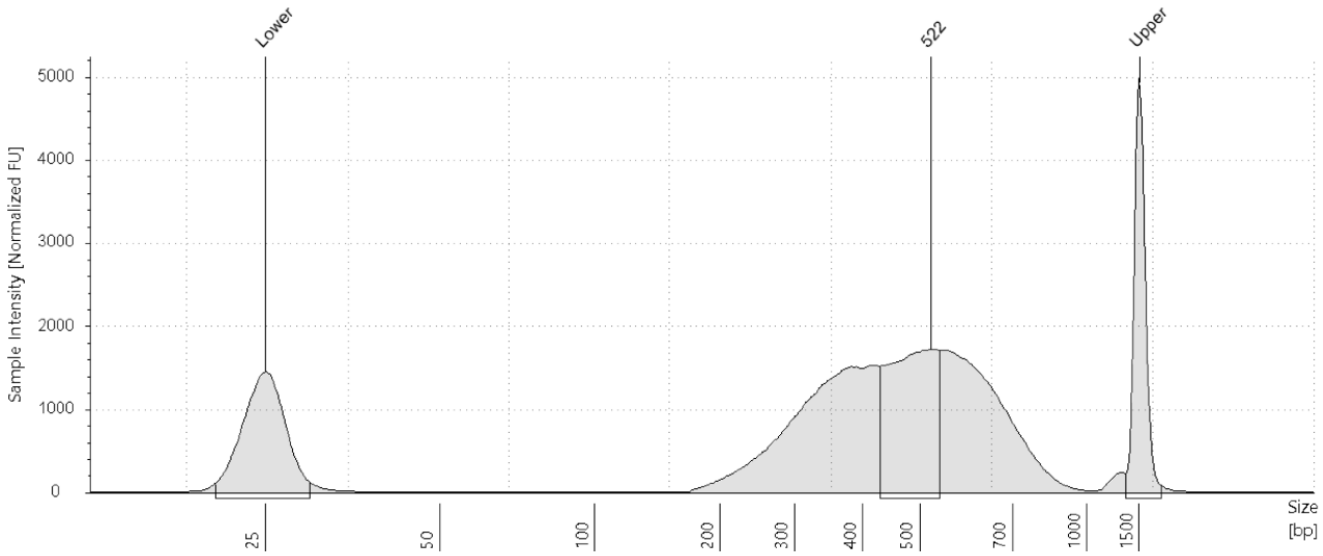

Fig S3. Target-Enriched Tn5 library size.
The size of the target-enriched Tn5 library pool as determined by Agilent Tapestation run.

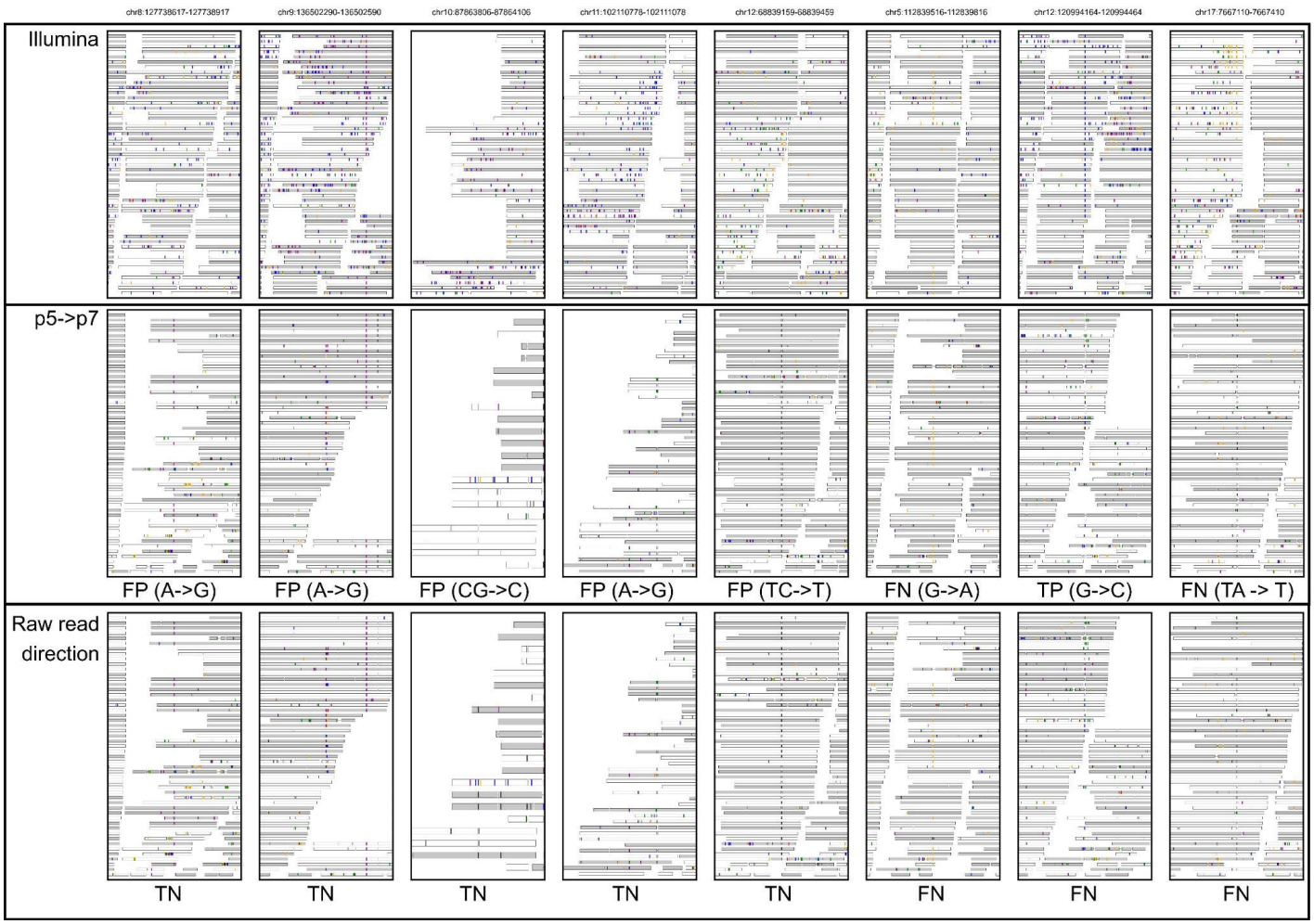

Fig S4: Read context around R2C2/Pepper-Deepvariant miscalls. Subsampled Illumina as well as R2C2 read alignments are shown in genome browser style visualizations around variant calls where R2C2/Pepper-Deepvariant disagreed with Illumina/Deepvariant. R2C2 data and variant calls are shown in both their original orientation (center: p5->p7) as well as reoriented direction (bottom: Raw read direction). Illumina variant call (e.g. A->G) and R2C2 variant call status (FP - False Positive, FN - False Negative, TP - True Positive, TN - True Negative) is indicated for both orientations. In the read alignments, mismatches are marked by lines colored by the read base (A - orange; T - green; C - blue; G - purple). Insertions are shown as gaps in the alignments while deletions are shown as black lines. “Plus” strand alignments are shown with a white background, while “Minus” strand alignments are shown with a grey background.

Splint oligos

```
>UMI_Splint_1_F_Next_A
GATCTCGGTGGTCGCCGTATCATTTGAGGCTGATGAGTTCCATANNNNNTATATNNNNNATCACTACTTAGTTTTTTGATAGCTTCAAGCCAGAGTTGTCTTTTTCTC
TTTGCTGGCAGTAAAAG
>UMI_Splint_1_R_Next_B
ATCTCGTATGCCGTCTTCTGCTTGAAAGGGATATTTTCGATCGCNNNNNATATANNNNNTTAGTGCATTTGATCCTTTTACTCCTCCTAAAGAACAACCTGACCCAGC
AAAAGGTACACAATACTTTTACTGCCAGCAAAGAG
>UMI_Splint_2_F_Next_A
GATCTCGGTGGTCGCCGTATCATTTGCCGGTTGGGTATCAATAANNNNNTATATNNNNNATTGCCTTTATTCTATCTACTTAGTTTTGGCGATGTAGTCTACCTATCC
TGATGCTGAATAAAGGC
>UMI_Splint_2_R_Next_B
ATCTCGTATGCCGTCTTCTGCTTGAATTAGGTTCTAGGATCACGNNNNNATATANNNNNCTGCCATCGAAAATTTTTCACCCGTAACAAGAACTTACAACTCTCTGAC
GCCTATATCATGAAGGCCTTTATTCAGCATCAGGA
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Tn5 oligos

```
Tn5ME-R 5'-[phos]CTGTCTCTTATACACATCT-3'
Tn5ME-A (Illumina FC-121-1030): TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
Tn5ME-B (Illumina FC-121-1031): GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
```

```
Nextera_Primer_A1   AATGATACGGCGACCACCGAGATCTACAC [i5 index] TCGTCGGCAGCGTCAGATG
Nextera_Primer_B1   CAAGCAGAAGACGGCATACGAGAT [i7 index] GTCTCGTGGGCTCGGAGATGTGTAT
```

Custom blocking oligos for target-enriched Tn5 library prep

```
>NextA_F_Blocking
AATGATACGGCGACCACCGAGATCTACAC  IIIIIIII  TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG/3ddC/
>NextA_RC_Blocking
CTGTCTCTTATACACATCTGACGCTGCCGACGA  IIIIIIII  GTGTAGATCTCGGTGGTCGCCGTATCATT
>NextB_F_Blocking
CAAGCAGAAGACGGCATACGAGAT  IIIIIIII  GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG/3ddC/
>NextB_RC_Blocking
CTGTCTCTTATACACATCTCCGAGCCCACGAGAC  IIIIIIII  ATCTCGTATGCCGTCTTCTGCTTG
```

Table S1: Custom oligos

All the oligos used in this study are shown. Spaces in the sequences are only intended to make sequences easier to read

	Read format	Reads/ flowcell	Gbases/ flowcell	Reagents ($)	Reads/ $	Mbases/ $	Machine (k$)
Illumina iSeq 100	150PE	4	1.2	582	6873	2.06	19.9
Illumina MiniSeq	150PE	25	7.5	1750	14286	4.29	65
Illumina MiSeq	300PE	25	15	1750	14286	8.57	99
Illumina NextSeq 550	150PE	400	120	5256	76104	22.83	250
ONT MinION (R2C2)	200-1000SE	4-9	3	650	6154-1385	4	1
ONT PromethION (R2C2)	200-1000SE	20-45	15	1100	18182-36364	13.64	10-75

Table S3: Output and Cost characteristics of R2C2 compared to benchtop Illumina sequencers.

This table compares output and cost characteristics of several Illumina sequencers with R2C2 as performed on the ONT MinION and PromethION. Output can vary widely on ONT sequencers and is therefore given as a range. Costs are approximate and do not take instrument costs into account.