Supplementary Materials for

A nanopore interface for high bandwidth DNA computing

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Tables S1-S4

Figures S1-S7

Circuit #	Circuit Component	Sequence	Purification Method
0	Gate	AGAGTGTGGAGTTGATAGGAGAG	Standard Desalting
	Output	ACACCTTACTCTCTACTCTCCTATCAACTCCACATTTTTTNNNNNNTT/3BioTEG/	HPLC
	Input	CCTATCAACTCCACACTCTCACTAATTCTACATC	Standard Desalting
	Fuel	TACCTCATTCAAACTCTCTCCTATCAACTCCACA	Standard Desalting
	Quencher	/5IAbRQ/ACACCTTACTCTCTA	HPLC
	Fluorophore	AGAGTAGAGAGTAAGGTGT/36-FAM/	HPLC
1	Gate	AGAGAATAATGGTTGTAGGAGAG	Standard Desalting
	Output	CTTCTTATACCACACCTCTCCTACAACCATTATTTTTTTTNNNNNNCA/3BioTEG/	HPLC
	Input	CCTACAACCATTATTCTCTCAATCTACCAAACTC	Standard Desalting
	Fuel	ACAAATACCTCATCCCTCTCCTACAACCATTATT	Standard Desalting
	Quencher	/5IAbRQ/CTTCTTATACCACAC	HPLC
	Fluorophore	AGAGGTGTGGTATAAGAAG/36-FAM/	HPLC
2	Gate	AGAGTAAGTATAGAGGTGAAGAG	Standard Desalting
	Output	CAACTACAATCTCTCCTCTTCACCTCTATACTTATTTTTTNNNNNNTA/3BioTEG/	HPLC
	Input	TCACCTCTATACTTACTCTTCCTTCATCTTCTAC	Standard Desalting
	Fuel	TCTCCAATTTCAACTCTCTTCACCTCTATACTTA	Standard Desalting

	Quencher	/5IAbRQ/CAACTACAATCTCTC	HPLC
	Fluorophore	AGAGGAGAGATTGTAGTTG/36-FAM/	HPLC
3	Gate	AGAGAGGATTAGGATAGTGAGAG	Standard Desalting
	Output	AACCACATTAACCTTCTCTCACTATCCTAATC CTTTTTTTNNNNNNCC/3BioTEG/	HPLC
	Input	CACTATCCTAATCCTCTCTAAACCTTACCACC AC	Standard Desalting
	Fuel	CCTTCTCAACTCCTCCTCTCACTATCCTAATCC T	Standard Desalting
	Quencher	/5IAbRQ/AACCACATTAACCTT	HPLC
	Fluorophore	AGAGAAGGTTAATGTGGTT/36-FAM/	HPLC
4	Gate	AGAGGGTGTTTAGAGTTTAAGAG	Standard Desalting
	Output	TTATCCAACTCACTACTCTTAAACTCTAAACA CCTTTTTTNNNNNNAT/3BioTEG/	HPLC
	Input	TAAACTCTAAACACCCTCTAATAACACCTCCT AA	Standard Desalting
	Fuel	CTCTTCTTTCCAAACCTCTTAAACTCTAAACA CC	Standard Desalting
	Quencher	/5IAbRQ/TTATCCAACTCACTA	HPLC
	Fluorophore	AGAGTAGTGAGTTGGATAA/36-FAM/	HPLC
5	Gate	AGAGAAAGTGATAAGATGGAGAG	Standard Desalting
	Output	TCTTTCACCTCACATCTCTCCATCTTATCACTT TTTTTTTNNNNNNCA/3BioTEG/	HPLC
	Input	CCATCTTATCACTTTCTCTATTACTTCCTACAC C	Standard Desalting

	Fuel	ATCCTCCTTCCATCCCTCTCCATCTTATCACTTT	Standard Desalting
	Quencher	/5IAbRQ/TCTTTCACCTCACAT	HPLC
	Fluorophore	AGAGATGTGAGGTGAAAGA/3Cy5Sp/	HPLC
6	Gate	AGAGGGTATTAGTTAGGTAAGAG	Standard Desalting
	Output	TCCATTTCATTTCACCTCTTACCTAACTAATACCTTTTTTNNNNNNAA/3BioTEG/	HPLC
	Input	TACCTAACTAATACCCTCTCTCCATAACATTCCA	Standard Desalting
	Fuel	ACTTCTAACAACTACCTCTTACCTAACTAATACC	Standard Desalting
	Quencher	/5IAbRQ/TCCATTTCATTTCAC	HPLC
	Fluorophore	AGAGGTGAAATGAAATGGA/36-FAM/	HPLC
7	Gate	AGAGTGTTAGTAGTAGAGTAGAG	Standard Desalting
	Output	AAATTCTATCCACTCCTCTACTCTACTACTAACATTTTTTNNNNNNCC/3BioTEG/	HPLC
	Input	ACTCTACTACTAACACTCTTCTACATCCACATCT	Standard Desalting
	Fuel	CACTCAATAACTACCCTCTACTCTACTACTAACA	Standard Desalting
	Quencher	/5IAbRQ/AAATTCTATCCACTC	HPLC
	Fluorophore	AGAGGAGTGGATAGAATTT/36-FAM/	HPLC
8	Gate	AGAGGTATAAAGGAGTTTGAGAG	Standard Desalting
	Output	TAACTCTACCACAAACTCTCAAACTCCTTTATACTTTTTTNNNNNNTA/3BioTEG/	HPLC

	Input	CAAACTCCTTTATACCTCTCTCTACTCATCTTCC	Standard Desalting
	Fuel	CCACCTCCATCTATACTCTCAAACTCCTTTATAC	Standard Desalting
	Quencher	/5IAbRQ/TAACTCTACCACAAA	HPLC
	Fluorophore	AGAGTTTGTGGTAGAGTTA/3Cy5Sp/	HPLC
9	Gate	AGAGTGGTAAGGTAGTTAAAGAG	Standard Desalting
	Output	CTAACAAACTTTACCCTCTTTAACTACCTTACCATTTTTTNNNNNNTC/3BioTEG/	HPLC
	Input	TTAACTACCTTACCACTCTACATTCCTTCTAATC	Standard Desalting
	Fuel	ATTTACATCTCAACCCTCTTTAACTACCTTACCA	Standard Desalting
	Quencher	/5IAbRQ/CTAACAAACTTTACC	HPLC
	Fluorophore	AGAGGGTAAAGTTTGTTAG/3Cy5Sp/	HPLC

Table S1: Table of seesaw gate, output strand, input strand, fuel strand, quencher-labeled strand, and fluorophore-labeled strand sequences for all ten circuits used in kinetics analysis and multiplexing experiments. Iowa Black RQ from IDT is used as the quencher molecule and either 6-FAM or Cy 5 is used as the fluorophore in the fluorescent reporter complexes. The nanopore barcode region (red) can be substituted with any barcode from **Table S2**.

Barcode #	Set A	Set B	Set C
0	CAAATA	GGGTTC	/iSpC3/CATAC
1	TCATAC	TGATTG	T/iSpC3/ATAC
2	ATATCT	AGAGTT	TC/iSpC3/TAC
3	CTCCAC	AGAGGA	TCA/iSpC3/AC
4	ATCTAA	ATATCA	TCAT/iSpC3/C
5	CTCAAA	TTCTGT	TCATA/iSpC3/
6	AAATAC	AGCCTC	/iSpC3/CATA/iSpC3/
7	TCCAAC	GATACT	T/iSpC3/AT/iSpC3/C
8	CAAAAC	TCTCTG	TC/iSpC3//iSpC3/AC
9	ACCTCC	AATCAA	T/iSpC3//iSpC3/TAC
10	-	TGGAAG	TCA/iSpC3//iSpC3/C
11	-	GCACAT	TCAT/iSpC3//iSpC3/
12	-	-	T/iSpC3//iSpC3//iSpC3/AC
13	-	-	AA/iSpC3/CAA

Table S2: Table of all explored output strand barcodes organized into Set A (randomly selected), Set B (based on predictive model) and Set C (contains abasic sites). Abasic sites are denoted as /iSpC3/ (C3 Spacer phosphoramidite modification from IDT) .

Circuit Component	**Sequence**	**Purification Method**
Gate	TGAGTGTGATTGTGTTATGAGTG	Standard Desalting
Output	CAACATATCAATTCACTCATAACACAATCACATTTTTT TCATACCA/3BioTEG/	HPLC
Input	CATAACACAATCACACTCACCACCAAACTTCA	Standard Desalting
Fuel	CACTAACATACAACACTCATAACACAATCACA	Standard Desalting
Quencher	/5IAbRQ/CAACATATCAATTCA	HPLC
Fluorophore	TGAGTGAATTGATATGTTG/3Cy5Sp/	HPLC

Table S3: Table of seesaw gate, output strand, input strand, fuel strand, quencher-labeled strand, and fluorophore-labeled strand sequences for clamped circuit from **Figure S1**. Iowa Black RQ from IDT is used as the quencher molecule and Cy 5 is used as the fluorophore in the reporter complex. Barcode A1 (red) from **Table S2** was used in this circuit's output strand.

Probe Component	Sequence	Purification Method
let-7a Probe		
Input	UGAGGUAGUAGGUUGUAUAGUU	HPLC
Helper	GAGTGTAGTGGAAGTTGGAG	PAGE
Bottom	AACTATACAACCTACTACCTCACTCCAACTTCCACTACACTC/36-FAM/	HPLC
Top	/5IAbRQ/GAGTGTAGTGGAAGT	HPLC
Output	TGGAGTGAGGTAGTAGGTTTTTTTTTGGAAGTT/3BioTEG/	HPLC
let-7c Probe		
Input	UGAGGUAGUAGGUUGUAUGG	HPLC
Helper	TGAGGTATGGAGTGAGTGGA	PAGE
Bottom	AACCATACAACCTACTACCTCATCCACTCACTCCATACCTCA/36-FAM/	HPLC
Top	/5IAbRQ/TGAGGTATGGAGTGA	HPLC
Output	GTGGATGAGGTAGTAGGTTTTTTTTAA/iSpC3/CAATT/3BioTEG/	HPLC
let-7e Probe		
Input	UGAGGUAGGAGGUUGUAUAGUU	HPLC
Helper	TGAGGTATGGAGTGAGTGGA	PAGE
Bottom	AACTATACAACCTCCTACCTCATCCACTCACTCCATACCTCA/3Cy5Sp/	HPLC
Top	/5IAbRQ/TGAGGTATGGAGTGA	HPLC
Output	GTGGATGAGGTAGGAGGTTTTTTTTT/iSpC3//iSpC3//iSpC3/ACTT/3BioTEG/	HPLC

Table S4: Table of let-7 miRNA input, helper strand, bottom strand (with fluorophore), top strand (with quencher), and output strand (with barcode) for the two-step let-7 detection probes. The

barcode region is represented in red. Barcodes B10, C13, and C12 from Table S2 were used for the let-7a, let-7c, and let-7e probes respectively.

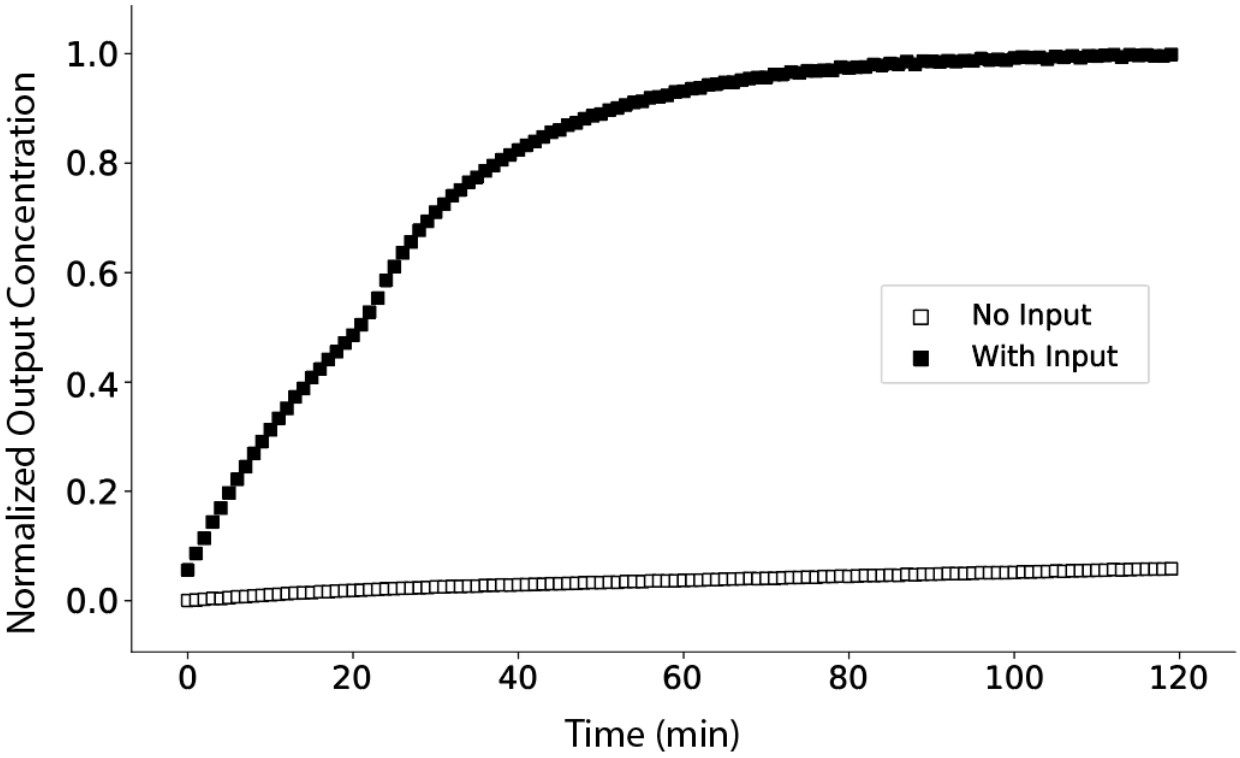


Figure S1: Kinetics of a clamped catalytic DSD circuit measured on a fluorospectrometer. Addition of clamp domains mitigate circuit leakage caused by blunt end stacking.

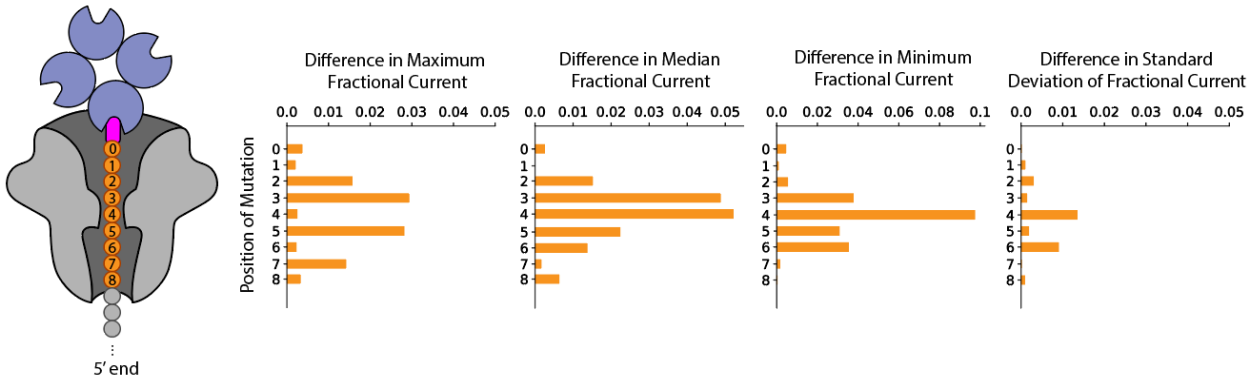


Figure S2: Each plot depicts the change in either maximum, median, minimum, or standard deviation of nanopore fractional current elicited by a single-nucleotide mutation at each position on the strand's barcode.

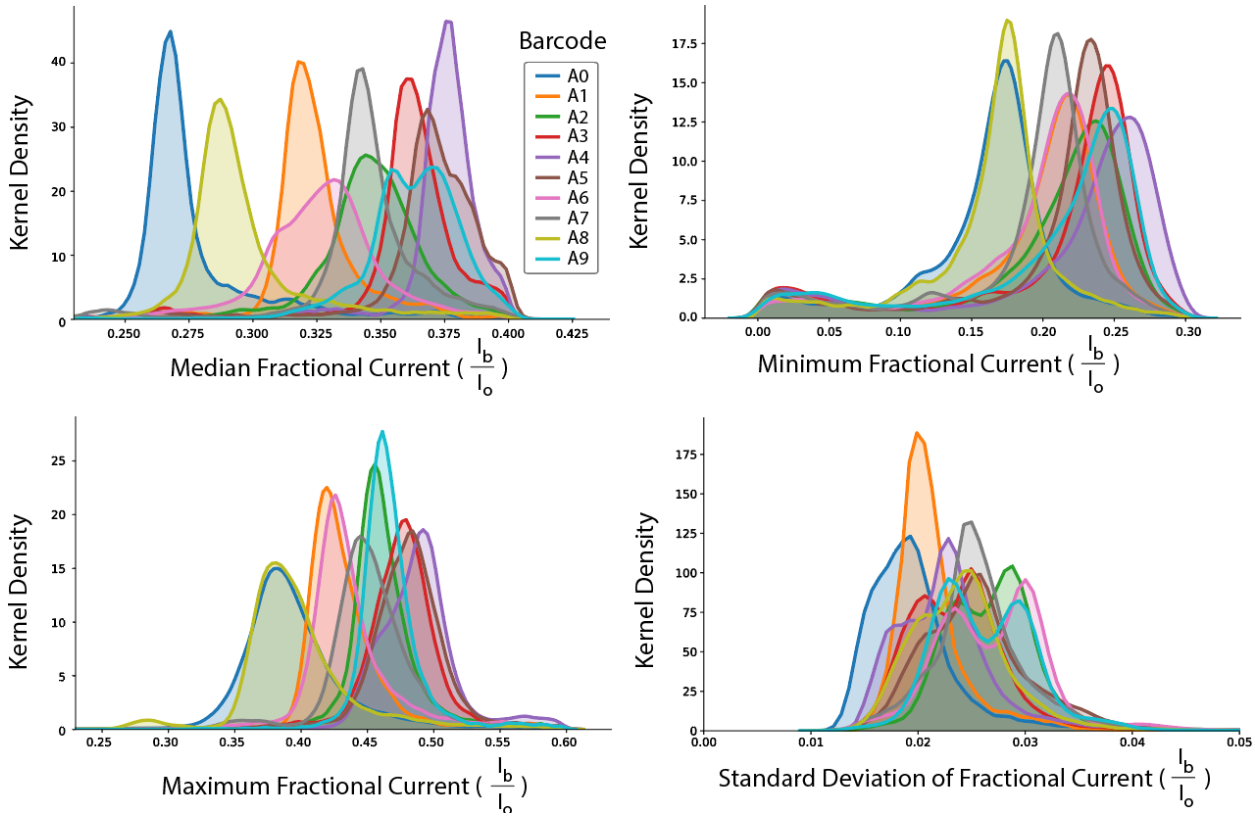


Figure S3: Distributions of median, minimum, maximum, and standard deviation of nanopore fractional current for each barcode in Set A. Each distribution is composed of ~14500 data points.

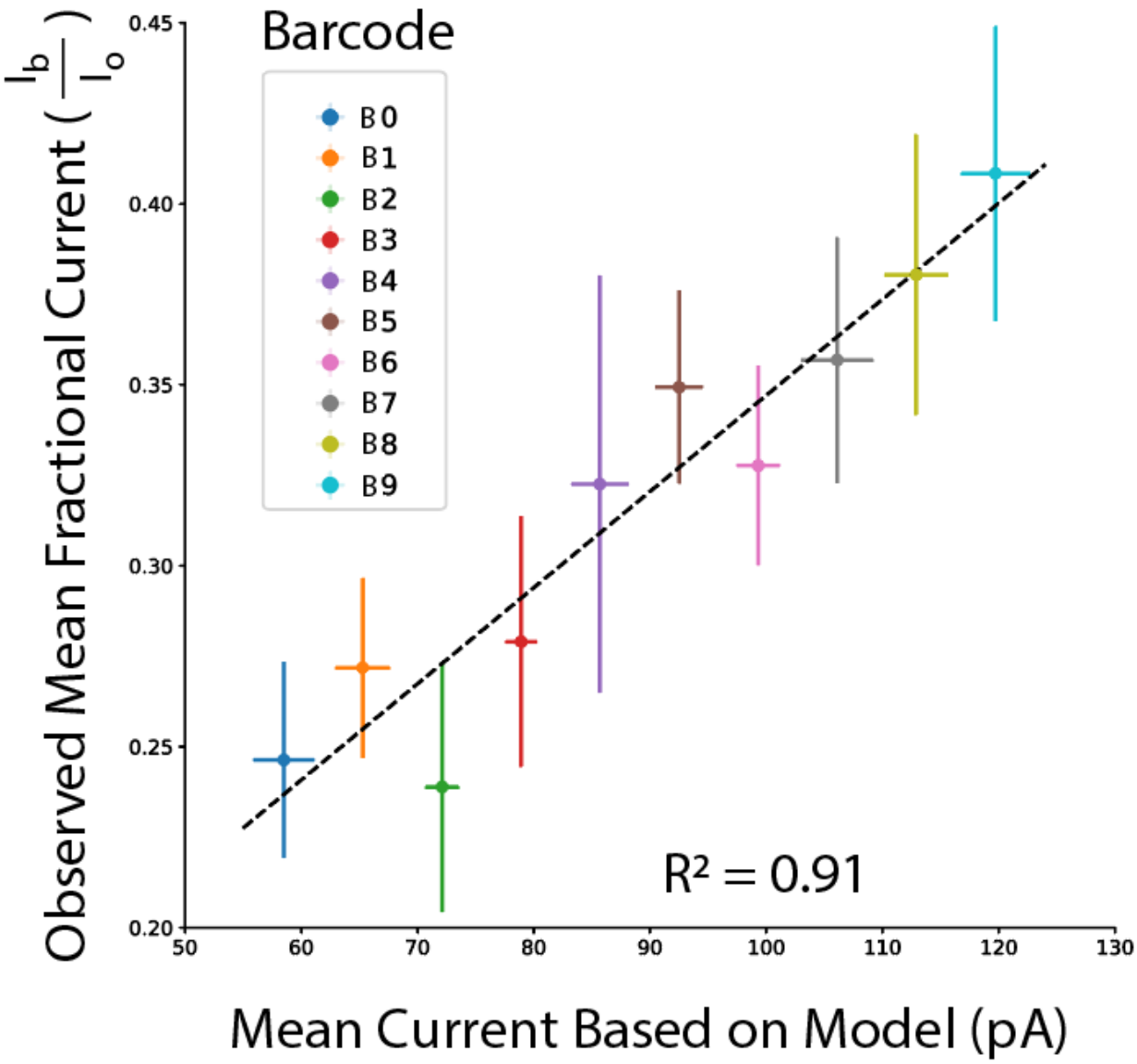


Figure S4: Correlation of observed mean fractional currents to the mean current from predictive model for barcodes B1-B9. Vertical and horizontal error bars show standard deviation for observed mean fractional current and mean current based on model respectively.

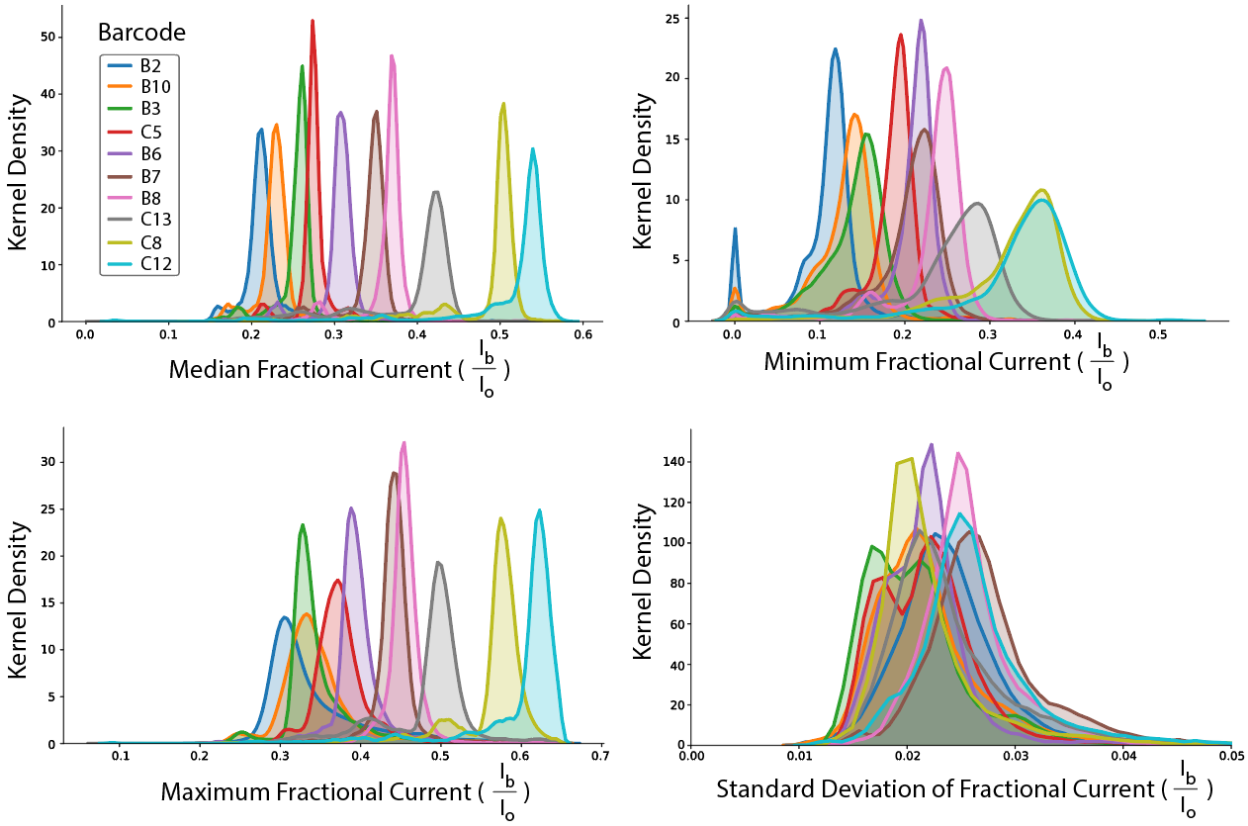
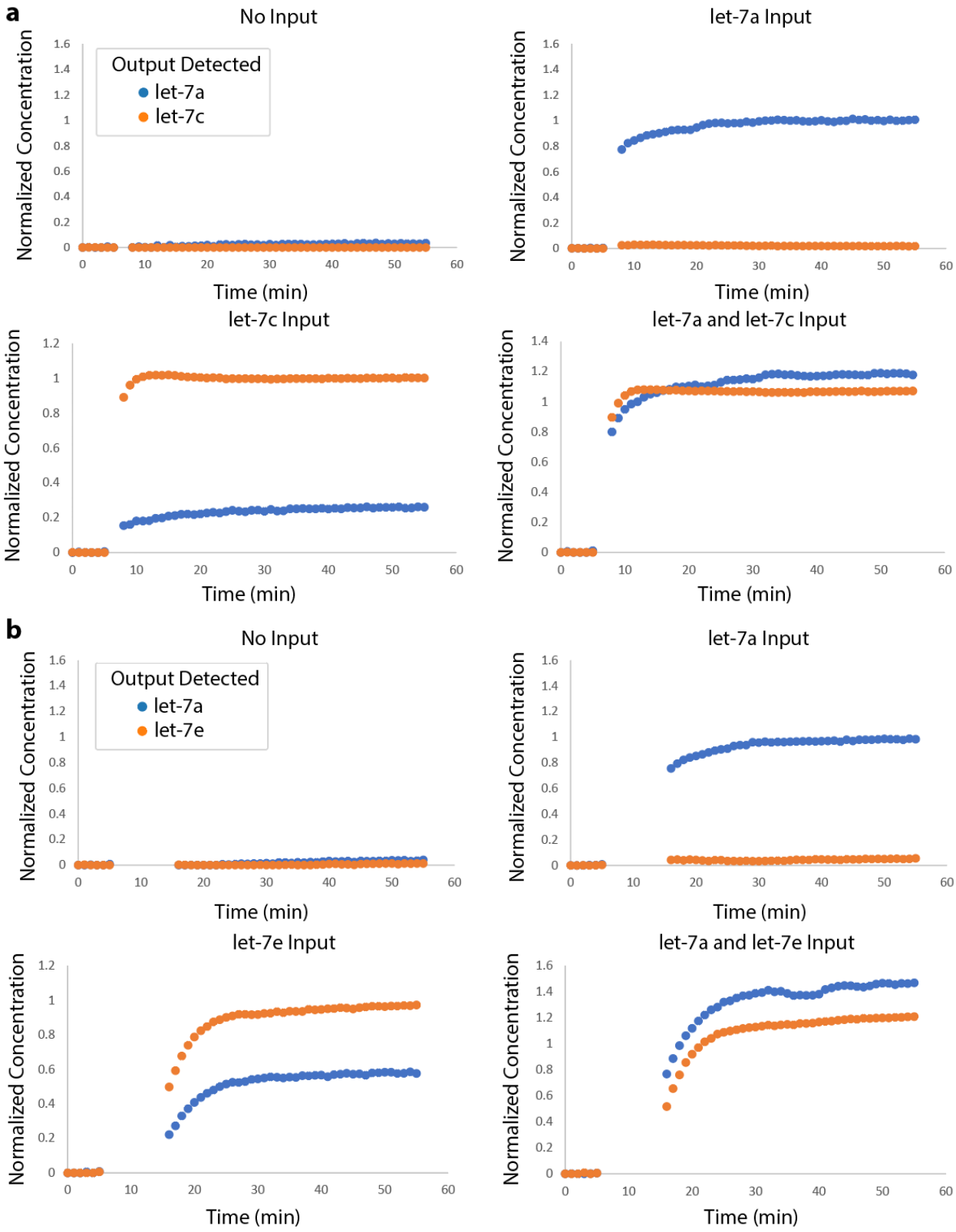


Figure S5: Distributions of median, minimum, maximum, and standard deviation of nanopore fractional current for each barcode in the selected set of orthogonal barcodes used for multiplexing experiments. Each distribution is composed of ~13000 data points.


a
No Input
Output Detected
let-7a
let-7c
Normalized Concentration
Time (min)
let-7a Input
let-7c Input
let-7a and let-7c Input
b
No Input
Output Detected
let-7a
let-7e
let-7a Input
let-7e Input
let-7a and let-7e Input

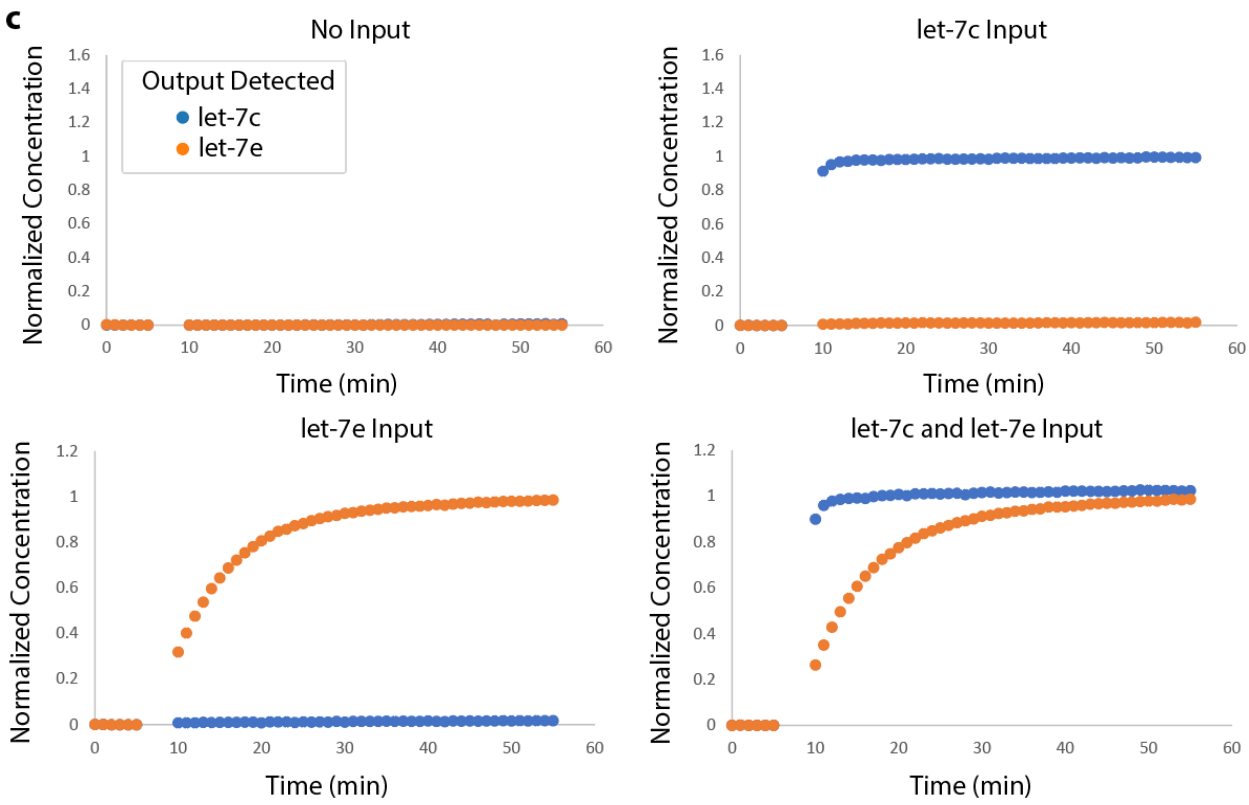


Figure S6: Normalized concentrations of let-7 probe output measured by fluorospectrometer. **a)** let-7a and let-7c probes, **b)** let-7a and let-7e probes, and **c)** let-7c and let-7e probes were multiplexed and their response to the introduction of miRNA input strands was measured. Each probe was present at 100 nM, each helper strand at 130 nM, each input strand at 50 nM, and streptavidin at 1200 nM.

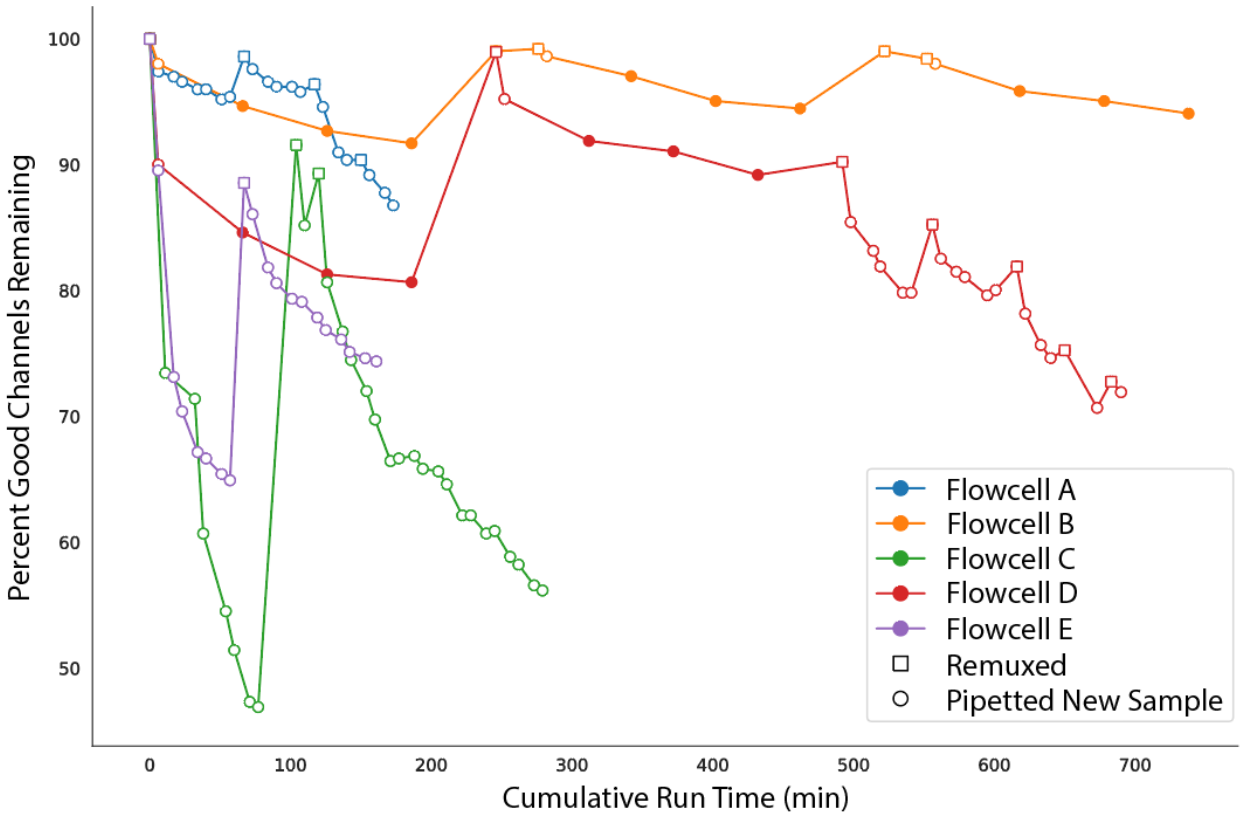


Figure S7: Plot showing the number of good channels on MinION R9.4.1 flow cells over run time. Each flow cell (colored line) begins with 400-512 good channels. Channel loss occurs at a higher rate when new samples (circles) are pipetted into the flow cell more frequently. Remuxing (squares) of the flow cell recovers channels.