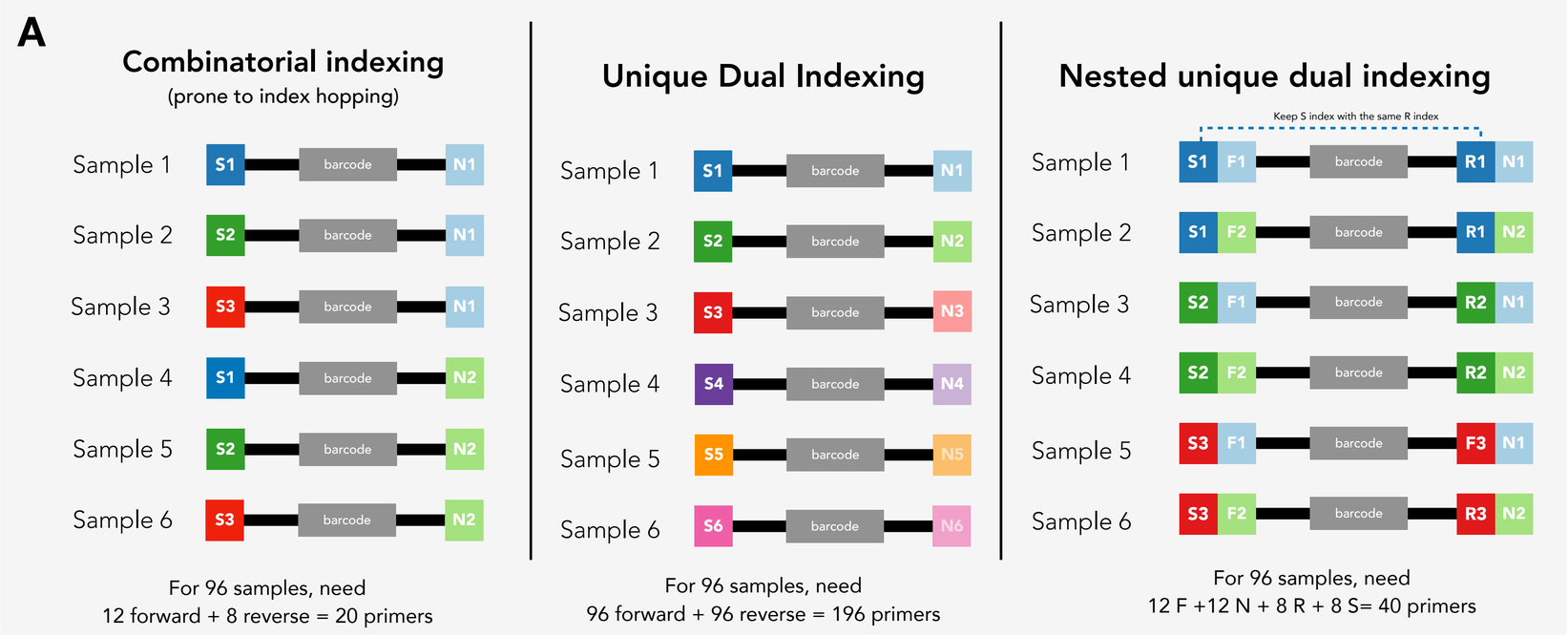
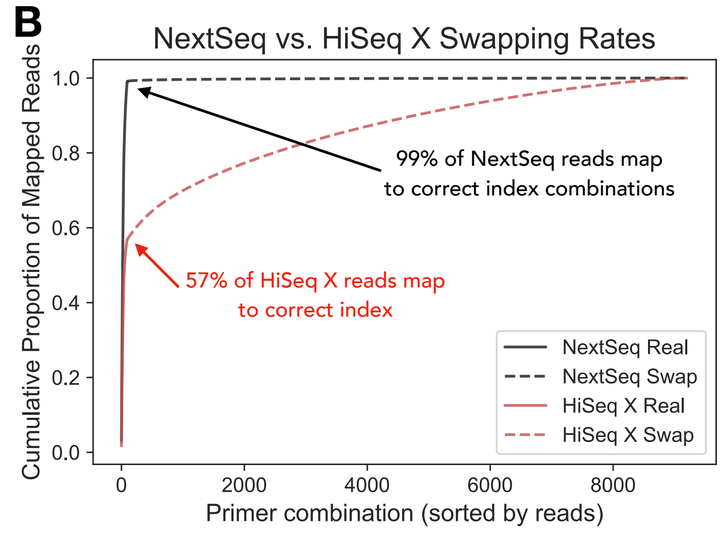
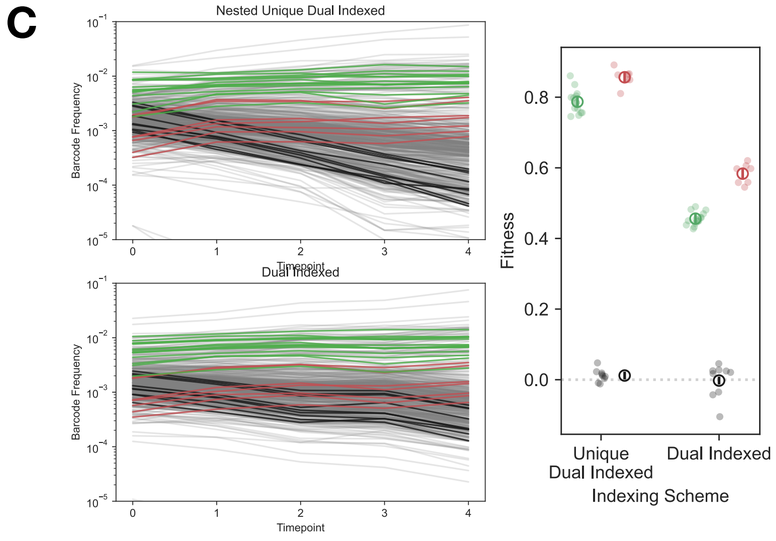
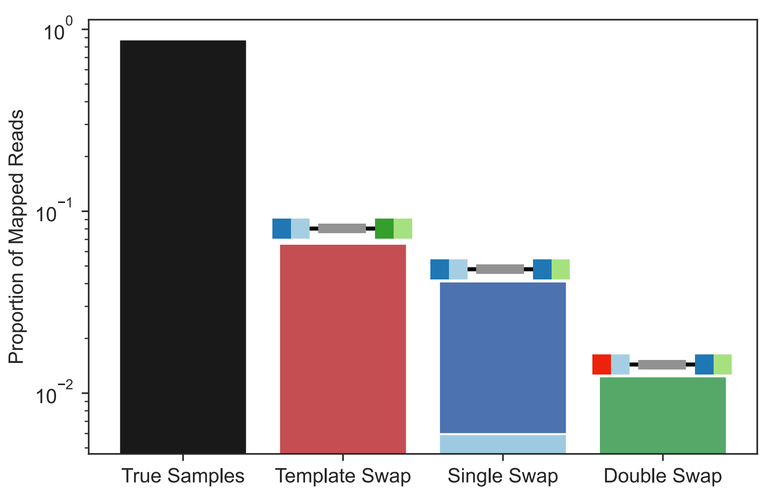

A
Combinatorial indexing
(prone to index hopping)
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
S1
S2
S3
barcode
N1
N2
For 96 samples, need
12 forward + 8 reverse = 20 primers
Unique Dual Indexing
S4
S5
S6
N3
N4
N5
N6
For 96 samples, need
96 forward + 96 reverse = 196 primers
Nested unique dual indexing
Keep S index with the same R index
F1
F2
F3
R1
R2
R3
For 96 samples, need
12 F +12 N + 8 R + 8 S= 40 primers

D
Mechanisms for index hopping
"Index Hopping"
"Template Swapping"
Free-floating indices get incorporated
Homology across amplicons creates chimeric reads
Read out: single-index swaps
Read out: combinations of otherwise correct pairs


B
NextSeq vs. HiSeq X Swapping Rates
Cumulative Proportion of Mapped Reads
Primer combination (sorted by reads)
99% of NextSeq reads map
to correct index combinations
57% of HiSeq X reads map
to correct index
NextSeq Real
NextSeq Swap
HiSeq X Real
HiSeq X Swap


C
Nested Unique Dual Indexed
Dual Indexed
Barcode Frequency
Timepoint
Fitness
Unique
Dual Indexed
Dual Indexed
Indexing Scheme


E
Proportion of Mapped Reads
True Samples
Template Swap
Single Swap
Double Swap