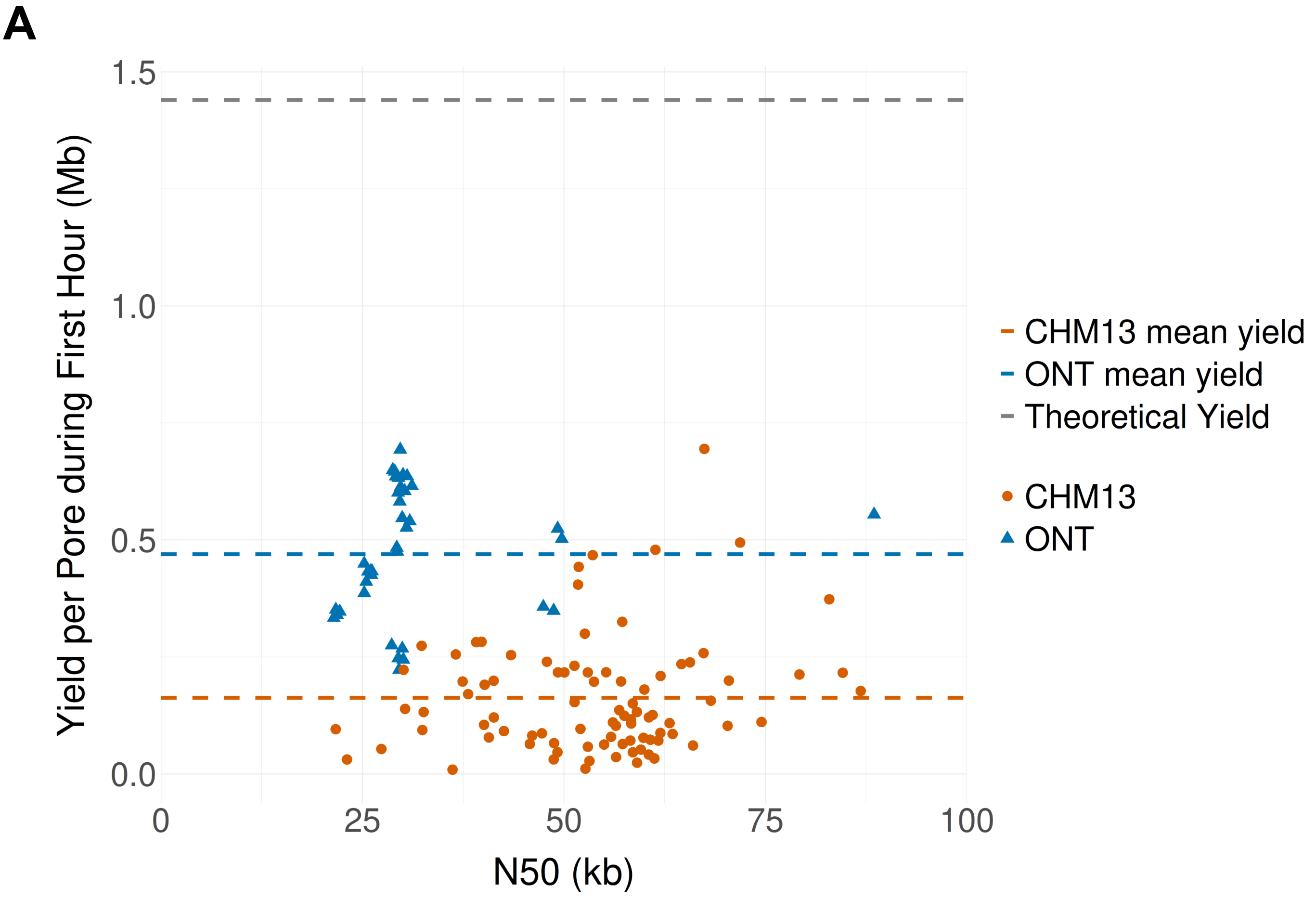
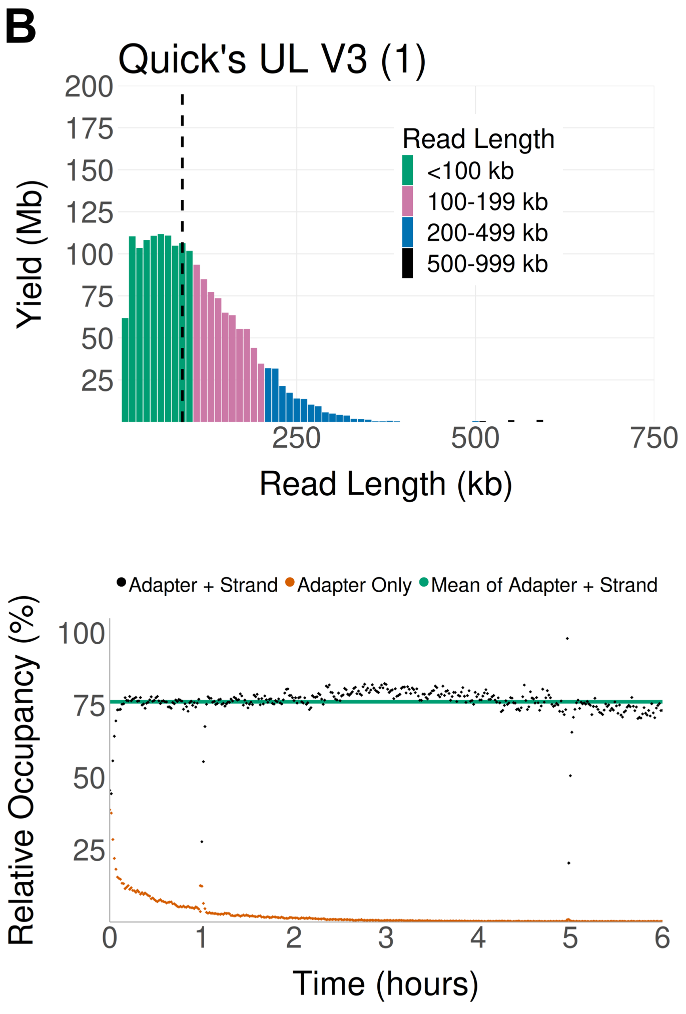
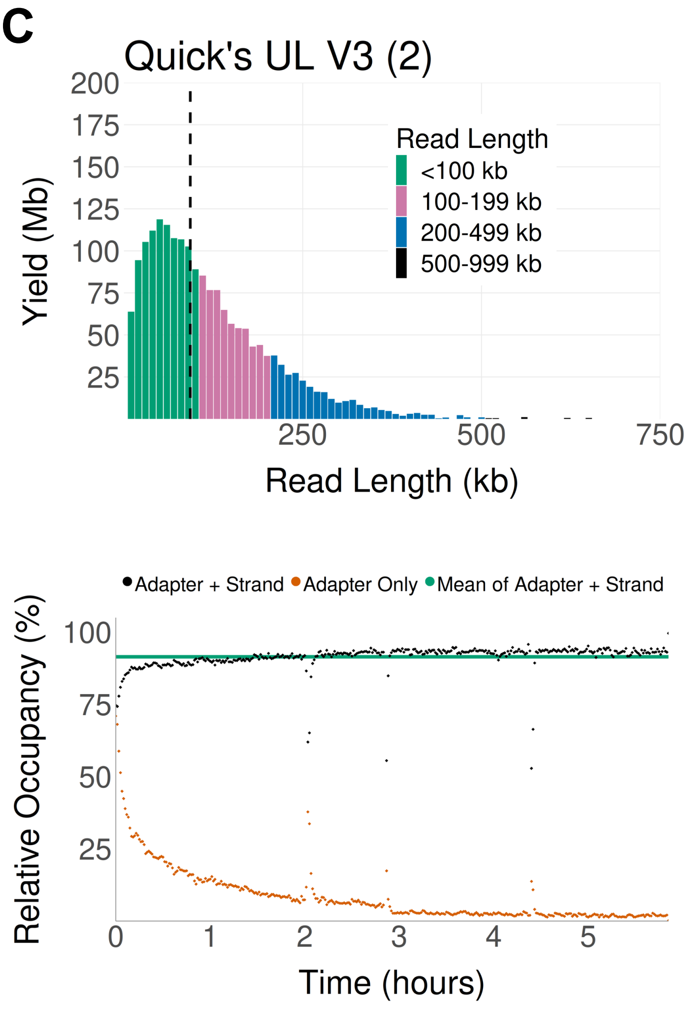
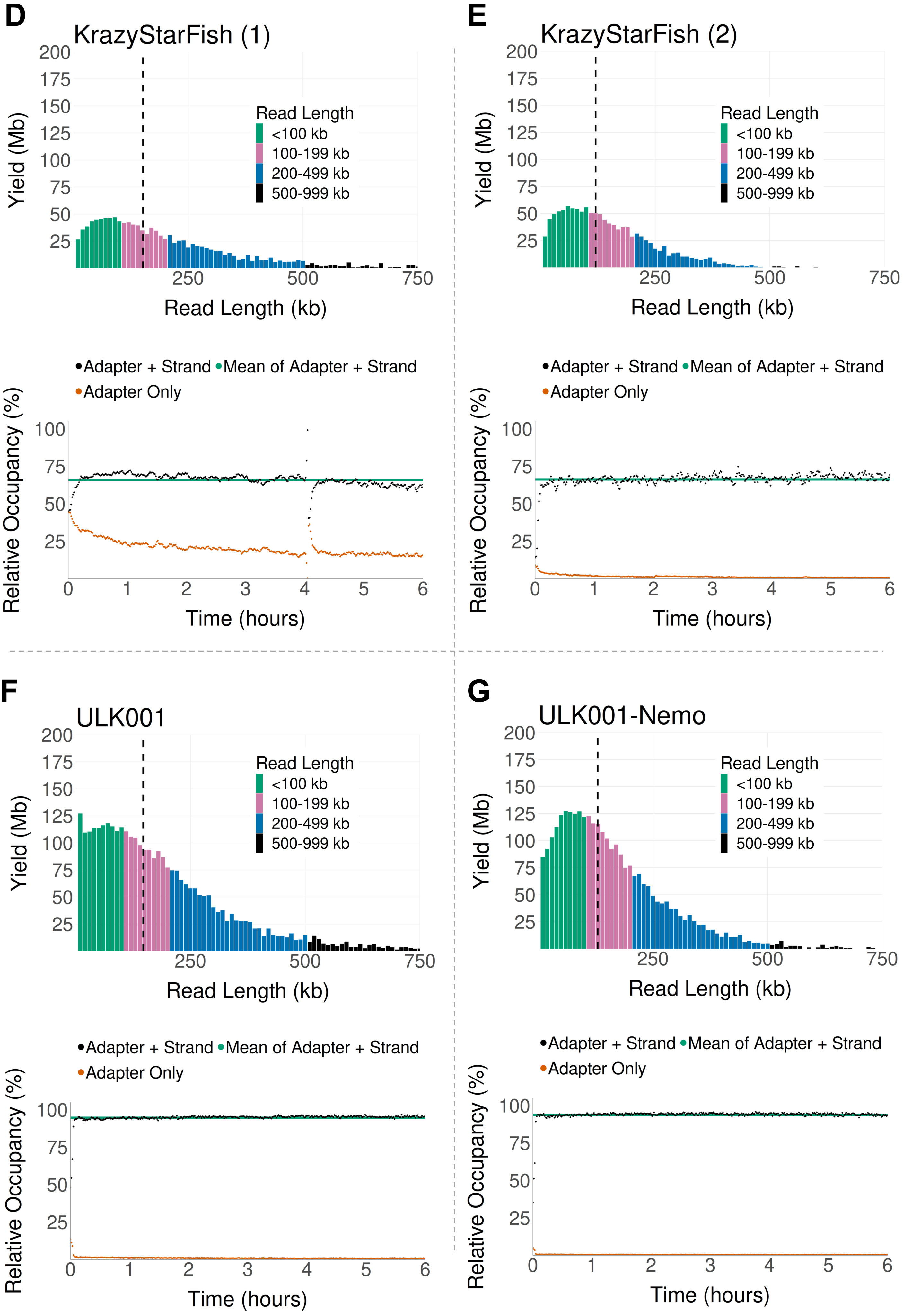

A
Yield per Pore during First Hour (Mb)
1.5
1.0
0.5
0.0
0
25
50
75
100
N50 (kb)
CHM13 mean yield
ONT mean yield
Theoretical Yield
CHM13
ONT


B
Quick's UL V3 (1)
Yield (Mb)
200
175
150
125
100
75
50
25
250
500
750
Read Length (kb)
Read Length
<100 kb
100-199 kb
200-499 kb
500-999 kb
Adapter + Strand
Adapter Only
Mean of Adapter + Strand
Relative Occupancy (%)
100
75
50
25
0
1
2
3
4
5
6
Time (hours)


C
Quick's UL V3 (2)
Yield (Mb)
200
175
150
125
100
75
50
25
250
500
750
Read Length (kb)
Read Length
<100 kb
100-199 kb
200-499 kb
500-999 kb
Adapter + Strand
Adapter Only
Mean of Adapter + Strand
Relative Occupancy (%)
100
75
50
25
0
1
2
3
4
5
Time (hours)


D
KrazyStarFish (1)
Yield (Mb)
200
175
150
125
100
75
50
25
250
500
750
Read Length (kb)
Read Length
<100 kb
100-199 kb
200-499 kb
500-999 kb
Adapter + Strand
Mean of Adapter + Strand
Adapter Only
Relative Occupancy (%)
100
75
50
25
0
1
2
3
4
5
6
Time (hours)
E
KrazyStarFish (2)
F
ULK001
G
ULK001-Nemo

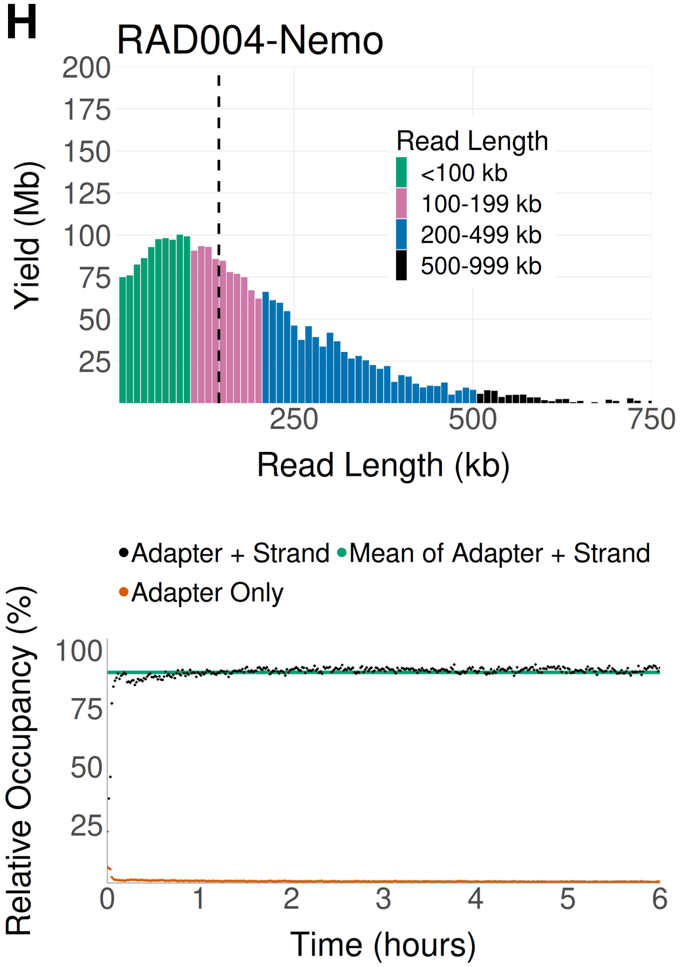


Figure S1. Comparison of sequencing outputs of early ultra-long (UL) library preparation protocols. Sequencing yields of the CHM13 ultra-long data (Logsdon et al. 2021) were lower on average compared to publicly available ONT data (GM24385 and GM24631) obtained from ligation-based libraries (A). We previously developed two protocols based on the RAD004 kit: Original Quick's UL v3 (B-C) and KrazyStarFish (KSF) (D-E). New protocols based on Nemo clean-up were developed afterward, with comparable outputs to the control ULK001 protocol (F-H).

(B-H) Upper panels = read length distribution with N50 value shown as a black dashed line; lower panels = timelapse of relative pore occupancy. All data show 6 hours of run time. All libraries were prepared from GM12878 cells, loaded on MinION flow cells, and sequenced on the GridION platform.