Illumina Unamplified Indexed Library Construction: An Automated Approach

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DISCLAIMER

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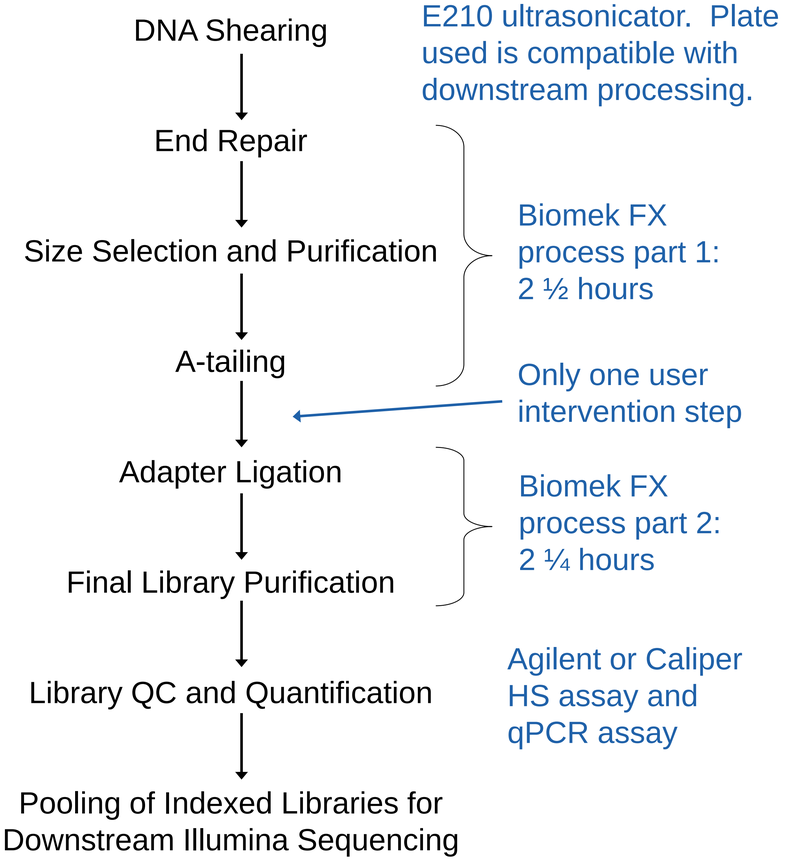
Introduction

Manual library construction is a limiting factor in Illumina sequencing. Constructing libraries by hand is costly, time-consuming, low-throughput, and ergonomically hazardous, and constructing multiple libraries introduces risk of library failure due to pipetting errors. The ability to construct multiple libraries simultaneously in automated fashion represents significant cost and time savings.

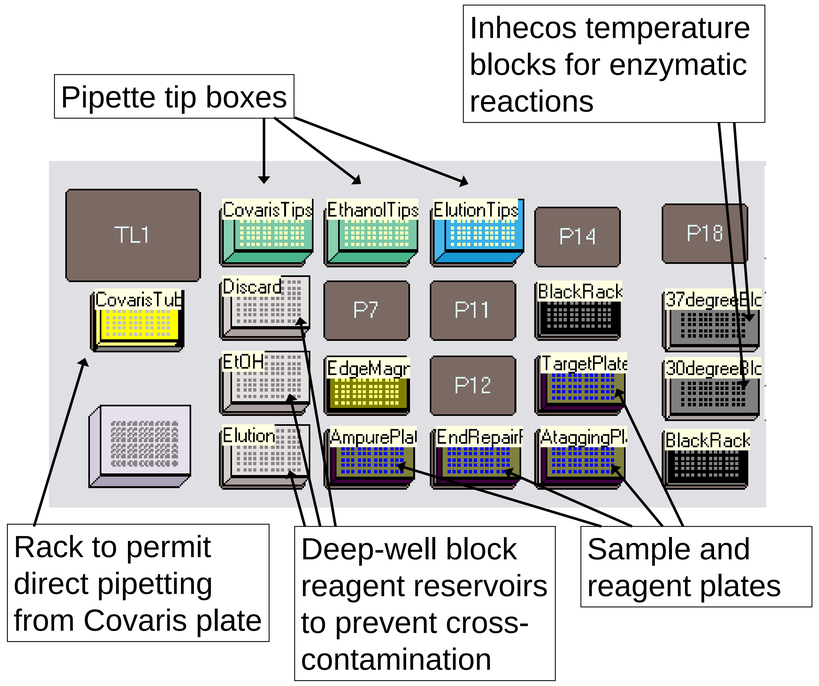
Here we present a strategy to construct up to 96 unamplified indexed libraries using Illumina TruSeq reagents and a Biomek FX robotic platform. We also present data to indicate that this library construction method has little or no risk of cross-contamination between samples.

Workflow



Following DNA shearing, a single operator can construct 96 libraries and set up QC in a single day.

Instrument Layout

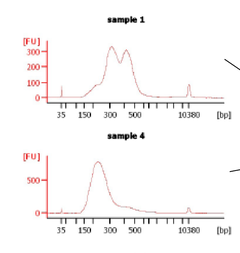


Proof of Principle Experiment

24 unamplified indexed TruSeq libraries were constructed using this method and subsequently pooled and sequenced in a 1x36 run on an Illumina GAii instrument. Sequence data was analyzed for potential cross-contamination between adjacent libraries.

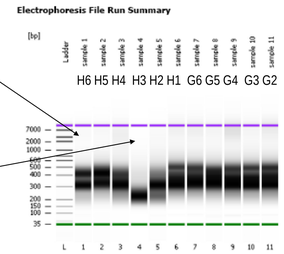
Row \ Column	1	2	3	4	5	6
E	E.coli K12 Adapter #9	E.coli K12 Adapter #8	E.coli K12 Adapter #7	E.coli K12 Adapter #12	E.coli K12 Adapter #11	E.coli K12 Adapter #10
F	E.coli K12 Adapter #3	Saccharomonospora azurea Adapter #2	E.coli K12 Adapter #1	Actinopolyspora iraqiensis Adapter #6	E.coli K12 Adapter #5	Saccharomonospora saliphila Adapter #4
G	E.coli K12 Adapter #12	E.coli K12 Adapter #11	E.coli K12 Adapter #10	E.coli K12 Adapter #9	E.coli K12 Adapter #8	E.coli K12 Adapter #7
H	E.coli K12 Adapter #6	Cycloclasticus pugetii Adapter #5	E.coli K12 Adapter #4	Acetobacterium sp. WL Adapter #3	E.coli K12 Adapter #2	Sporomusa sp. KB1 Adapter #1

Library QC Data

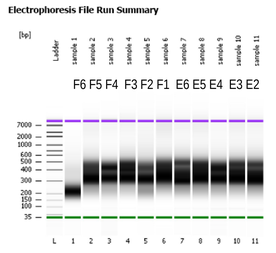


Top: Successful library: electropherogram shows mono- and duo-adapter peaks

Bottom: Failed library: electropherogram shows no adapter ligation



Agilent HS data for libraries in rows H and G



Agilent HS data for libraries in rows F and E

Both libraries constructed with Adapter #4 failed, leading us to conclude that Adapter #4 in the kit lot used was faulty, rather than the method failing.

Pool #1: Libraries in rows H and G, except failed library H3 was not included.
Pool #2: LIbraries in rows F and E, except failed library F6 was not included.

Sequence data analysis

From each pool, reads were sorted by index sequence, then mapped against the reference genomes of S. azurea (library F2), A. iraqiensis (library F4), and E. coli (control libraries). The percentage of reads mapping to each reference genome are shown in the table below:

	1	2	3	4	5	6	
E	0.09%	0.06%	0.08%	0.06%	0.07%	0.06%	S_azurea
E	0.23%	0.19%	0.21%	0.19%	0.21%	0.20%	A_iraqiensis
E	95.54%	95.74%	95.41%	95.37%	95.88%	95.55%	E.coli
F	0.05%	85.40%	0.07%	4.64%	0.06%	0.07%	S_azurea
F	0.19%	4.19%	0.20%	79.97%	0.19%	0.20%	A_iraqiensis
F	95.78%	1.27%	95.20%	1.96%	95.38%	95.47%	E.coli
G	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	S_azurea
G	0.19%	0.20%	0.19%	0.19%	0.19%	0.19%	A_iraqiensis
G	95.31%	95.85%	95.41%	95.45%	95.67%	95.35%	E.coli
H	0.05%	0.01%	0.05%	0.03%	0.05%	0.05%	S_azurea
H	0.19%	0.02%	0.19%	0.03%	0.19%	0.04%	A_iraqiensis
H	95.58%	0.35%	95.41%	0.17%	95.43%	0.43%	E.coli

For each index there are a handful of reads that mapped to the “wrong” reference genome. When the mapping is redone excluding reads from ribosomal DNA, the instances of “wrong” mapping are not seen. This indicates that the reads mapping to unexpected reference genomes are not a result of cross-contamination during library construction, but rather just representative of highly conserved regions of ribosomal DNA.

Reads corresponding to Index #4 were observed in both pools, despite the fact that no library constructed with Adapter #4 was included in either pool. We hypothesize that this is the result of contamination of kit reagents, and was not introduced during library construction.

Conclusion

We have successfully demonstrated the ability to construct up to 96 Illumina unamplified indexed libraries in high-throughput fashion, and that the risk of cross-contamination in the library construction process is minimal. We have high hopes of being able to scale up this process for use in a production sequencing environment.

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