An Optimal Strategy for Internalizing Ultra-high Throughput Plasmid Sequencing

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Abstract

Most synthetic biology companies are laser-focused on scaling proprietary genome engineering technologies and developing the biological content that serves as the primary driver of their growth and enterprise value. Many companies decided it was simpler to outsource plasmid sequencing rather than investing in the buildout of a high throughput DNA sequencing lab. However, an outsourced (or a suboptimal in-house) sequencing strategy can become the slow step that constrains the growth of a company's lead pipeline. Fortunately, recent developments in NGS and library prep have made it possible for labs to go from bacterial colonies to sequence-verified clones within 24 hours.[1] seqWell has developed a robust one-step library preparation method for ultra-high throughput plasmid sequencing, which:

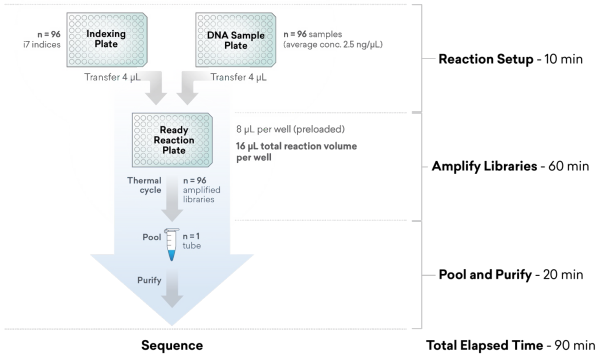
- Reduces plasmid sequencing costs by 4-fold
- Increases throughput and reduces turn-around time
- Allows one lab technician to generate libraries and sequence data from 1,536 plasmids in under 24 hours

Methods

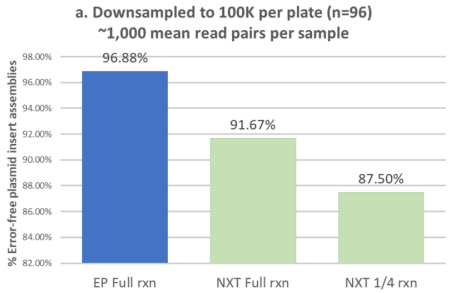
We conducted an objective comparison of seqWell's new ExpressPlex™ technology to a commonly used library prep method (Illumina Nextera® XT at full and ¼ reaction scales). Miniprepped plasmid DNA (pDONR-201/221 clones) from a commercial source which spanned between 2,561 and 10,020 bp in total plasmid length was used as the DNA input for both methods. The GC% of the insert DNA was 19 - 75% and was originally cloned from a diverse set of microorganisms, including *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Vibrio cholerae* and *Yersinia pestis*.

After sequencing on the Illumina MiSeq® sequencing platform, the output from each 96-well plate of plasmids was down-sampled to 100K total read pairs and de novo assembled, which translated to an average output of 1,042 read pairs per clone. Overall "success" for each method was defined as the percent yield of complete and error-free de novo plasmid insert assemblies obtained.

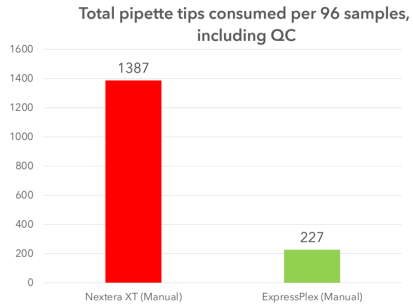
ExpressPlex Workflow



Results



Plasmid assembly success rate



Reduction in plastic waste

Conclusions

- Using ExpressPlex, one research technician can generate libraries and sequence 1,536 plasmids in under 24 hours.
- Due to auto-normalization of read output, 4X more plasmids can be sequenced on a single run and successfully assembled with Express Plex as compared to Nextera XT.
- Plastic waste is reduced by 4 – 5-fold.
- The speed, simplicity and reliability of the auto-normalizing ExpressPlex technology make it an ideal library prep solution for ultra-high throughput plasmid sequencing.

Auto-normalized read balance: 1,536 ExpressPlex variable input (4 – 40 ng) plasmid libraries on one sequencing run