Ultra-High Throughput Sequencing for Synthetic Biology Discovery

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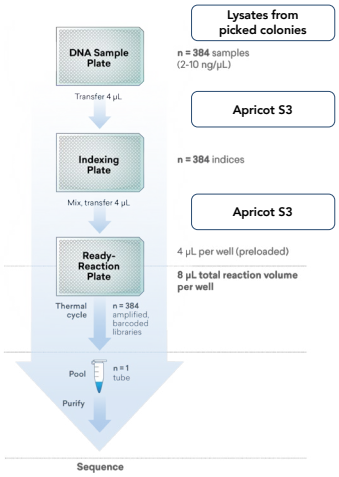
Introduction

Industrial-scale protein engineering and gene editing techniques have simultaneously necessitated and fueled breakthroughs in highly multiplexed molecular biology. The growth rate of NGS data output has far-outpaced development of the technologies necessary to power the Design-Build-Test-Learn (DBTL) cycle.

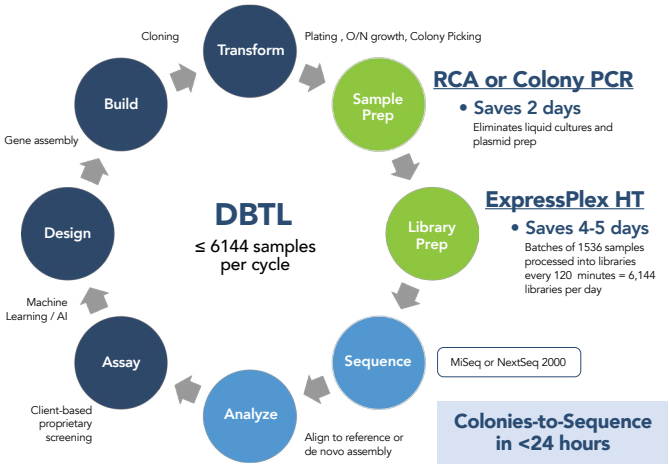
We show that starting from bacterial colonies, several thousand plasmids can be sequenced on a single Illumina NextSeq 2000 run and bioinformatic analysis completed by the next day. We leverage a scalable, modular pipeline that includes colony picking, liquid handling, DNA sequencing and bioinformatic analysis. Rolling Circle Amplification (RCA) or direct PCR from colonies is substituted for conventional bacterial culturing and plasmid purification. Integration of an auto-normalizing, one-step library preparation technology provides 6,144 indexes in a convenient 384-well, assay-ready configuration (384 wells x 16 plates). Our benchmarking comparison against other workflows demonstrate that this automated pipeline shortens the typical synthetic biology DBTL cycle from weeks down to hours.

ExpressPlex™ HT workflow (384-well format)

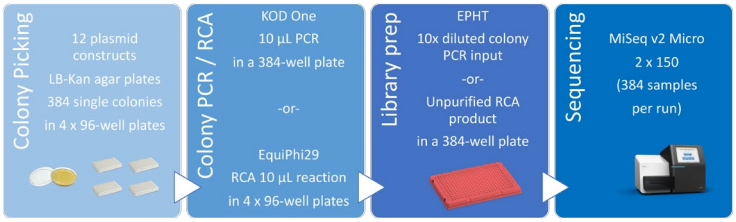
- **Fast**
  Rolling Circle Amplification (EquiPhi29™) and ExpressPlex HT library prep workflow enable 24-hour plasmid sequencing TAT (from colony to sequence).
- **Highly multiplexed**
  Auto-normalizes across all 6,144 barcode combinations on a single sequencing run.
- **Automation-friendly**
  Easy to stamp out libraries with conventional liquid handlers (96- or 384-tip pipetting heads).



Accelerating the Design-Build-Test-Learn Cycle



Performance of ExpressPlex HT with RCA and colony PCR

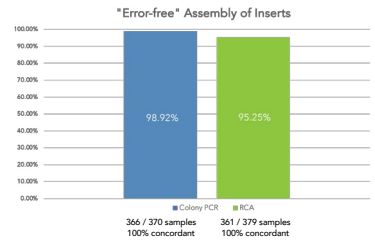


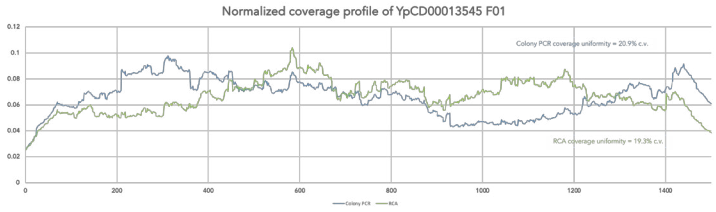
Plasmid constructs used in this study

Clone	Insert size / bp	PCR amplicon size / bp	Full plasmid size / bp	Vector
ScCD00013271	5508	5746	8058	pDONR221
ScCD00013266	5052	5290	7602	pDONR221
ScCD00013267	5052	5290	7602	pDONR221
ScCD00013263	4995	5233	7545	pDONR221
ScCD00008904	4962	5200	7512	pDONR221
YpCD00016721	2016	2254	4566	pDONR221
VcCD00060963	1776	2014	4326	pDONR221
YpCD00013559	1719	1957	4269	pDONR221
YpCD00016351	1554	1792	4104	pDONR221
VcCD00025726	1539	1777	4089	pDONR221
YpCD00013828	1524	1762	4074	pDONR221
YpCD00013545	1512	1750	4062	pDONR221

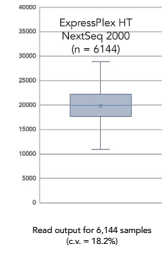
- 384 E. coli colonies were picked from LB agar kanamycin plates into four 96-well PCR plates.
- Plasmids were amplified directly from colony lysates using RCA (EquiPhi29™, Thermo Fisher), or colony PCR (KOD One™, Toyobo).
- 384-plex libraries were prepared from unpurified RCA and colony PCR reactions using ExpressPlex HT kits (seqWell, Inc.) on the Apricot S3 Liquid Handler (SPT Labtech).
- Sequencing was conducted on the MiSeq™ sequencing platform on a Micro v2 kit (384 samples per run), or, on a NextSeq 2000 P1 300 cycle kit (6,144 samples per run).

Results





Read depth comparison across insert DNA: Colony PCR vs. RCA



Read output for 6,144 samples (c.v. = 18.2%)

Summary and Conclusions

- ExpressPlex HT library prep kits enable 6,144 samples to be prepared and sequenced within 24 hours, greatly shortening the DBTL Cycle.
- For high throughput sequence verification, colony PCR and RCA are fast, robust alternatives to plasmid purification.
- **Contact info@seqwell.com for more information.**