Miniaturized library preparation and large adapter sets for fast, cost-effective and high-throughput **Next Generation Sequencing**



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ABSTRACT

Next-generation sequencing is an incredible tool for the discovery and validation of biomarkers, understanding the biology of diseases, assisting protein and antibody engineering, metagenomics and many more applications. While the sequencing cost per base is significantly decreased by high throughput sequencer, library preparation cost becomes a larger portion of the total costs. Additionally, maximizing the sample number on each flow cell and balance of data usage of each sample during multiplexed sequencing, which is often limited by the number of Unique Dual Index(UDI) adapters and sample normalization, also significantly affect the cost of NGS.

Here we used GenScript's GenNature library preparation kit, a 2-step kit based on tagmentation, and compared different automation systems when handling miniaturized reaction volume, then adopted a protocol with the most reliable passing rate for low-depth sequencing. Utilizing our large UDI adapter sets, we demonstrated that we are able to sequence over 5000 samples each run, and reduce the total sequencing cost, including library preparation and sequencing cost by >80%. We also tested the limits of miniaturization library prep and large adapter sets for deep amplicon sequencing and showed even 1/2 volume can still be applicable for minimal depth over 1000x, with the average depth of 5800X on NextSeq550.

Miniaturized library preparation by GenScript's GenNature 2 step DNA library preparation kit

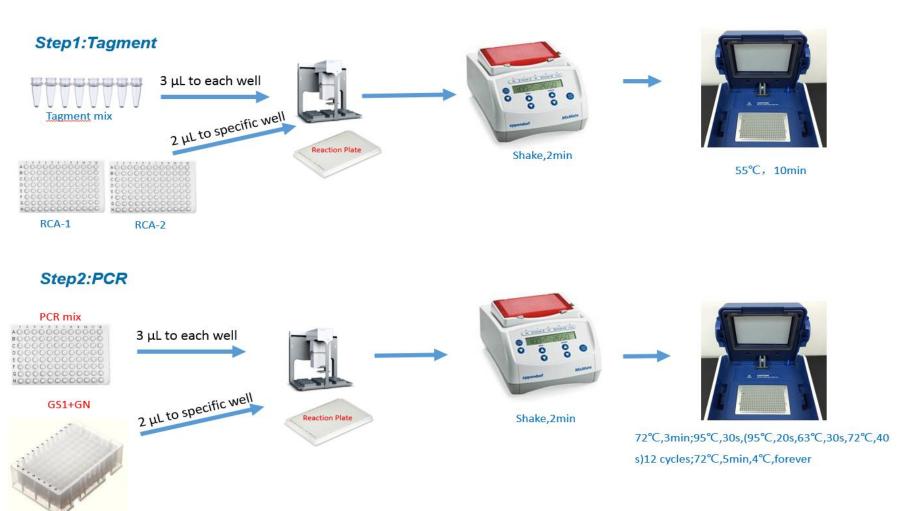
96 total unique fragments

# of passing			•			
Automation	unique	Passing	Approximate	ation		Total
system	elements	rate	reaction size	time	PCR time	time
Echo	55	57.3%	1/25	40min	62min	102min
Agilent Bravo	96	100.0%	1/5	31min	52min	83min
Agilent+Echo	87	90.6%	1/10	31min	65min	96min
Echo Index	87	90.6%	1/5	30min	58min	88min

Liquid handler used in the workflow

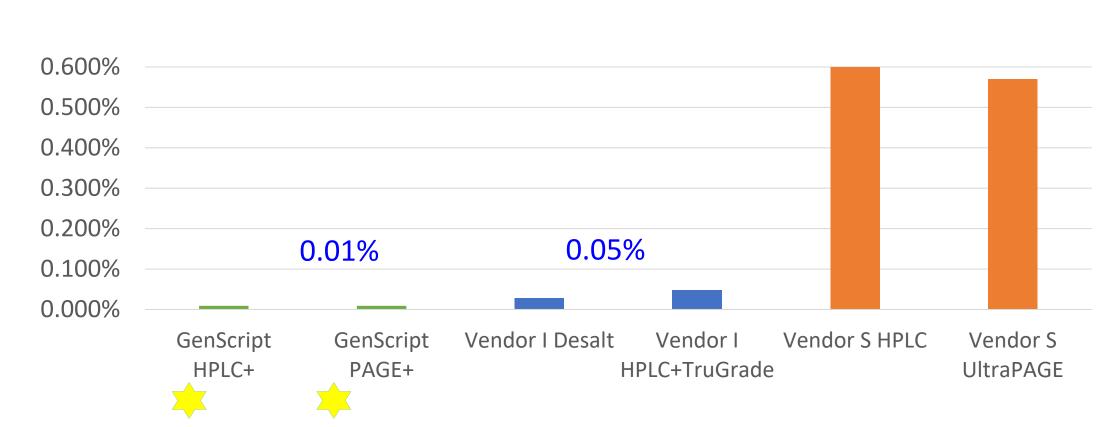
- Agilent Bravo
- Labcyte Echo 555

1 of the 4 platforms tested with the best result



Up to 96x96 UDI Adapter sets enhance sequencing capacity and reduce cost





Custom Index design for large UDI adapter sets

- ➢ GC balance
- ➤ Length for high-plex but match the sequencer
- ➤ Hamming and Levenstein
- Nucleotide diversity (color balance)

GenNature Kit	Standard library prep system	Miniaturized library prep
DNA input	>25ng	15~25ng
Tagmentation	30ul	5ul
PCR system	50ul	10ul

1/5 Library prep and sequencing cost, thousands of sample per run

# of samples	>30x Passing rate	Library prep cost		Sequencing cost		Total Cost per sample		
		Standard library + adaptor	Miniature library + adaptor	384 adapters pairs	up to 9216 adapter pairs	Standard workflow	HT miniaturized workflow	Savings
1140	98.86%	\$26	\$5	\$4	\$0.3	\$30.4	\$5.50	82%
1398	94.78%	\$26	\$5	\$4	\$0.2	\$30.4	\$5.44	82%
1900	98.79%	\$26	\$5	\$4	\$0.2	\$30.4	\$5.38	82%
3420	93.95%	\$26	\$5	\$4	\$0.1	\$30.4	\$5.30	83%
5642	85.78%	\$26	\$5	\$4	\$0.1	\$30.4	\$5.26	83%

- *Calculated based on GenNature library prep and NextSeq 500/550 v2.5 Kits
- **GenNature is library prep kit by tagmentation, price calculated based on the listing price

Conclusions

Miniaturized library and high throughput multiplex sequencing with large adapter set are:

- Easy adaptation to existing liquid handler and current workflow.
- Fully automatable and fast turnaround time
- No additional PCR/barcode to bias the sample
- Multiplex large number of sample to save sequencing cost, large adaptor set available
- > Less redundant data, easier data analysis

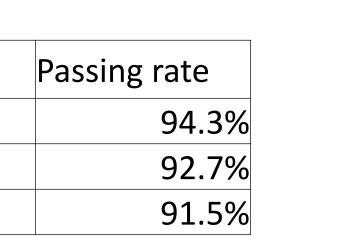
Miniaturized library +1000 adapter sets for deep sequencing of 1000X minimal depth

Total 1000 samples ~5 kb region 27 amplicon per sample, 150-250bp, total 27x1000 amplicons NextSeq 500/550 Mid Output v2 kit • Library prep: 1/2 volume Min Depth Passing rate Custom UDI Adapters: 3/10 rxn >500x 94.3%

Sequencing: 2 NextSeq runs

 Final data size: 80G Minimum depth: >1000X

92.7% >1000x 91.5%







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