

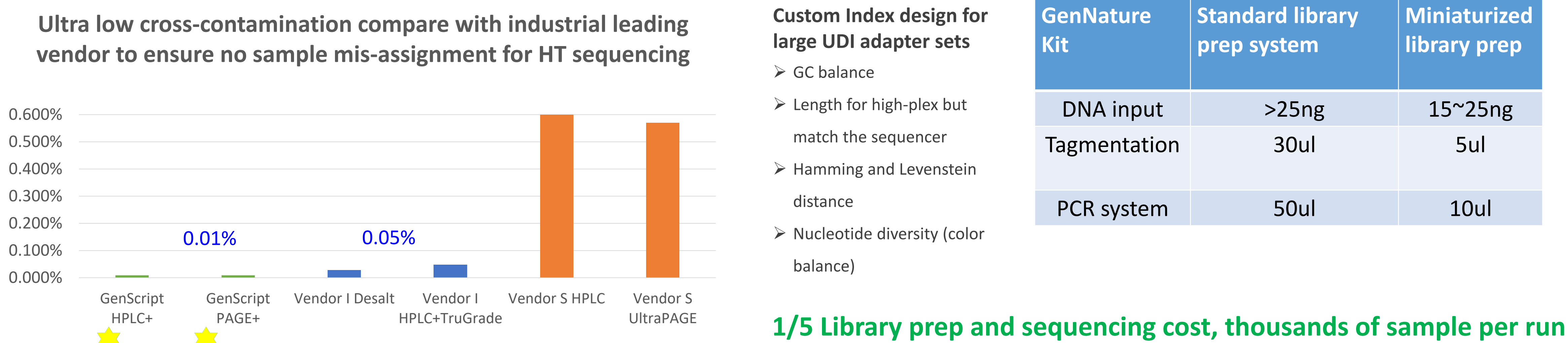
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ABSTRACT

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.

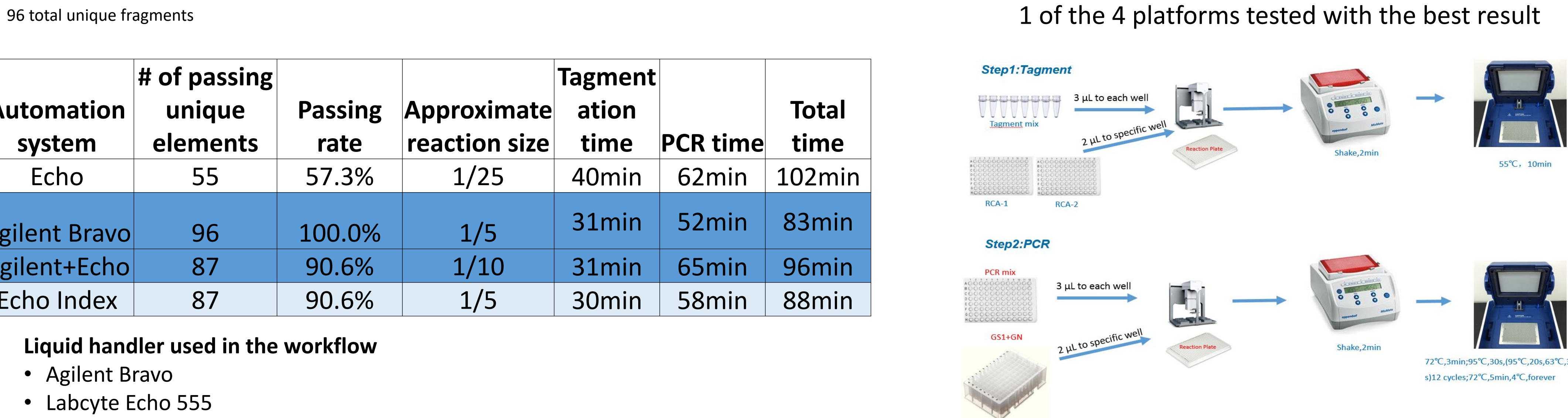
Up to 96x96 UDI Adapter sets enhance sequencing capacity and reduce cost



# of samples	>30x Passing rate	Library prep cost		Sequencing cost		Total Cost per sample		Savings
		Standard library + adaptor	Miniature library + adaptor	384 adapters pairs	up to 9216 adapter pairs	Standard workflow	HT miniaturized workflow	
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

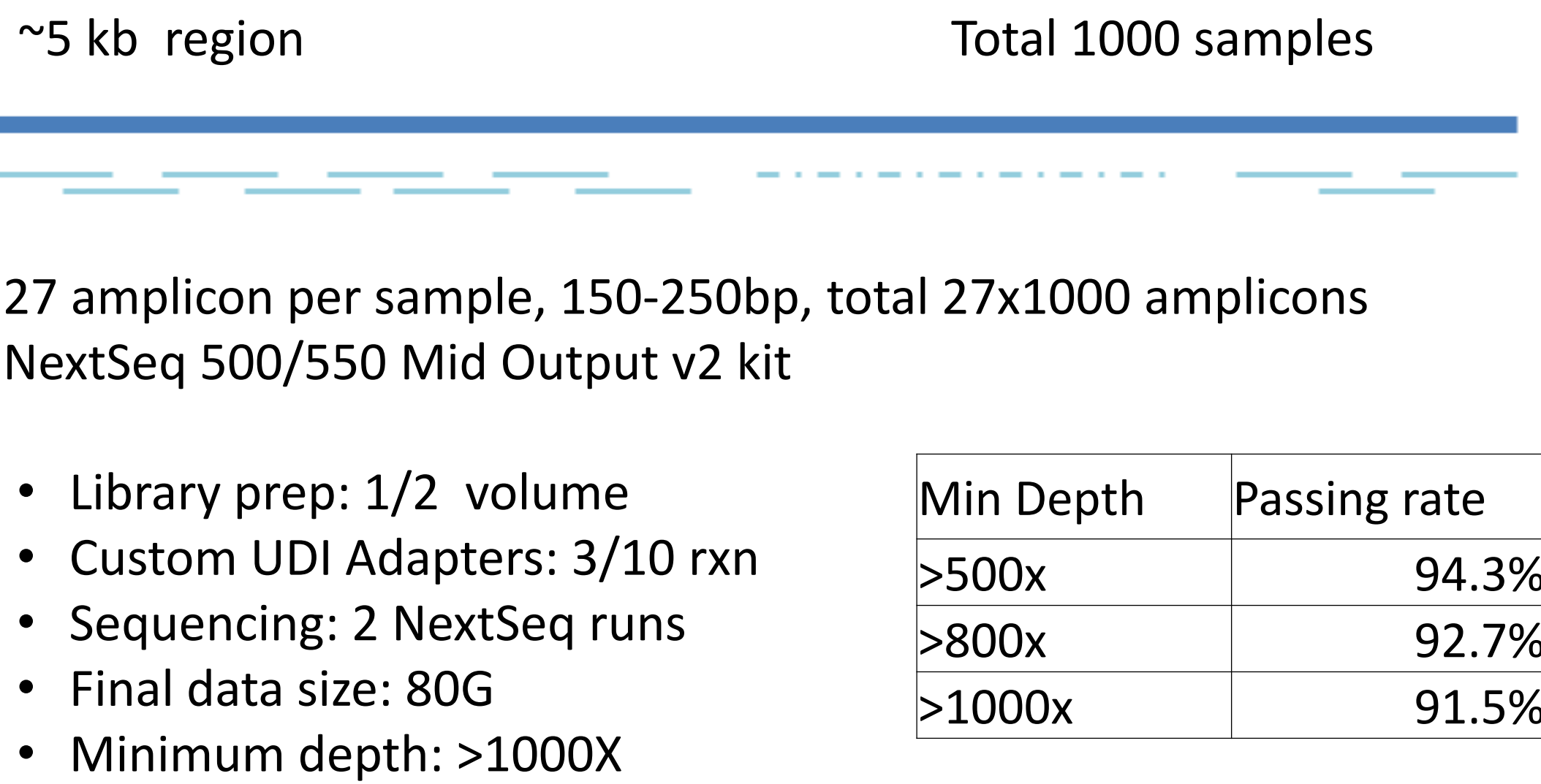
Miniaturized library preparation by GenScript’s GenNature 2 step DNA library preparation kit



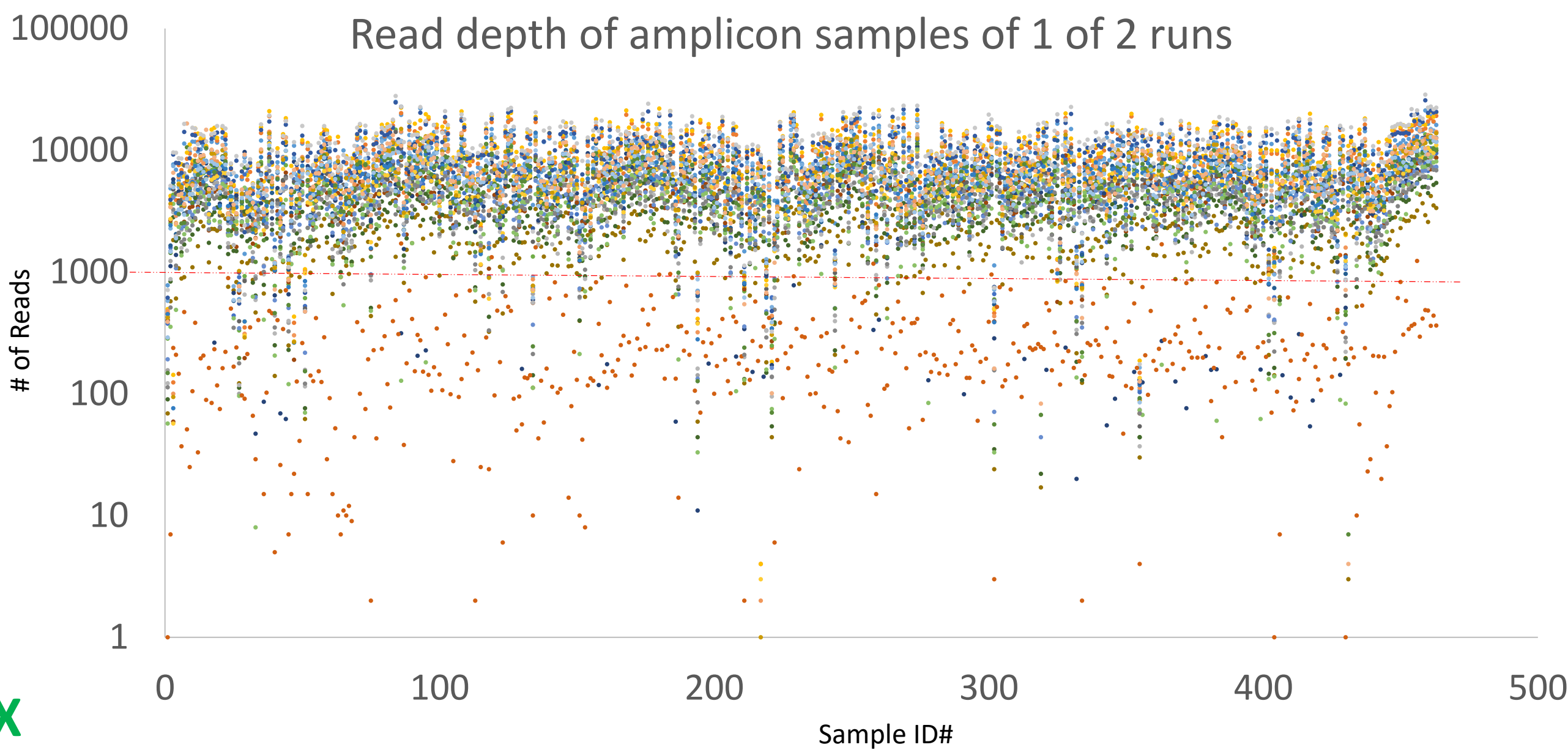
Conclusions

- 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



50% cost savings with >90% passing rate over 1000X



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