

An efficient, tunable solution for pre-capture multiplexing in targeted sequencing via novel chemistry and semiconductor DNA synthesis technology

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ABSTRACT

To help improve target capture efficiency and fulfill high-throughput sequencing for different research or diagnostic needs, we developed a series of target enrichment solution including:

- GenFisher hybridization and wash kit that is compatible with different types of hybridization probes, ssDNA or dsDNA, and up to 24-plex pre-capture pooling
- A novel target enrichment technology, the GenFisher capture probes by semiconductor DNA synthesis technology with the ability to evolve based on test-learn cycle.
- TRUE universal blocker using proprietary chemistry, to provide highly efficient, cost-effective hybrid capture, regardless of index length

High compatibility hybridization and wash kit with 24-plex pre-capture pooling

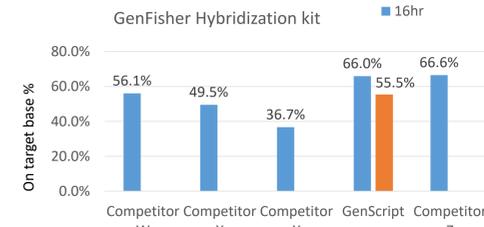


Figure 1. GenFisher 16hr Hybridization kit is compatible with both dsDNA probe and ssDNA probes. 1hr fast hybridization kit allows 1 day workflow for library preparation and target enrichment without compromising capture efficiency. All data without considering flanking regions when calculating on-target %.

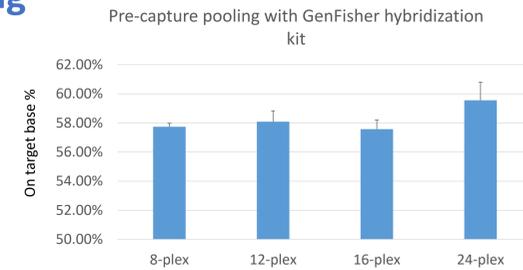


Figure 2. GenFisher Hybridization kit allows up to 24-plex pre-capture pooling with good consistency among different samples.

Tunable target enrichment by semiconductor technology

Semiconductor technology offers a fast and cost-effective way to synthesize capture probes panel for target enrichment. Moreover, since the sequence and abundance of each probe on the chip can be precisely controlled, the panel performance can be evolved based on NGS data feedback.

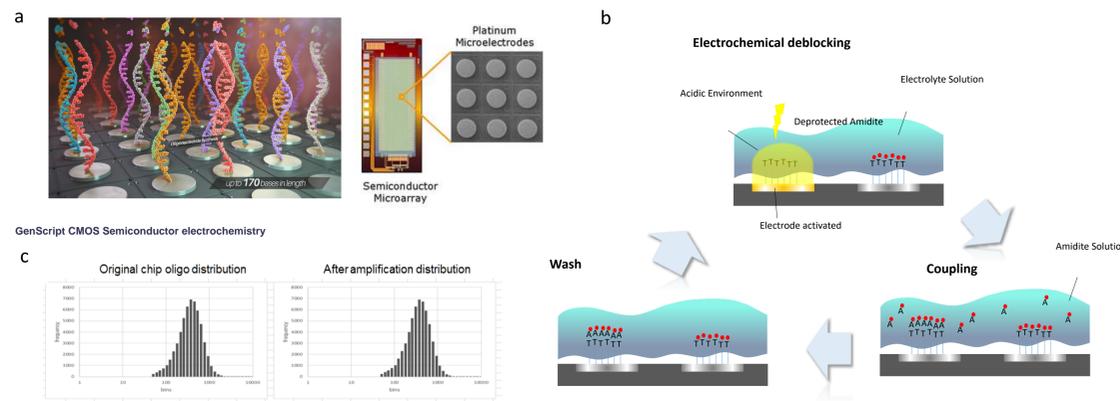


Figure 3. GenScript used Semiconductor DNA Synthesis platform

- GenScript-CustomArray CMOS semiconductor chip
- Electrochemical synthesis of DNA oligo in transient micro-reactors
- NGS analysis concludes 99.7% coverage. Interdecile # < 2.5

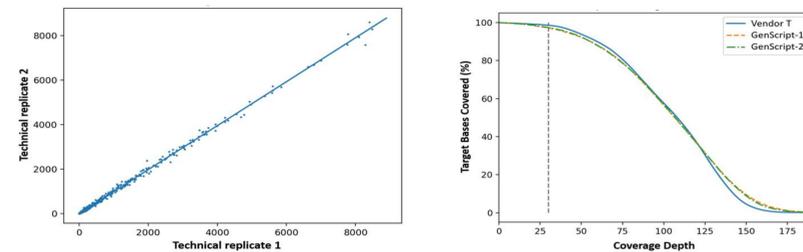


Figure 4. Comparison of semi-conductor based dsDNA hybridization probe panel with column-based ssDNA probe panel. ~8000 120nt probes covering a 600kb target region.

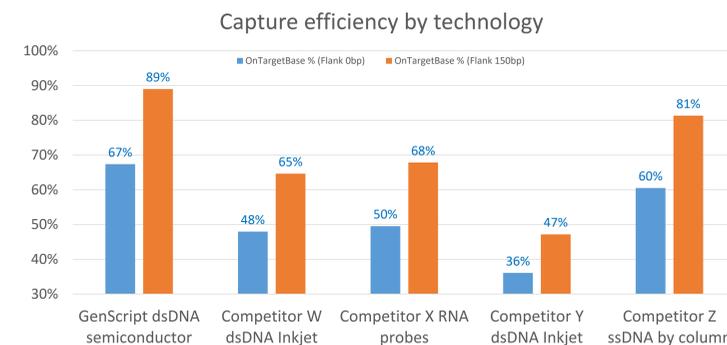


Figure 5. Comparison of different technology. Semiconductor chip synthesized GenFisher custom hybridization probes panel provides better capture efficiency compared to other vendors' panels using single-stranded DNA, RNA or inkjet synthesized double-stranded DNA. All data are average of two independent technical replicates.

Simplified pre-capture target enrichment workflow with TRUE Universal blockers

To enable the maximum pooling potential of targeted NGS libraries, here we introduce a novel design and chemistry to hybrid capture blockers, an essential component in the experimental workflow to block the permissive interactions between library adapters and target probes, and adapters themselves, both of which lead to wasteful sequencing. The conventional blockers, whose sample index-matching portion uses either degenerate bases or universal pairing bases, depends on the length of the sample index for the libraries, dramatically impeding the flexibility of index-blockers pairing. Our new invention, GenFisher Universal Blockers, create a novel solution to the annealing between sample indexes on libraries and blockers regardless of the index length.

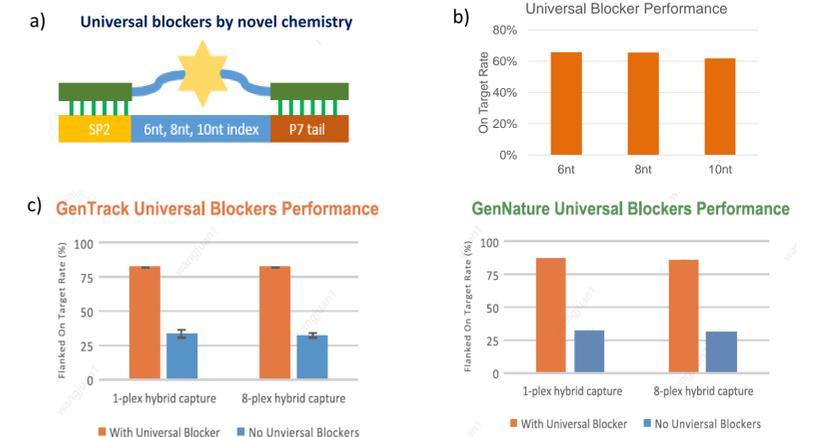


Figure 6. GenFisher Universal blocker makes pre-capture pool easier

- GenFisher Universal Blocker uses novel chemistry to block indexes of different lengths.
- Hybridization capture by GenFisher Hybridization kit and Universal Blockers show similar on target rate regardless of index length
- 1-plex vs 8-plex pre-capture pooling with and without Universal Blockers. Multiplexing has no effect on the efficiency of blockers.



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