Comprehensive Microbial Community Characterization Using an Expanded 16S rRNA Hybridization Capture Probe Set

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16S rRNA Capture: Short Read Sequencing

Method Advantages

- **Reduced bias** — hyb-cap reduces PCR-driven taxonomic biases
- **Cost-effective** — lower sequencing depth required vs. shotgun metagenomics
- **Full 16S recovery** — all variable regions captured for improved taxonomic assignment
- **Strong rare-taxa detection** — outperforms amplicon and shotgun approaches
- **Standard Illumina workflow** — compatible with existing short-insert library prep protocols

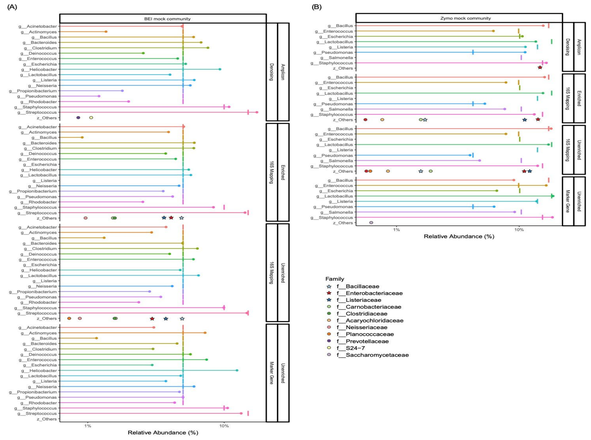


Figure 1. Short-read capture performance across mock community samples. Panel A is multi-species, even mix mock community. Panel B is a multi-species, staggered mix mock community.

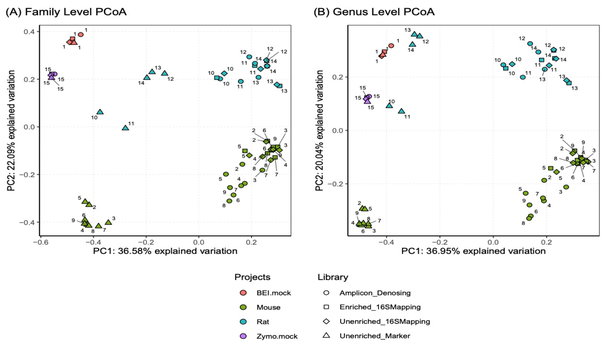


Figure 2. Taxonomic composition comparison: capture vs. amplicon vs. shotgun.

16S-Capture: Long Read Sequencing

Full-Length 16S Recovery with Flanking Regions from Mixed Samples

myBaits 16S capture of long-insert libraries (2–10 kb fragments) built from mixtures of several bacterial taxa and host DNA recovers **complete, contiguous 16S rRNA gene sequences** plus 1–2 kb of flanking sequence:

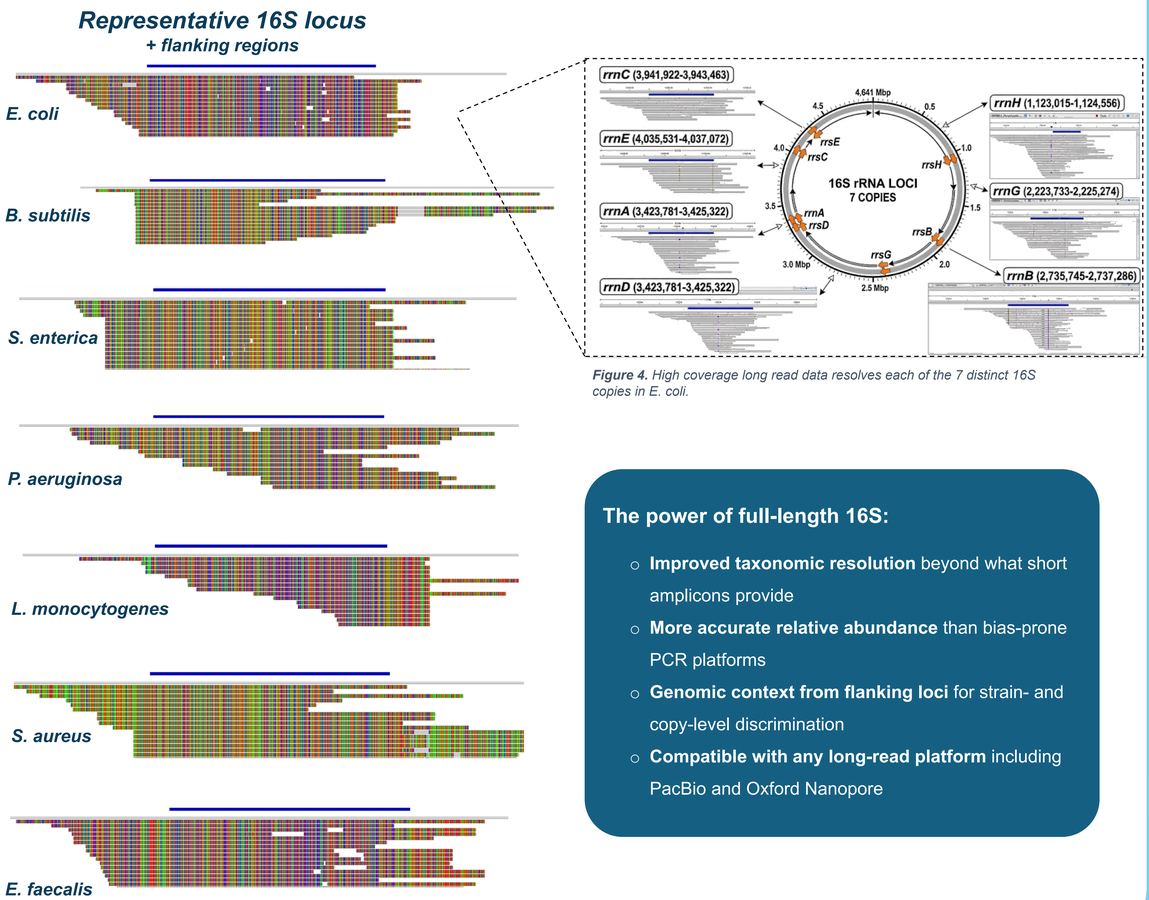


Figure 4. High coverage long read data resolves each of the 7 distinct 16S copies in E. coli.

The power of full-length 16S:

- **Improved taxonomic resolution** beyond what short amplicons provide
- **More accurate relative abundance** than bias-prone PCR platforms
- **Genomic context from flanking loci** for strain- and copy-level discrimination
- **Compatible with any long-read platform** including PacBio and Oxford Nanopore

Figure 3. Combined 16S long read alignments, with flanking regions, for 8 species in a mock community.

16S-Capture v2: Updated Database

Updated 16s Catalog Kit coming soon!

- **Expanded database** — Includes all bacteria and archaea sequences (SILVA138.2 database)
- **Increased performance** — Higher enrichment rate than previous versions
- **Optimized for newest myBaits chemistry** – Updated reagents make for more efficient capture
- **Compatible with the Arbor Biosciences Library Preparation Kit** – Streamlined end-to-end workflow
- **Versatile and customizable configuration -** Panels in the Microbial Collection are modular; order as many different panels as you want, in the quantities you need for each

Features	Silva 138.2 (used for 16S v2)
Total reference sequence count	>500,000 nonredundant SSU references; millions in full SILVA release
Update cadence	Actively curated and periodically updated
Taxonomic depth at lower ranks	Greater genus- and species-level granularity where possible
Phylogenetic breadth across Tree of Life	Much broader phylogenetic span across Bacteria and Archaea
Representation of uncultured organisms	Many uncultured lineages included
Inclusion of recently discovered clades	Continuously expanded to include newly recognized lineages
Sequence redundancy management	NR99 maintains high diversity while reducing exact redundancy

Table 1. Features of database used for v2 bait design.

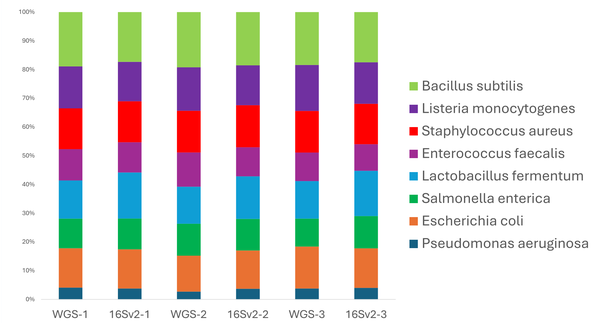
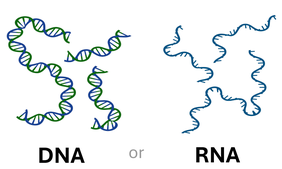
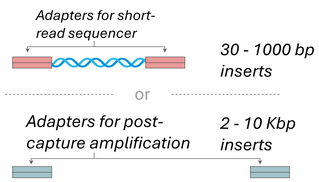


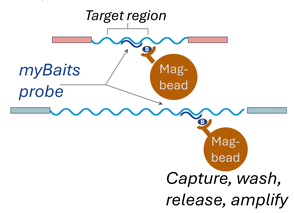
Figure 5. Enrichment of 16S sequences in mixed samples using 16S-capture v2 exhibits improved specificity thanks to updated myBaits design and synthesis strategies

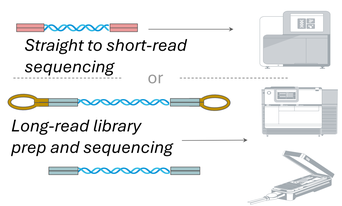
How does hybridization capture work?

myBaits hybridization capture (hyb-cap) is compatible with virtually any nucleic acid substrate and any style of sequencing. Whether genomic (DNA) or transcriptomic (RNA) material, of short (50-1000 bp) or long (2,000-10,000 bp) fragments, hyb-cap can retrieve and reconstruct the initial molecular frequency and sequence composition of a substrate. Probe sets initially designed for one or another substrate are also generally compatible with alternative substrates, because most probe designs are tiled across targets and can tolerate moderate levels of sequence mismatch.









1 Targeted sequencing with hyb-cap can start with virtually any nucleic acid source, whether high molecular weight or degraded, RNA or DNA, enzymatically or chemically deaminated, chromatin-accessible or proximity-ligated.

2 Material can be taken directly to adapter ligation, or sheared beforehand to match the requirements of the sequencer. This forms a sequencing-ready library, or dsDNA precursor that can later be taken to platform-specific prep.

3 myBaits hybridization capture probes, designed for your specific application, anneal to the target fraction of the denatured library, and then sequestered onto magnetic beads through biotin-streptavidin complex formation.

4 Captured library is released from beads and typically amplified. Short-read sequencers: final product is ready to template a flowcell. Long-read sequencers: platform-specific adapters are typically added.

16S-Capture: Long Read Sequencing

- Hybridization capture eliminates PCR amplification bias, delivering more accurate community composition and relative abundance estimates across >95% of on-target reads
- Full-length 16S recovery with flanking regions enables strain- and copy-level resolution on both short- and long-read sequencing platforms
- Expanded v2 probe set covers >500,000 nonredundant SSU references (SILVA 138.2), spanning bacteria and archaea, which includes uncultured and newly recognized lineages
- Without needing to select primers for a specific subset of variable regions,16S-Capture is a cost-effective alternative to shotgun metagenomics - lower sequencing depth required, compatible with standard NGS library prep, and one kit supports all sequencer types without primer selection
- Modular panel design allows simultaneous capture of 16S alongside antimicrobial resistance genes, respiratory viruses, and other targets from a single library

Beyond the Microbiome…

We have myBaits hybridization capture kits for:

- Antimicrobial resistance genes
- Respiratory Viruses
- Cryptosporidium spp.
- Any custom kit you need!
- And many many more!

ORDERING INFO

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