A universal targeted sequencing system for any high-throughput sequencing platform

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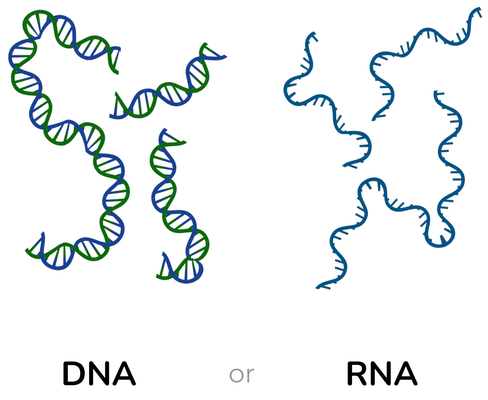
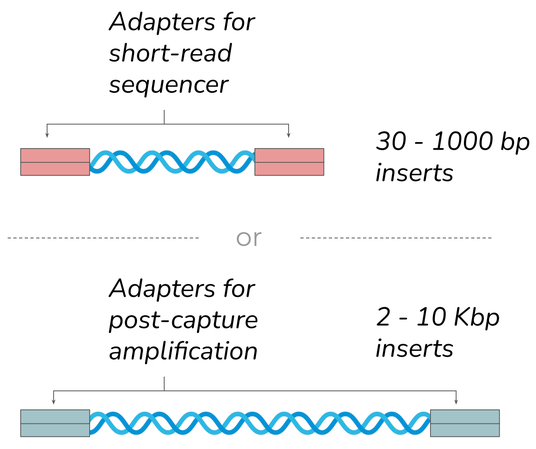
PAG30 | Booth #310

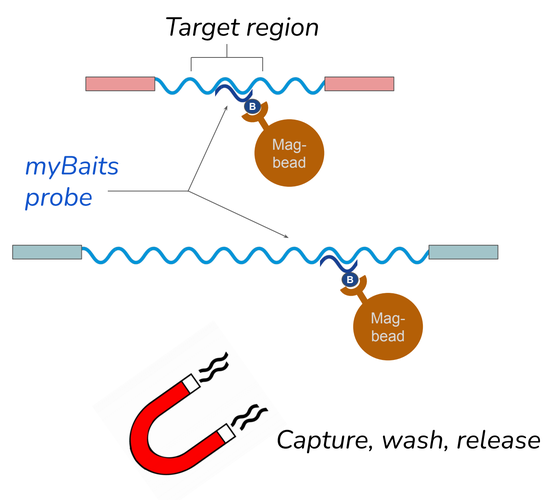
With the recent launch of multiple novel high-throughput sequencing (HTS) platforms, the landscape of HTS workflow options is richer than ever before. Choosing a targeted sequencing solution that is compatible with sequencing on any current or future HTS platform is important for maximizing the utility of a given assay. The myBaits® hybridization capture system from Daicel Arbor Biosciences is by design universally compatible with virtually any HTS workflow, whether short- or long-read. With highly versatile custom probe design algorithms and platform-agnostic protocol options, myBaits is a robust solution for achieving any DNA or RNA targeted sequencing need for any species or sample type. In this poster, we highlight the technical features of myBaits that permit its unique versatility in the modern HTS landscape, including data examples demonstrating its universal application with both short- and long-read HTS platforms relevant to the plant and animal genomics community.

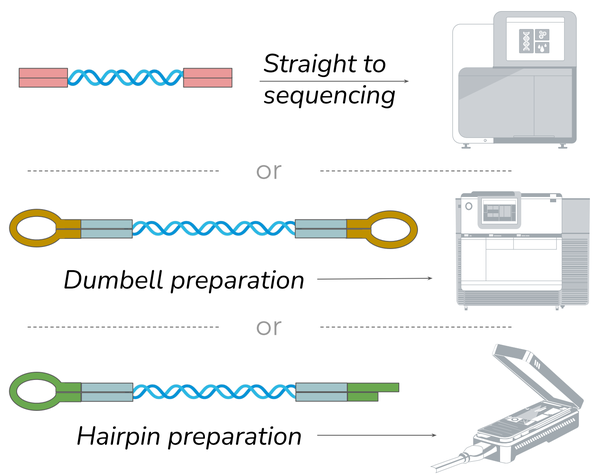
Short or long, DNA or RNA

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.

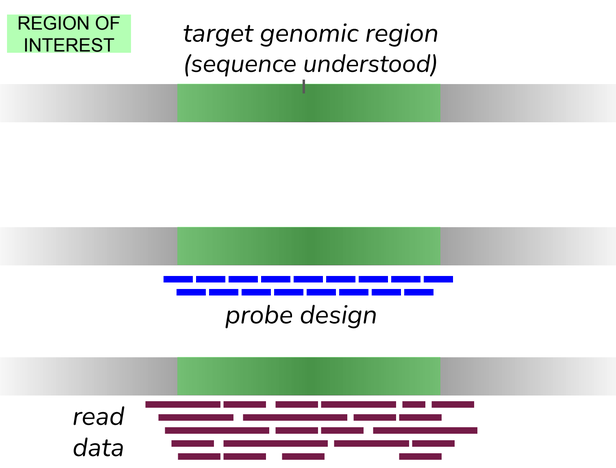
2 Material can be taken directly to adapter ligation, or sheared beforehand to match the requirements of the sequencer. This forms a sequencing-ready library, or dsDNA precursor that can later be taken to platform-specific prep.

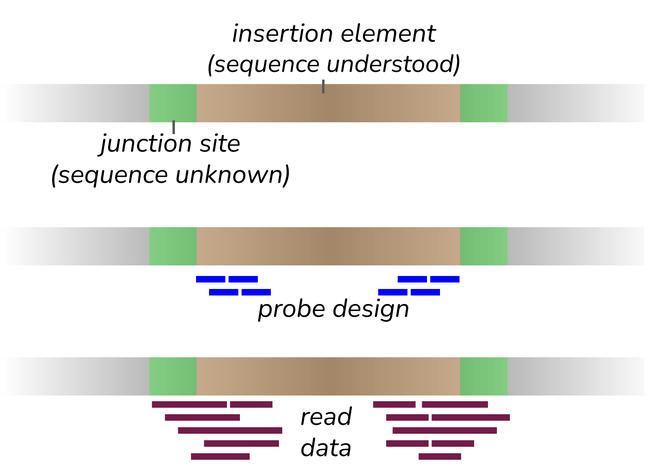
3 myBaits hybridization capture probes, designed for your specific application, anneal to the target fraction of the denatured library, and then sequestered onto magnetic beads through biotin-streptavidin complex formation.

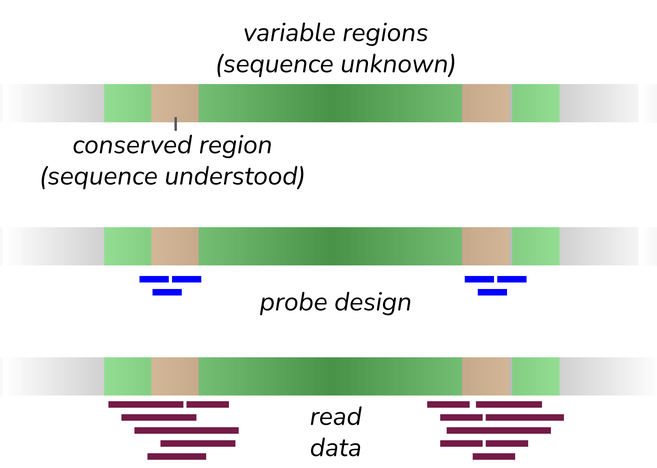
4 Captured library is released from beads and typically amplified. Short-read sequencers: final product is ready to template a flowcell. Long-read sequencers: platform-specific adapters are typically added.

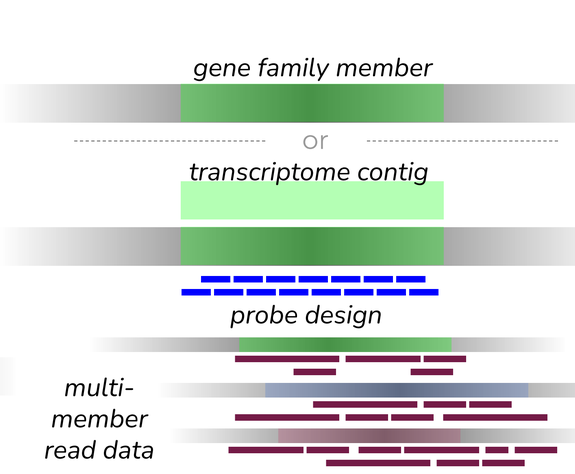
A multitude of target types

Hybridization capture with myBaits relies solely on a single probe hybridization to a target, which contrasts from PCR which requires at least two hybridization events in close proximity. This extremely simple system, combined with inherently high tolerance of nucleic acid hybridization of moderate levels of sequence mismatch, makes hyb-cap among the most versatile targeted sequencing tools. Whether you need to detect natural or artificially-induced single mutations, resolve the location of a novel element or junction event, or discover altogether new genomic regions, hyb-cap probe and experimental design can adapt - thanks to the extensive experience of the myBaits design team at Arbor.









1 **TARGET REGION RESEQUENCING**
The most common use case for hyb-capture is to retrieve up to hundreds of megabases of genomic space for detecting known and novel variation, whether those mutations are natural, EMS-induced, or gene editing-induced.

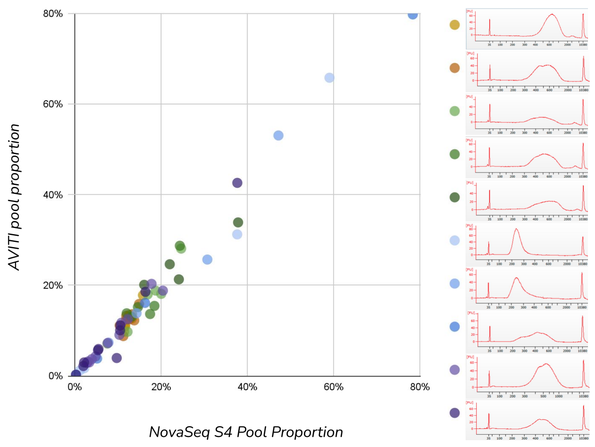
2 **INSERTION SITE DETERMINATION**
In e.g. transgene insertion confirmation, genome location is critical to learn. Molecules overlapping the insertion junction can be captured by targeting just the transgene. Long-insert library prep and sequencing increases power.

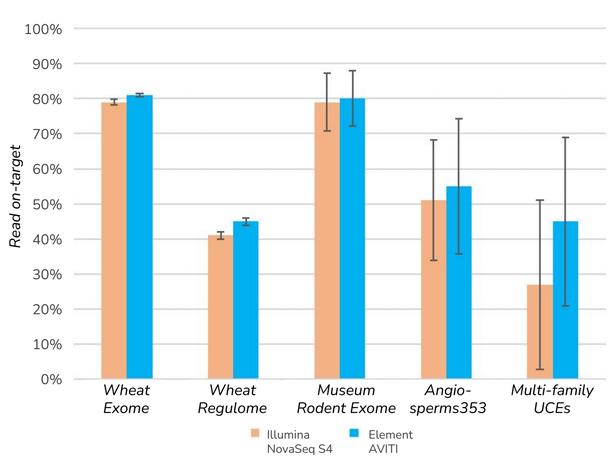
3 **FLANK SEQUENCING**
For the researcher who is trying to discover or characterize altogether new variation, targeting known motifs but sequencing adjacent regions powers phylogenetics across vast evolutionary timescales.

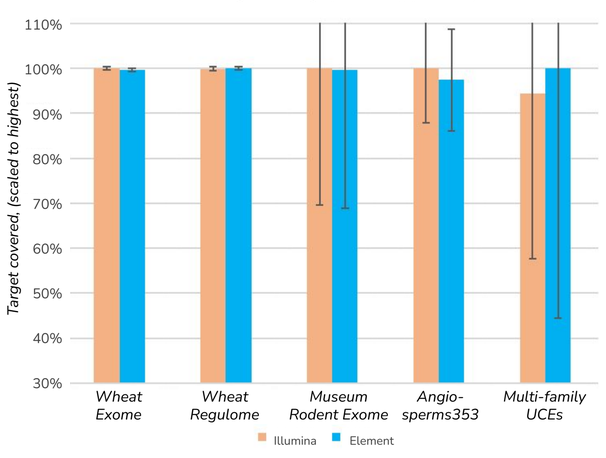
4 **GENE FAMILIES AND ISOFORMS**
Multiple gene family members or isoforms can be retrieved with the same probes. This is effective for e.g. characterizing disease resistance genes in crops with large or highly repetitive genomes (RenSeq).

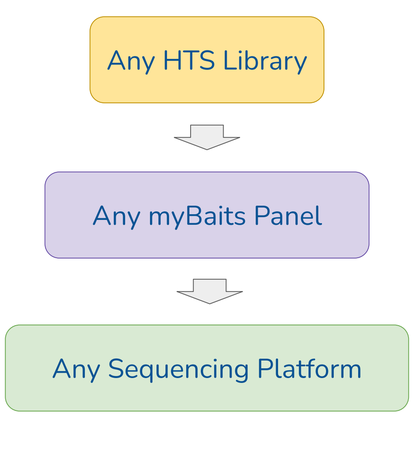
Ready for new sequencers

In 2022, several new short-read sequencers and chemistries were announced, released, and/or made available to new regional markets. These include new systems from Element Biosciences®, Singular Genomics®, Ultima Genomics®, and PacBio®, new chemistry from Illumina®, and broader availability of platforms from MGI®. Each of these remains fundamentally compatible with hybridization capture as a pre-sequencing complexity reduction technology. We recently tested the new Element AVITI® system with several of our most popular myBaits hybridization capture panels (e.g., Wheat Exome, Wheat Regulome, Angiosperms-353, and others). In each case, the conversion to Element sequencing was seamless and returned excellent performance.









1 **SUPERB LIBRARY REPRESENTATION**
Despite a range of insert lengths among the 66 libraries on the sequencing run, desired sequencing depth was obtained without changing the index content or pooling design prior to sequencing compared to the original Illumina runs.

2 **HIGH SPECIFICITY**
The AVITI data was even more enriched for target sequences compared to NovaSeq®, whether for region resequencing (first 3 assays) or flank sequencing (second 3).

3 **COMPREHENSIVE LOCUS RETRIEVAL**
Even when controlling for the higher specificity of the AVITI data, the amount of target space retrieved or novel sequence space reconstructed *de novo* was comparable or improved with AVITI.

4 **MAXIMUM VERSATILITY**
Virtually any HTS library built from DNA and/or RNA can be enriched for targets regions of interest with myBaits, and sequencing on virtually any HTS platform currently on the the market.

References

Wheat Exome - IWGSC Press Release. Oct 4 2019. "IWGSC Wheat Exome v1 now available for bread and durum wheat from Arbor Biosciences". https://www.wheatgenome.org/Resources/ Genomics-Tools/Wheat-Exome-v1

Wheat Regulome - Zhang J et al. (2022). A second-generation capture panel for cost-effective sequencing of genome regulatory regions in wheat and relatives. The Plant Genome. https://doi.org/10.1002/tpg2.20296

Angiosperms353 - Johnson MG et al. (2019). A Universal Probe Set for Targeted Sequencing of 353 Nuclear Genes from Any Flowering Plant Designed Using k-Medoids Clustering. Systematic Biology. https://doi.org/10.1093/sysbio/syy086

RenSeq - Jupe F et al. (2013). Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. The Plant Journal. https://doi.org/10.1111/tpj.12307

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