EXTENDED EXOME CAPTURE:
An efficient sequencing method to target expressed regulatory regions

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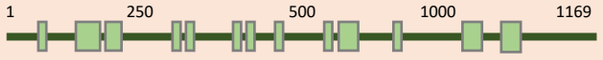
Background

For non-model organisms, sequencing methods are mostly limited to whole genome sequencing (WGS) or reduced representation. WGS is costly, yet low-cost methods such as GBS only sequence a fraction of the genome. Recently Expressed Exome Capture Sequencing[1] began to resolve this problem, but still limits the sequencing to mostly exons and surrounding areas. In order to specifically target regulatory regions of the genome, we take advantage of larger fragments through Tn5 tagmentation to optimize this approach. This extends the captured regions to include surrounding introns and intergenic regions, thus targeting regulatory regions of expressed genes

Objective

Develop a protocol for sequencing regulatory regions of DNA, to target regions of the genome likely undergoing selection.



For example, above the LDH-b gene schematic with intron/exon boundaries. By using > 1kb pieces of DNA we capture the entire gene and flanking regions

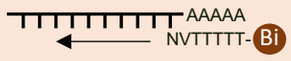
Methodology

We developed a method based on EECseq[1]. We enhanced this method using tagmentation and larger fragment sizes to capture more of the intergenic and promoter regions associated with genes. RNA was isolated from multiple tissues and used to generate biotinylated cDNA capture probes. Isolated genomic DNA was then tagmented with a mutated $Tn5_{R27S,E54K,L372P}$ transposase[2] purified in-house to produce larger fragments (>1kb). Genic DNA was then captured with the biotinylated probes. In order to sequence the final libraries, the fragments were tagmented again to ~500 bp.

Step One: Probe Generation

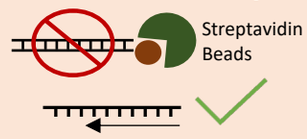
Expose Tissue or Organism to enrich mRNA library

Isolate mRNA to make cDNA probes



Reverse-transcription with biotinylated NVT primer

Normalize cDNA pool



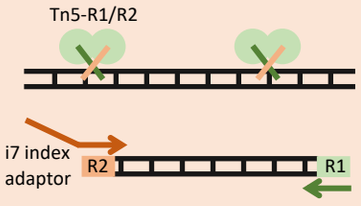
Denature and reanneal ds-cDNA. Capture ds-cDNA with SA beads. Retain normalized ss-cDNA pool & extend to make dsDNA

Amplify final Probes

Fragment probes to ~750 bp
Ligate Adaptors
PCR with biotinylated primers

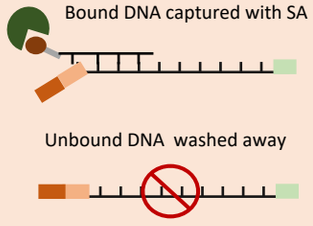
Step Two: Genomic DNA isolation and Library Preparation

Tn5 Tagment gDNA & add i7 adaptors



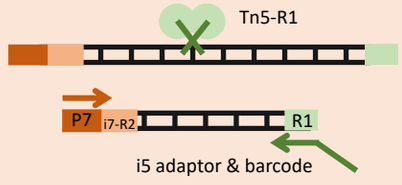
Tagment genomic DNA with R1/R2 loaded Tn5 variant to 1-4 kb fragments
Amplify to add Illumina i7 indices/adaptors. Pool at plate level

Hybridization and Capture



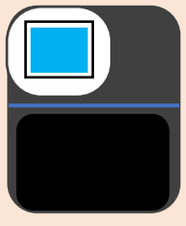
Tagmented DNA is hybridized with biotinylated probes & captured with SA beads. Unbound non-genic DNA is washed away

Tn5 Tagment & add i5 adaptors



Tagment hybridized DNA with R1 loaded Tn5 to 300-700 bp fragments for sequencing

Illumina Sequencing



Conclusions and Next Steps

- Tagmentation reduces the time and simplifies the protocol
- Larger fragments can be generated with the Tn5 variant
- Hybridization efficiently captures expressed genes while not capturing non-genic DNA, verified by gene specific PCR amplification
- Future work once sequencing data is available will provide insight to the genomic coverage of this method and the polymorphisms present in these expressed genic regions

Acknowledgments

We'd like to thank J. Puritz for helpful discussion about initial experiment design and K. Remans for providing the Tn5 plasmid and assistance with Tn5 purification protocol. Funding provided by National Science Foundation

Learn More

Check out the Full Protocol

References: 1. Puritz JB, Lotterhos KE. Expressed exome capture sequencing: A method for cost-effective exome sequencing for all organisms. Mol Ecol Resour. 2018 Nov;18(6).
2. Hennig BP, et al. Large-Scale Low-Cost NGS Library Preparation Using a Robust Tn5 Purification and Tagmentation Protocol. G3 (Bethesda). 2018 Jan 4;8(1):79-89.