Step One: Expose Fish

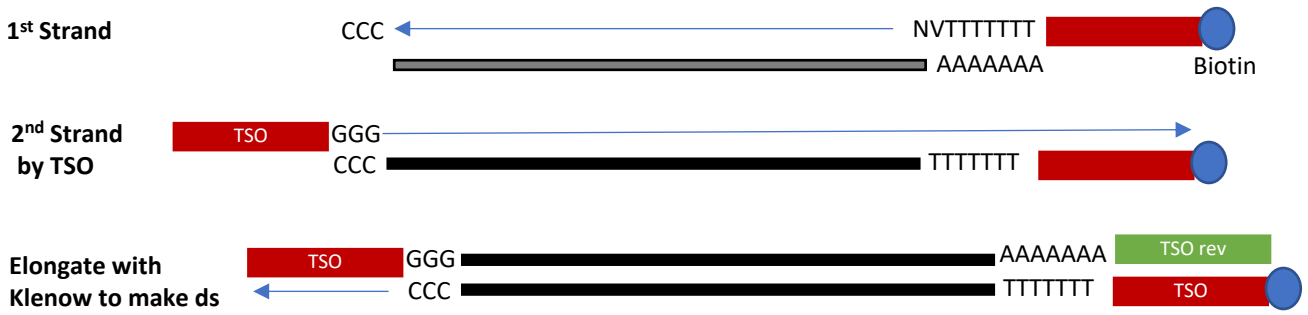
Expose Fish and Harvest Tissues

Step Two: RNA isolation

Isolate RNA with preferred method

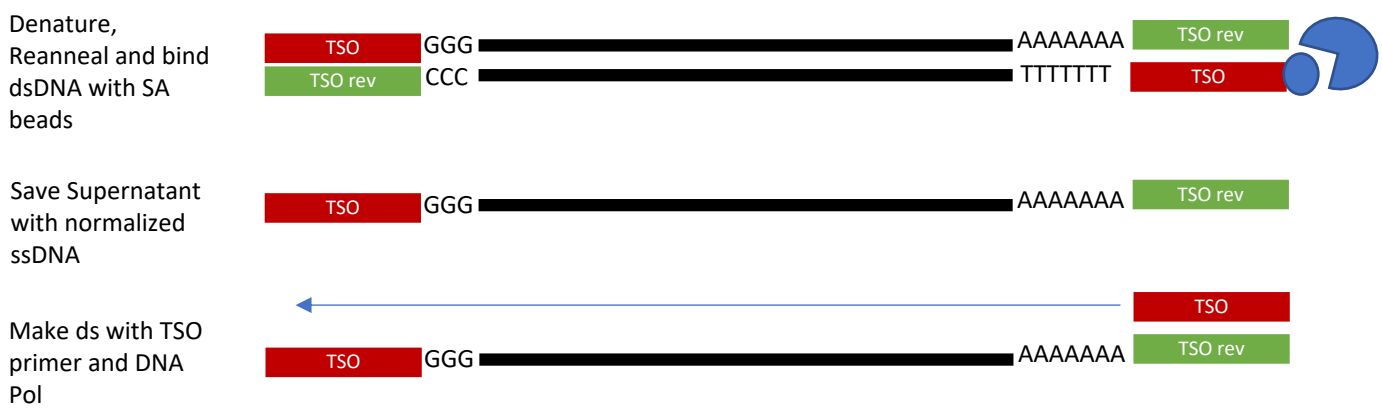
Step Three: cDNA Synthesis

Synthesize cDNA using reverse transcriptase, Bi-NVT primer and Transcript Switching Oligo (TSO)



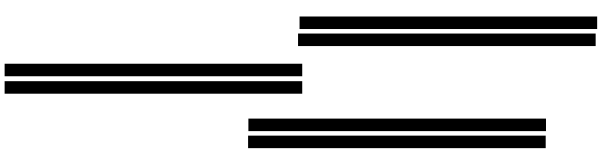
Step Four: Normalize

Denature and Reanneal, then bind to Streptavidin (SA)



Step Five: Fragment to 750bp

Cut with Fragmentase to 750bp

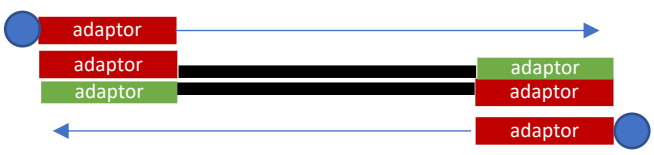


Step Six: Adaptor Ligation

End Repair, A-Tail and Ligate PCR Adaptors

Step Six: Final PCR to enrich and add biotin

Can be repeated indefinitely for as much probe as needed. Uses 5' Biotinylated primer necessary for DNA capture



Final Probe Product

Step One: Isolate gDNA

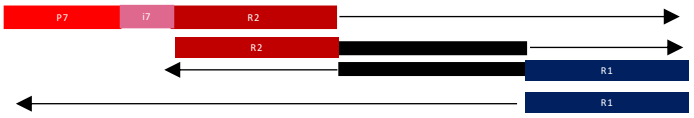
Isolate genomic DNA with magnetic bead protocol

Step Two: Tagmentation

Tagment to 1-4kb using R1 and R2 with Tn5 variant

Step Three: Elongate and add i7 indexes by PCR

PCR with P7-i7-R2 and R1 primers and Pool at the plate level



Step Four: Hybridize and Capture

Mix with biotinylated cDNA probes and capture with SA and elute ssDNA

Step Five: PCR to elongate and make dsDNA

Elongate with P7 and R1 primers to make ds

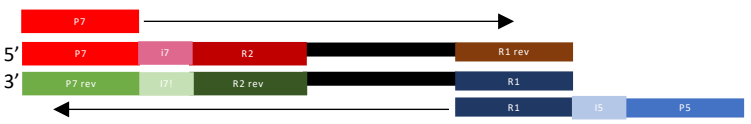
Step Six: Tagmentat to ~500bp

Fragment DNA with Tn5 to 500 bp for sequencing

Product:

Step Eight: Elongate and add i5 indexes by PCR

PCR with R1-i5-P5 primers and P7 primer.



Step 9: Sequence with Illumina

Product