Required Third Party Reagents:

- SuperScript III™ Reverse Transcriptase
- OneTaq® 2X Master Mix with Standard Buffer
- SPRIselect or equivalent DNA/RNA Purification Beads (also known as SPRI beads)
- Optional: RNaseH
- Optional: RNaseOUT™ Recombinant Ribonuclease Inhibitor

Recommended Input Materials

- 1000ngs of crude or purified total cellular RNA
- A260/A280 = 1.9-2.2
- Provided in nuclease-free water (must be free of residual ethanol)
- No RNA Fragmentation
- No RNA Enrichment/Selection/Depletion required
- RIN>6.0
- IMPORTANT: Do not use carrier RNA during RNA purification (this is usually poly(A)-oligomers)

Order from: www.baseclick.eu

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Poly(A)-ClickSeq™

Directed 3'-end RNAseq for gene expression and polyadenylation analysis

Simple and Affordable
Next-Generation Sequencing, powered by Click-Chemistry

A partnership with baseclick GmbH and ClickSeq Technologies LLC

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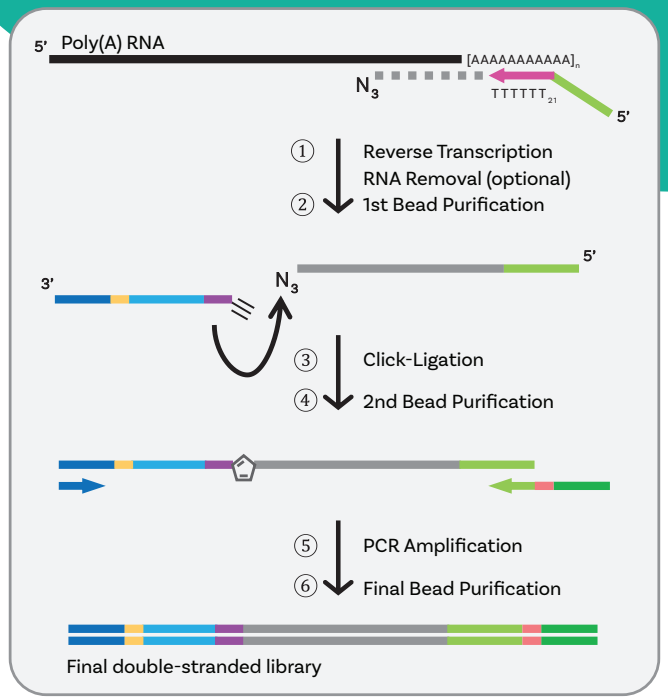


Total Turnaround Time

2 - 3 hrs Wait Time | <2 hrs Hands On

30 - 60 min | 35-40 min

15 min | 30 min

80 - 100 min | 40 min

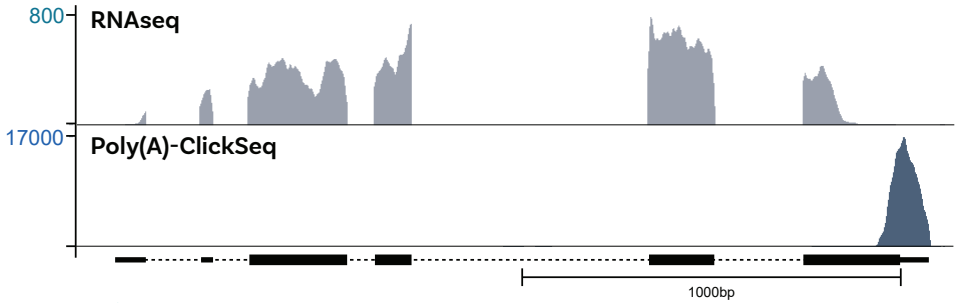
Poly(A)-ClickSeq Library Prep Kit Overview

1. Total cellular RNA is reverse transcribed using an oligo-dT(21) primer containing a partial Illumina p7 sequencing adapter. Reverse transcription is performed in the presence of azido-nucleotides that stochastically terminate cDNA synthesis upstream of poly(A)-tail.
2. cDNA is purified using SPRI magnetic beads.
3. Click-chemistry is used to chemically ligate the Illumina p5 sequencing adapter
4. Click-ligated cDNA is purified using SPRI beads.
5. PCR fills the remainder of the i7 indexing adapter and amplifies the amount of dsDNA library
6. A final bead purification and size selection yields sequencing-ready libraries.

Applications

- Captures any polyadenylated RNAs
- mRNA sequencing and quantification
- Gene expression analysis
- Poly(A)-site discovery
- Alternative Polyadenylation (APA) Analysis

Read data is concentrated at boundary of 3'UTR and poly(A)-tails:



Benefits

- No fragmentation steps required
- No enrichment/depletion steps required. Removes potential biases and reduces cost, time and loss of samples.
- No enzymatic ligation steps, reduces artifactual recombination
- Highly degraded and/or fragmented RNA can be processed.
- Reduced sample input, as little as 100ng Total Cellular RNA required.
- Libraries generated in ~6 hours.
- Unique Molecular Identifiers (UMIs) available
- 10-20M reads per sample is sufficient for most applications.