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TAGseq vs RNAseq

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Sponsored by Lexogen

What is TagSeq

- Generic name for 3' biased transcriptome sequencing
- Standard RNAseq has a bias associated with transcript length
- In TagSeq methods one transcript -> one read
- Interested in Differential Gene Expression AND not splice variation, transcript sequence, etc.

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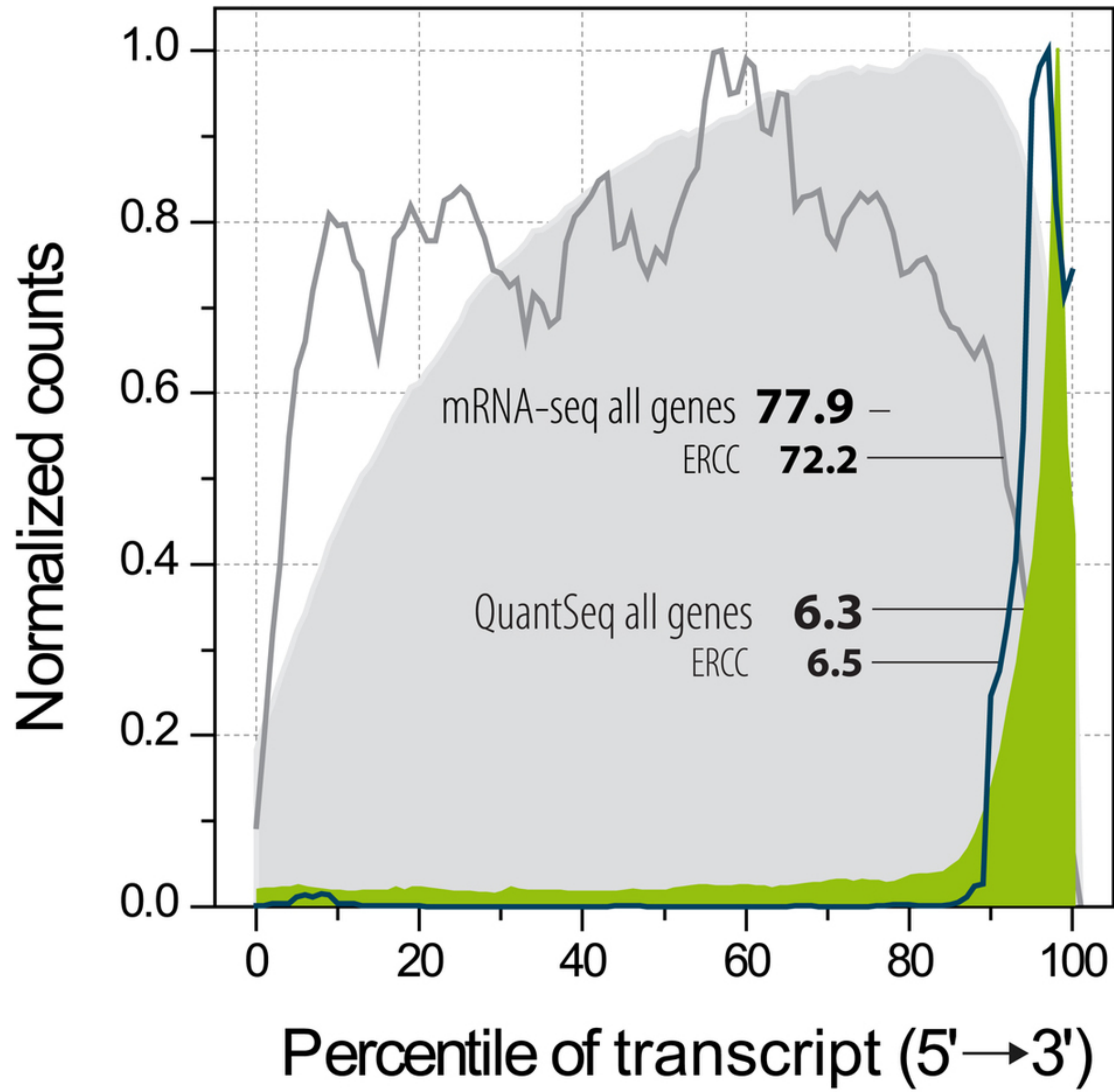
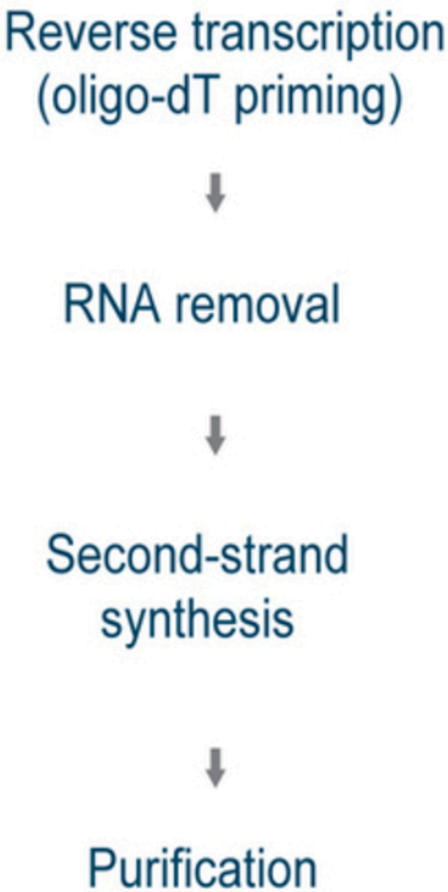
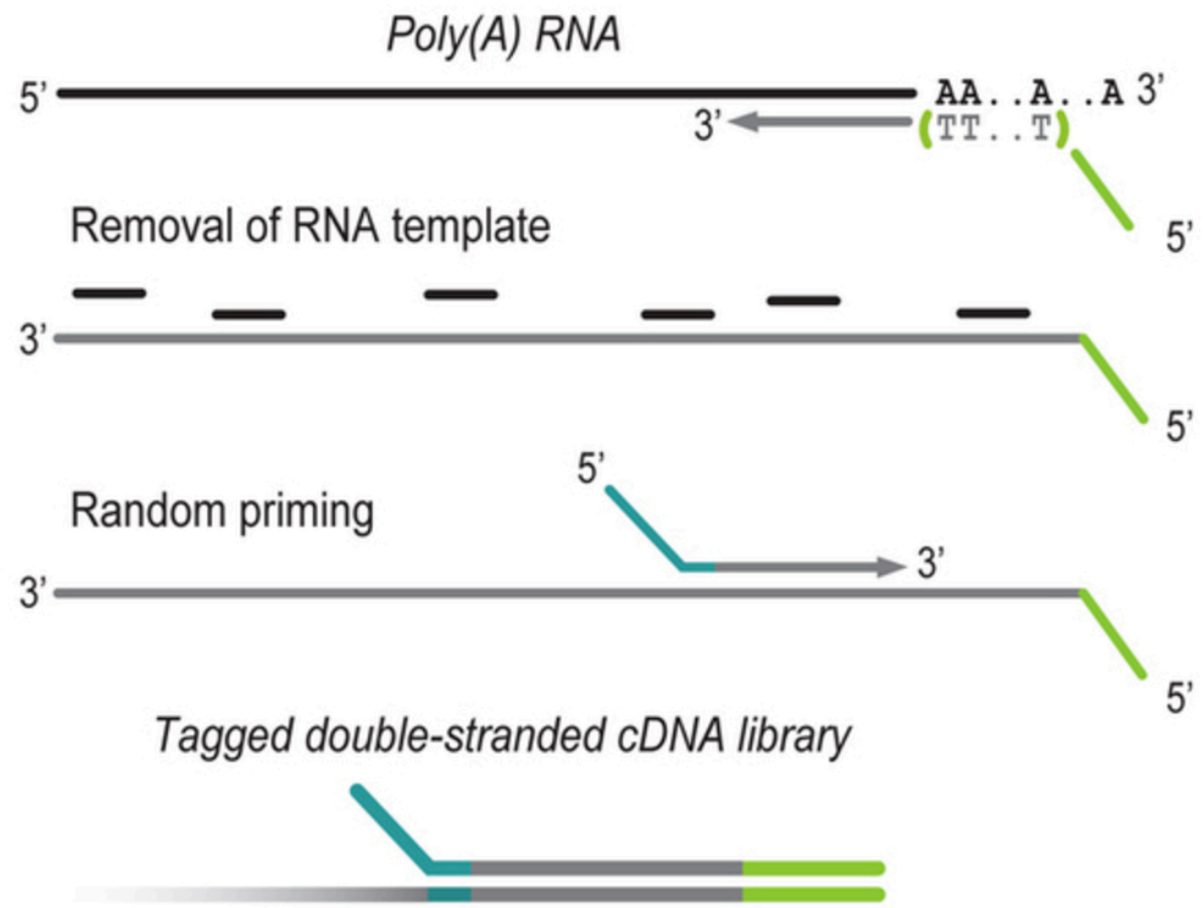


Lexogen QuantSeq

Lexogen sponsored a break during the workshop

Protocol





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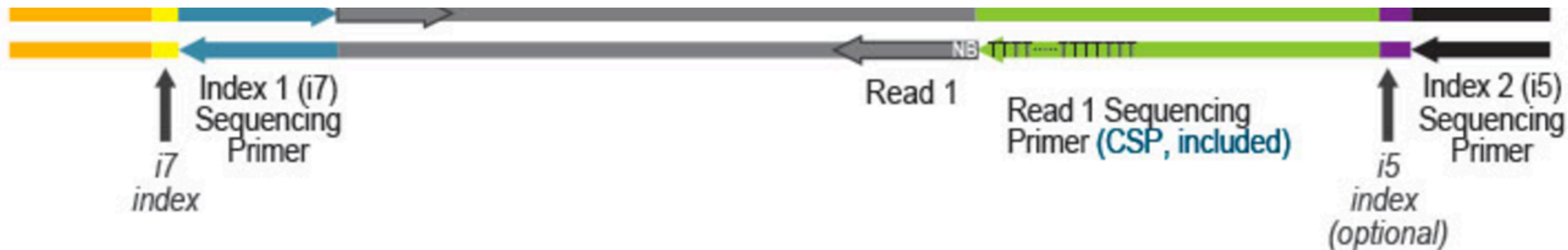
Lexogen Quant-Seq FWD/REV

Read orientation for QuantSeq FWD

Read orientation for QuantSeq REV with CSP



Benefits

- Low input and low quality samples (say as low as 100pg)
- Faster library preparation protocol, means cheaper libraries
- Strand specific
- Can pinpoint 3’ to polyA junctions and obtain accurate information about the 3’ UTR (REV kit)
- Best suited for gene counting
- Significant cost saving through multiplexing, need less sequencing per sample, comparable to microarrays
- Single read sequencing is sufficient

Disadvantages

- Data does not contain any transcript splicing information
- Requires (sort of) a reference genome with good annotation (plus known UTRs)
- Only applicable to Eukaryotic samples (requires polyA)
- protocol is a (a bit) more sensitive to chemical contaminants (spin column cleaned RNA samples are recommended)

Bioinformatics

- When sequencing only the interior (non-polyA) read
  - Need to trim off the first 11bp that represents the random primer
  - Align with STAR, which will soft clip off any potential adapter and span any intron-exon gap you may land in
- When sequencing pairs, can follow the same protocol as ’normal’ RNAseq paired end, but need to also trim that same 11bp, now possibly on read2
- Rest is the same as standard RNAseq

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