RNA sequencing and its applications

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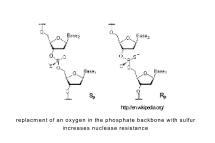## RNA sequencing is a method to measure mRNA expression of all genes in the cell (transcriptomics)

we recently used RNA-seq to find enzymes that break apart plant biomass
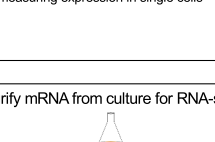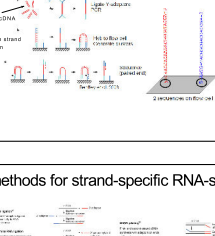

Today we will focus on 3 applications in RNA sequencing

1 strand-specificity

2 promoter mapping

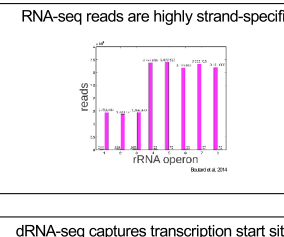3 measuring expression in single cells
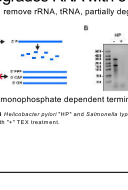
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purify mRNA from culture for RNA-seq
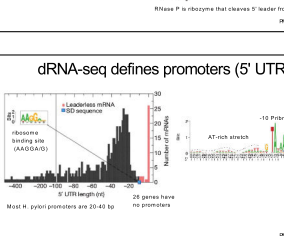

Deplete ribosomal RNA (rRNA)

depletion of 23S (2904 bp) and 16S (1542 bp) bands

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standard Illumina library preparation and sequencing

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phosphorothioate nucleotides are nuclease resistant

replacement of an oxygen in the phosphate backbone with sulfur increases nuclease resistance

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Illumina sequencing

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methods for strand-specific RNA-seq

Levin et al, 2010

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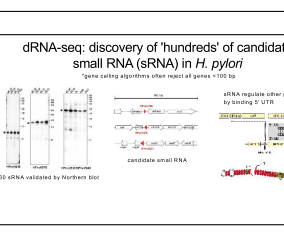
each method has positives and negatives

Levin et al, 2010

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dUTP sequencing library preparation
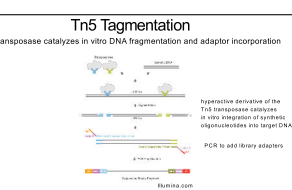
RNA-seq reads are highly strand-specific

rRNA operon

Nicolas et al, 2012

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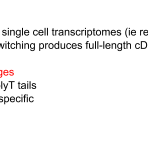
dRNA-seq captures transcription start sites

*enrichment of 5' PPP RNA (at bacterial primary transcripts have 5' PPP)

TEX degrades RNA with 5' phosphate

remove rRNA, tRNA, partially degraded mRNA

TEX=5' monophosphate dependent terminator exonuclease

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dRNA-seq enrichment of mRNA TSS

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dRNA-seq defines promoters (5' UTR)

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dRNA-seq: antisense transcription in 46% H. pylori ORFs

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dRNA-seq: discovery of 'hundreds' of candidate small RNA (sRNA) in H. pylori

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Smart-seq single cell transcriptomics

Locked nucleic acid (LNA)

Base

increased thermal stability of nucleic acid duplexes = shorter primers with higher specificity

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Tn5 Tagmentation

transposase catalyzes in vitro DNA fragmentation and adapter incorporation

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Smart-seq summary

Advantages
-analyze 96 single cell transcriptomes (ie regions of a tumor)
-template-switching produces full-length cDNA

Disadvantages
-requires polyT tails
-not strand-specific