University of Glasgow | College of Medical, Veterinary & Life Sciences

Transcriptomics

Graham Hamilton
Glasgow Polyomics

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Outline

Introduction to sequencing technologies

- High throughput sequencing
- Overview of the sequencing platforms

Experimental design

Analysis of transcriptomics data

Case studies

- Drosophila study
- No genome available

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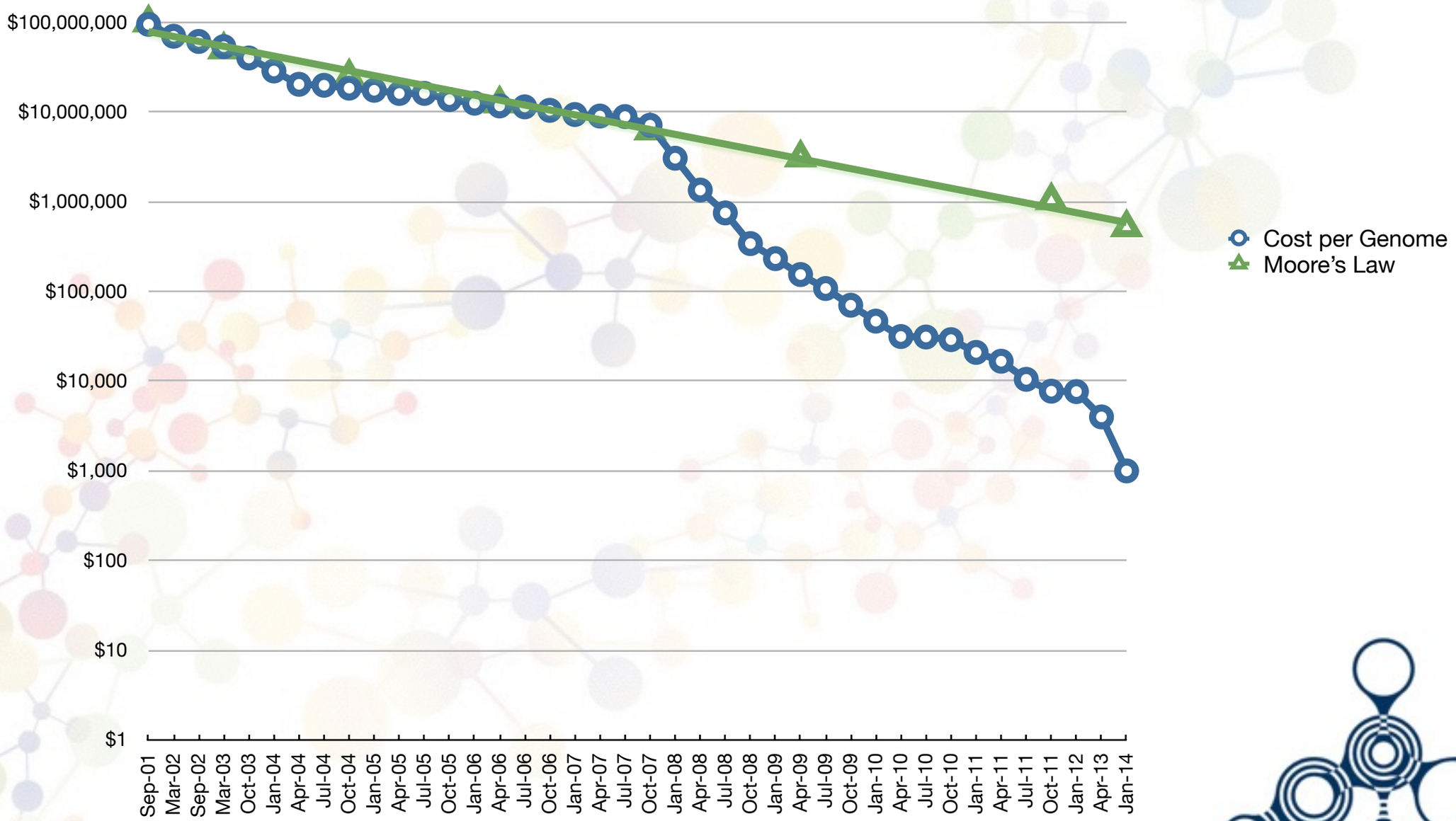
Cost of sequencing



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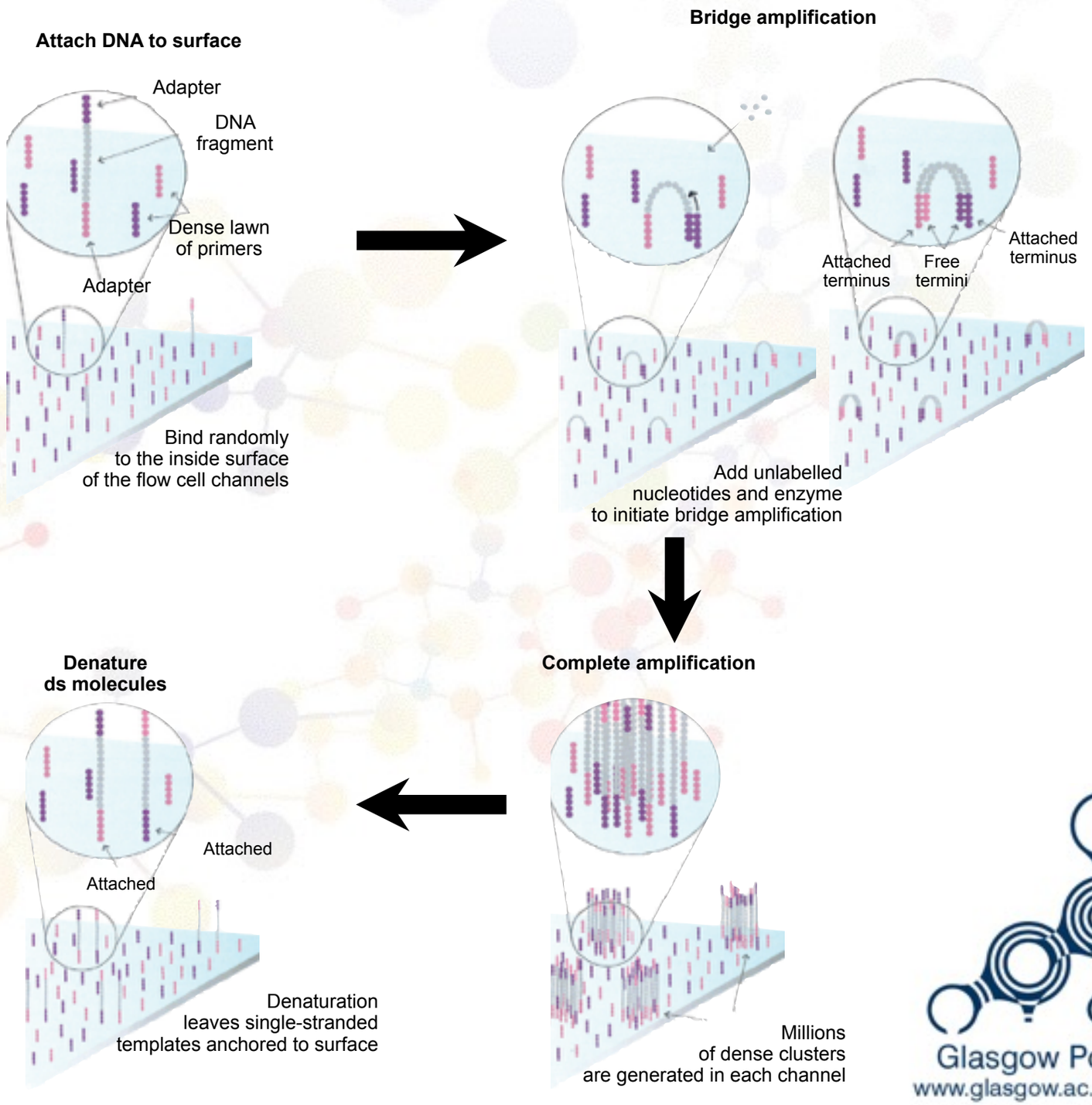
Illumina sequencing

- Cluster generation



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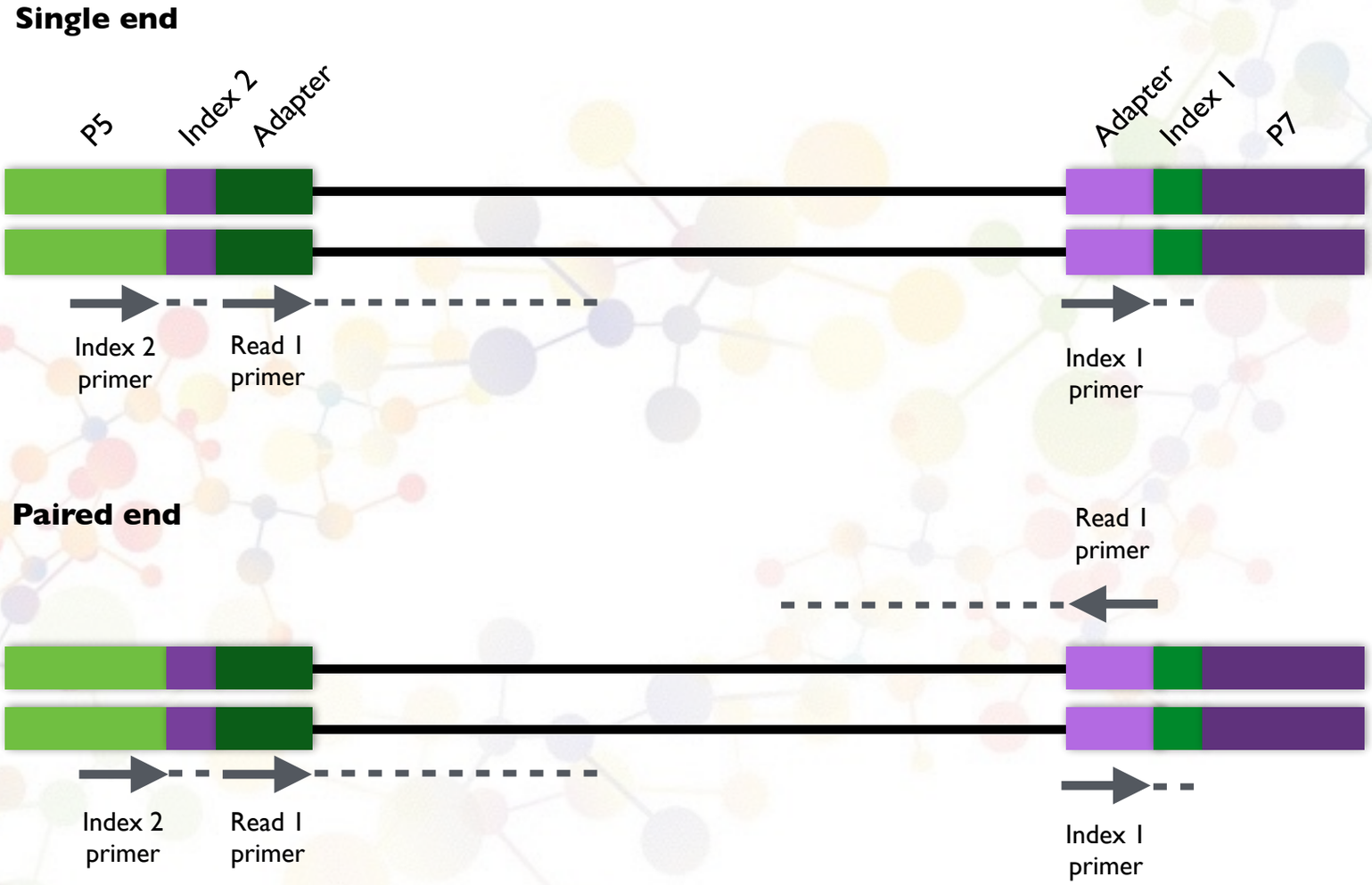
Paired vs Single end sequencing



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Illumina

- Base by base sequencing
- Reads all the same length
- Short reads
- High quality

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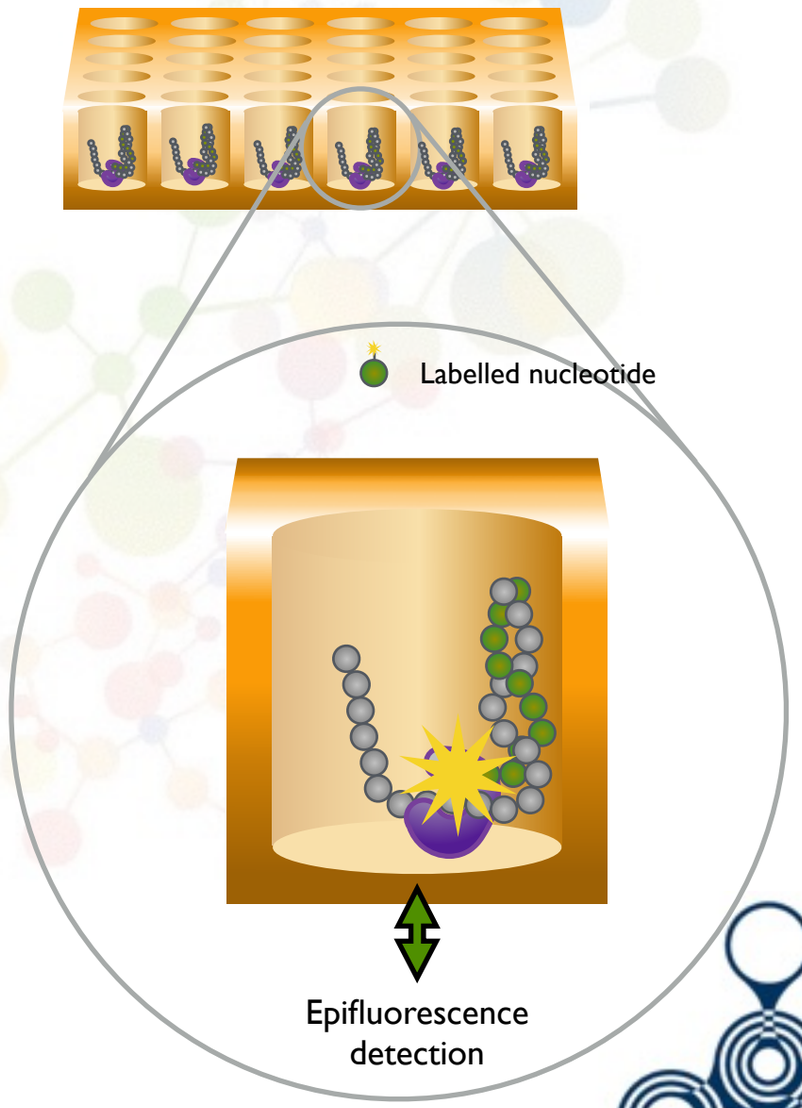
Pacific Biosciences

Real time single molecule sequencing

Long reads up to 20kb

Direct sequencing of modified bases e.g. methylated Cytosine



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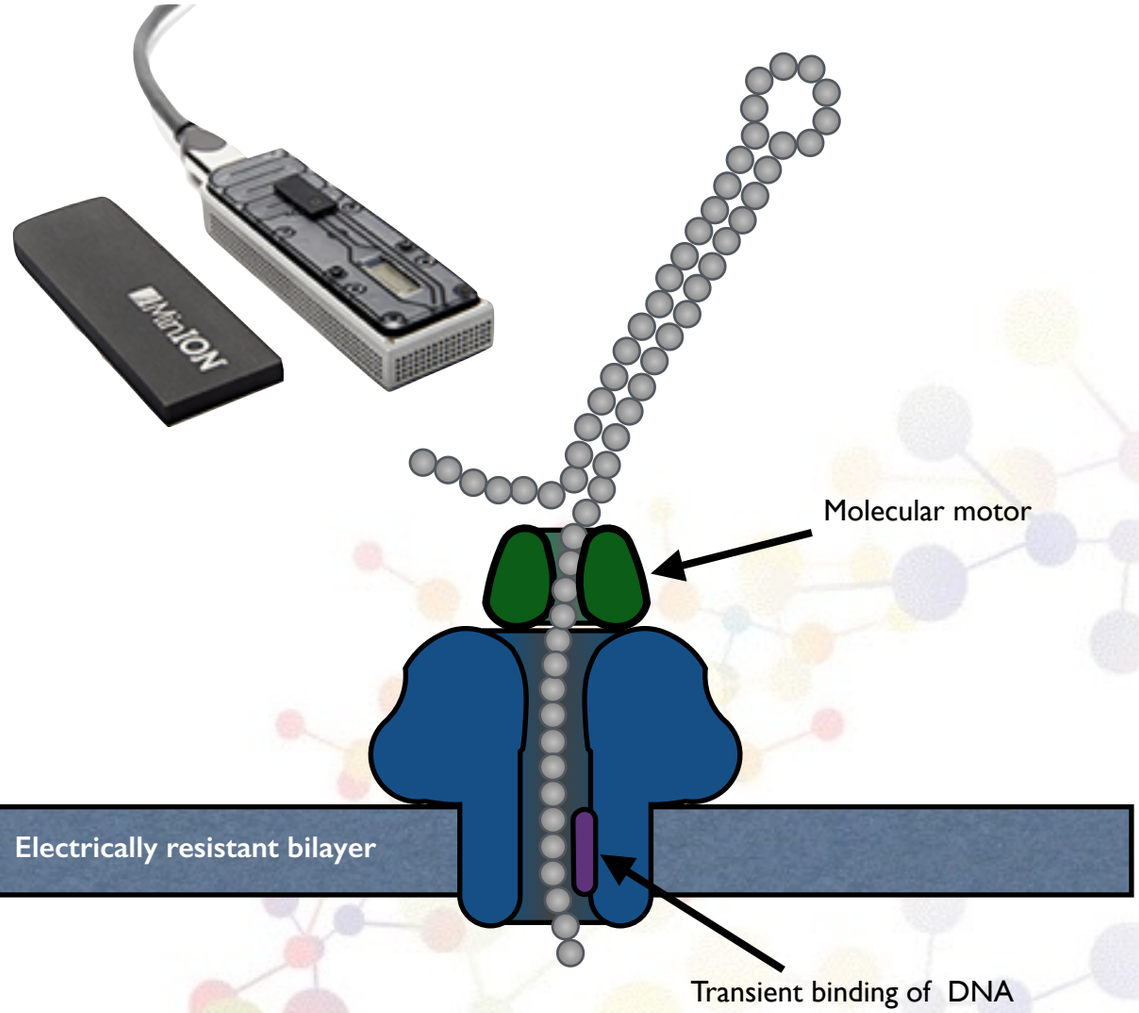
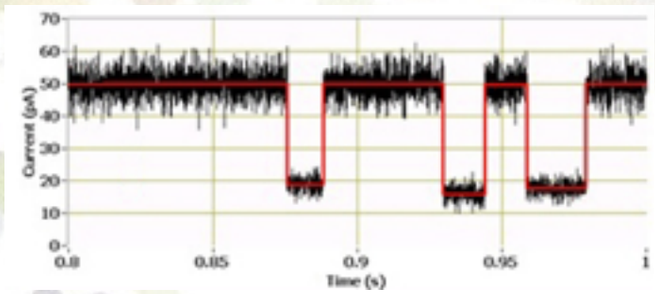
Oxford Nanopore





Real time single molecule sequencing

Long read lengths
10's of kilo bases

Can detect modified DNA bases

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Many applications

Total RNA

micro RNA

Immuno precipitated RNA

Template preparation & Sequencing

Data processing, base calling & storage

Mapping to reference genome

***De novo* assembly**

Transcript differential expression

Novel transcript discovery

Differential isoform usage

micro RNA analysis

RIP-Seq analysis

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Experimental design

- Key to an expression study
- Ensure that the questions can be answered within experimental constraints
  - Cost
  - Availability of RNA

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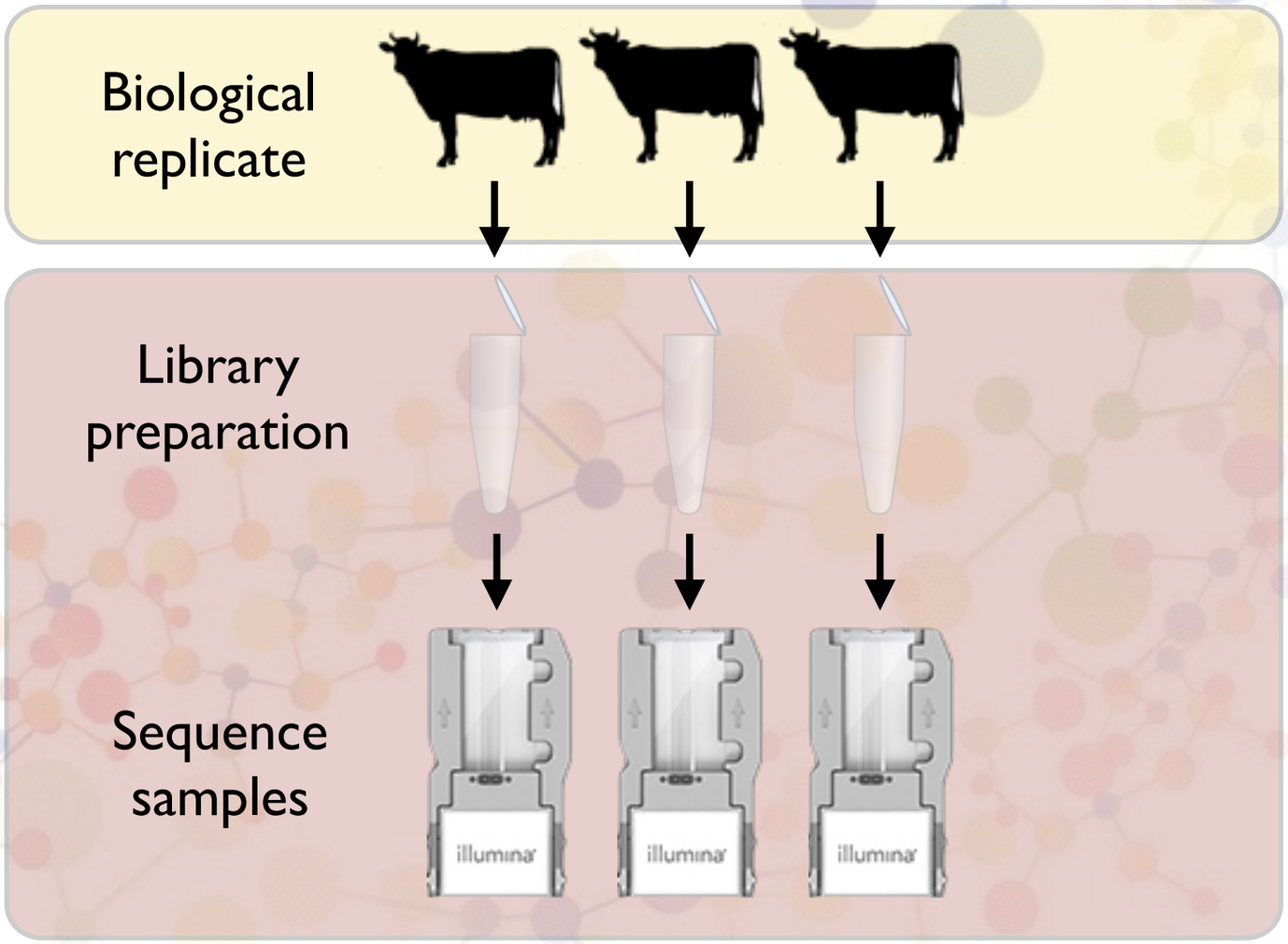
Variation in RNA-Seq experiments

- Biological variation
- Technical vaiation

Levels of variation



Flowcell effect < Library prep effect << Biological effect

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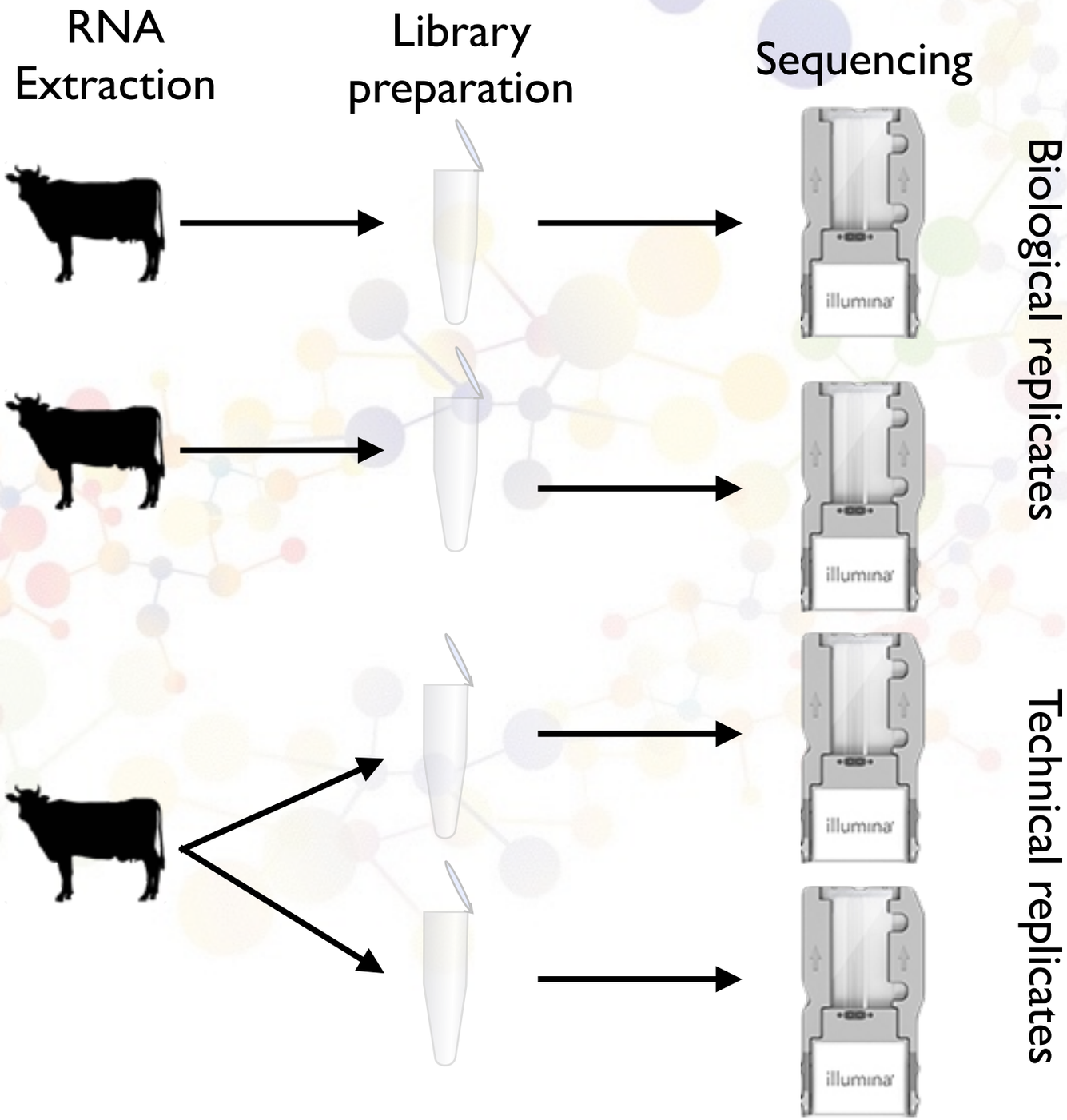
Replicates



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Replicates

- Biological replication is essential
  - How many replicates?
- Technical variation
  - Barcode and multiplex

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Sequencing depth

- Difficult to predict
- Estimates can be based on the length of the transcriptome and the expected dynamic range of transcript abundances
- Greater sequence depth increases sensitivity to detect smaller changes and lower expressed transcripts

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What do I need to provide?

Poly A selection

- 1 µg total RNA
- Prepare using tri reagent or Qiagen Total RNA kit
- Recommend paired end sequencing

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Ribosomal reduction

- 10ng to 400ng ribosomal reduced RNA
- Ribosomal reduction kits from Qiagen, Illumina, Thermo, NEB etc
- Needs optimisation
- Prepare using tri reagent or Qiagen Total RNA kit
- Recommend paired end sequencing
- More reads

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Alignment

Alignment-Tophat

Genomic
reference
sequence

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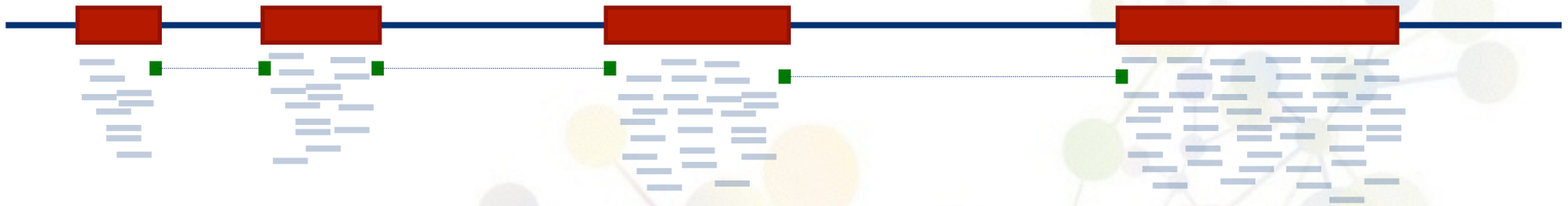
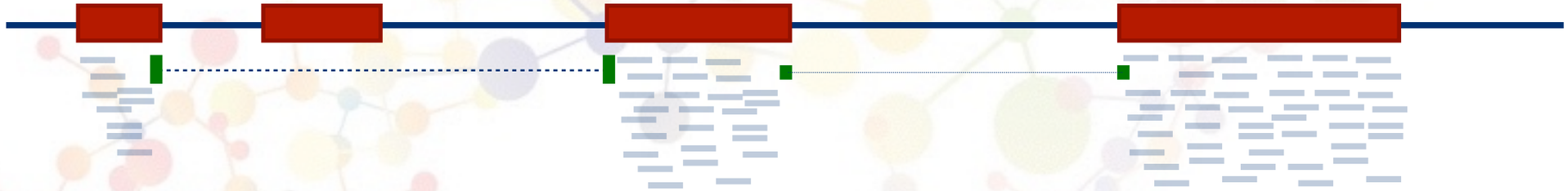
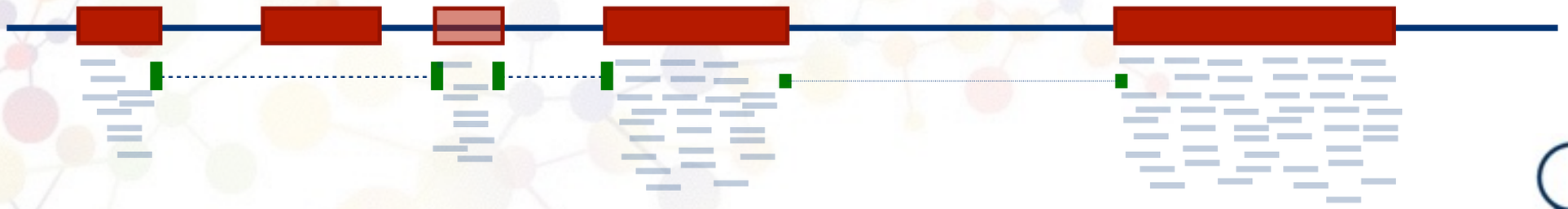
Alignment-Tophat



Exon skipped



Novel exon



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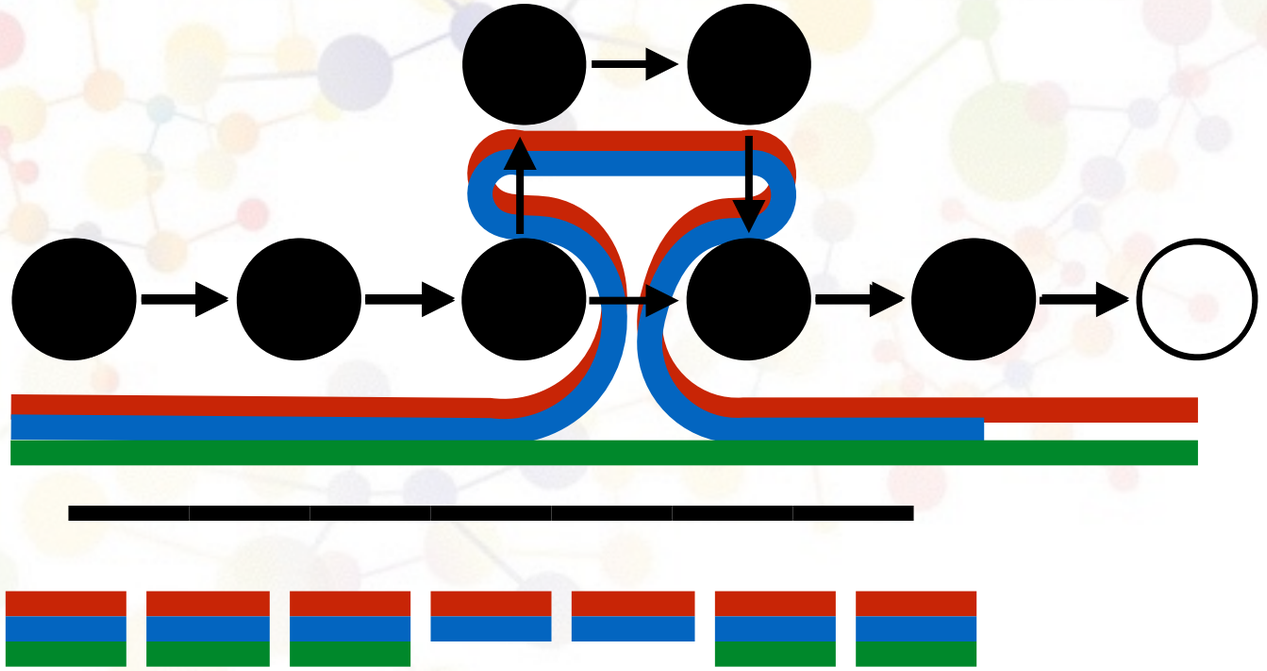
HISAT

- Similar to Tophat
- Two types of index
  - An index of the whole genome
  - Multiple indexes of overlapping ~64kb chunks of the genome

Kallisto



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Differential expression

- Several sources of variability
- Batch effects
- Over dispersion
- Uncertainty in count origin

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Software tools

Cufflinks - Differential analysis at transcript level

DESeq2 - Pools information across genes. Shrinkage estimation for dispersion and fold changes

Voom - Transforms transcript count data to logCPM and maps each observation to the mean variance

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Novel insecticide target discovery by RNA Sequencing

Background

Insects dominant life form

Disease vectors

- malaria, yellow fever, sleeping sickness

15-25% loss to GDP worldwide

Few new pesticides

Must not harm pollinators

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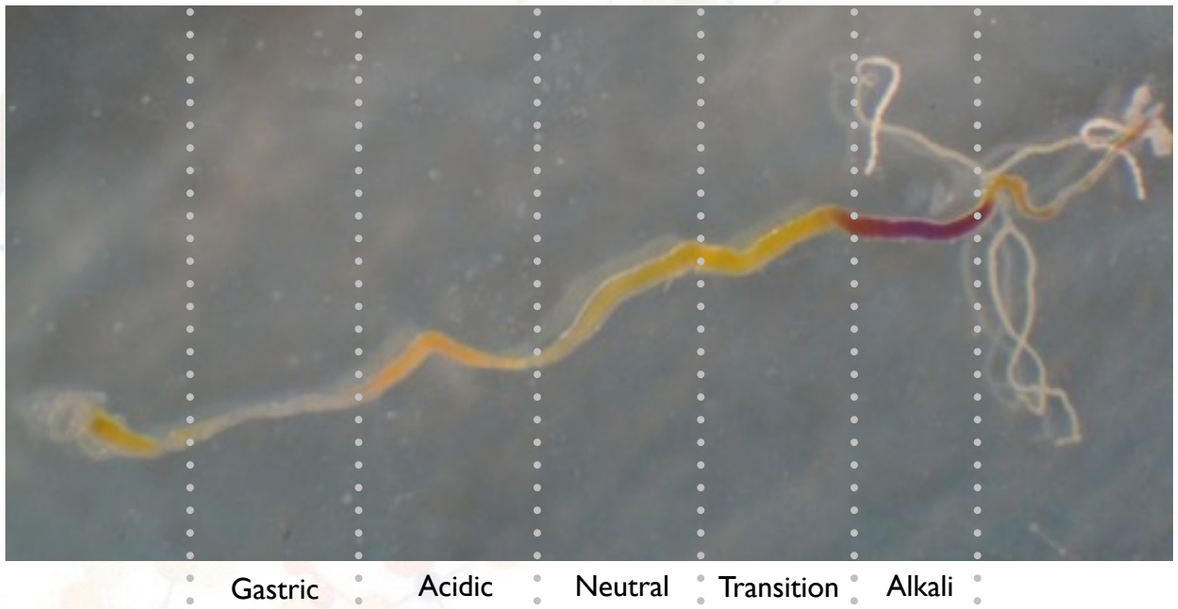
Transcriptomics

- RNA isolated from 5 distinct regions of the digestive tract
- Samples were sequenced
- Compare sample to discover differently expressed transcripts.



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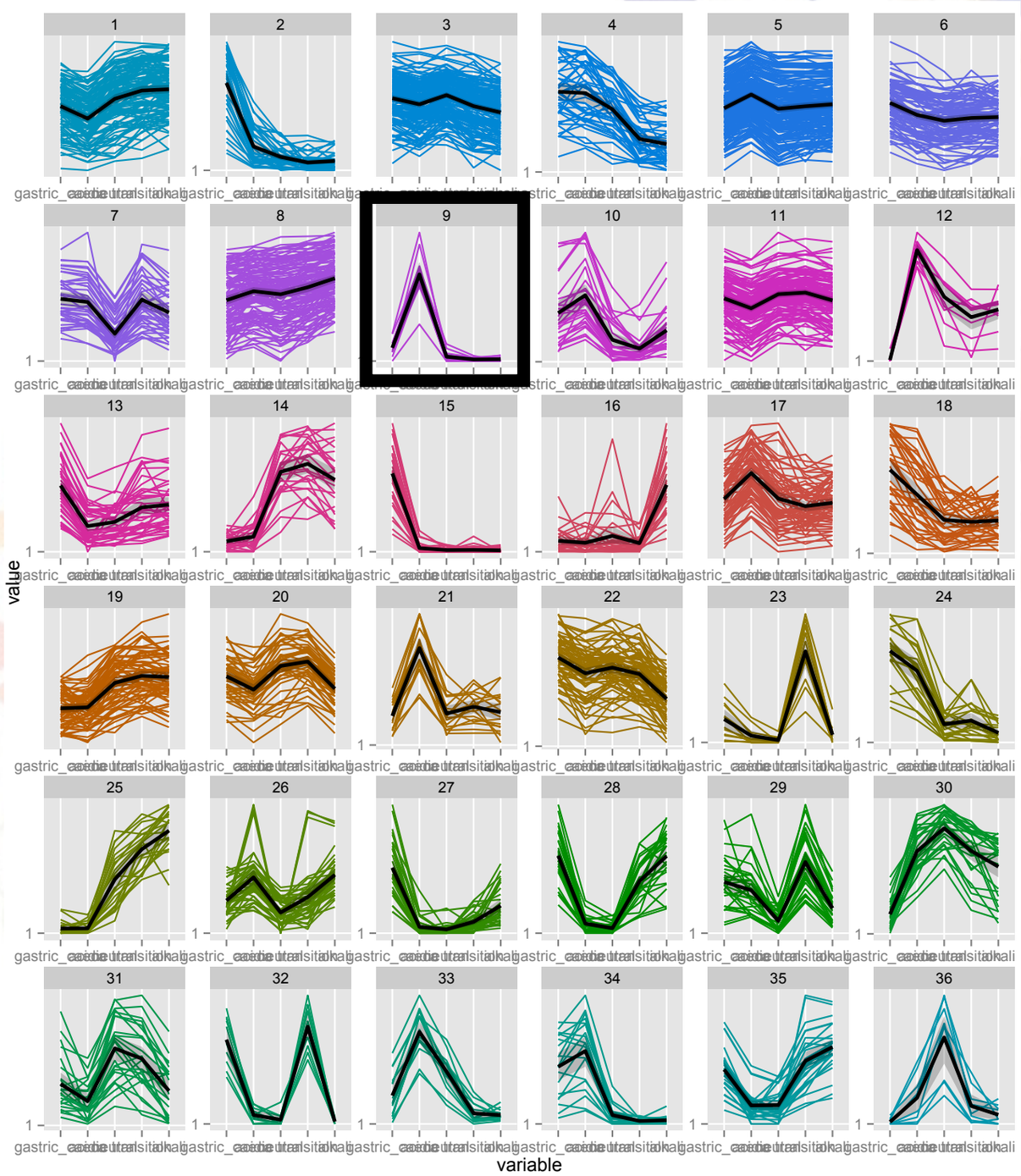

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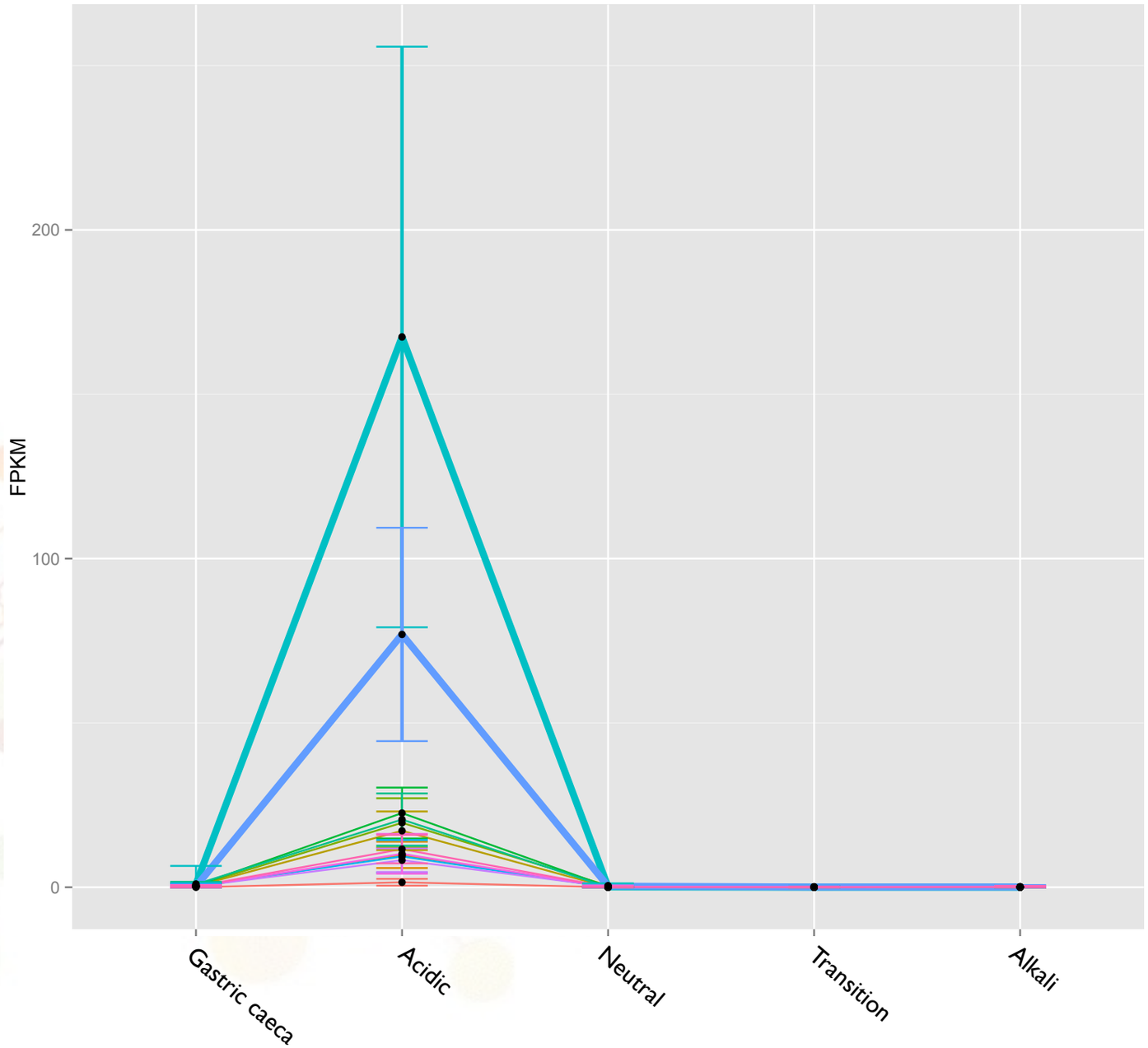

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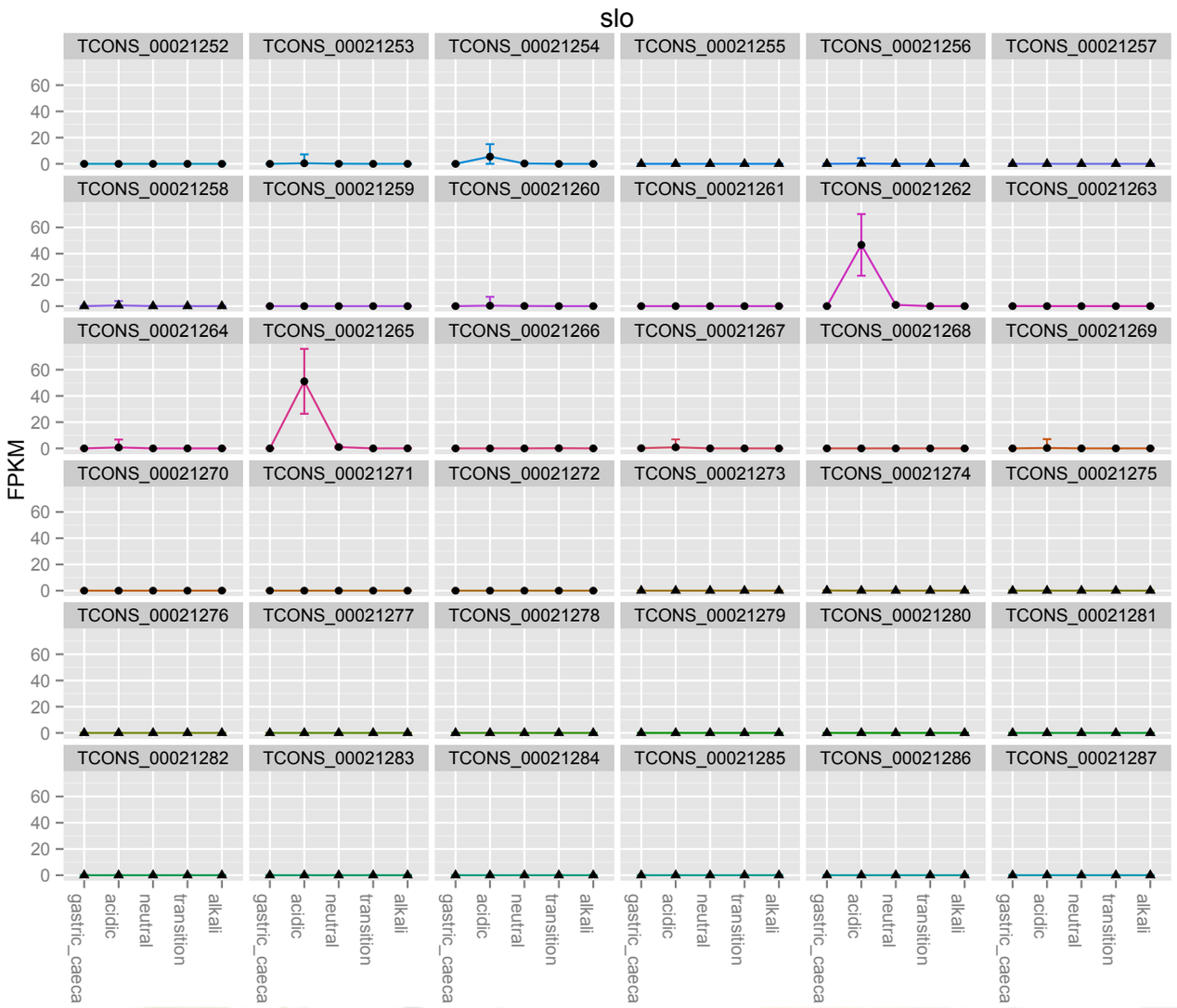
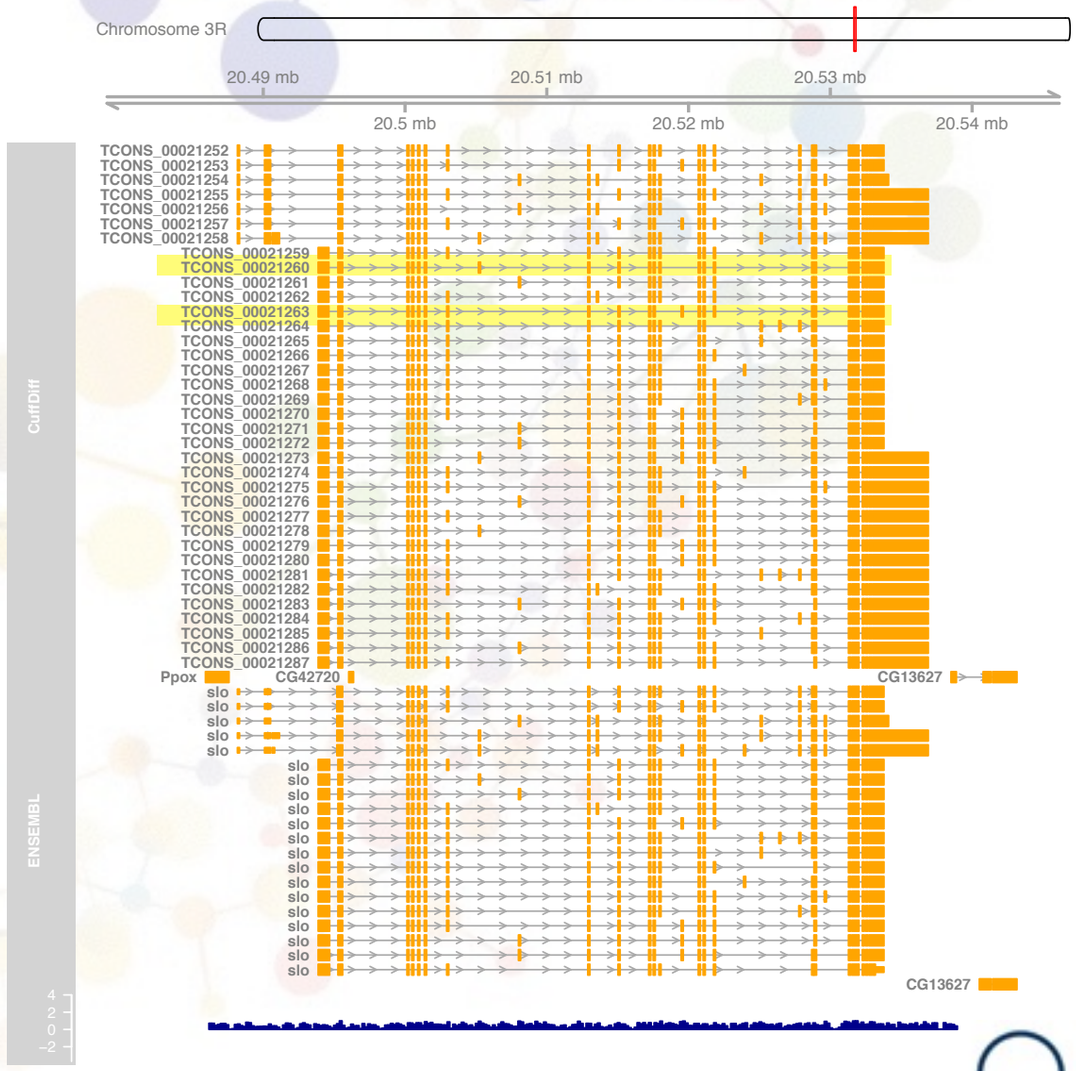
RNA-Seq





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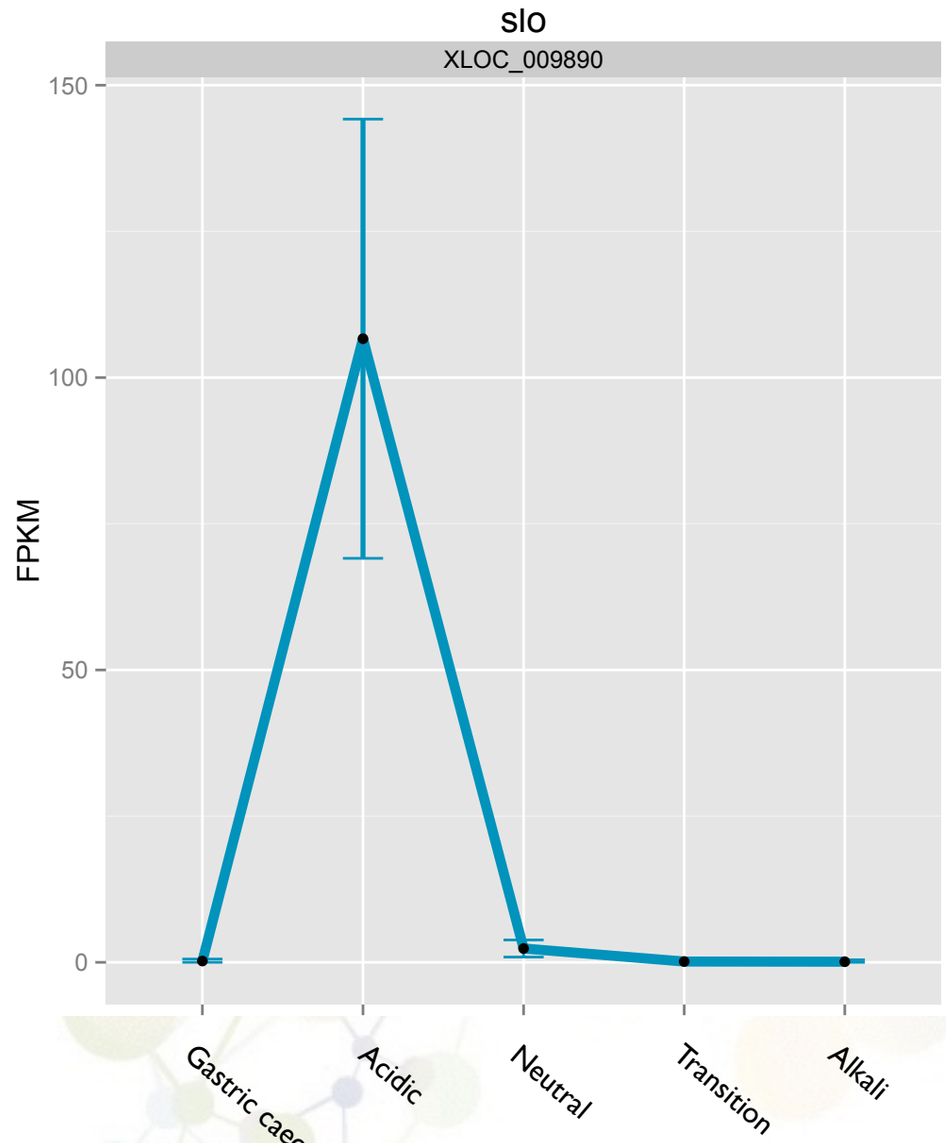
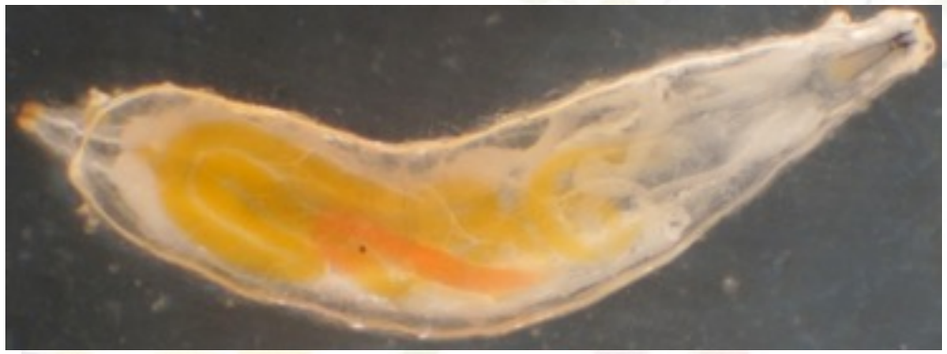
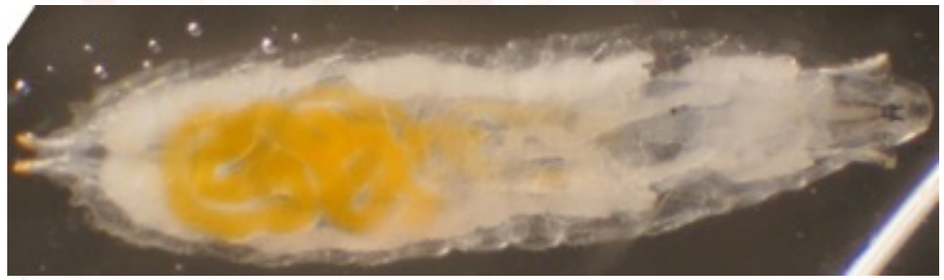
What does this transcript do?





Control



Slowpoke RNAi

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RNA-Seq without a reference

Trinity-
Transcriptome assembly

Sequence RNA

Align to a related reference genome

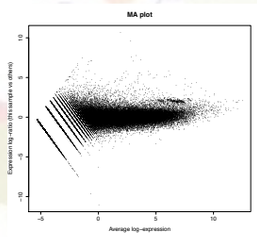
de novo assembly

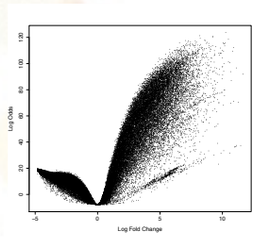
Assembled transcriptome

Annotation

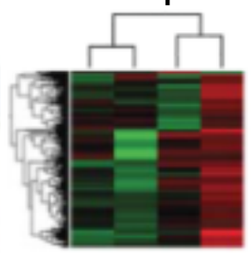
Abundance estimation

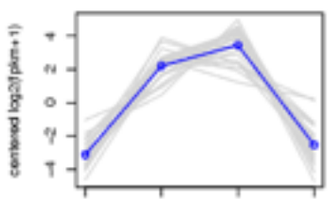
Identify differentially expressed transcripts





Expression patterns, transcript clusters





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