Gene expression workflow for Cryptococcus hybrid samples

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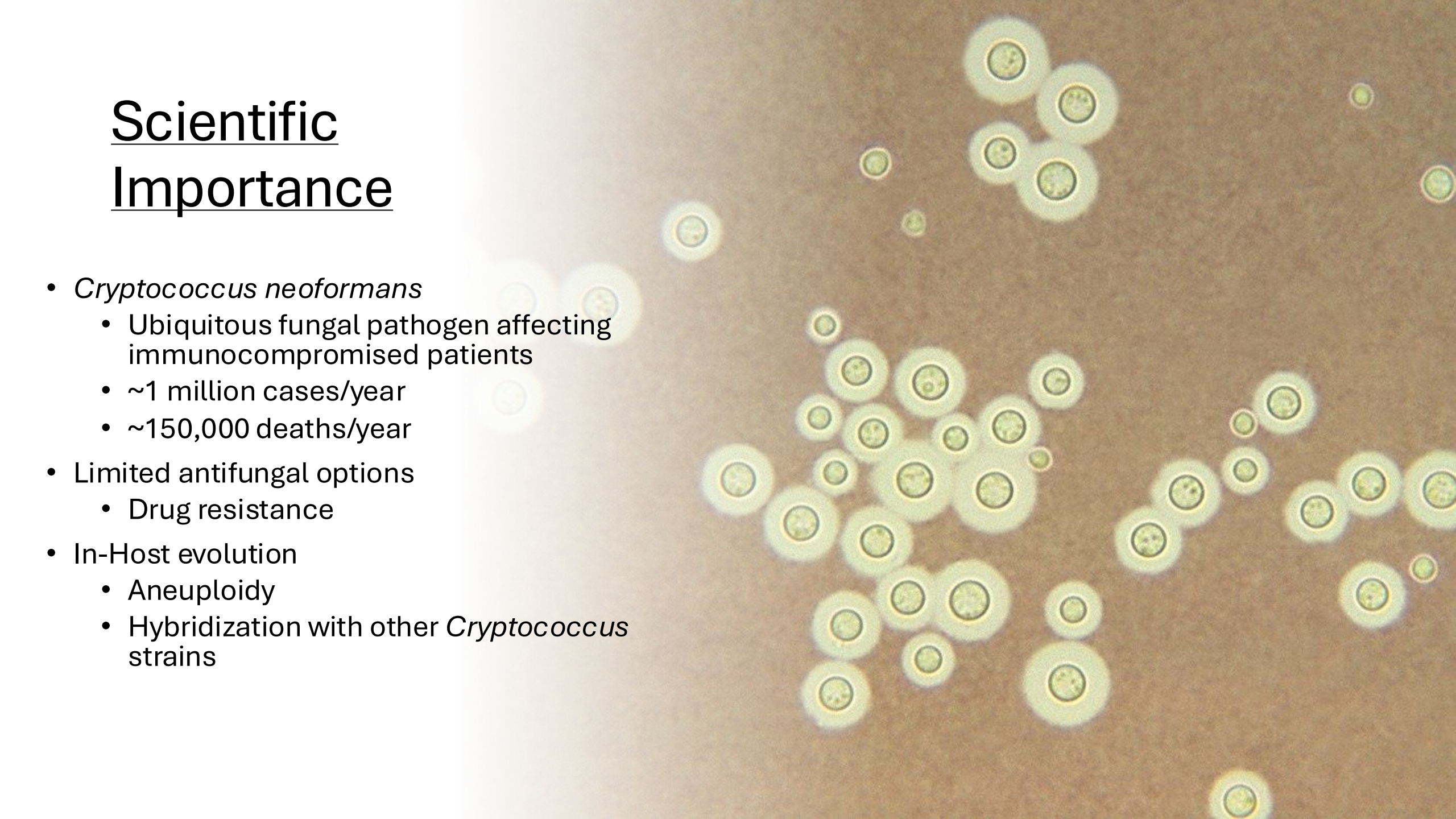
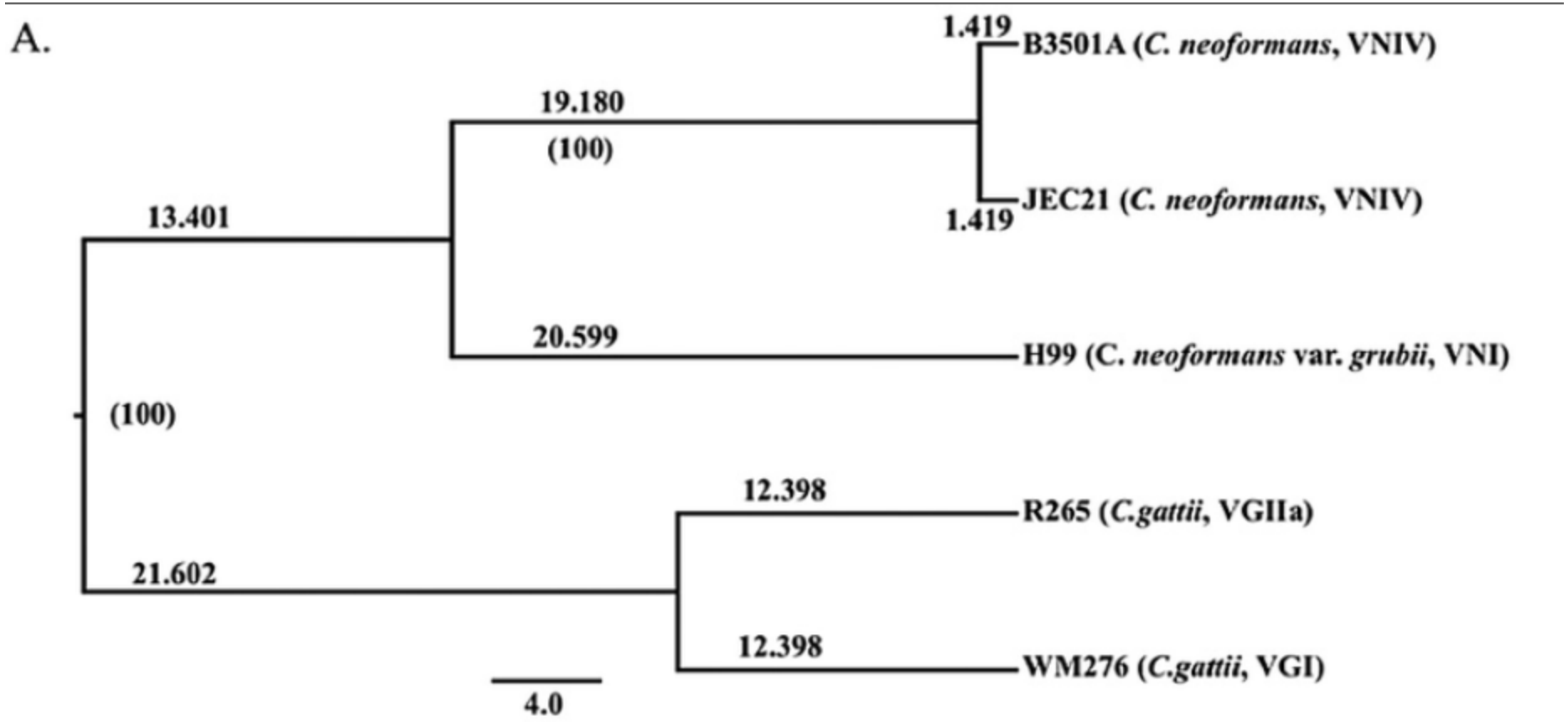
Scientific Importance

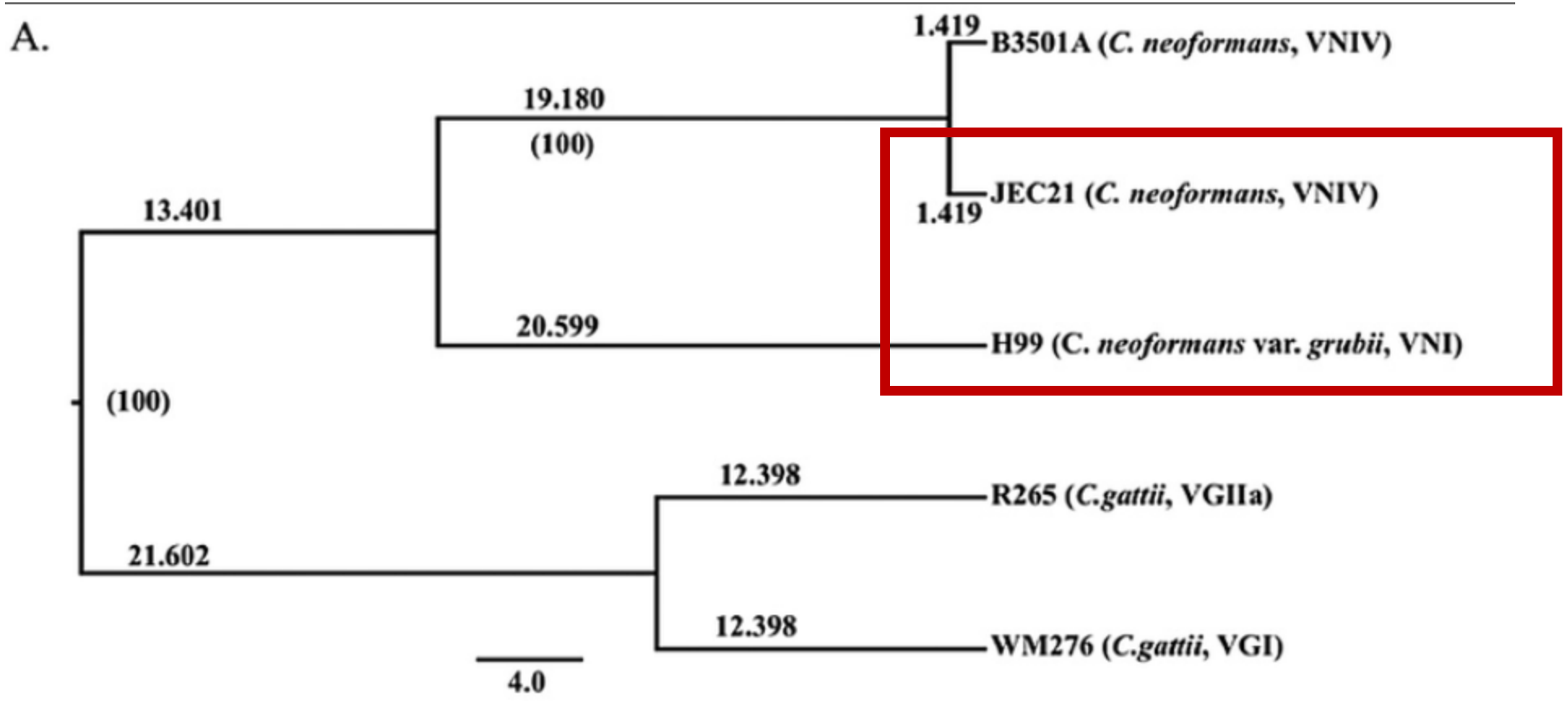
- *Cryptococcus neoformans*
  - Ubiquitous fungal pathogen affecting immunocompromised patients
  - ~1 million cases/year
  - ~150,000 deaths/year
- Limited antifungal options
  - Drug resistance
- In-Host evolution
  - Aneuploidy
  - Hybridization with other *Cryptococcus* strains



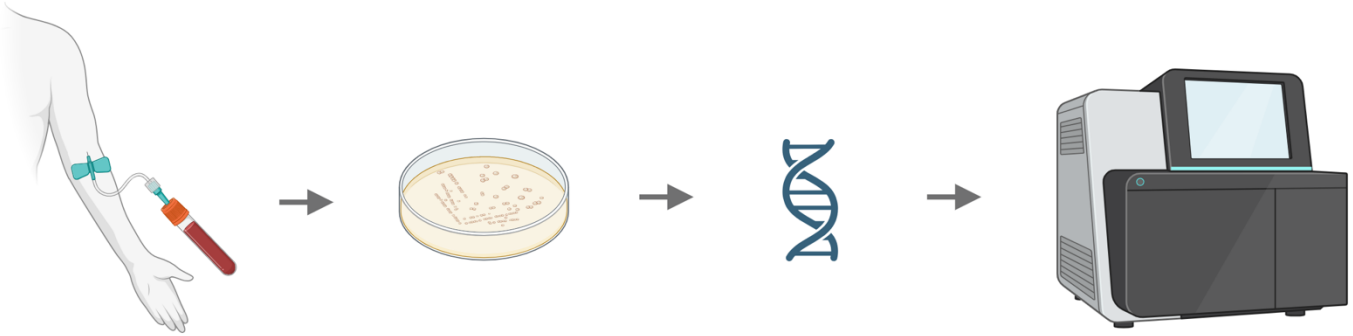
Cryptococcal Hybrids



Cryptococcal Hybrids



Previous analysis of clinical *Cryptococcus* samples

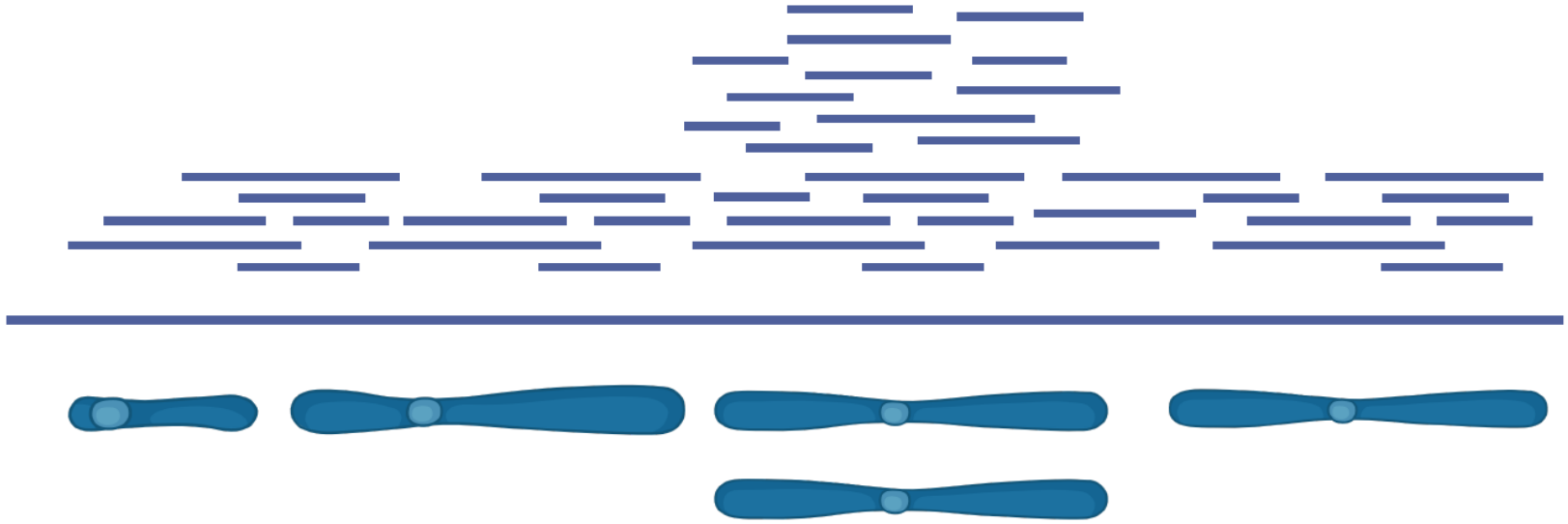


- Preserved cultures isolated and sequenced with **Illumina short-read sequencing**

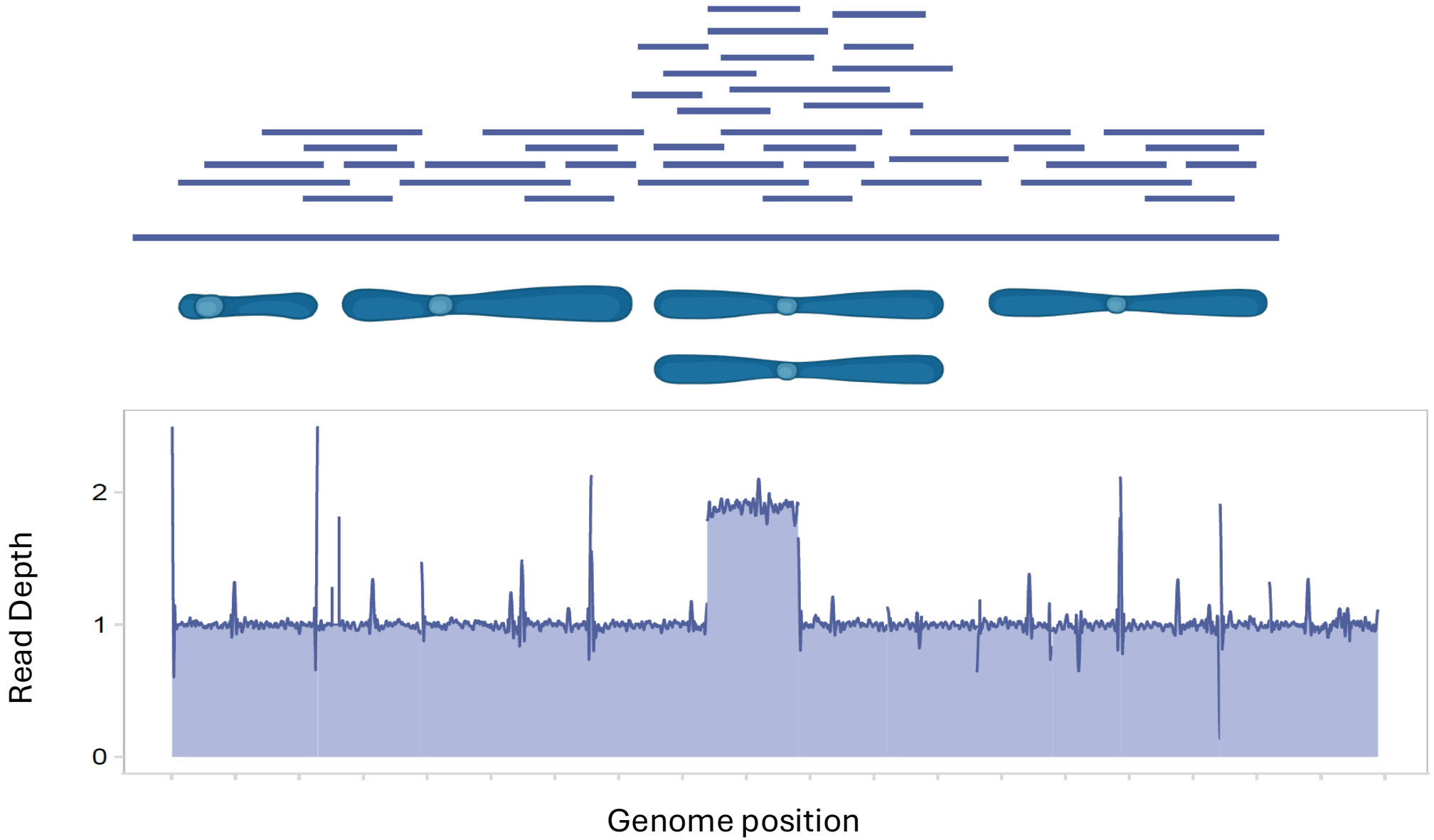
- Sample reads mapped to genomes of all sequenced *Cryptococcus* strains

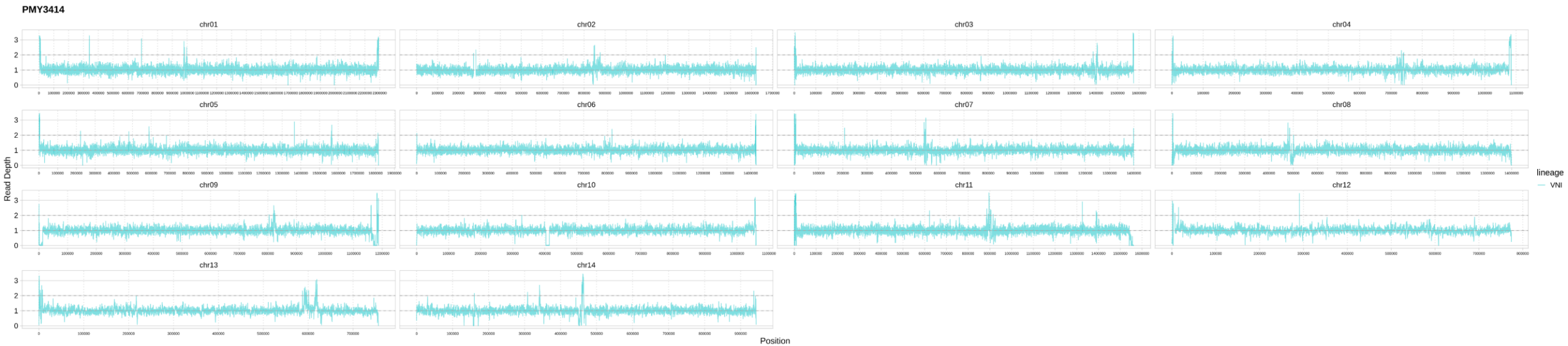
- **Read depth analysis** to find structural variants

Genomic read depth analysis



Genomic read depth analysis

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chr03
chr04
chr05
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chr10
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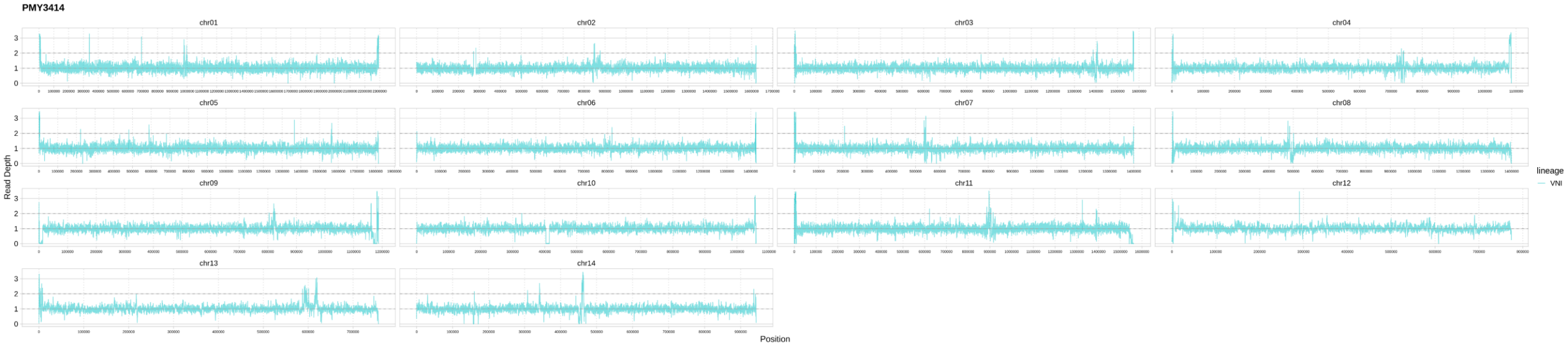
chr14
Read Depth
Position
lineage
VNI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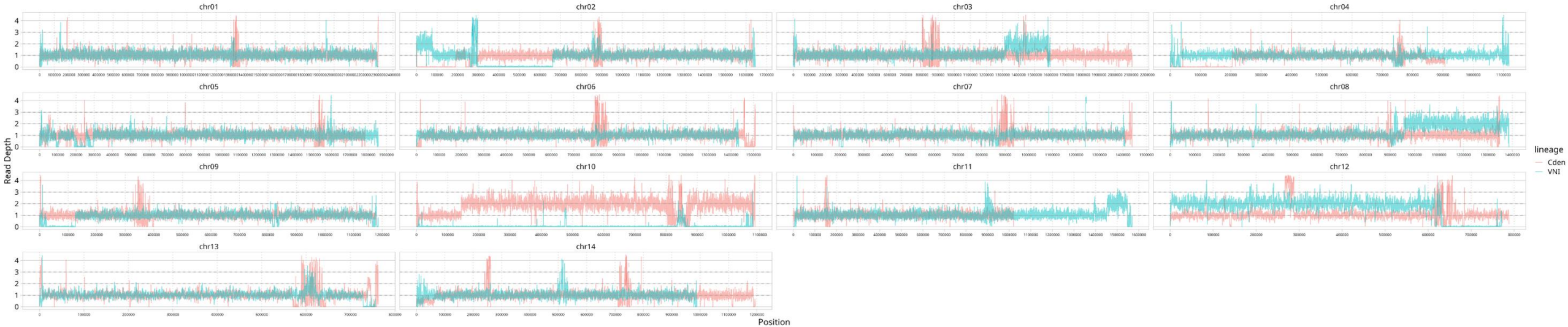
PMY3439



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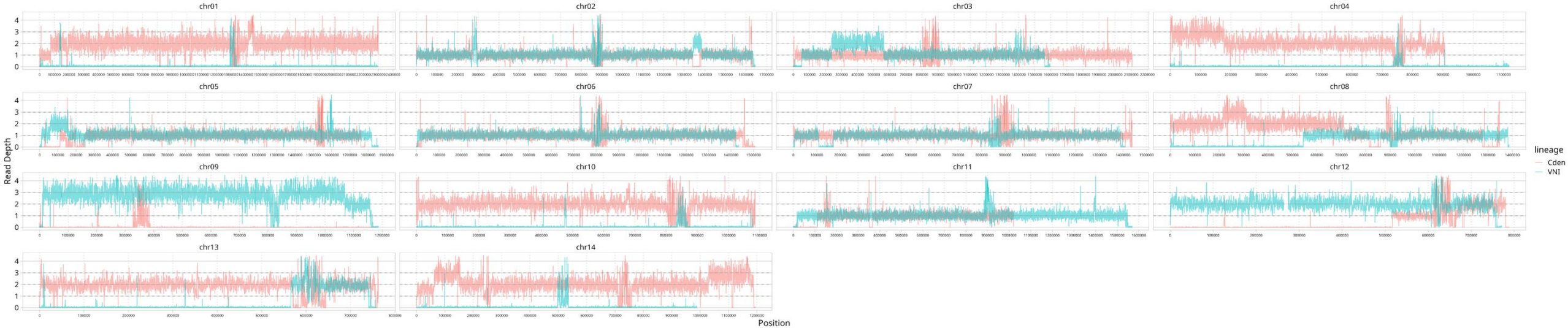

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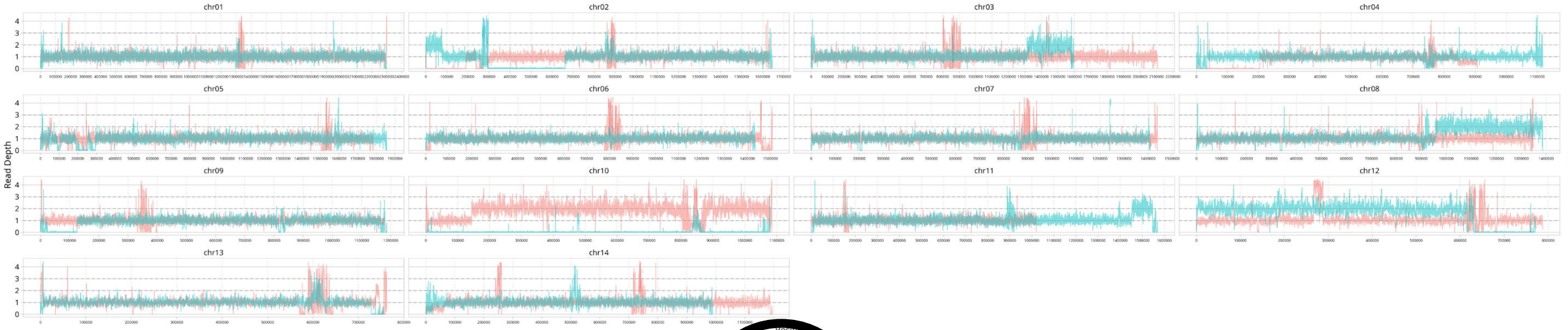

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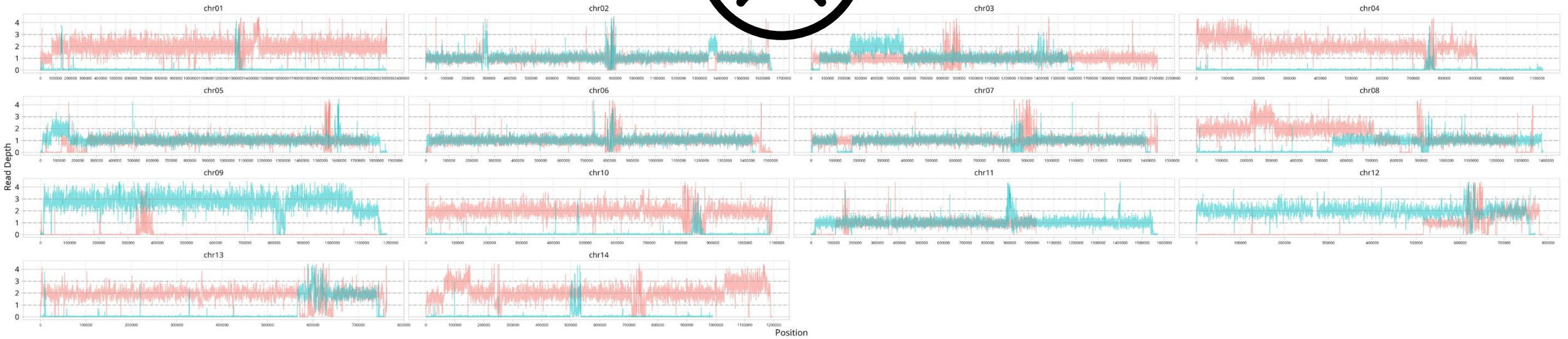
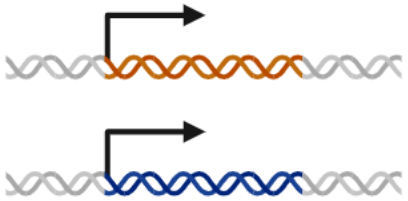
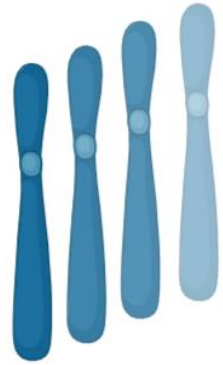


Main Questions

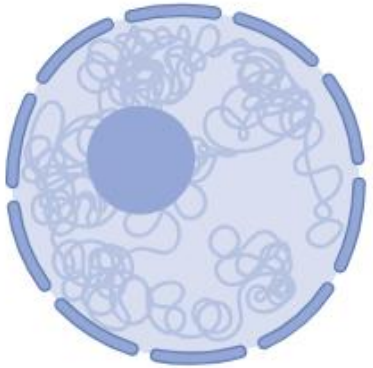
- 1) How are hybrid cryptococcus samples behaving transcriptionally?
  - Are parental haplotypes separately regulated?
  - Cis/trans effects?
  - Differences from synthetic hybrids?
  - Isoforms?



- 2) How do ploidy changes affect transcriptional regulation in clinical cryptococcus samples?
  - Linear increase in expression?
  - Differences in hybrids/nonhybrids?

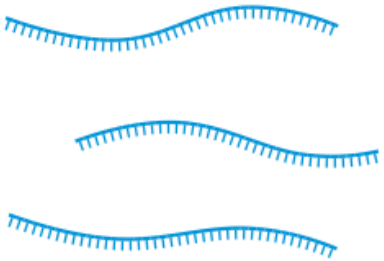


- 3) How do chromosomal rearrangements affect transcriptional regulation in hybrid samples?
  - Cis/trans changes?
  - Physical interactions?

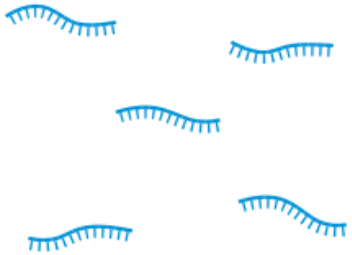


RNA-sequencing overview

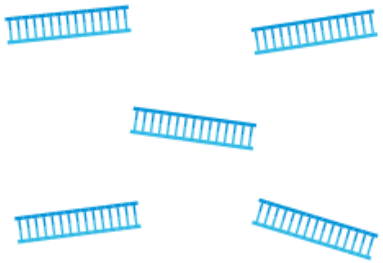
1 **Isolate RNA from samples**



2 **Fragment RNA into short segments**

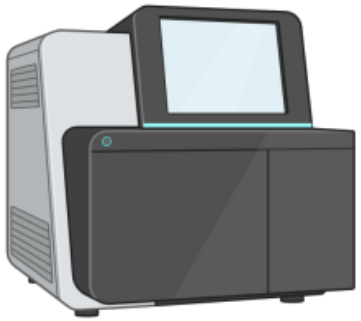


3 **Convert RNA fragments into cDNA**

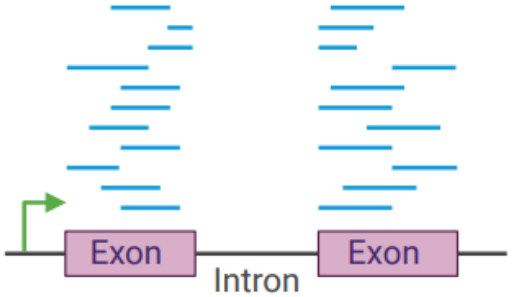


4 **Ligate sequencing adapters and amplify**

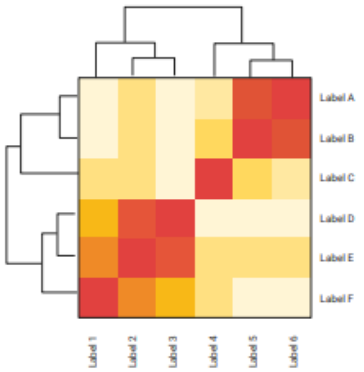
5 **Perform NGS sequencing**



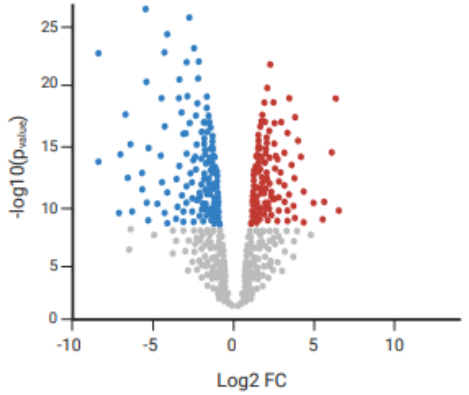
6 **Map sequencing reads to the transcriptome/genome**



7 **Correct and normalize transcript counts**



8 **Identify differential gene expression**



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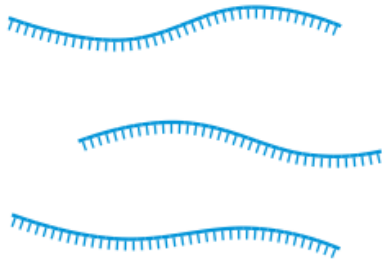

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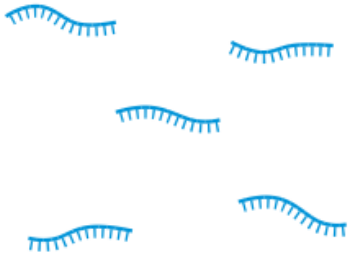

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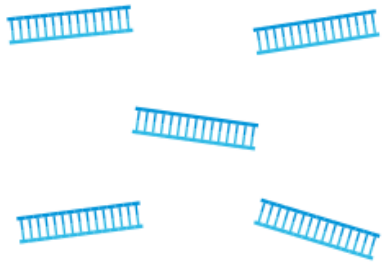

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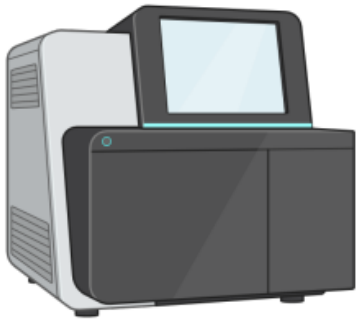

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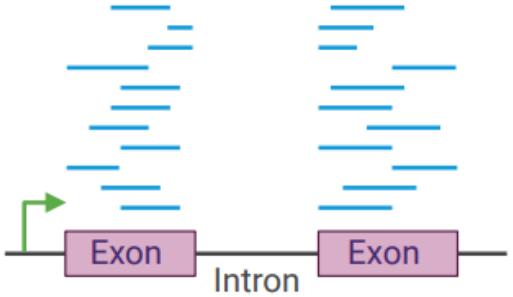

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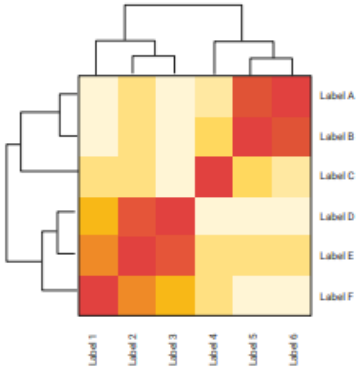

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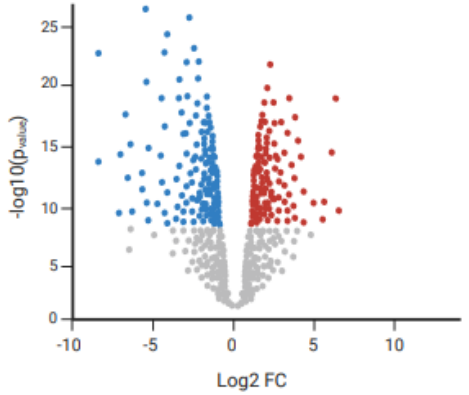


Analysis tools

- 1) How are hybrid cryptococcus samples behaving transcriptionally?
  - Quality control: **FastQC, Trimmomatic, Cutadapt, Skewer**
  - Read mapping/alignment: **STAR, Stampy, Bowtie2**
  - Differential expression: **DESeq2, edgeR**

- 2) How do ploidy changes affect transcriptional regulation in clinical cryptococcus samples?
  - Normalization: **RSEM, edgeR**

- 3) How do chromosomal rearrangements affect transcriptional regulation in hybrid samples?
  - de novo assembly: **Trinity, Trans-ABySS, SOAPdenovo-Trans, BUSCO, DETONATE**

Data formats

- 1) How are hybrid cryptococcus samples behaving transcriptionally?
  - RNA-sequencing
    - **fastq** raw reads and **fasta** reference genome
    - **SAM/BAM** files – alignment mapping
    - **GFF/GTF** files – gene annotations, isoform information
    - Output = **Count matrix (csv)**

- 2) How do ploidy changes affect transcriptional regulation in clinical cryptococcus samples?
- 3) How do chromosomal rearrangements affect transcriptional regulation in hybrid samples?
  - **Metadata (csv/tsv)** – hybrid status, ploidy estimates, known translocations
  - **Count matrix** and **Normalized count matrix**
  - Differential gene expression output = **Volcano plots, etc.**
  - Hi-C / methylation information?

Computing needs and workflow

- Many small genomes (19 Mb) Many intermediate files that can be very large (BAM/SAM, BED, GFF)
  - Both temporary and long term storage needed

- Workflow structure in **Snakemake**
  - **Conda** environments for software version consistency
  - Modular workflow + small genomes = not as much working memory needed
  - Already tested and works well on local cluster

Decisions

- Isoforms
  - May depend on sequencing platform and cost
    - Long + short reads?
  - Likely to be complicated in messy hybrids
  - May need preliminary data first

- Alignment of reads
  - Mostly reference-based
  - May also create de novo genome assemblies

- Differential expression
  - Looking for specific genes or general patterns?
  - What are the significance cutoffs?

Statistical challenges

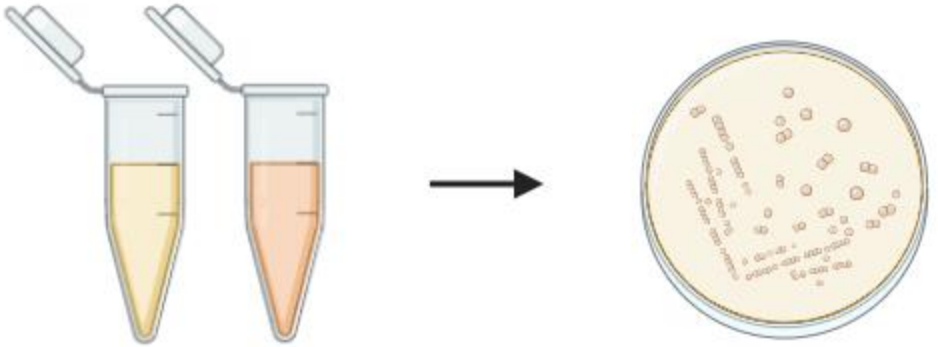
- Normalization and differential expression
  - How to deal with ploidy changes?

> "The **overall expression** of diploids was remarkably similar to that of the haploids in the same condition (**r=0.94, 0.93** for S. cerevisiae and S. paradoxus, respectively) and the **between-species differences** were also highly correlated between the haploids and diploids (**r=0.82**), suggesting that the results are not affected by ploidy level"

- Recombination + structural variation
  - Unknown area of research
  - May vary by strain and mating type

Statistical challenges

- Synthetic hybrids vs. clinical hybrids
  - Creating *in vitro* hybrids = better cis/trans calling, compare early/established hybrids
    - Both parents known and available
    - May not be stable or 'realistic'
  - Clinical strains may not have clear parental strains available
    - Could split haplotypes and treat as parents?
    - Could use closest sequenced strain to each haplotype?



Future Plans

- Testing on example data
  - Choosing software tools
  - Testing with/without parents included
  - Testing with imputed parents from offspring haplotypes

- Finding more information on current software
  - Isoform information
  - Cis/trans calling
  - Short + long read sequencing
  - Hybrid organisms

- Narrowing goals
  - Looking for specific genes (drug resistance)
  - Patterns temporally/geographically