From RNA-seq reads to gene models

Biodiversity Genomics Academy 2024

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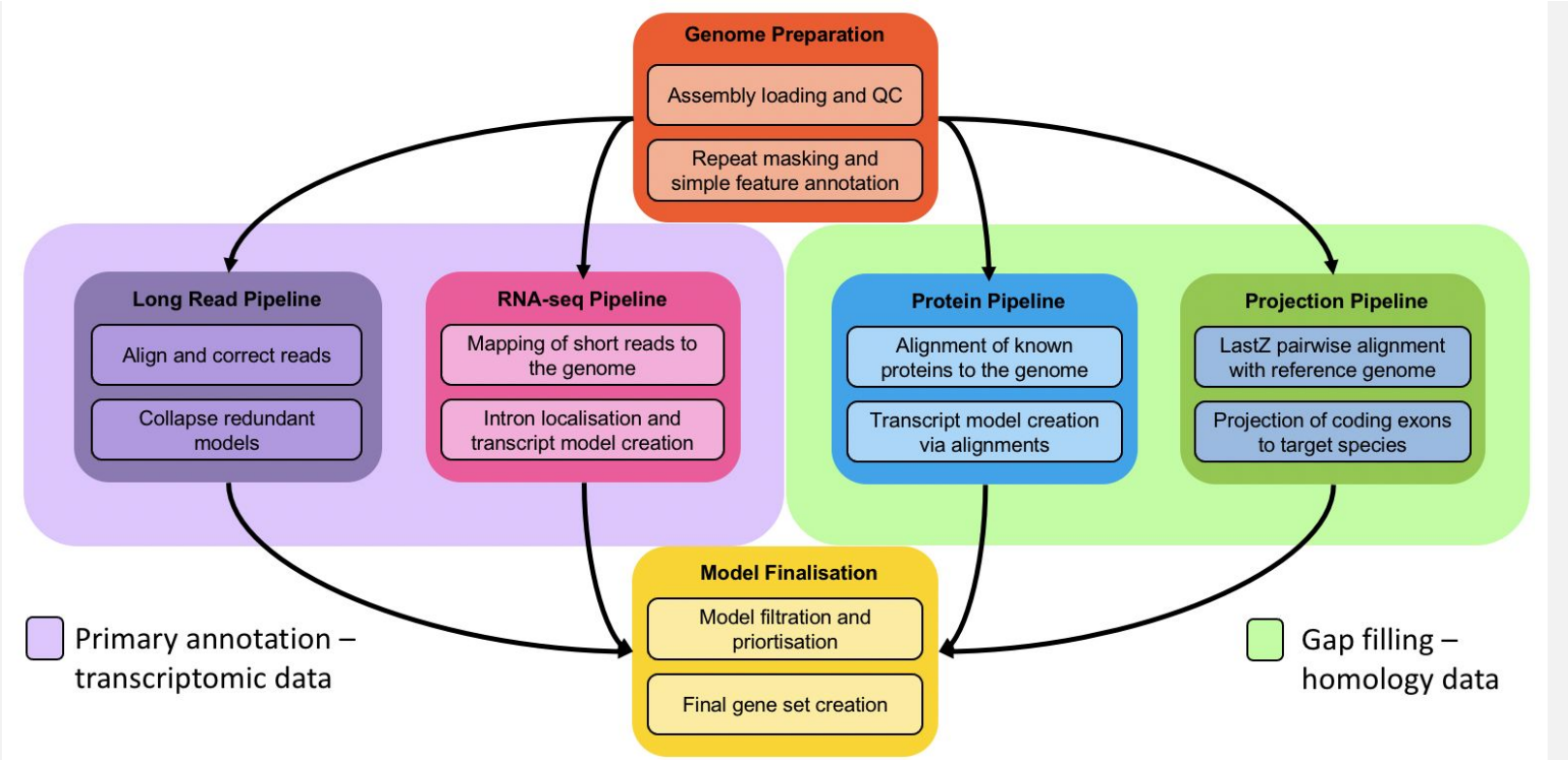
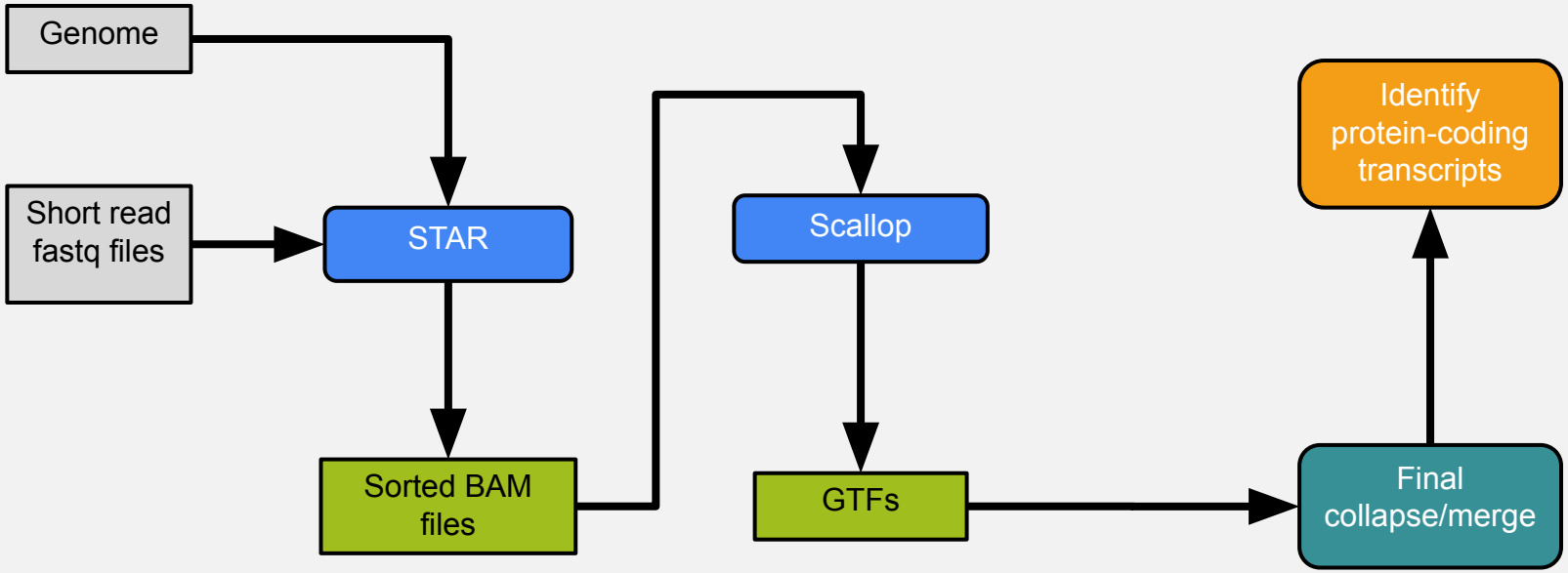
EMBL-EBI

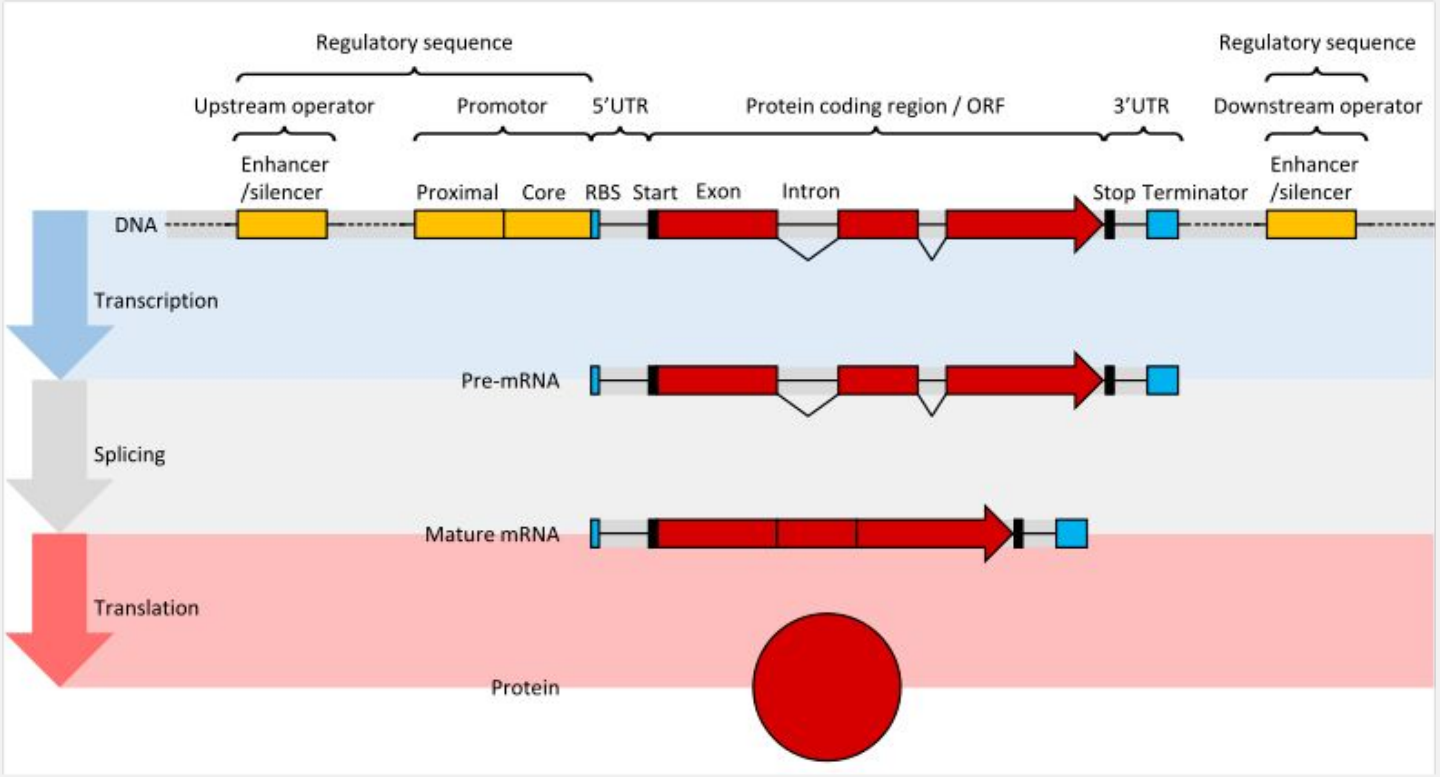
introduction - genome annotation



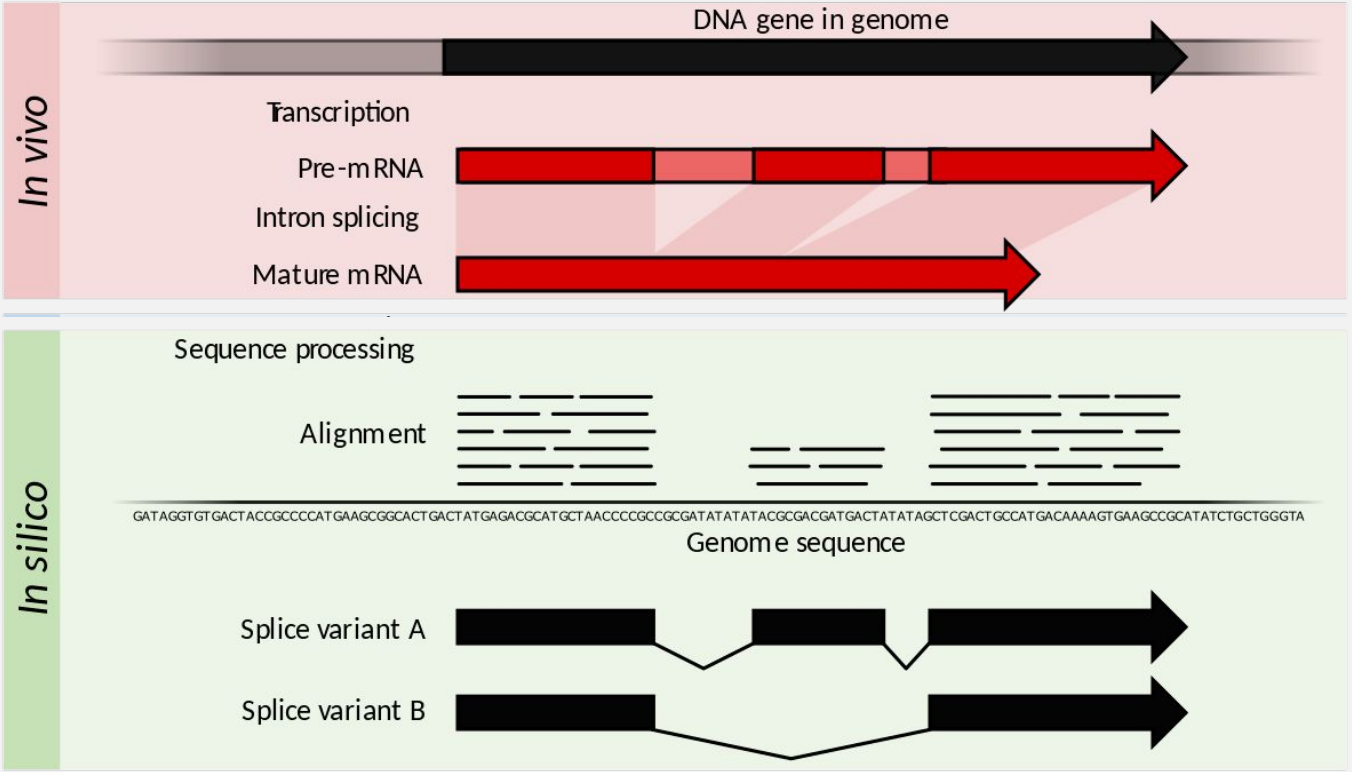
introduction - genome annotation



introduction - transcription and RNA-seq



introduction - transcription and RNA-seq



introduction - data

- Annotating_the_genome_with_rnaseq_data.pdf
- Fastq/
  - 2cell_chr12_R1.fastq
  - 2cell_chr12_R2.fastq
  - 6hpf_chr12_R1.fastq
  - 6hpf_chr12_R2.fastq
- Genome/
  - Danio_rerio.GRCz11.dna.chromosome.12.fa

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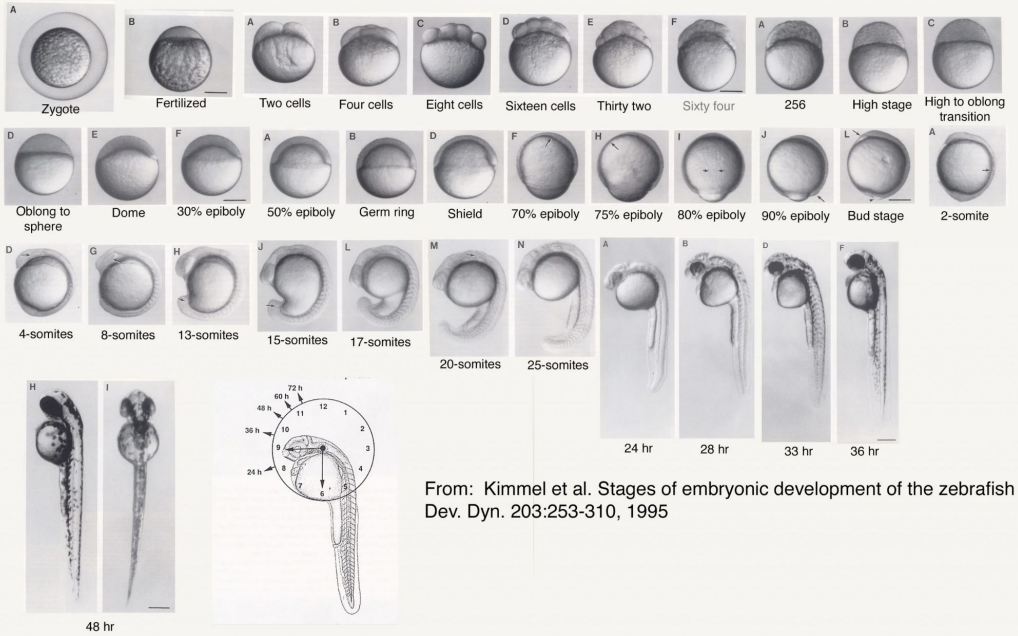

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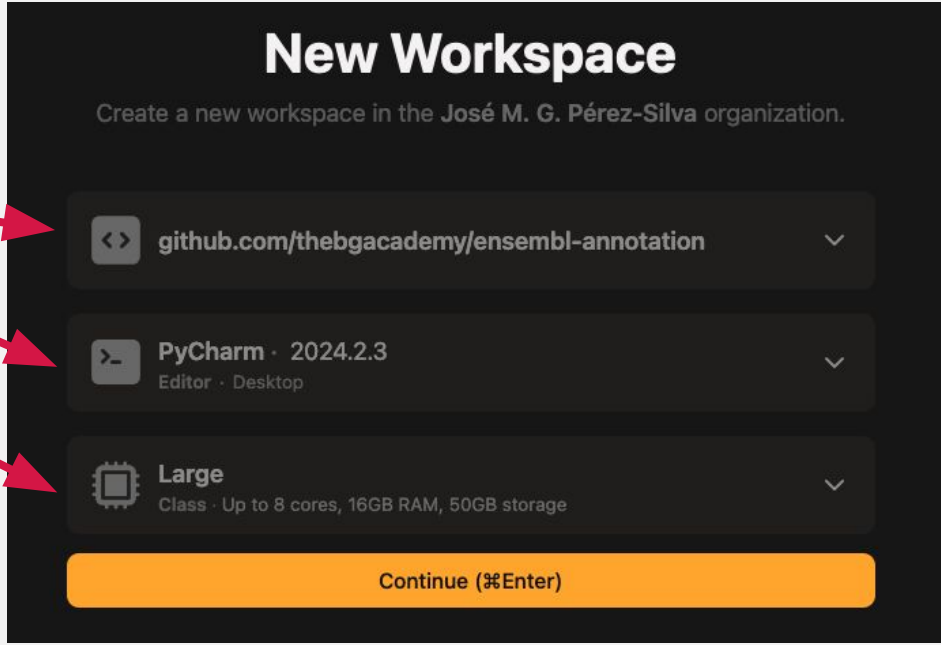
introduction - tools

- STAR: Spliced Transcripts Alignment to a Reference ©
  - “Ultrafast universal RNA-seq aligner”
- SCALLOP:
  - “Accurate reference-based transcript assembler”
- SAMTOOLS:
  - “Suite of programs for interacting with high-throughput sequencing data”

let’s get starting

- Access: https://gitpod.io/#https://github.com/thebgacademy/ensembl-annotation
- From the options:
  - Leave this
  - Choose your favourite editor
  - Choose “Large” in the third



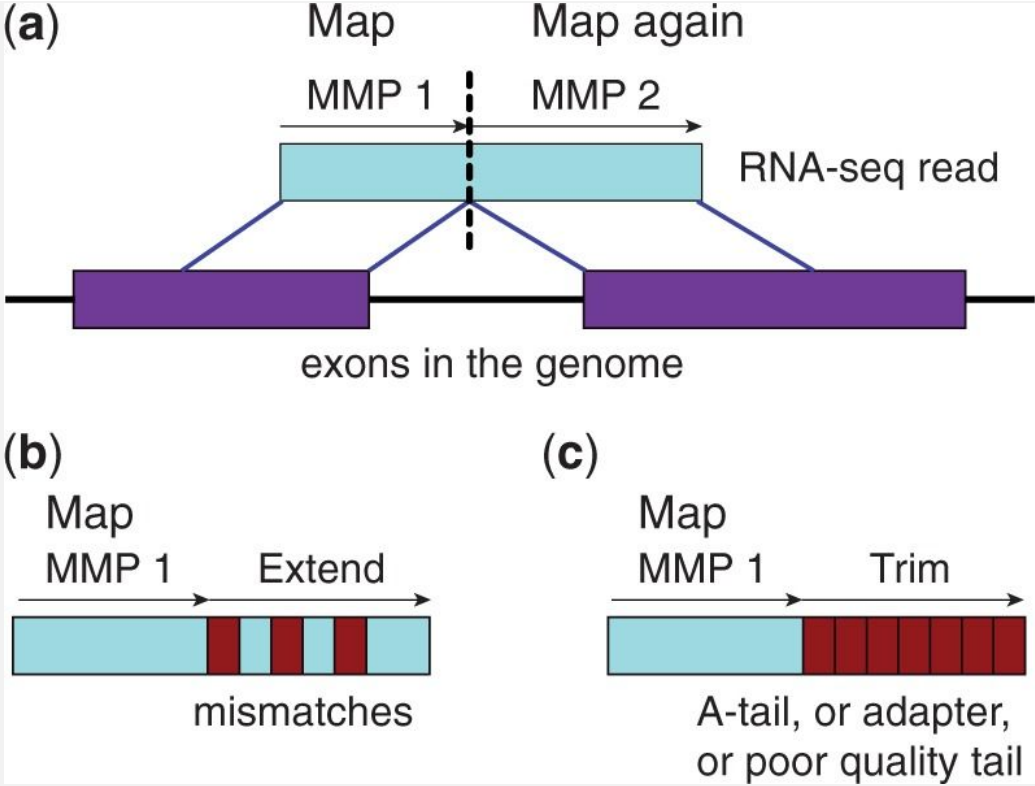
let’s get starting

- Wait while it loads. This may take a second or two.
- Navigate the files in the left, open **`ensembl-annotation`**, then

  `Annotating_the_genome`, and PREVIEW **`instructions.md`** by left-clicking it.
- You should now have a markdown document with instructions.

STAR

- Maps over splices, mismatches and excludes unwanted seqs
- Requires a genome index
- Docs available in github page
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3530905/



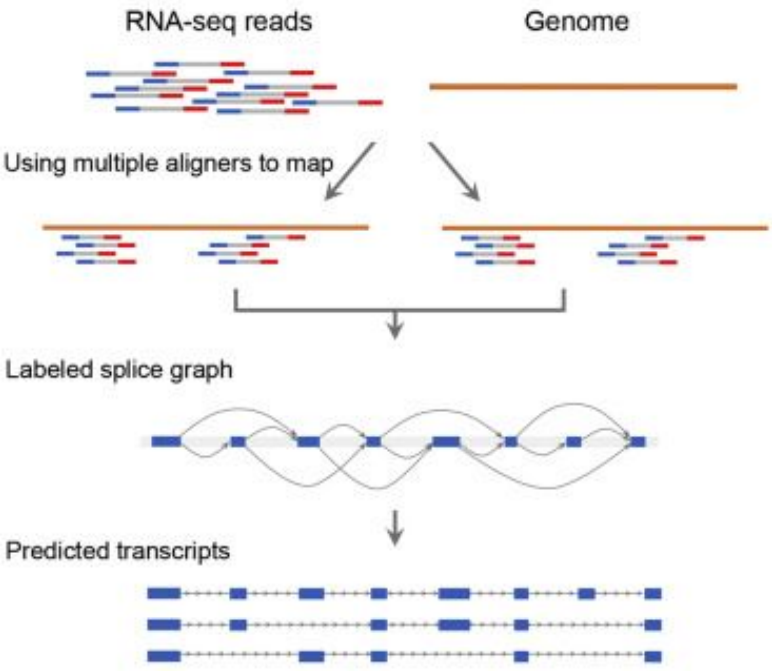
SAMtools

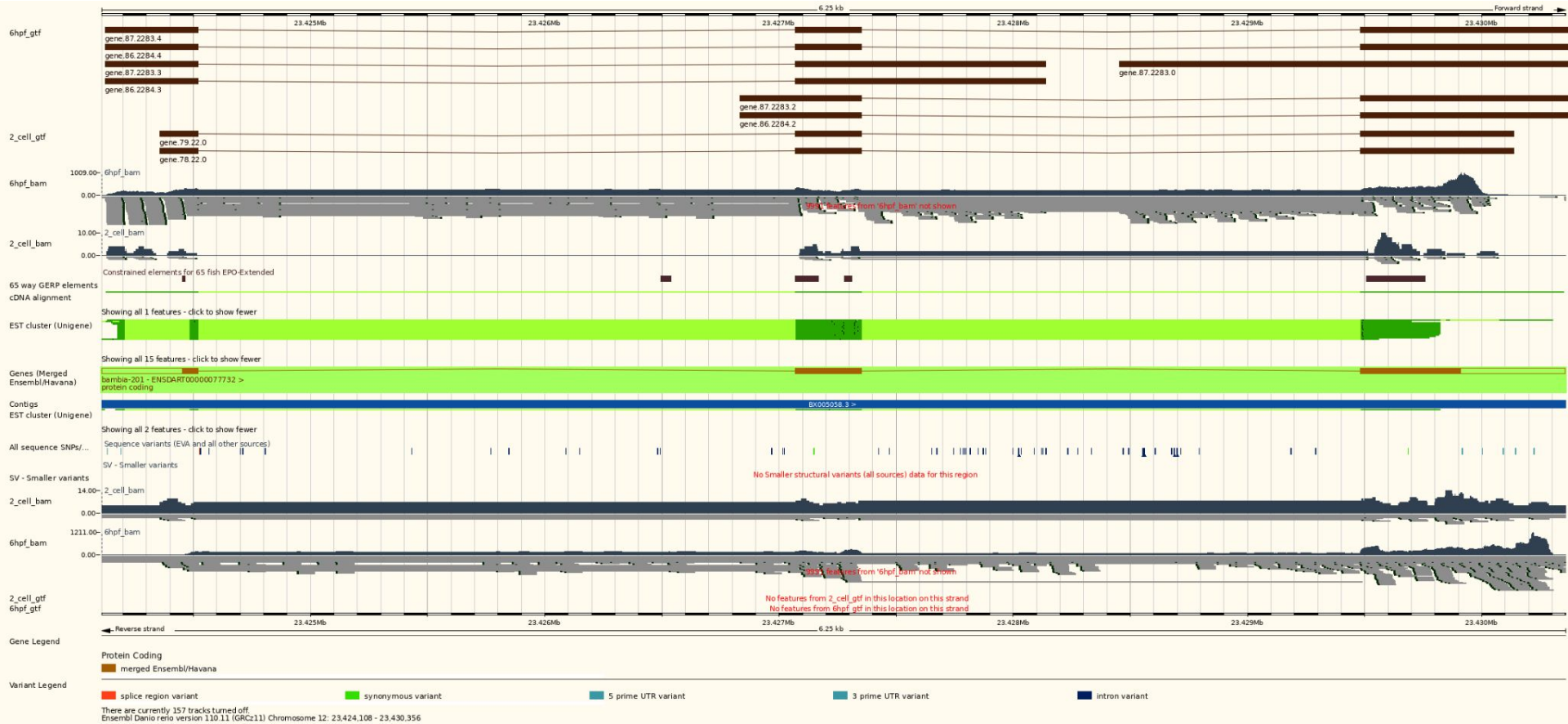
- A suite of software for various tasks:
  - Fastq to BAM/CRAM
  - WGS/WES mapping to variant calls
  - Filtering of VCF files
  - Several, BAM-workflow related
- Docs available in github page
- https://pubmed.ncbi.nlm.nih.gov/33590861/

```
samtools addreplacerg -r 'ID:fish' -r 'LB:1334' -r 'SM:alpha' -o output.bam input.bam
samtools ampliconclip -b bed.file input.bam
samtools ampliconstats primers.bed in.bam
samtools bedcov aln.sorted.bam
samtools calmd in.sorted.bam ref.fasta
samtools cat out.bam in1.bam in2.bam in3.bam
samtools collate -o aln.name_collated.bam aln.sorted.bam
samtools consensus -o out.fasta in.bam
samtools coverage aln.sorted.bam
samtools cram-size -v -o out.size in.cram
samtools depad input.bam
samtools depth aln.sorted.bam
samtools dict -a GRCh38 -s "Homo sapiens" ref.fasta
samtools faidx ref.fasta
samtools fasta input.bam > output.fasta
samtools fastq input.bam > output.fastq
samtools fixmate in.namesorted.sam out.bam
samtools flags PAIRED,UNMAP,MUNMAP
samtools flagstat aln.sorted.bam
samtools fqidx ref.fastq
samtools head in.bam
samtools idxstats aln.sorted.bam
samtools import input.fastq > output.bam
samtools index aln.sorted.bam
samtools markdup in.algnsorted.bam out.bam
samtools merge out.bam in1.bam in2.bam in3.bam
samtools mpileup -C50 -f ref.fasta -r chr3:1,000-2,000 in1.bam in2.bam
samtools phase input.bam
samtools quickcheck in1.bam in2.cram
samtools reference -o ref.fa in.cram
samtools reheader in.header.sam in.bam > out.bam
samtools reset -o /tmp/reset.bam processed.bam
samtools samples input.bam
samtools sort -T /tmp/aln.sorted -o aln.sorted.bam aln.bam
samtools split merged.bam
samtools stats aln.sorted.bam
samtools targetcut input.bam
samtools tview aln.sorted.bam ref.fasta
samtools view -bt ref_list.txt -o aln.bam aln.sam.gz
```

scallop

- Splice graph
- Docs available in github page
- https://www.nature.com/articles/nbt.4020




6.25 kb
Forward strand
6hpf_gtf
gene:87.2283.4
gene:86.2284.4
gene:87.2283.3
gene:86.2284.3
gene.87.2283.0
gene.87.2283.2
gene.86.2284.2
2_cell_gtf
gene.79.22.0
gene.78.22.0
6hpf_bam
1009.00
0.00
6hpf_bam
2933 features from '6hpf_bam' not shown
2_cell_bam
10.00
0.00
2_cell_bam
65 way GERP elements
Constrained elements for 65 fish EPO-Extended
cDNA alignment
EST cluster (Unigene)
Showing all 1 features - click to show fewer
Genes (Merged Ensembl/Havana)
Showing all 15 features - click to show fewer
bambia-201 - ENSDART00000077732 >
protein coding
Contigs
BX005058.3 >
EST cluster (Unigene)
Showing all 2 features - click to show fewer
All sequence SNPs/...
Sequence variants (EVA and all other sources)
SV - Smaller variants
SV - Smaller variants
No Smaller structural variants (all sources) data for this region
14.00
2_cell_bam
0.00
2_cell_bam
1211.00
6hpf_bam
0.00
6hpf_bam
2933 features from '6hpf_bam' not shown
2_cell_gtf
No features from 2_cell_gtf in this location on this strand
6hpf_gtf
No features from 6hpf_gtf in this location on this strand
23.425Mb
23.426Mb
23.427Mb
23.428Mb
23.429Mb
23.430Mb
Reverse strand
6.25 kb
Gene Legend
Protein Coding
merged Ensembl/Havana
Variant Legend
splice region variant
synonymous variant
5 prime UTR variant
3 prime UTR variant
intron variant
There are currently 157 tracks turned off.
Ensembl Danio rerio version 110.11 (GRCz11) Chromosome 12: 23,424,108 - 23,430,356

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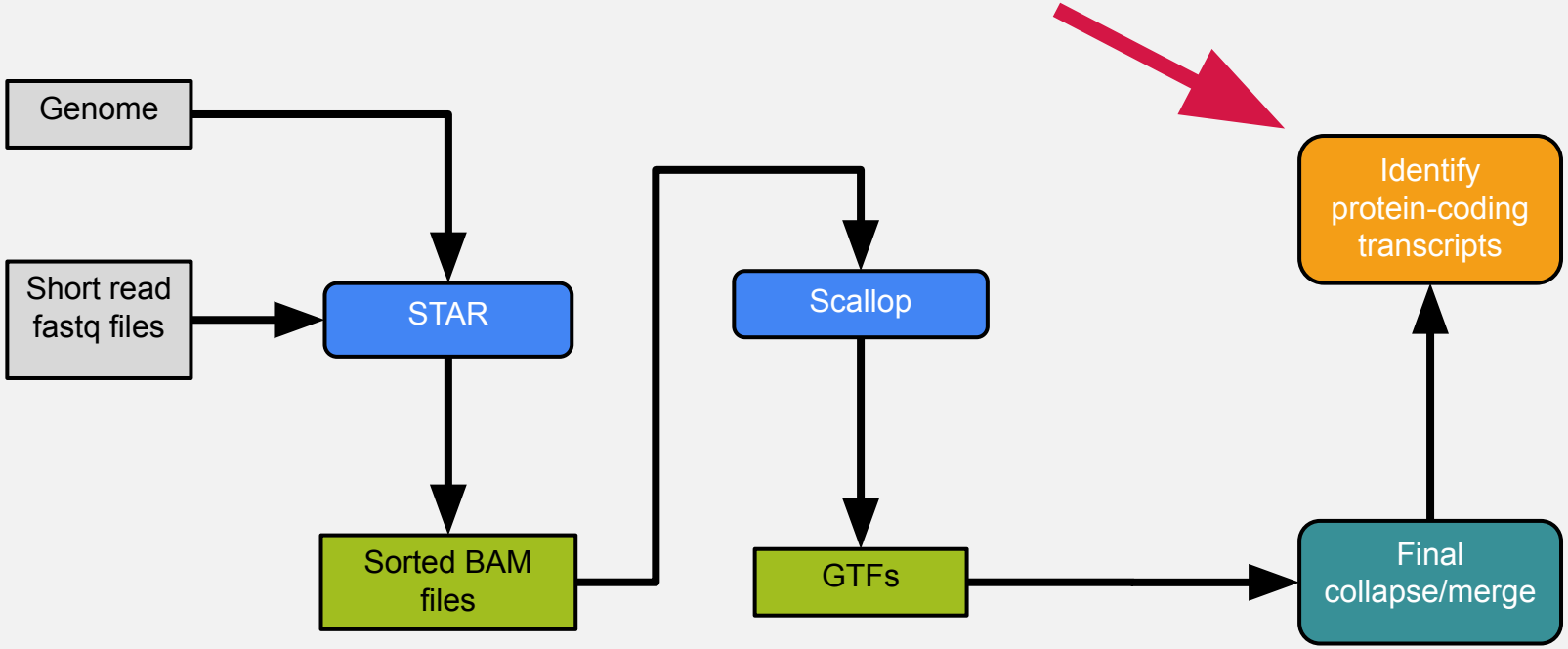
what comes next?

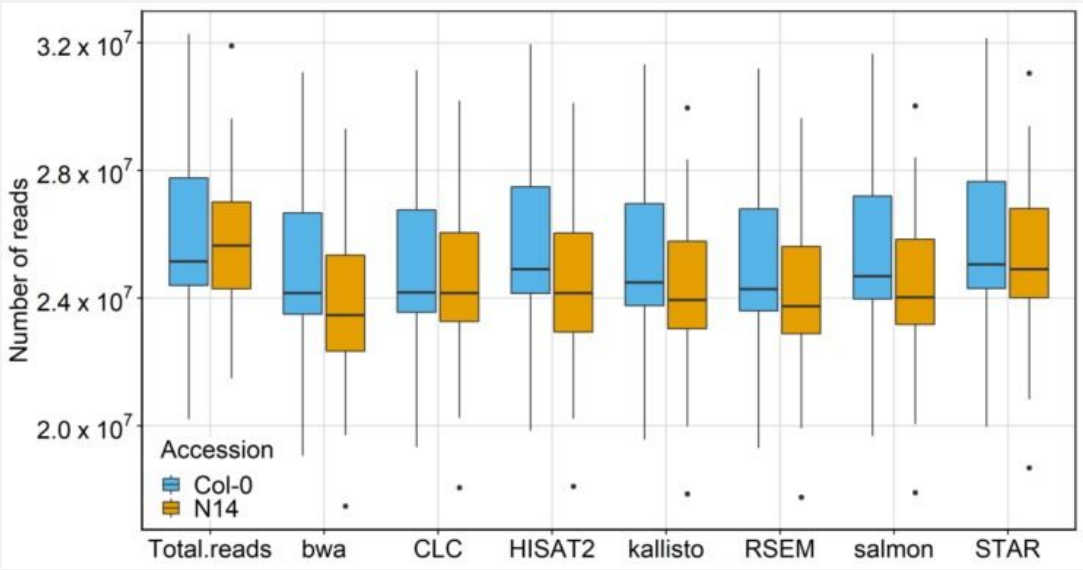


what comes next?

- There are tons of transcripts (m, snc, lnc, r, …)
- We must differentiate among them
- Translation, gene model generation, final geneset

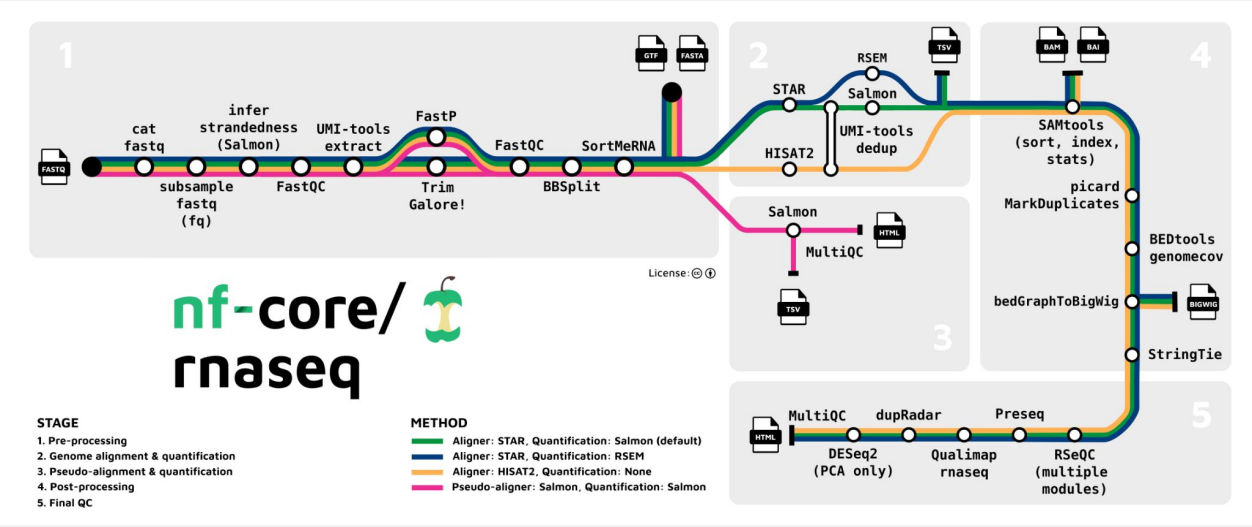
overview

- Simplified show of 1 process among many in our pipeline
- Alternatives:
  - HISAT2, salmon
  - StringTie2



overview

- A different alternative:
  - NextFlow/nf-core
- Different functionalities and uses
- Modules and pipelines



The Eukaryotic Annotation Team

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Open Targets

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TRANSFORMING GENOMIC MEDICINE INITIATIVE

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