Short Read Workshop Day 4
Trimming, Mapping, IGV

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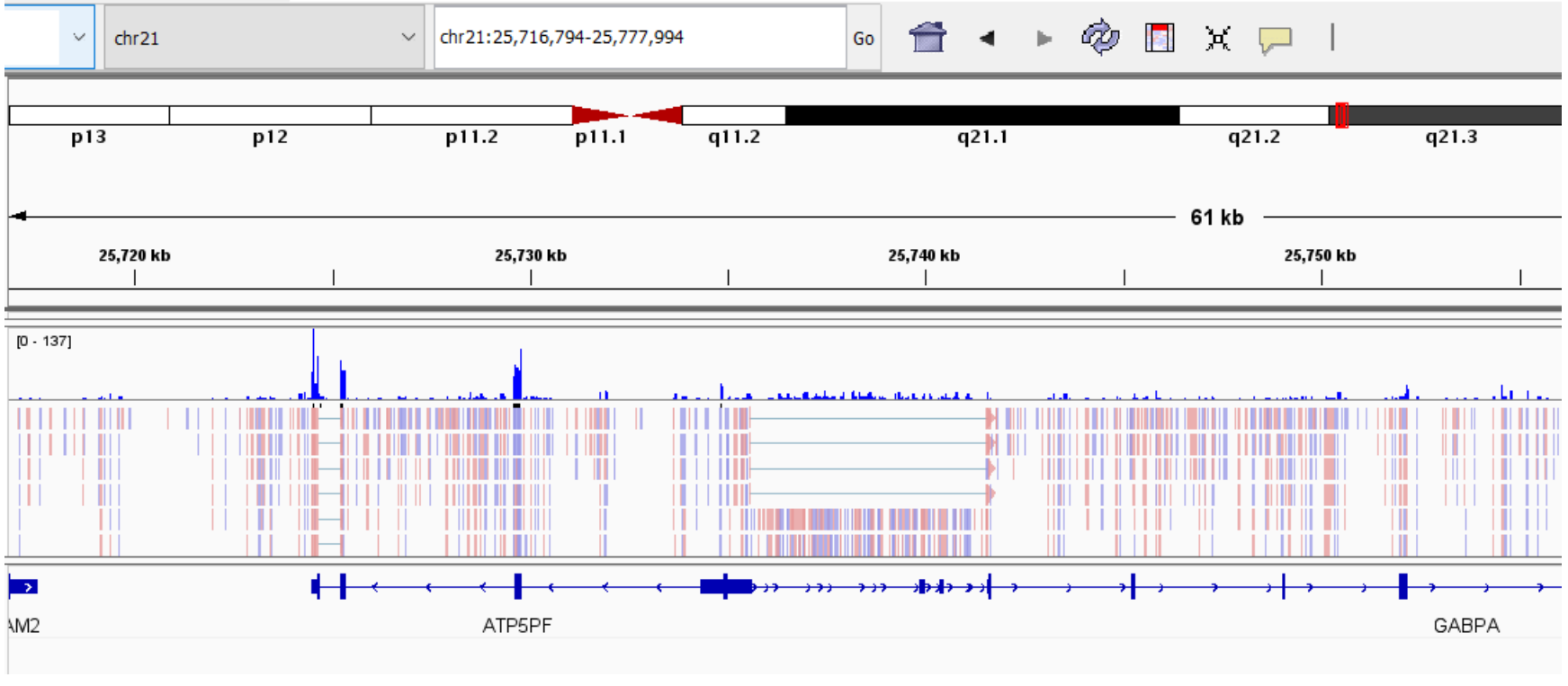
2023

Day 4 overview

- Trimming fastq files
- Mapping fastq files
- More about mapped file formats
- Visualizing mapped files

Goal of the Day

View sequencing data as reads aligned to a genome



Trimming/mapping recap

50 base read:
TAGGCTAACTCTGTAGCCCCAGGTACCATGCATAATTGACCAGGATATAG

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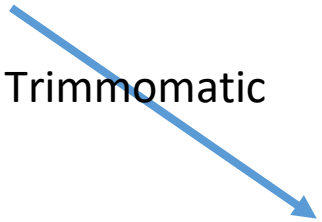

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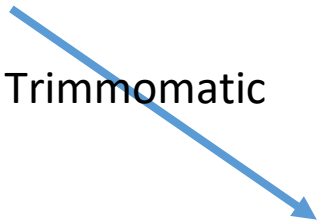

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TAGGCTAACTCTGTAGCCCCAGGTACCATGCATAATTGAC

Genome:
AGCTTCGGATCGATCGACTGACTAGGCTAACTCTGTAGCCCCAGGTACCATGCATAATTGACCGCGATTACGAC
TCGAAGCCTAGCTAGCTGACTGATCCGATTGAGACATCGGGGTCCATGGTACGTATTAACTGGCGCTAATGCTG

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Trimmomatic

40 base trimmed read:
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HISAT2

Genome:
AGCTTCGGATCGATCGACTGACTAGGCTAACTCTGTAGCCCCAGGTACCATGCATAATTGACCGCGATTACGAC
TCGAAGCCTAGCTAGCTGACTGATCCGATTGAGACATCGGGGTCCATGGTACGTATTAACTGGCGCTAATGCTG

IGV

AGCTTCGGATCGATCGACTGACTAGGCTAACTCTGTAGCCCCAGGTACCATGCATAATTGACCGCGATTACGAC
TCGAAGCCTAGCTAGCTGACTGATCCGATTGAGACATCGGGGTCCATGGTACGTATTAACTGGCGCTAATGCTG

Variables

Variables - defining

variablename=

```
a=5

gene_name=ACTB

filepath=/Users/<username>/sr2023/day04/scripts

filelist=$(ls .)
```

Variables – evaluating (calling)

$variablename

echo $a

grep $gene_name <filename>

trim_script=“$filepath”/d4_trim_qc.sbatch
OR
trim_script=${filepath}/d4_trim_qc.sbatch

wc $filelist

Variables – evaluating (calling)

$variablename

echo $a

grep $gene_name <filename>

trim_script="$filepath"/d4_trim_qc.sbatch
OR
trim_script=${filepath}/d4_trim_qc.sbatch

wc $filelist

Several ways of evaluating:

$a

${a}

"$a"

These differ slightly, and you will see us use them all in scripts

Trimming fastq files with Trimmomatic

- Follow Trimomatic worksheet to:
- Create Day4 directories
- Edit script to run Trimmomatic
  Input: fastq files with full-length reads
  Output: fastq files with trimmed/filtered reads
- Extra: Edit the d4_fastqc.sbatch script to run pre-trim qc and compare the plots/stats to post-trim

How do you trim polyA regions from both sides of reads?

How do you trim polyA regions from both sides of reads?

- Make a new fasta file with a polyA segment, or append to the Illumina adapter file, if writeable
- >polyA

  AAAAAAAAAAAAAAAAAAAAAAAAA
- ILLUMINACLIP:<new fasta file>:2:30:10

Mapping fastq files with HISat2

- Follow Mapping/IGV worksheet to:
- Rsync the mapping script
- Edit script and run HISAT2

  Input: trimmed fastq files

  Outputs: .sam, .bam, .sorted.bam, .bam.bai files
- Visualize BAM file on the IGV web app

Homework

Day 4 Homework – FASTQC, trimming, mapping, IGV

The assessment tomorrow will run many of the same steps as this homework. These steps are essential in ALL short read data processing.