SAMtools and BEDtools

Folder: gencommand\_proj2\_data

Module 2 Exam – Command Line Tools for Genomic DS

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First, a couple of introductory comments. A BAM file contains alignments for a set of input reads. Each read can have 0 (none), 1 or multiple alignments on the genome. These questions explore the relationships between reads and alignments.

The number of alignments is the number of entries, excluding the header, contained in the BAM file, or equivalently in its SAM conversion. To find the number of alignments, we can apply (‘%’ denotes the terminal prompt):

% samtools flagstat athal\_wu\_0\_A.bam

which will list the number of alignments on line 1. An alternate method would be to count the number of lines in the converted SAM file (header excluded):

% samtools view athal\_wu\_0\_A.bam | wc -l

Note that, if the file was created with a tool that includes unmapped reads into the BAM file, we would need to exclude the lines representing unmapped reads, i.e. with a ‘\*’ in column 3 (chrom):

% samtools view athal\_wu\_0\_A.bam | cut –f3 | grep –v ’\*’ | wc -l

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Description automatically generated with low confidence

An alignment with an unmapped mate is marked with a ‘\*’ in column 7. Note that the question asks for alignments, not reads, so we simply count the number of lines in the SAM file with a ‘\*’ in column 7:

% samtools view athal\_wu\_0\_A.bam | cut –f7 | grep –c ‘\*’

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Deletions are be marked with the letter ‘D’ in the CIGAR string for the alignment, shown in column 6:

% samtools view athal\_wu\_0\_A.bam | cut –f6 | grep –c ‘D’

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Description automatically generated with low confidence

Alignments with the read’s mate mapped to the same chromosome are marked with a ‘=’ in column 7:

% samtools view athal\_wu\_0\_A.bam | cut –f7 | grep –c ‘=’

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Description automatically generated

A spliced alignment will be marked with an ‘N’ (intron gap) in the CIGAR field:

% samtools view athal\_wu\_0\_A.bam | cut –f6 | grep –c ‘N’

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We first need to construct the reduced set, i.e. to extract from the original set only those alignments in the specified region. For this, we need to sort and index the file:

% samtools sort athal\_wu\_0\_A.bam athal\_wu\_0\_A.sorted

This will create the file ‘athal\_wu\_0\_A.sorted.bam’. We then index this file:

% samtools index athal\_wu\_0\_A.sorted.bam

This will create the index file ‘athal\_wu\_0\_A.sorted.bam.bai’ in the current directory. Lastly, we extract alignments in the specified range:

% samtools view –b athal\_wu\_0\_A.sorted.bam “Chr3:11777000-11794000” > athal\_wu\_0\_A.region.bam

The option ‘-b’ will generate output in BAM format. The resulting BAM file will be sorted, so it can be indexed directly if needed.Common pitfalls: make sure to specify the correct reference sequence (‘Chr3’, not ‘chr3’) and exclude ‘,’ when representing the query coordinates. Also, make sure to use the sorted and index BAM file. To determine the number of alignments in the new (region) file, we can use the same commands as for Q1, e.g.:

% samtools flagstat athal\_wu\_0\_A.region.bam

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% samtools view athal\_wu\_0\_A.region.bam | cut –f7 | grep –c ‘\*’

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% samtools view athal\_wu\_0\_A.region.bam | cut –f6 | grep –c ‘D’

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Description automatically generated

Incorrect: 150913

% samtools view athal\_wu\_0\_A.bam | cut –f7 | grep –c ‘=’

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Description automatically generated

% samtools view athal\_wu\_0\_A.bam | cut –f6 | grep –c ‘N’

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Description automatically generated with low confidence

This information can be found in the header of the BAM file. Starting with the original BAM file, we use samtools to display the header information and count the number of lines describing the sequences in the reference genome:

% samtools view –H athal\_wu\_0\_A.bam | grep –c “SN:”

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Description automatically generated with medium confidence

The length information is stored alongside the sequence identifier in the header (pattern ‘LN:seq\_length’):

% samtools view –H athal\_wu\_0\_A.bam | grep “SN:” | more

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Description automatically generated

The program name is listed in the ‘@PG’ line in the BAM header (pattern ‘ID:program\_name’):

% samtools view –H athal\_wu\_0\_A.bam | grep “^@PG”

The ‘^’ sign in the search pattern tells the grep function to match the pattern ‘@PG’ at the start of the line.

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Description automatically generated

This information is shown in column 1 of the first alignment record in the SAM file:

% samtools view athal\_wu\_0\_A.bam | head -1 | cut –f1

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Description automatically generated with medium confidence

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Description automatically generated with low confidence

We start by running BEDtools on the alignment set restricted to the specified region (Chr3:11777000-11794000) and the GTF annotation file listed above. To allow the input to be read directly from the BAM file, we use the option ‘-abam’; in this case we will need to also specify ‘-bed’ for the BAM alignment information to be shown in BED column format in the output:

% bedtools intersect –abam athal\_wu\_0\_A.region.bam –b athal\_wu\_0\_A\_annot.gtf –bed -wo > overlaps.bed

This will create a file with the following format: Columns 1-12 : alignment information, converted to BED format Columns 13-21 : annotation (exon) information, from the GTF file Column 22 : length of the overlapAlternatively, we could first convert the BAM file to BED format using ‘bedtools bamtobed’ then use the resulting file in the ‘bedtools intersect’ command. To answer the question, the number of overlaps reported is precisely the number of lines in the file (because only entries in the first file that have overlaps in file B are reported, according to the option ‘-wo’):

% wc –l overlaps.bed

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The size of the overlap is listed in column 22 of the ‘overlaps.bed’ file. To determine those longer than 10 bases, we extract the column, sort numerically in decreasing order, and simply determine by visual inspection of the file the number of such records. For instance, in ‘vim’ we search for the first line listing ‘9’ (‘:/9’), then determine its line number (Ctrl+g). Alternatively, one can use grep with option ‘-n’ to list the lines and corresponding line numbers:

% cut –f22 overlaps.bed | sort –nrk1 > lengths

Or

% cut –f22 overlaps.bed | sort –nrk1 | grep –n “^9” | head -1

For the latter, the last ”10” line will be immediately above the first “9”, so subtract 1 from the answer.

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Columns 1-12 define the alignments:

% cut –f1-12 overlaps.bed | sort –u | wc -l

Potential pitfalls: Multiple reads may map at the same coordinates, so the information in columns 1-3 is insufficient. The minimum information needed to define the alignments is contained in columns 1-5, which include the read ID and the flag, specifying whether this is read 1 or read 2 in a pair with the same read ID).

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Columns 13-21 define the exons:

% cut –f13-21 overlaps.bed | sort –u | wc -l

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Description automatically generated with low confidence

This question simply asks for the number of transcripts in the annotation file, since the BED format would represent each transcript on one line. This information can be obtained from column 9 in the GTF file:

% cut –f9 athal\_wu\_0\_A.annot.gtf | cut –d ‘ ‘ –f1,2 | sort –u | wc -l