### disbamer.sh

### \$ bash disbamer.sh 43 | less –S \$ bash disbamer.sh 43

Was a read number supplied?

\$# : Number of arguments passed to bash script

We looking for one input only!

```
if [[ $# -ne 1 ]]; then
    echo messages
    exit 2
fi
```

## Is **samtools** loaded?

Try samtools, output to null, and redirect error to stdout, if we get output then show message > command -v samtools >/dev/null 2>&1
 || { echo >&2 "- Is samtools
 loaded? "; exit 1; }

#### Extract Read from SAM file.

run samtools, use sed to get line requested, and put read in to var bamdata > bamdata=\$(samtools view sam.lnk |
 sed "\${1}q;d")

#### Get read data fields.

Use awk to get fields from bamdata. Bash 'read' puts output into variable : seqD

> read seqD <<< \$(echo "\$bamdata"| awk
 '{print \$10}')
# Repeat for flag region position
 sequence cigar ...</pre>

### Check sequence field.

Secondary alignments don't hold sequence

Sam data only has sequence in primary/supplementary mapped reads.

```
> if [ "$seqD"== "*"]; then
  echo "No sequence data : secondary?"
  exit 1
fi
```

## disbamer.sh

> bash disbamer.sh 43 | less -S

# Using CIGAR calculate length of Reference sequence required

awk script

Use awk script to calculate. Use bash 'read' to assign result. to variable, returns '\*' if error

> read seqlengthD <<< \$(awk -v
 cigA="\$cigarD"-f cigtoRefLen.awk)</pre>

# Obtain reference matching sequence for read

awk script

Send bash variables for 'region, position and length' to the awk script; return sequence > read seqrefD <<< \$(awk -v
 regA="\$regionD"-v posA="\$positionD"-v
 lenA="\$seqlengthD"-f getrefseq.awk
 ref.lnk)</pre>

# Display read alongside Reference with indels marked

awk script

Send bash variables for 'cigar, read and reference sequences' to the awk script for display

> awk -v cigA="\$cigarD"-v seqA="\$seqD"-v
 refA="\$seqrefD"-f viewread.awk

# cigtoRefLen.awk

Using CIGAR calculate length of Reference sequence required

# read -r seqlengthD <<< "\$(awk -v cigA="\$cigarD" -f cigtoRefLen.awk)"

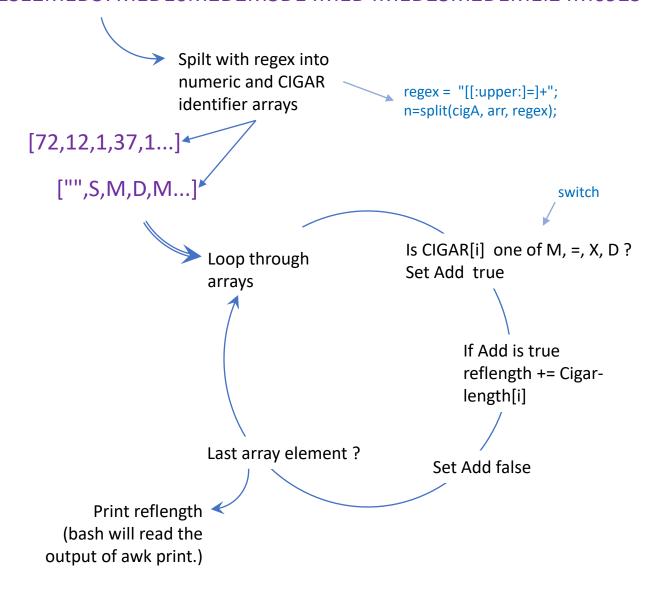
Input:

Output:

Cigar String,

Ref Length

#### 72S12M1D37M1D10M2D2M3D14M1D4M1D23M2D1M1I24M692S



# getrefseq.awk

# Obtain reference matching sequence for read

Input:

Region

Output:

• Ref Sequence

read -r seqrefD <<< \$(awk -v regA="\$regionD" -v
posA="\$positionD" -v lenA="\$seqlengthD" -f
getrefseq.awk ref.lnk)</pre>

#### Start length Set REGION (chromosome to search for) **BEGIN** Set begin position { initialise } Set end position **Print Ref** exit awk reads reference line by line switch Stage 3 Stage 1 Stage 2 Stage 4 Count bases Search for > Count length Find Region Find Start Find End Region not found **END** {Error eval > stderr} **Print Output** read by disbamer script Reference File format: >CM000663.2 chr 1, GRCh38 NNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNN ttccAATACCTCTGAAAAAACTGatccaaa region >KI270706.1 contig chr 1, GRCh38 GAATTCAGCTGAGAAGAACAGGCAAG TAATTTAAGATTTTCTCCCCCTACgtaatt

ACTTGCTGTTTAAGGAACTAATTAAAC

## viewread.awk

### Display read alongside Reference with indels marked

#### awk -v cigA="\$cigarD" -v seqA="\$seqD" -v refA="\$seqrefD" -v outview="\$nopipe\_out" -f viewread.awk

#### Input:

- · Cigar String,
- Read Sequence (seq) and
- Reference sequence
- nopipe out

5M1I3M2D7M2I6M TCCCCCTACTAATTACGCTGTT

TCCCCTACAGTAATTACGGTCTT

Spilt Cigar String via regex into numeric and CIGAR identifier arrays

step through CIGAR arrays

switch

M: Add matching sequence to results

=: Add matching sequence to results

X: Add matching sequence to results (mismatches)

D: Add cigar element length of dashes "-" to results

I: Add quoted sequence to results (these are not in Reference)

N: Add cigar element length of N's "N" to results

P: Add cigar element length of P's "P" to results

S: If first Cigar then set start of result sequence to end of clip. Otherwise increment over clip.

H: do nothing hard clip sequences are not in sam file.

[CIGAR] [length]

CIGAR

Sequence, showing inserts and deletions. Also gather

Build a copy of the Read

statistics.

- Matches
- Deletions
- Insertions
- Clip

For [M, =, X, D] Build position string. For I (inserts) build string with quotes.

deletion Read is now: TCCCC'C'TAC--TAATTAC'TT'GCTGTT

insertions

For each char in formatted read Format Reference sub sequence to align to our read. Build an indicator string of deletions, mismatches and additions.

': add insert indicator '+' into reference and indicator for length of insert

-: reference remains as is, indicator string has dashes

Otherwise : reference remains as is, indicator string has 'x' if reference value does not match read.

If output is being piped "|" then output will print on a single line. (ie. for viewing with less –S)

Otherwise will print 80 base pairs per line to terminal, automatically set by bash variable \$nopipe out.

#### Output:

