disbamer.sh

```
$ bash disbamer.sh 43 | less –S
$ bash disbamer.sh 43
```

Is output being piped?

```
if [ -t 1 ] ; then nopipe_out=terminal; fi;
```

Determines output style.

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 …
```

Cont…

disbamer.sh

> bash disbamer.sh 43 | less -S

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
```

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)
```

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)
```

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

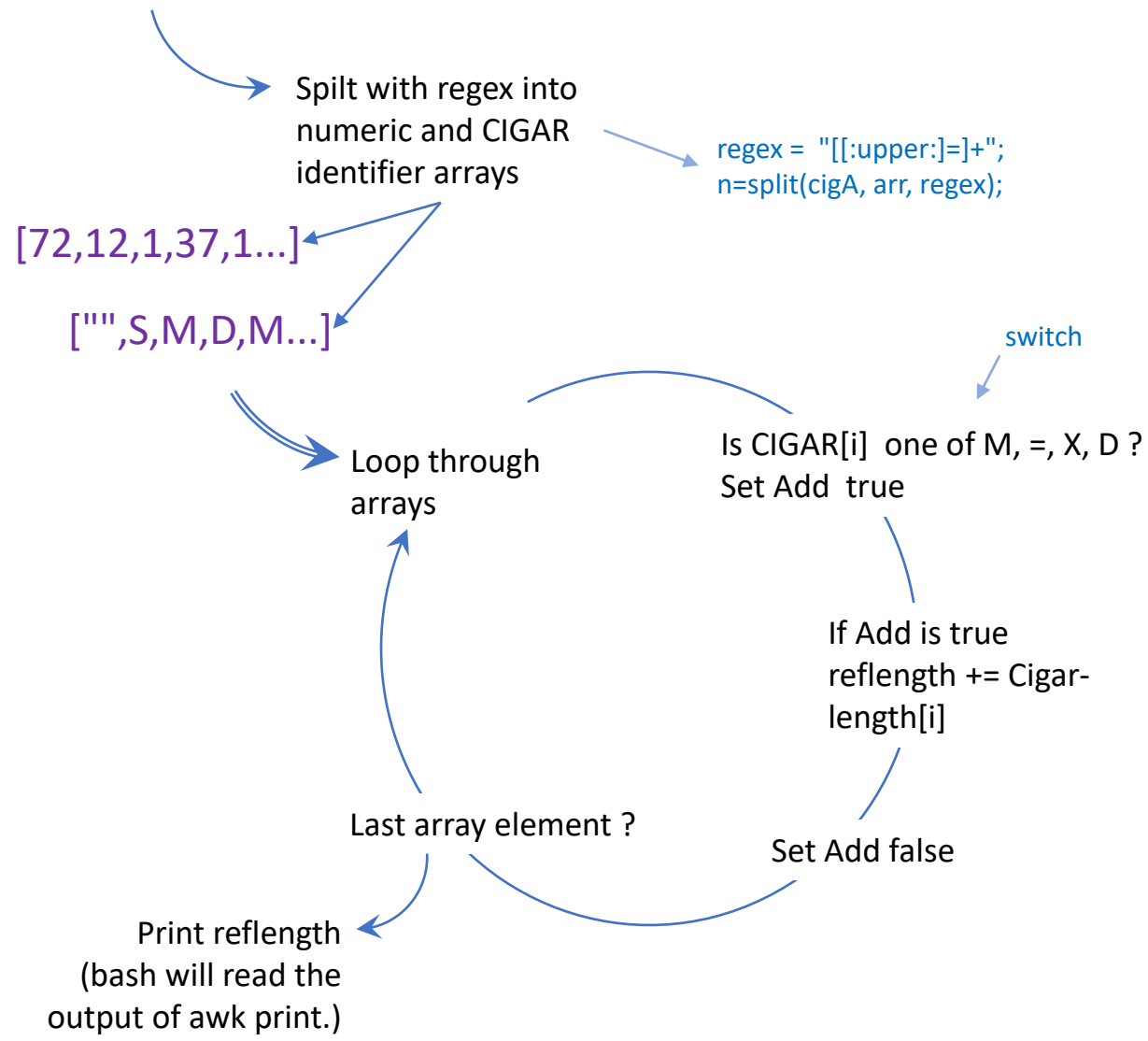
Using CIGAR calculate length of Reference sequence required

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

Input:
- Cigar String,

Output:
- Ref Length

72S12M1D37M1D10M2D2M3D14M1D4M1D23M2D1M1I24M692S



Note: All code is in a BEGIN block, and only operates on passed variable (via –v)

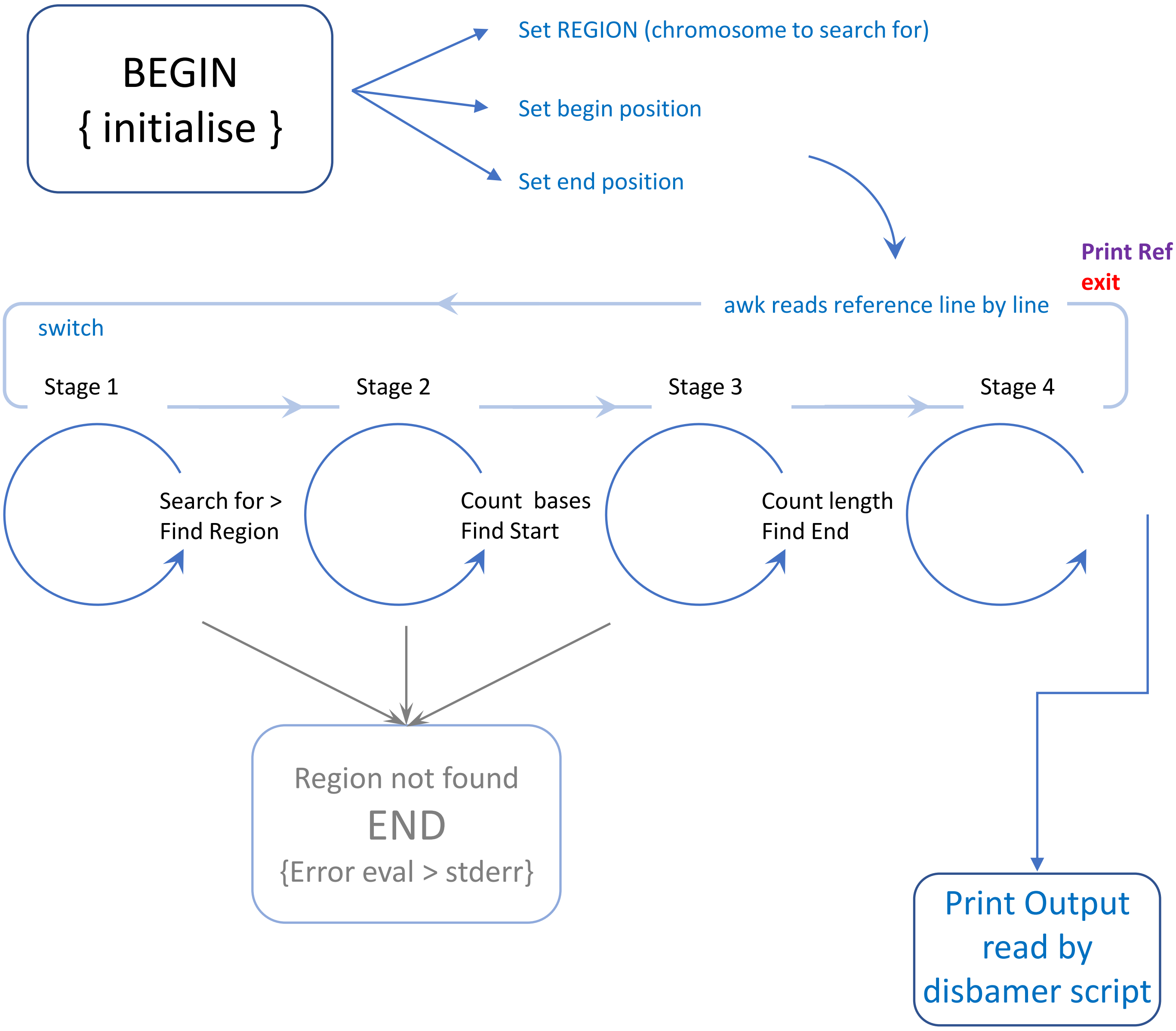
getrefseq.awk

Obtain reference matching sequence for read

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

Input:
- Region
- Start
- length

Output:
- Ref Sequence



Reference File format:

```
>CM000663.2 chr 1, GRCh38
NNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNN
ttccAATACCTCTGAAAAAACTGatccaaa
:
>KI270706.1 contig chr 1, GRCh38
GAATTCAGCTGAGAAGAACAGGCAAG
TAATTTAAGATTTTCTCCCCCTACgtaatt
ACTTGCTGTTTAAGGAACTAATTAAAC
:
```

region: KI270706.1
start: TCCCCCTACgtaatt
end: ACTTGCTGTT

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 [CIGAR] [length]

switch CIGAR

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.

Build a copy of the Read Sequence, showing inserts and deletions. Also gather statistics.

- Matches
- Deletions
- Insertions
- Clip

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

Read is now:

deletion

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:

```
READ       TCCCC'C'TAC--TAATTAC'TT'GCTGTTTT
Reference  TCCCC'+'TACAGTAATTAC'++'GGTCTTTT
Indicator  .....'+'...--.......'++'.x.x....
Position   .........|10.......|20.......|30
```