1. Long read basecalling with guppy (version 6.1.5)\*

See [here](https://denbi-nanopore-training-course.readthedocs.io/en/latest/basecalling/basecalling_1.html) and [here](https://community.nanoporetech.com/docs/prepare/library_prep_protocols/Guppy-protocol/v/gpb_2003_v1_revaq_14dec2018/setting-up-a-run-configurations-and-parameters) for how to use guppy.

2. Long read alignment with Minimap2 (version 2.24.0)\*

Splice aware alignment for dRNA-seq Oxford Nanopore datasets (annotation guided):

minimap2 -ax splice -k14 -C5 --junc-bed annotation.bed ref.fa reads.fq > alignment.sam

3. SAM file preparation with SAMtools (version 1.17.0)

Removal of secondary and supplementary alignments:

samtools view -F 256 -F 2048 -h alignment.sam > primary.sam

Conversion of primary alignment SAM file to sorted BAM file:

samtools view -bS primary.sam > primary.bam

samtools sort primary.bam > primary\_sorted.bam

Pass to bam\_filtration.R script to isolate EBV reads. Convert output to SAM file:

samtools view -h ebv\_reads.bam > ebv\_reads.sam

Remove header from EBV SAM file:

samtools view ebv\_reads.sam | cut -f 1-10 > ebv\_reads\_headerless.sam

\*Parts one and two are optional and can vary depending on the dataset(s) you are using. However, part three must be followed to ensure the SAM file is properly prepared for pipeline use.