

ONT long read  
fast5 format

Illunima short read  
fastq format

Preprocessing

**Basecalling**  
Guppy

**Trim and demultiplex**  
Porechop

Isoform detection

**Isoform detection**  
FLAMES

Map and quantify

**Map to genome**  
minimap2

**Map to transcriptome**  
minimap2

**Map to genome**  
Subread

**Gene-level quantify**  
featureCounts

**Transcript-level quantify**  
minimap2

**Gene-level quantify**  
Subread-featureCounts

**Exon-level quantify**  
Subread-featureCounts

Differential analysis

**Differential gene expression**  
limma-voom

**Differential transcript usage**  
DRIMSeq, limma-diffSplice

**Differential gene expression**  
limma-voom

**Differential splicing**  
limma-diffSplice