

Example pipeline

1. Run STAR (must build genome first)

```
STAR --genomeDir /path/to/star/genome --runThreadN 4 --readFilesIn paired_reads_1.fastq  
paired_reads_2.fastq --outSAMtype BAM SortedByCoordinate --outWigType wiggle --outWigNorm None --  
outFileNamePrefix aligned_reads
```

This command will generate the following files:

All reads in the form of a BAM file

* aligned_reads_STARAligned.sortedByCoord.out.bam

Coverage (wig) file for uniquely mapped reads only

* aligned_reads_STARSIGNAL.Unique.str1.out.wig # (-) strand coverage

* aligned_reads_STARSIGNAL.Unique.str2.out.wig # (+) strand coverage

Coverage (wig) file for multi-mapped and uniquely mapped reads

* aligned_reads_STARSIGNAL.UniqueMultiple.str1.out.wig # (-) strand coverage

* aligned_reads_STARSIGNAL.UniqueMultiple.str2.out.wig # (+) strand coverage

Splice junction counts

* aligned_reads_STARSJ_out.tab # Canonical splice junction counts file needs to be converted to bed format
appropriate for circleVis processing (see below)

2. Run star_sj_convert on canonical splice junction file

```
star_sj_convert aligned_reads_STARSJ_out.tab
```

This command will generate the following file:

```
* aligned_reads_STARSJ_out.tab.canonical.bed # Final canonical splice junction file
```

3. Run find_circ to generate backsplice junction calls

(See https://github.com/marvin-jens/find_circ for software and usage information)

```
# The output of find_circ will include a tsv file of backsplice junctions (name specified by user. For this example,  
# we will call the find_circ output file:
```

```
* my_sample_splice_sites.bed
```

4. Run find_circ_convert on find_circ junction file

```
find_circ_convert my_sample_splice_sites.bed
```

This command will generate the following file:

```
* my_sample_splice_sites.bed.circles.bed # Final backsplice junction file
```

```
# Uniquely mapped coverage will be used for this example, but it is up to the user to choose unique or unique +  
# multi
```

5. Using a GTF file with the same chromosome names as the genome used for your alignment (i.e. if chromosome 1 is labeled 'chr1' in the STAR genome, it must also be labeled 'chr1' in the GTF file you use. It cannot be 'chr1' in one file and '1' in the other.), run circbuild.

```
circbuild --gtf Homo_sapiens.GRCh38.92.gtf --wigneg aligned_reads_STARSignal.Unique.str1.out.wig --wigpos aligned_reads_STARSignal.Unique.str2.out.wig --splicejunction aligned_reads_STARSJ_out.tab.canonical.bed --circlejunction my_sample_splice_sites.bed.circles.bed --output my_sample_name
```

This command will generate the following file:

* my_sample_name.db

6. Run circplot using your gene of interest

```
circplot --database my_sample_name.db --gene EGFR
```

This command will generate the following files:

Open either one of them with any web browser

* EGFR_ENST00000275493.svg

* EGFR_ENST00000275493.html