

Example pipeline

1. Run STAR (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

Uniquely mapped reads only

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

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

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

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