IRFinder-S

- Download IRFinder-S image from https://github.com/RitchieLabIGH/IRFinder/releases
- IRFinder-S can be executed using singularity: *singularity run IRFinder*
- Step1: Build Reference (https://github.com/RitchieLabIGH/IRFinder/wiki/Build-Reference)
    - Can be done in three ways. I use "BuildRefProcess". You need to create a folder called "REF" and place the reference genome and annotation in this folder. You need to call the reference genome "genome.fa" and the annotation "transcritps.gtf".
- Step2: Quantify IR (https://github.com/RitchieLabIGH/IRFinder/wiki/Quantify-IR)
    - Can be done in three ways. I use "BAM". You need to provide the path to the "REF" folder, an output directory as well as the bam file.
    - You need to do the quantification for each replicate of your experiments
- Step3: Differential IR Analysis (https://github.com/RitchieLabIGH/IRFinder/wiki/Differential-IR-Analysis)
    - If you have >=3 replicates you can use the "deseq" mode
    - You define 2 groups (experiments that should be compared) and provide the paths to the output files of the replicates from the "Quantify IR" step. In addition, you provide an output directory
- You can checkout my Shell-scripts. In the corresponding project I have four experiments with each 3 replicates. In total, there are four pairwise comparisons that are of interest to us:
    - Download_ref_genome_and_anno.sh
        - Creates the "REF" directory, downloads the reference genome + annotation with the required names and unzips the two files
    - BuildRef.sh
        - Builds the reference. -j corresponds to the --sjdbOverhang STAR parameter and is read length – 1
    - QuantifyIR.sh
        - I use two for-loops. One for the experimental groups (experiments) and one for the replicates of each experiment group. The quantification is executed for each individual replicate.
    - DiffIR.sh
        - For each of the four pairwise comparisons the "Diff" mode is executed. With -g: you define the groups, which are followed by the paths to the quantification files of the individual replicates. Note that you can easily use wildcards.