Supplementary File V: Options of `cryosieve-csrefine`

The program `cryosieve-csrefine` is designed to automatically and sequentially execute a series of operations in CryoSPARC, namely `import particle stack`, `ab-initio`, `homogenous refinement` or `non-uniform refinement` jobs.

`--i`

The `--i` option specifies the input star file(s) or txt file(s). This parameter supports multiple input files, such as `--i a.star b.star c.star`. Wildcards can also be used, for example, `--i output/_iter?.star` will include `output/_iter0.star`, `output/_iter1.star` up to `output/_iter9.star` obtained in this protocol. Additionally, the input file can be txt file containing paths of star files, with each file listed on a separate line.

`--directory`

The `--directory` option specifies the directory of particles. If this option is not provided, the current directory is assumed as the default location.

`--o`

The `--o` option is used to specify the output summary file path. When running the program, it will generate a summary report for each particle stack in CSV format. This report includes information such as resolutions and B-factors estimated by CryoSPARC.

`--sym`

The `--sym` parameter specifies the molecular symmetry.

`--ref`

The `--sym` parameter specifies the path of initial reference model. If this option is not provided, the program will utilize CryoSPARC's `ab-initio` job to obtain an initial model.

`--repeat`

The `--repeat` option specifies the number of trials to be performed for each particle stack. It is important to note that CryoSPARC's estimation of resolution and B-factor includes some randomness. This option can be utilized if the user wishes to reduce noise in the results.

`--user`, `--project`, `--workspace` and `--lane`

These options specify the user (e-mail address), project, workspace and lane in CryoSPARC.

`--nu`

If the flag `--nu` is enabled, `cryosieve-csrefine` will perform non-uniform refinement instead of homogeneous refinement.

`--resplit`

If the flag `--resplit` is enabled, `cryosieve-csrefine` will activates the `force re-do GS split` option in CryoSPARC's homogeneous or non-uniform refinement. It is crucial to be aware of the risks associated with this option. For more details, please refer to Supplementary File IV.

`--workers`

By default, `cryosieve-csrefine` refines all star files in parallel, but the option `--workers` allows you to limit the number of jobs executed simultaneously.