Parameter set

Adjust observed SNV frequencies, if input is CCF or with CNV.

MEGA alignment

Make binomial replicates (use these replicates later)

For each replicate, generate new :ref and :alt counts using binomial distribution

Using MEGA alignment, make MP tree and infer ancestral sequences.

Remove redundant sequences

MEGA-CC (make MP tree and ancestral sequences; multiple MP trees can be produced)

Make new MEGA alignment with ancestral sequences for each MP tree

Select the MEGA alignment with smallest backward/parallel mutations

Compute clone frequencies (F).

Identify group of SNVs with issue (do this for each tumor)

Make matrix of MEGA alignment (M) and vector of SNV frequency (v\_obs)

Regression: M x F = v\_obs

Make MEGA alignment for each tumor (remove sequences with F=0)

Estimate SNV frequencies (v\_est) using M and F (M x F)

Group SNVs (SNV group) with identical v\_est

Compute v\_obs – v\_est for each SNV (do this for each SNV group)

Group SNVs into two subgroups (v\_obs – v\_est > 0 and v\_obs – v\_est < 0)

Keep subgroups when the mean of v\_obs – v\_est within a subgroup is significantly different from 0

Make MEGA alignment for subgroups of SNVs and add to the original MEGA alignment

Compute clone frequencies (F)

Add better sequences and remove bad sequences from the MEGA alignment (do this for each tumor)

Make new sequences by combining subgroups of SNVs with F>0 and add to the original MEGA alignment

Compute clone frequencies (F) and remove sequences with F=0.

Make new MEGA alignment by pooling all sequences from each tumor

Using MEGA alignment, make MP tree and infer ancestral sequences.

Fix backward/parallel mutations on new sequences and update MEGA alignment

Test if new MEGA alignment is better than the original one

Count the number of backward/parallel mutations for each new and original MEGA alignment

If the new MEGA alignment has smaller number, keep the new MEGA alignment. Then go back to “Add better sequences and remove bad sequences from the MEGA alignment (do this for each tumor)”

Otherwise, keep the original alignment.

Using MEGA alignment, make MP tree and infer ancestral sequences.

Compute clone frequencies (F).

Refine sequences (MEGA alignment), when users selected to do.

Remove mutations when tumor does not have mutations

Make MP tree and fix backward/parallel mutations if possible

Add ancestral sequences at the middle of branches

Identify group of SNVs with issue

Map SNV groups along MP tree and make additional ancestral sequences

For each binomial replicate, compute clone frequencies (F) using v\_obs and MEGA alignment (M)

Remove sequences when F=0 for most of binomial replicates

Compute clone frequencies (F)

Mask SNVs with parallel/backward mutations (optional)

Add unassigned mutations into sequences if possible (optional)

Combine similar sequences (optional)

Make output files (clone frequencies, tree, MEGA alignment, and such)