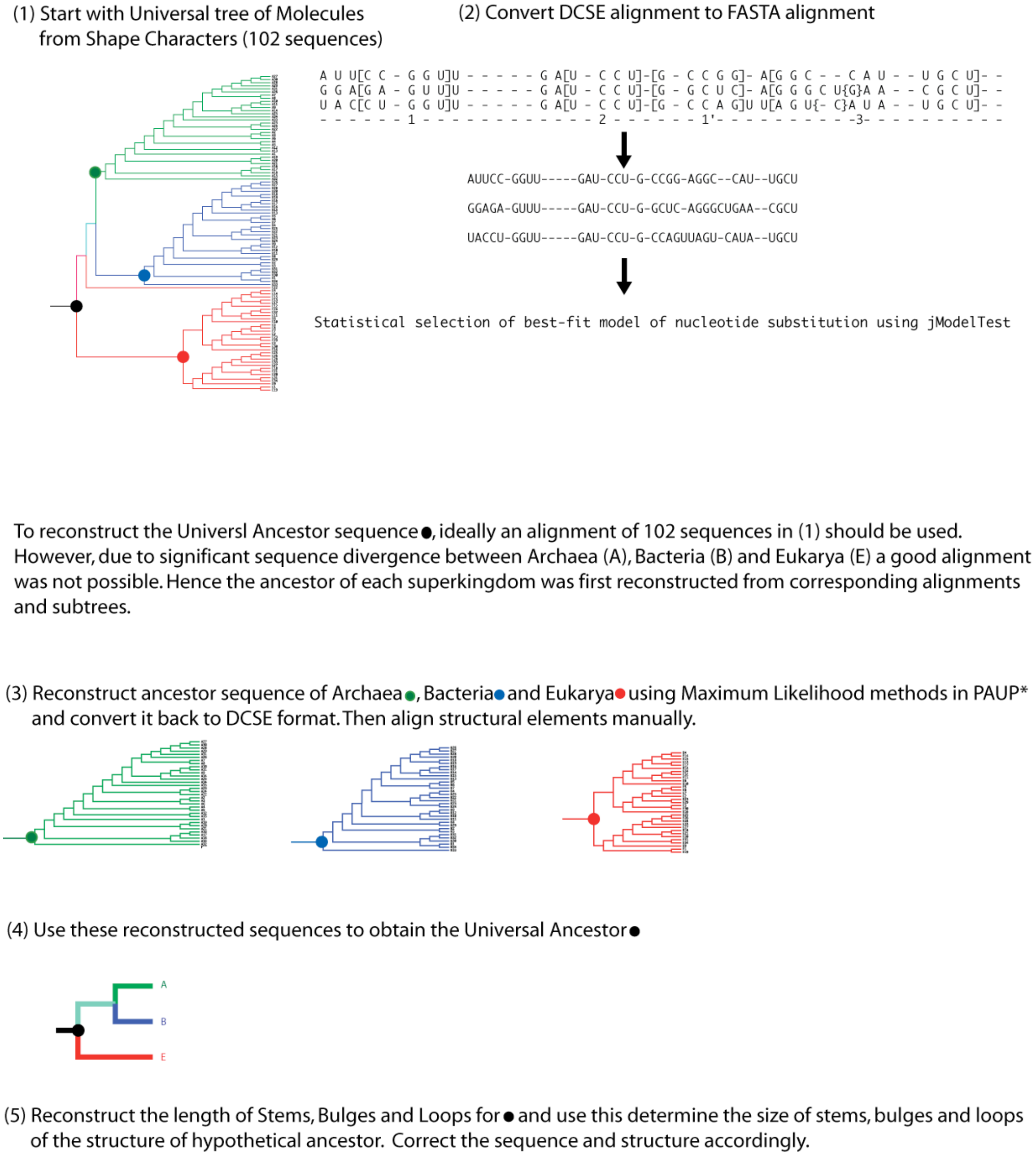

(1) Start with Universal tree of Molecules from Shape Characters (102 sequences)

(2) Convert DCSE alignment to FASTA alignment

```
A U U[C C - G G U]U - - - - - G A[U - C C U]-[G - C C G G]- A[G G C - - C A U - - U G C U]- -
G G A[G A - G U U]U - - - - - G A[U - C C U]-[G - G C U C]- A[G G G C U{G}A A - - C G C U]- -
U A C[C U - G G U]U - - - - - G A[U - C C U]-[G - C C A G]U U[A G U{- C}A U A - - U G C U]- -
- - - - - - 1 - - - - - - - - - - - - 2 - - - - - - 1'- - - - - - - - - -3- - - - - - - - - -
```

```
AUUCC-GGUU-----GAU-CCU-G-CCGG-AGGC--CAU--UGCU

GGAGA-GUUU-----GAU-CCU-G-GCUC-AGGGCUGAA--CGCU

UACCU-GGUU-----GAU-CCU-G-CCAGUUAGU-CAUA--UGCU
```

Statistical selection of best-fit model of nucleotide substitution using jModelTest

To reconstruct the Universl Ancestor sequence ●, ideally an alignment of 102 sequences in (1) should be used. However, due to significant sequence divergence between Archaea (A), Bacteria (B) and Eukarya (E) a good alignment was not possible. Hence the ancestor of each superkingdom was first reconstructed from corresponding alignments and subtrees.

(3) Reconstruct ancestor sequence of Archaea ●, Bacteria ● and Eukarya ● using Maximum Likelihood methods in PAUP* and convert it back to DCSE format. Then align structural elements manually.

(4) Use these reconstructed sequences to obtain the Universal Ancestor ●

(5) Reconstruct the length of Stems, Bulges and Loops for ● and use this determine the size of stems, bulges and loops of the structure of hypothetical ancestor. Correct the sequence and structure accordingly.

Figure S5. Overview of the reconstruction of hypothetical ancestral sequences and structures from rRNA. The flow chart describes the methods and data used to reconstruct ancestral rRNA molecules for remote homology analyses.