

Herein, we provided two separate sections to calculate GLS (Section 1) and GQS (Section 2) for every gene (see Fig. 1b). Both can handle gene trees with missing taxa.

Section 1: GLS calculation

For a given question, T1 is concatenated ML tree, whereas T2 is coalescent-based ASTRAL tree that differs from T1. ML2ASTRAL.tre contains T1 and T2 in this case.

- 1) Estimate site-wise loglk for alternative hypothesis (-z ML2ASTRAL.tre) using raxml (a) or iqtrees (b)

a) Estimate site-wise loglk for alternative hypothesis using raxml with option (-f G)

```
/home/shenx3/tools/standard-RAxML-master_8.2.0/raxmlHPC-PTHREADS-SSE3 -T 12 -f G -m GTRGAMMA -z
ML2ASTRAL.tre -s 37taxa_1245genes.fas.reduced -q 37taxa_1245genes_combine_orders.txt.reduced -n ML2ASTRAL --no-bfbs
```

b) Estimate site-wise loglk for alternative hypothesis using iqtrees with option (-wsl)

```
/gpf23/scratch/shenx3/tools/iqtrees-1.6.8-Linux/bin/iqtrees -nt 6 -st DNA -m GTR+G4 -z ML2ASTRAL.tre -wsl -s
37taxa_1245genes.fas.reduced --spp 37taxa_1245genes_combine_orders.txt.reduced -pre ML2ASTRAL
```

- 2) Passer site-wise log-likelihood file by GLS_parser_v1.pl (please make sure PERL has installed on your computer)

```
perl GLS_parser_v1.pl RAxML_perSiteLLs.ML2ASTRAL 37taxa_1245genes_combine_orders.txt.reduced ML2ASTRAL_GLS
```

Option Explanations

RAxML_perSiteLLs.ML2ASTRAL: Site-wise log likelihood file estimated from RAxML or IQ-TREE (see output in step 2a or 2b)

37taxa_1245genes_combine_orders.txt.reduced: Gene-based partition file in RAxML-style

ML2ASTRAL_GLS: Prefix for all output files

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- 3) Output file is ML2ASTRAL_GLS_table.txt. This table looks like below:

gene_id	tree_supported	tr1_GLS	tr2_GLS	df_GLS
9930021J03RIK_1_rat_final	tr1	-5904.88285	-5905.0219	0.139021
A2AB_1_rat_final	tr2	-3569.47893	-3568.9755	-0.503397

Note that:

- a) Characters in red are command lines

Section 2: GQS calculation

For a given question, T1 is concatenated ML tree), whereas T2 is coalescent-based ASTRAL tree that differs from T1.

- 1) Passer gene-wise coalescent-based signal by GQS_parser_v1.pl (please make sure PERL has installed on your computer)

```
perl GQS_parser_v1.pl gt=A4GNT_mer_final.fasta.treefile t1=ML_T1.tre t2=ASTRAL_T2.tre A4GNT_mer_final_GQS
```

```
##### Option Explanations #####
```

```
gt=A4GNT_mer_final.fasta.treefile: gene tree
```

```
t1=ML_T1.tre: concatenated ML tree
```

```
t2=ASTAL_T2.tre: coalescent-based ASTRAL tree
```

```
A4GNT_mer_final_GQS: Prefix for output file
```

```
#####
```

- 2) Output file is A4GNT_mer_final_GQS_table.txt. This table looks like below:

gene_id	tree_supported	tr1_GQS	tr2_GQS	df_GQS
A4GNT_mer_final.fasta.treefile	Tr1	62225	61535	690

Note that:

- a) Characters in red are command lines
- b) GQS_parser_v1.pl, A4GNT_mer_final.fasta.treefile, ML_T1.tre, and ASTRAL_T2.tre should be in the same directory.
- c) In this case, calculating GQS for each gene (it contains 37 tips) takes ~0.5 sec.
- d) Please do not delete the program folder, where it contains ASTRAL 5.6.3

Please let me know if you have any questions about them.

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