Mitochondrial molecular markers for US lineages of *P. infestans*

Brian J. Knaus, Javier F. Tabima and Niklaus J. Grünwald

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Contents

1 Samples

The sample includes data which was opportunistically gathered from previous publications as well as data which is not yet available to the public (Judelson, unpublished).

Sample	Count	Reference
T30-4	1	[4]
PIC99189 & 90128	2	[8]
13_a2	1	[2]
Yoshida et al.	13	[9]
Martin et al.	3	[7]
Judelson	8	NA
Total	28	

Note that both the Yoshida and Martin papers [9, 7] included ancient DNA in their analyses. Here we have omitted those samples and focused on modern samples. The sample 'T30-4' was the first sequenced genome and is considered the reference for nuclear work. This genome was assembled prior to high-throughput sequencing (i.e., Illumina and 454 technologies). The data presented here are not the sequences used for the paper but are part of a project by the Broad (http://www.broadinstitute.org/annotation/genome/phytophthora_infestans/MultiHome.html) to resequence this sample using Illumina and 454 technologies.

The Judelson data include a sample of US1 which was sampled at three different time points (us1_1, us1_2 and us1_3). We suspect that this was different samples and not necessarily the same clone. Therefore differences among these samples may either be due to biological or technical factors. It also includes a sample of US8 which has been characterized as having fungicide resistance [3]. This lineage was also sequenced by Martin et al. [7]. These are most likely different samples so differences among these samples may be interpreted as biological.

The Yoshida data includes one sample of *P. mirabilis* (p7722), this should jump out in the analyses.

For the mitochondrial data we used the type IIa form [1] because it was the longest sequence and we felt this would provide the best alignment.

For enigmatic reasons, not all of the samples from the Yoshida and Martin papers were actually available online. Therefore our numbers here do not match those presented in the papers.

2 Read mapping

Mapped reads to the type IIa mitochondrial reference "AY898627.1". Reads were mapped using bowtie2 [5], called variants, again using the type IIa mitochondrial reference (but a different format). SAMtools was used to call variants [6].

2.1 Preprocessing

To check which of the calls are good calls, the variant files were filtered by quality, read depth and mapping quality (Figure 1). For this we used an in-house R package Brian wrote called vcfR. The results of these statistics for the whole mitochondria can be seen in Figure 2. Note that quality here is for each individual call and ranges from 1-99. This is different from the per variant quality discussed above which ranges from 1-999. Most of these heterozygous sites are of low quality. The samples which included high quality heterozygote calls (p1362, p6096, p10650, p12204, p10127) were mostly from the Yoshida et al. [9] paper and were among the low sequencing depth samples they included. Because these samples are not among the US lineages we're interested in and because they are apparently of low sequencing depth I think we can ignore them for now. However, the sample nl07434 was among the high sequencing depth samples from this paper and is perhaps noteworthy. T30-4 was called as a heterozygote for one variant and is perplexinng.

```
## Before filtering:
## [1] 247
## After filtering:
## [1] 70
## gt.m2sfs is commented out
```

```
  Chromo
  Wide rows   (brows):  5
  Narrow rows (srows):  2
```

3 SNPs after filtering

After filtering we proceeded to look at which of the SNP's were supported after the filtering (Table 1).

Table 1: Diagnostic SNP positions for the mtDNA genome after filtering.

Position	SNP	Diagnostic for
7857	A/G	p10127, us11 and us1
8118	T/G	us1
20464	T/C	p17777, us22 and us8
22711	G/A	p17777, us22 and us8
26767	A/G	p10127, us11 and us1
28783	A/G	p10127, us11 and us1
36663	T/C	us8

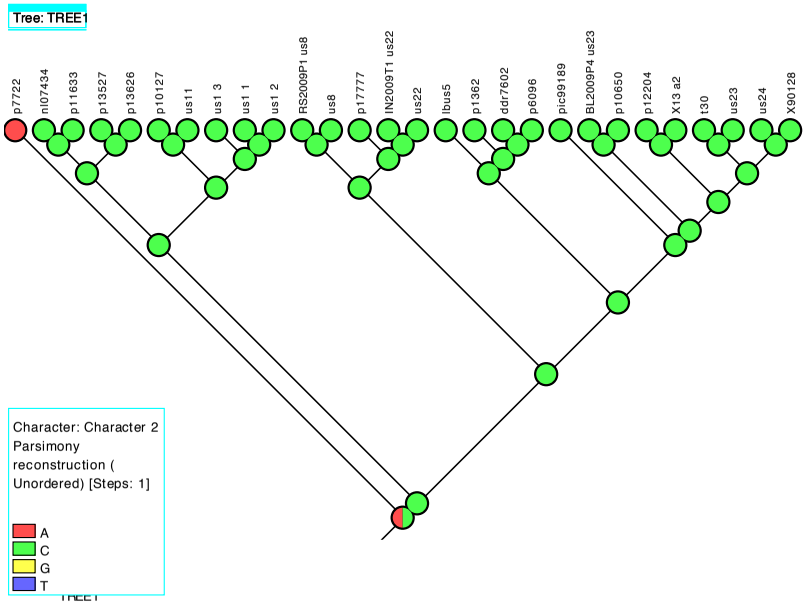
4 Diagnostic SNP mapping

4.1 Phylogenetic reconstruction

Using the whole genome alignment (28 sequences, 39870 nucleotides) we performed a whole-genome phylogeny using likelihood (RAxML) and bayesian inference (BEAST). We used RAxML using no partitions, 1000 bootstrap replicates, a GTR+I+G model of nucleotide evolution to obtain a bipartitioned tree with the boostrap values mapped to the branches. For BEAST, we specified `p7722` (*P.mirabilis*) as the outgroup. We used a `HKY+G+I` model of nucleotide substitutions, a strict molecular clock, a constant population size prior, UPGMA starting tree and 10 million markov Chains. The best tree is shown in Figure 3.

4.2 Mapping the SNP's in the BEAST tree

To map the SNP's found in the mtDNA genome to the coalescent tree, we used `Mesquite`. We did a removal of invariable regions and ancestral State reconstruction for all 31 SNPs using a parsimony reconstruction state (Figure **??**).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p11633
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p13626
p10127
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us1 1
us1 2
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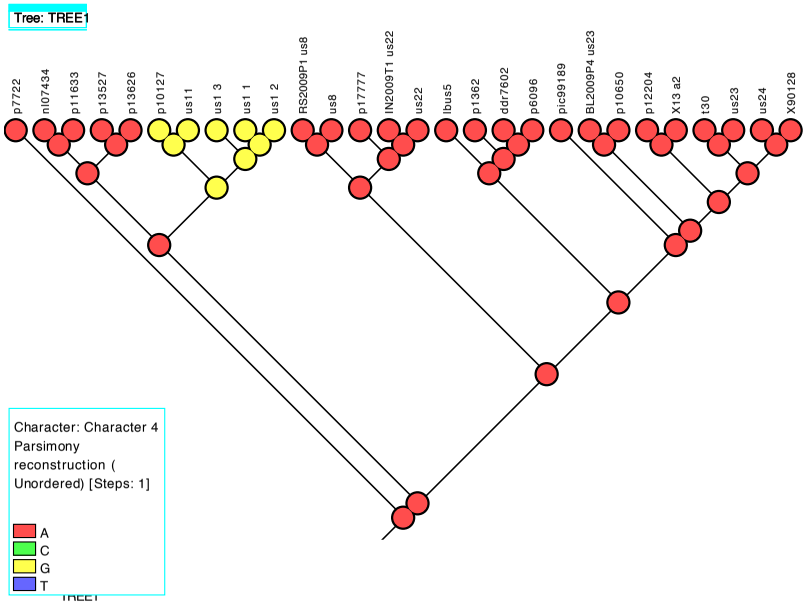
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 2
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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us22
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p1362
ddr7602
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pic99189
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p10650
p12204
X13 a2
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reconstruction (
Unordered) [Steps: 1]
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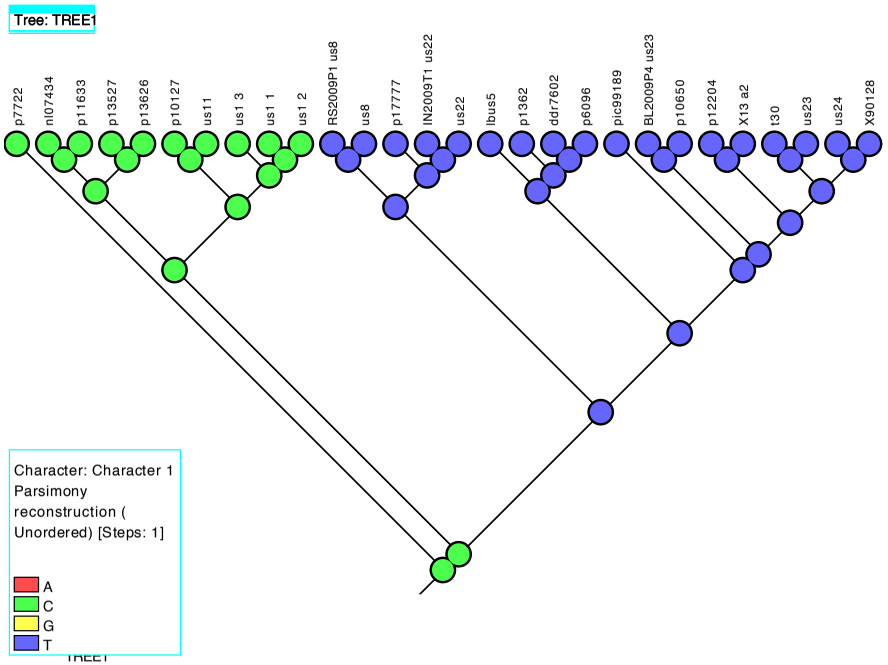
C
G
T
TREE1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p1362
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p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
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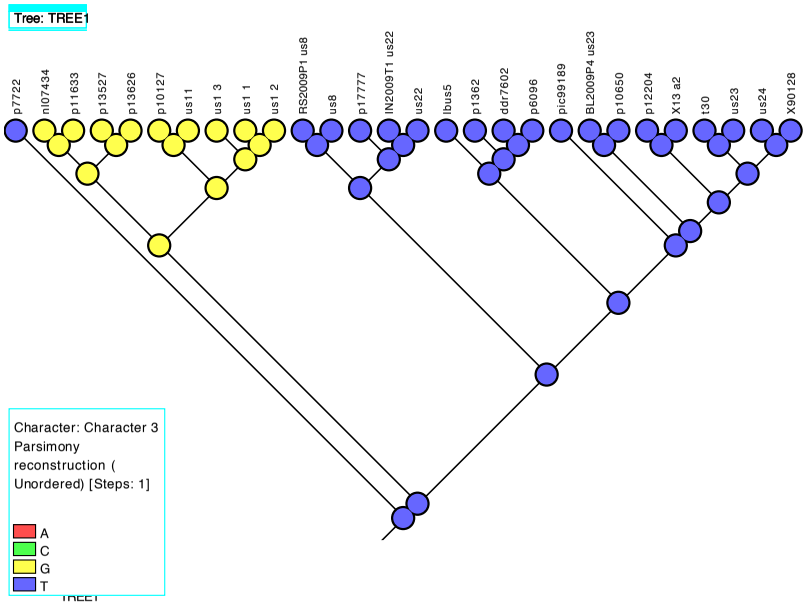
Character: Character 1
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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BL2009P4 us23
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Parsimony
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Unordered) [Steps: 1]
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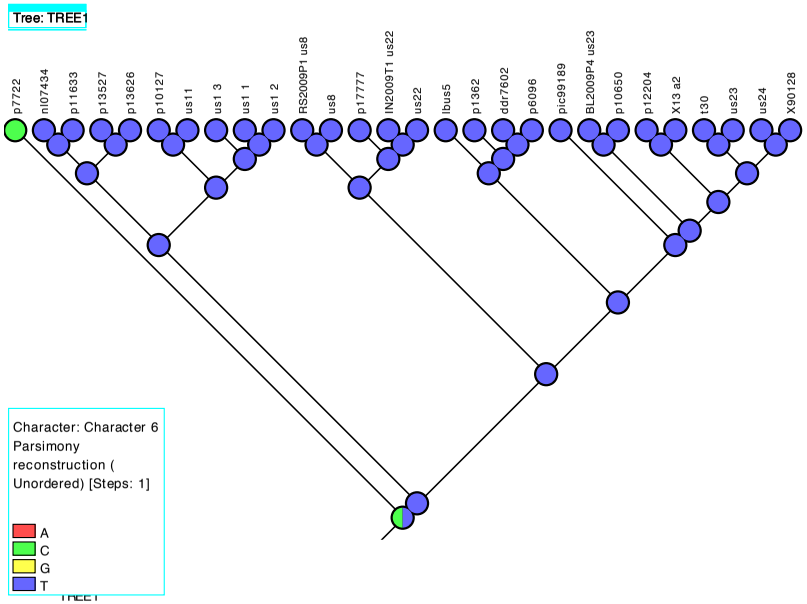
C
G
T
TREE1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IN2009T1 us22
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X13 a2
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Parsimony
reconstruction (
Unordered) [Steps: 1]
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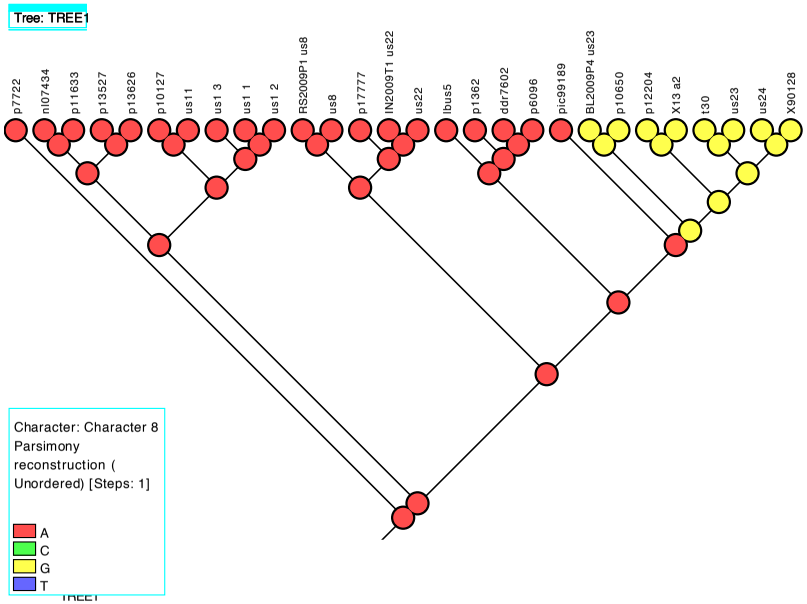
C
G
T
TREE1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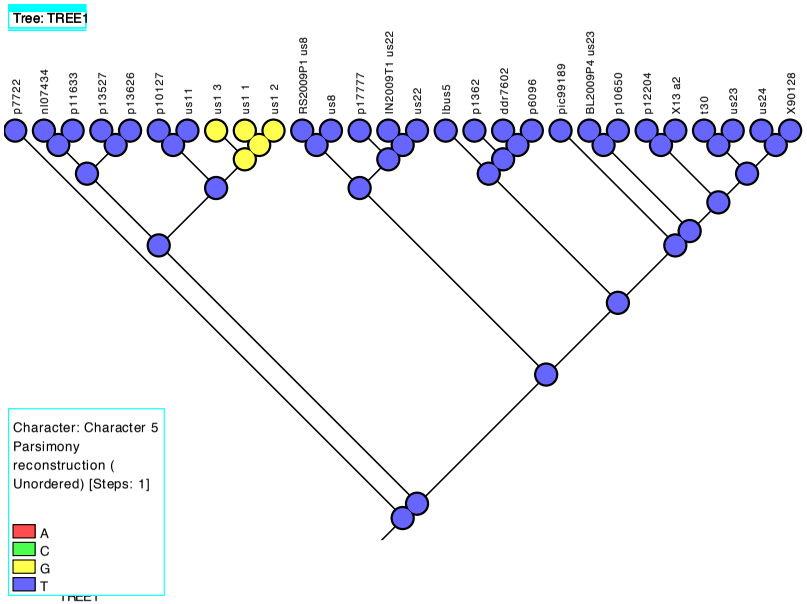
C
G
T
TREE1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pic99189
BL2009P4 us23
p10650
p12204
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reconstruction (
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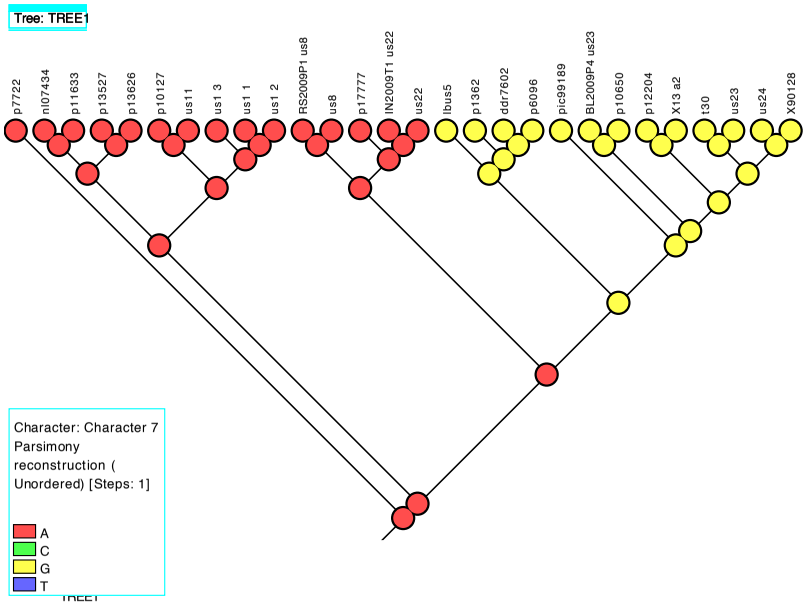
C
G
T
TREE1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IN2009T1 us22
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p1362
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pic99189
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p10650
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X13 a2
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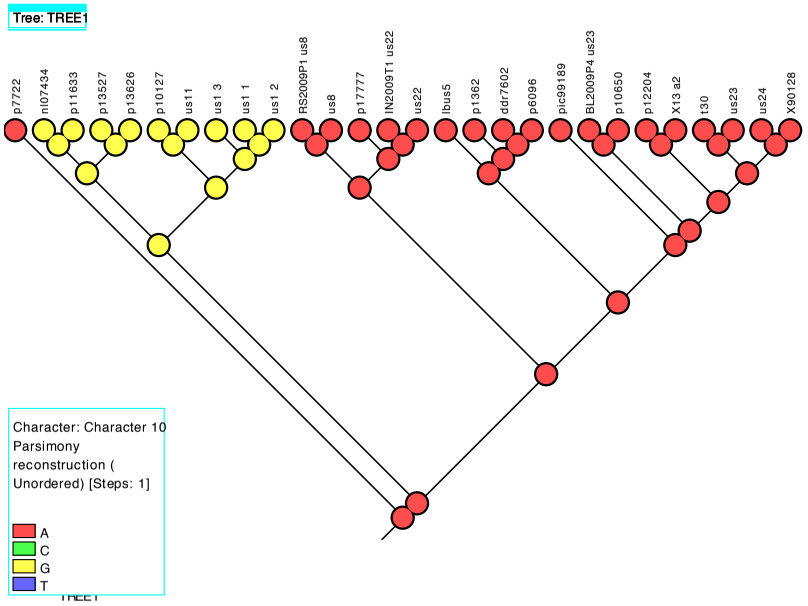
C
G
T
TREE1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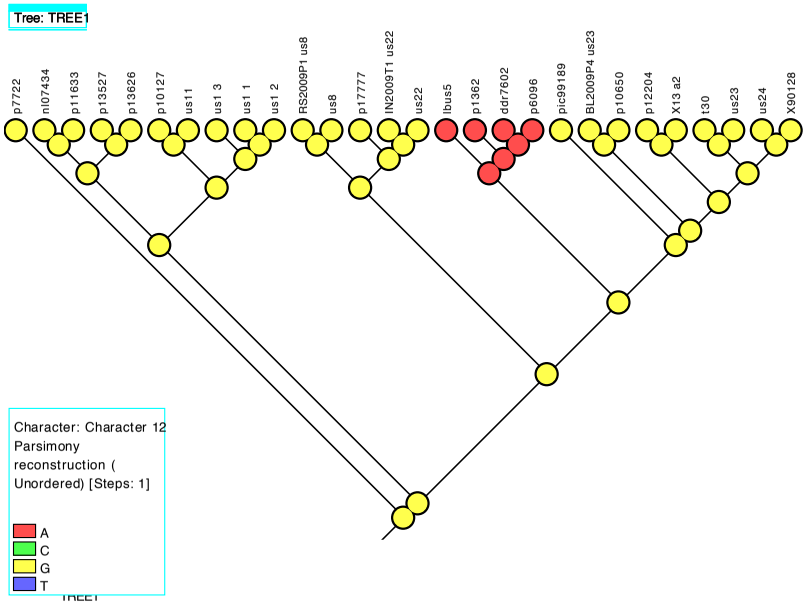
G
T
TREE1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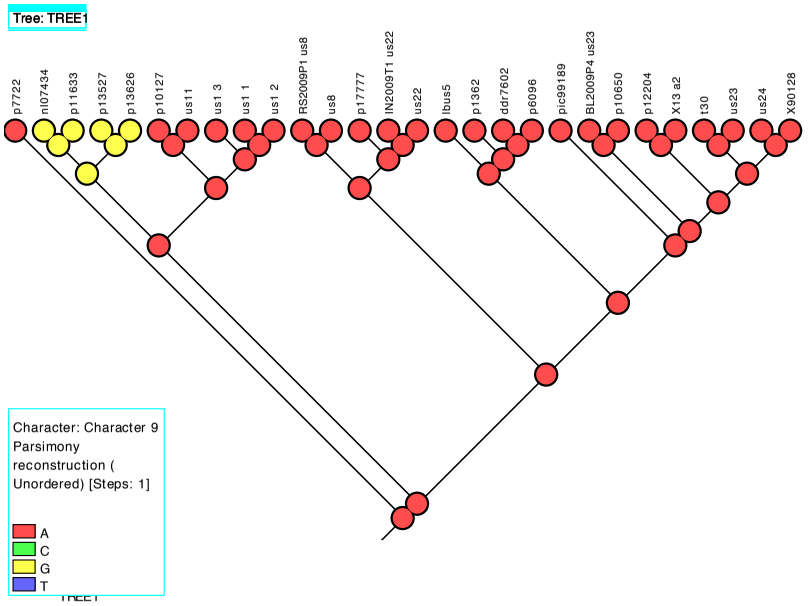
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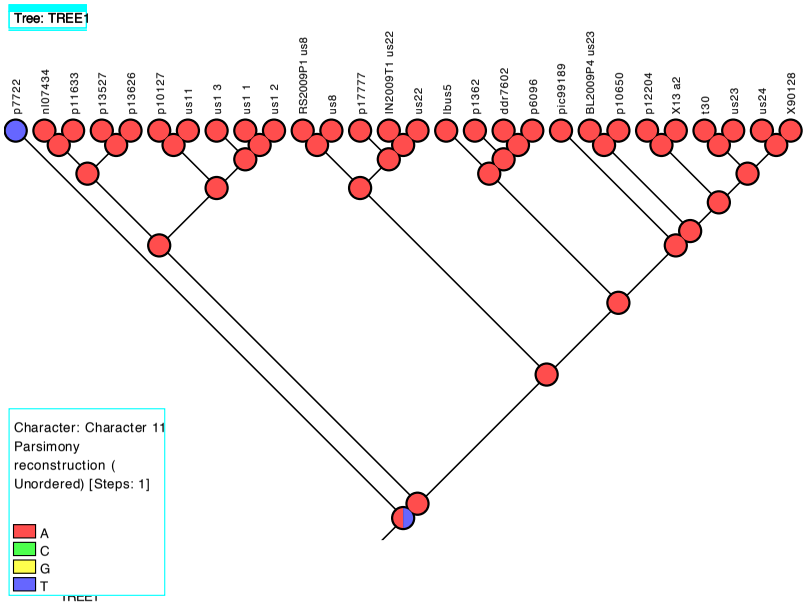
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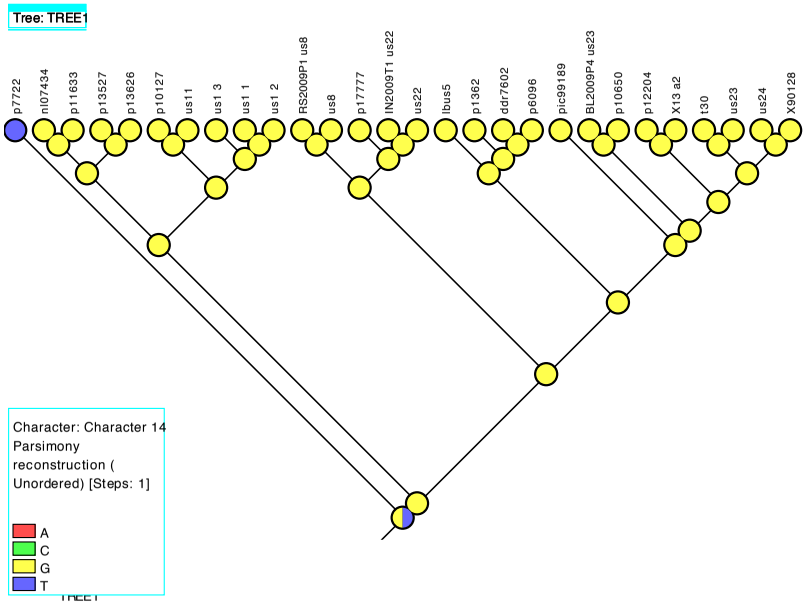
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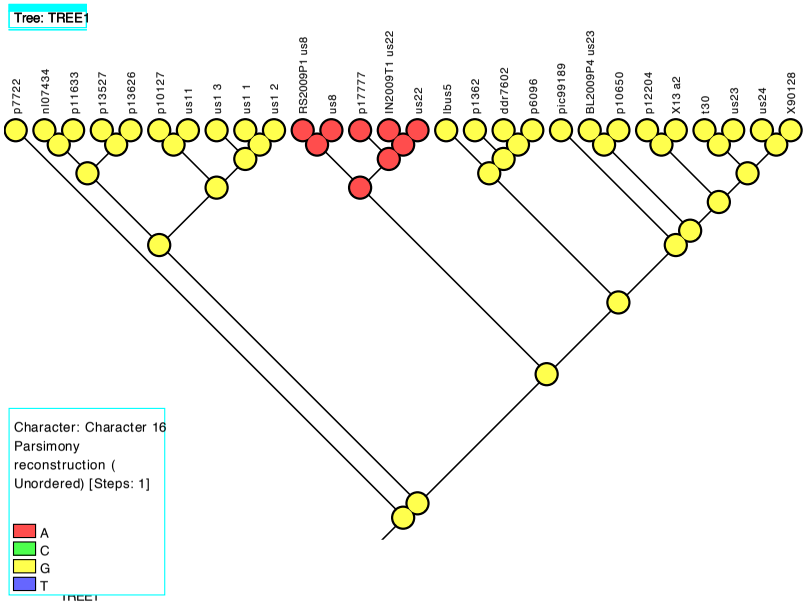
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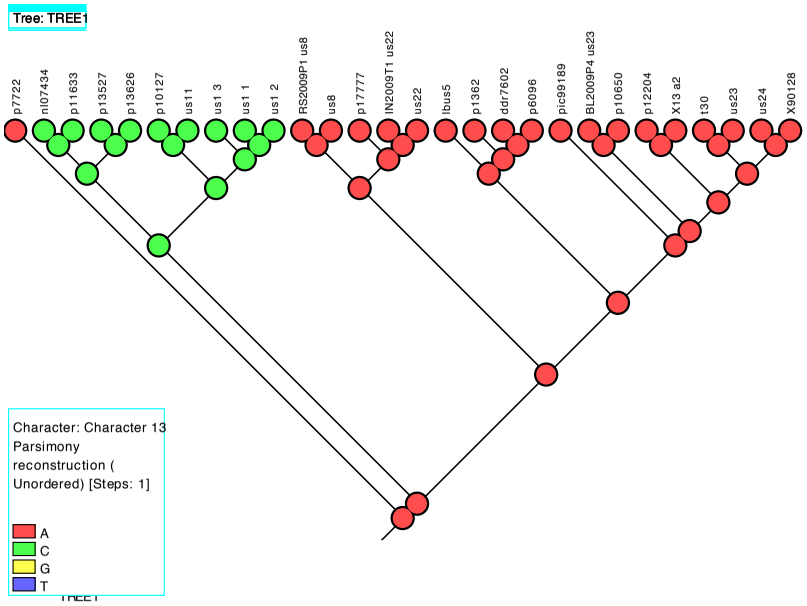
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Parsimony
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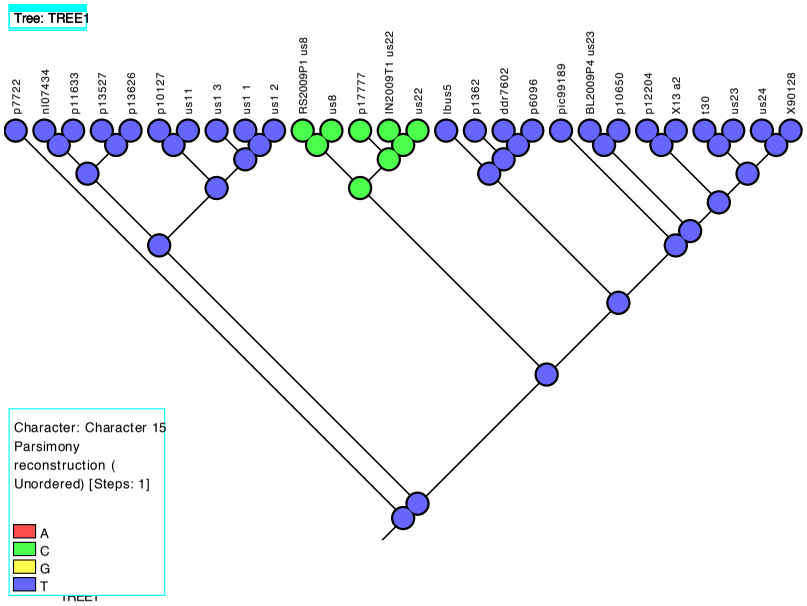
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p12204
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Unordered) [Steps: 1]
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G
T
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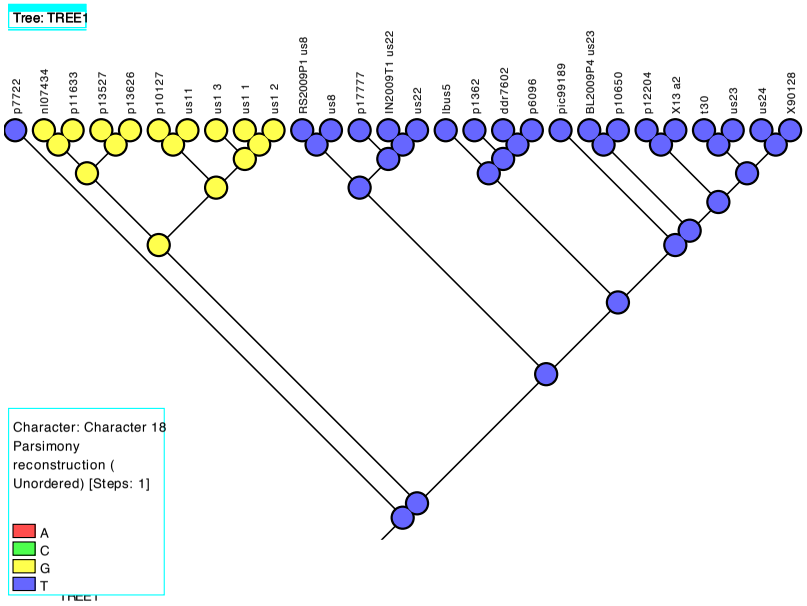
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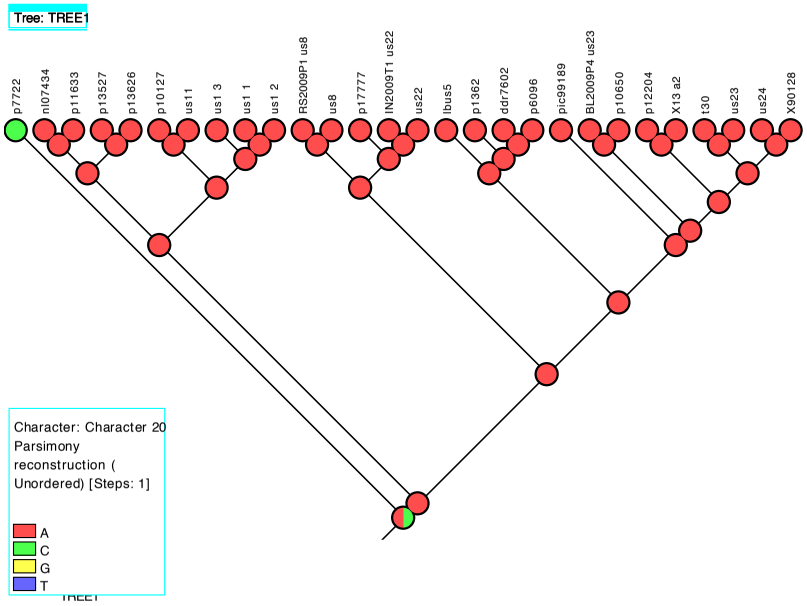
G
T
TREE1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X13 a2
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G
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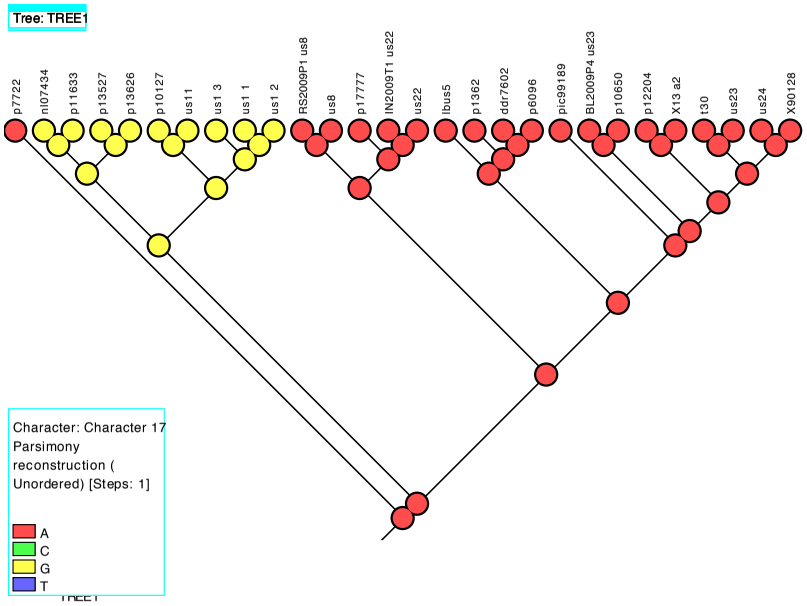
TREE1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p10650
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X13 a2
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X90128
Character: Character 17
Parsimony
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Unordered) [Steps: 1]
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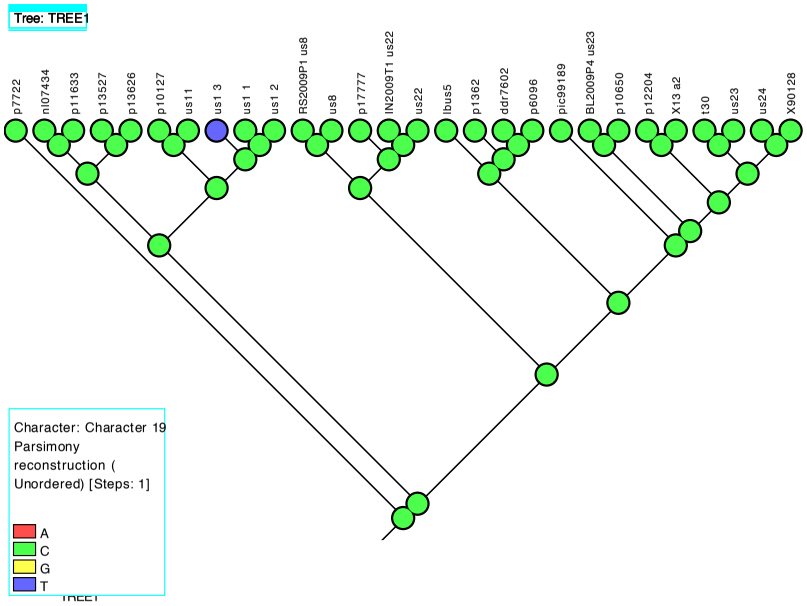
C
G
T
TREE1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us22
lbus5
p1362
ddr7602
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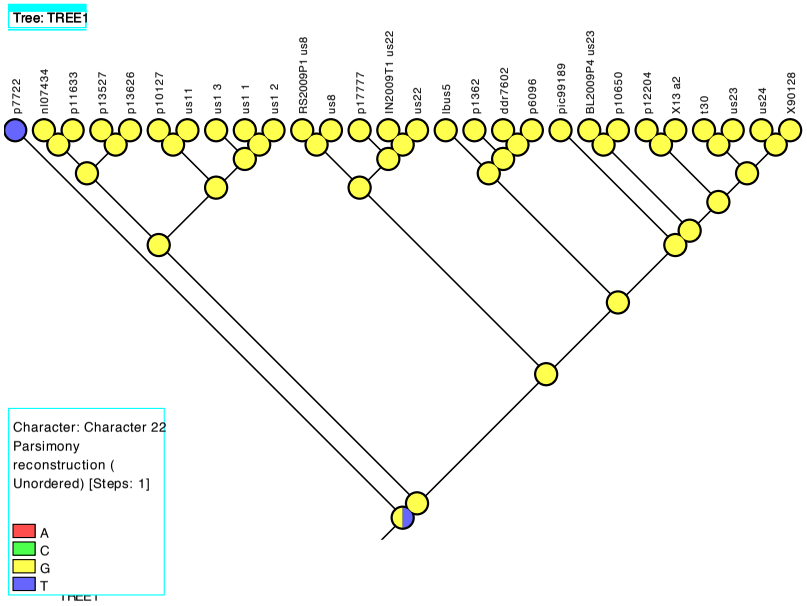
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 19
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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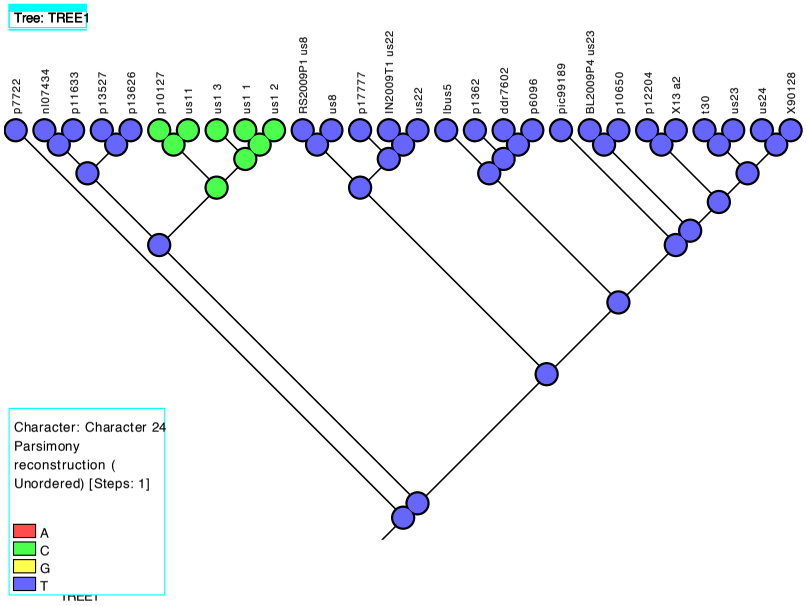
us11
us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 22
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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p13626
p10127
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us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
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us23
us24
X90128
Character: Character 24
Parsimony
reconstruction (
Unordered) [Steps: 1]
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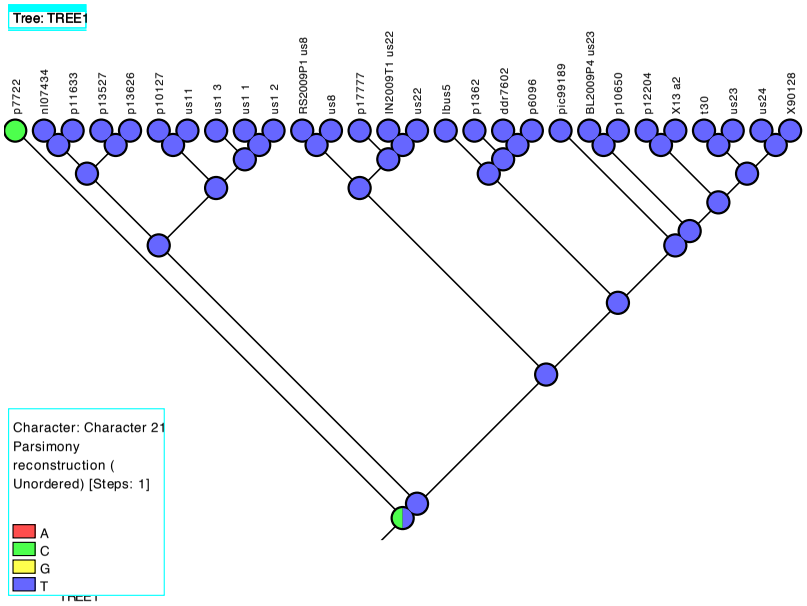
C
G
T
TREE1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us1 1
us1 2
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us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
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us23
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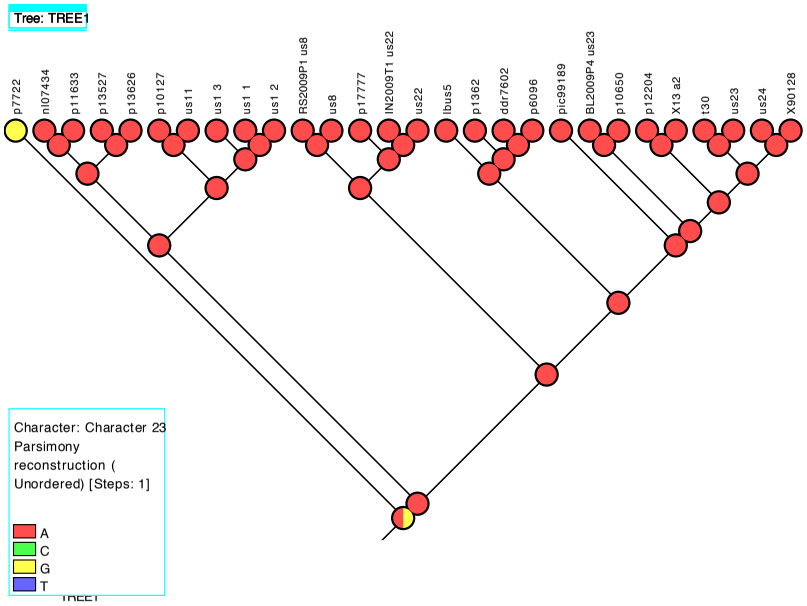
X90128
Character: Character 21
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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p10127
us11
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us1 1
us1 2
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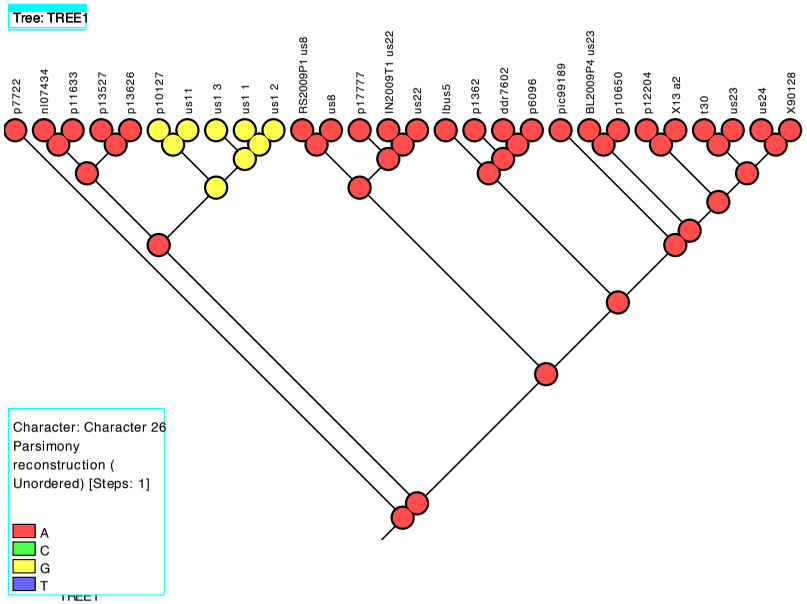
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 23
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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p7722
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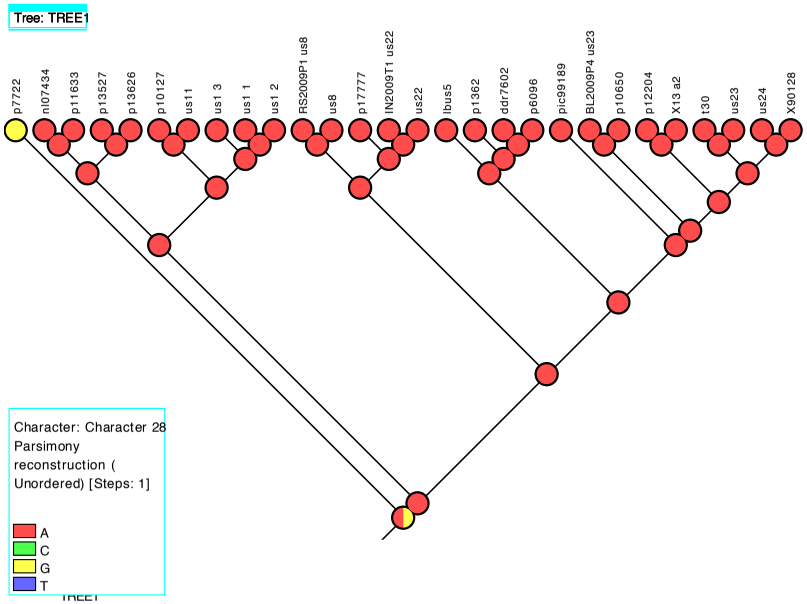
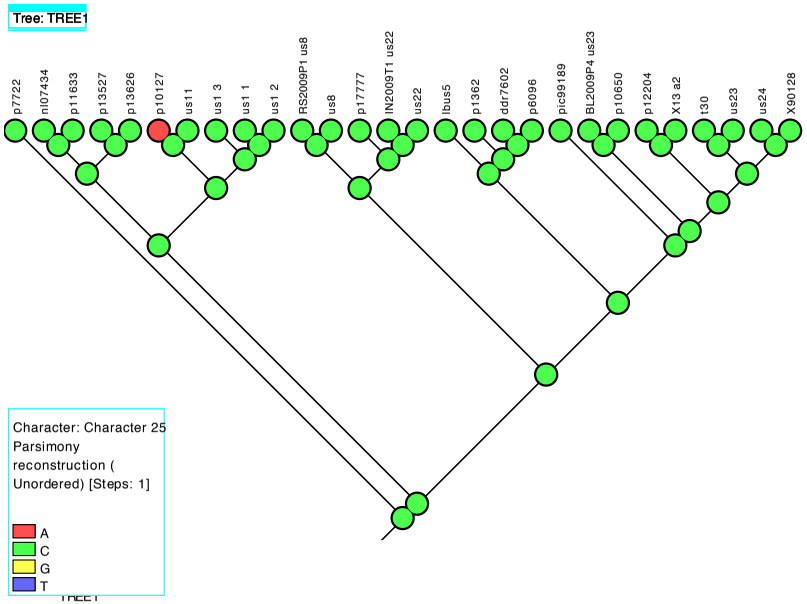
p13626
p10127
us11
us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 26
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1


Tree: TREE1
p7722
nl07434
p11633
p13527
p13626
p10127
us11
us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 28
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1


Tree: TREE1
p7722
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p11633
p13527
p13626
p10127
us11
us1 3
us1 1
us1 2
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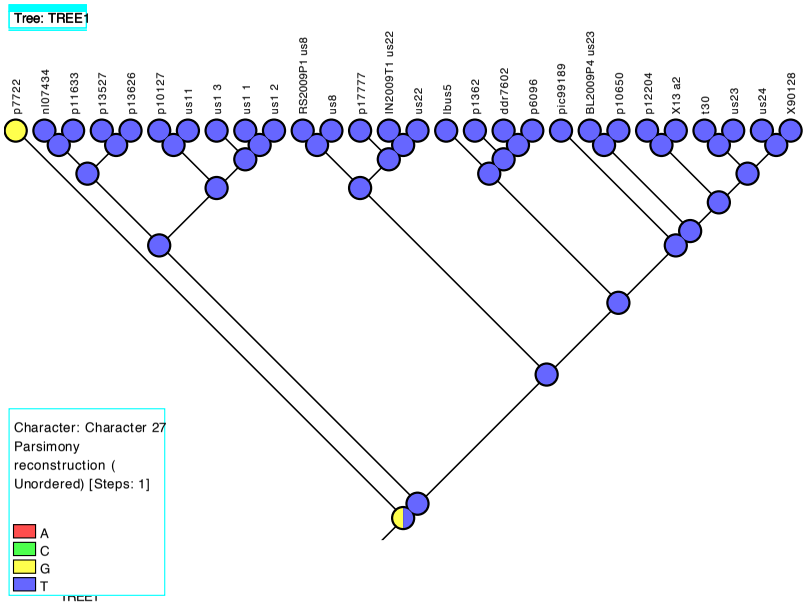
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 25
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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p13626
p10127
us11
us1 3
us1 1
us1 2
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p17777
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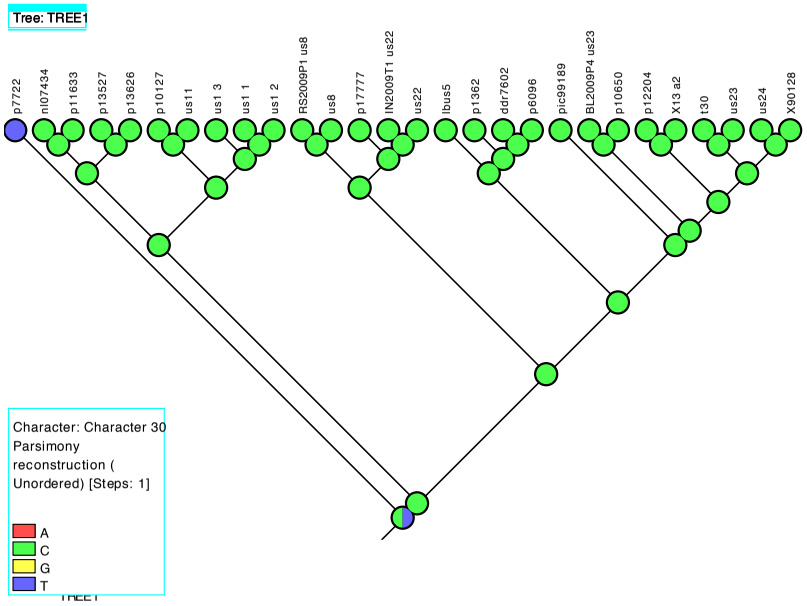
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 27
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1


Tree: TREE1
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nl07434
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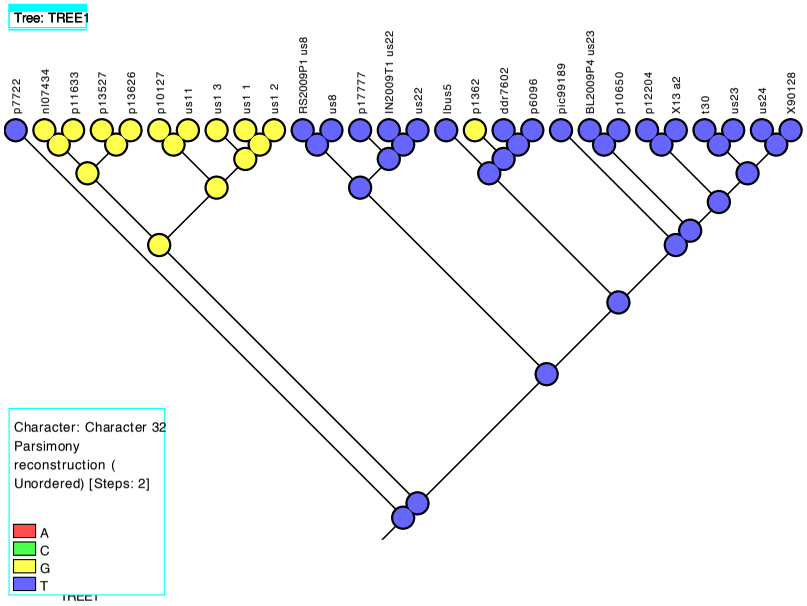
p13626
p10127
us11
us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 30
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1


Tree: TREE1
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nl07434
p11633
p13527
p13626
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us11
us1 3
us1 1
us1 2
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us8
p17777
IN2009T1 us22
us22
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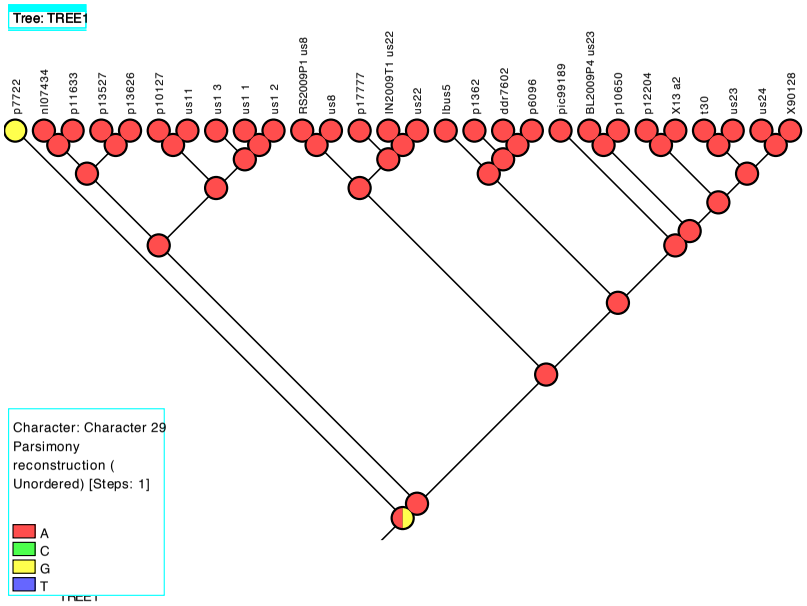
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 32
Parsimony
reconstruction (
Unordered) [Steps: 2]
A
C
G
T
TREE1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nl07434
p11633
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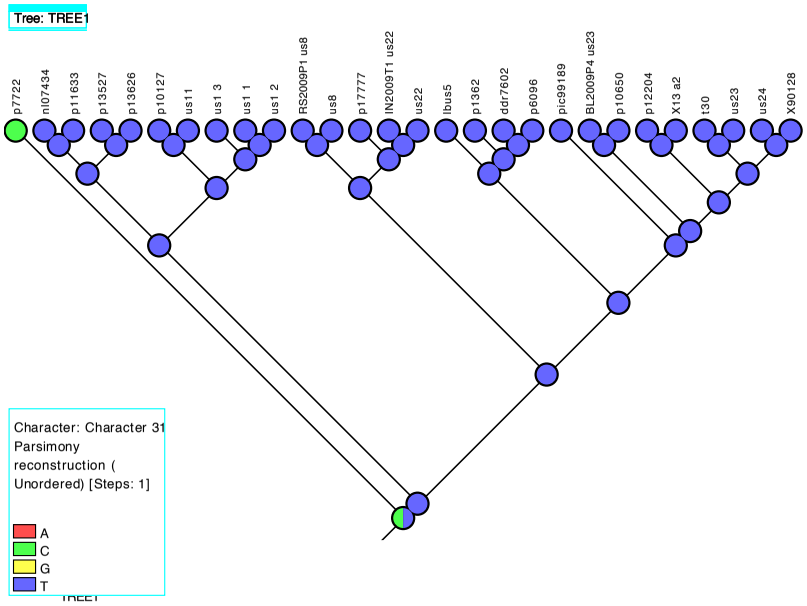
p13626
p10127
us11
us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 29
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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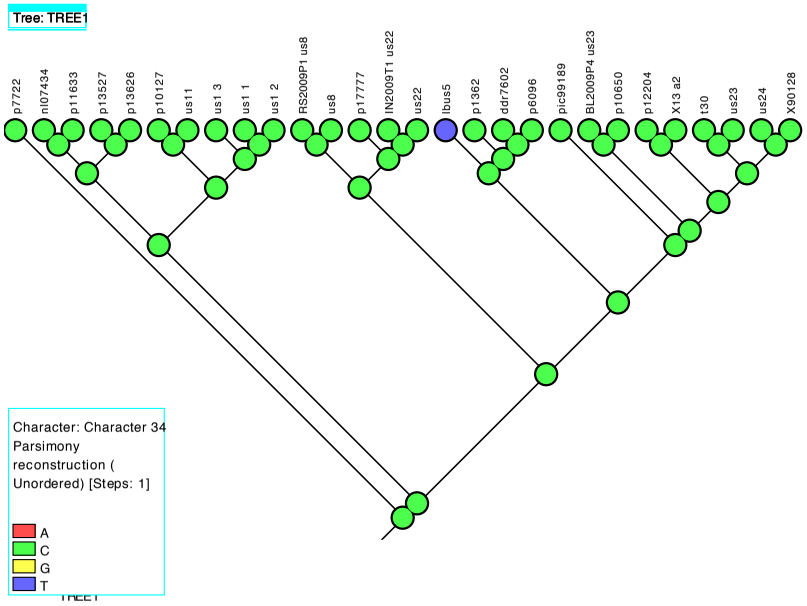
p10127
us11
us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 31
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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us1 2
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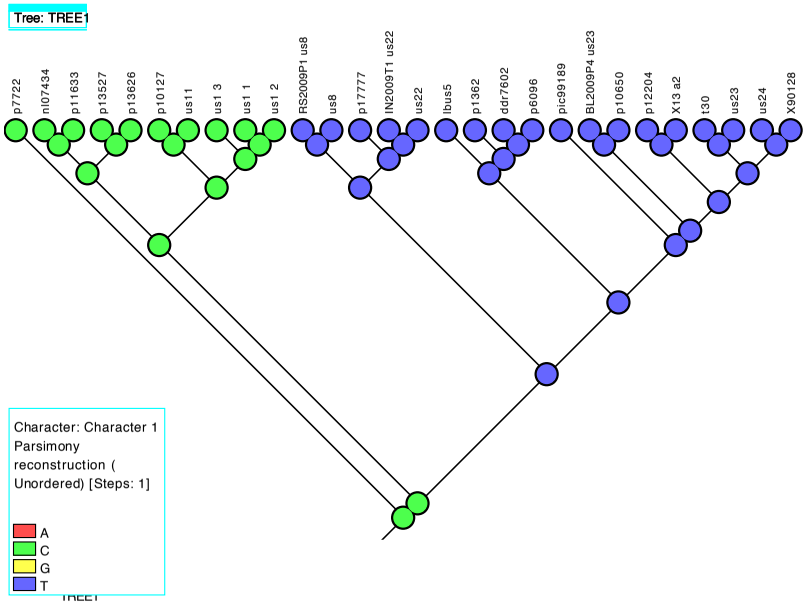
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 34
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1


Tree: TREE1
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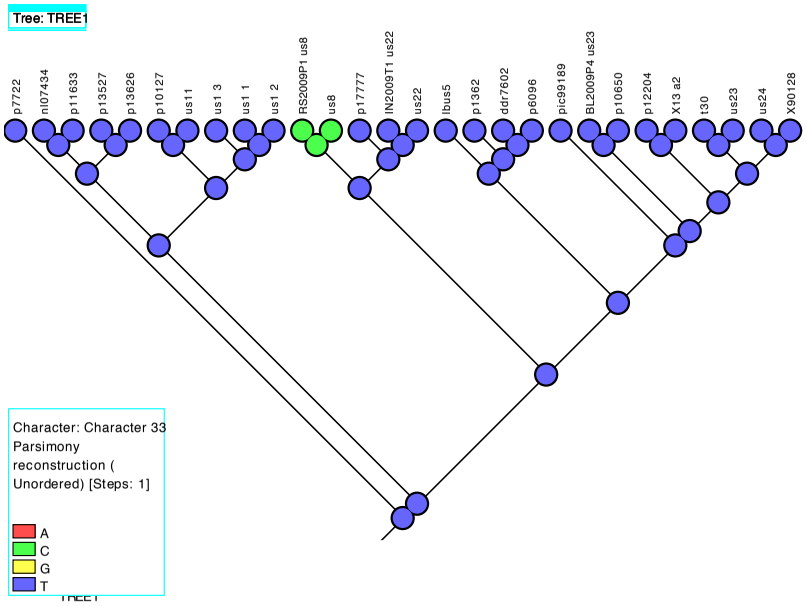
p10127
us11
us1 3
us1 1
us1 2
RS2009P1 us8
us8
p17777
IN2009T1 us22
us22
lbus5
p1362
ddr7602
p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 1
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1


Tree: TREE1
p7722
nl07434
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p13626
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us11
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us1 1
us1 2
RS2009P1 us8
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p17777
IN2009T1 us22
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pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
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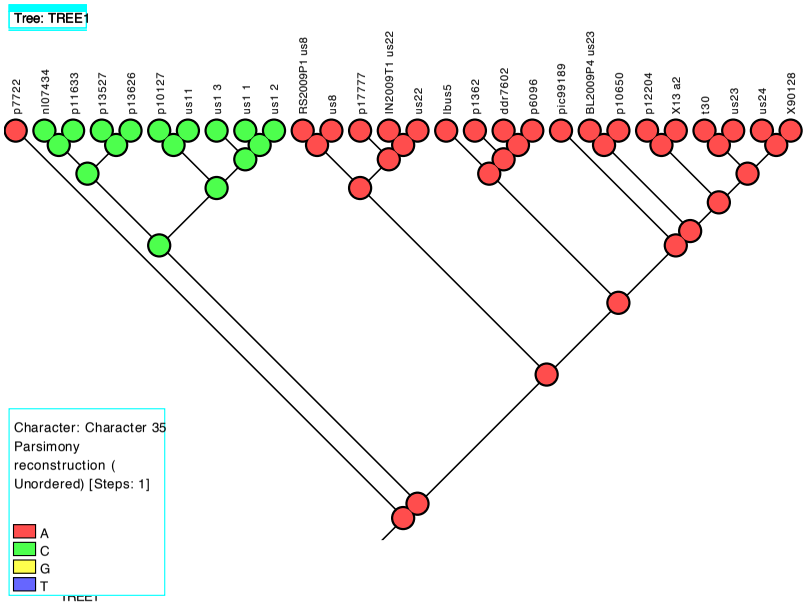
Character: Character 33
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1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us1 2
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p6096
pic99189
BL2009P4 us23
p10650
p12204
X13 a2
t30
us23
us24
X90128
Character: Character 35
Parsimony
reconstruction (
Unordered) [Steps: 1]
A
C
G
T
TREE1

5 Session Information

```
sessionInfo()

## R version 3.0.2 (2013-09-25)
## Platform: x86_64-apple-darwin10.8.0 (64-bit)
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] vcfR_0.1  knitr_1.5
##
## loaded via a namespace (and not attached):
## [1] ape_3.0-11      evaluate_0.5.1  formatR_0.10    grid_3.0.2
## [5] lattice_0.20-23 nlme_3.1-111    stringr_0.6.2   tools_3.0.2
```

References

[1] Cruz Avila-Adame, Luis Gómez-Alpizar, Victoria Zismann, Kristine M Jones, C Robin Buell, and Jean Beagle Ristaino. Mitochondrial genome sequences and molecular evolution of the Irish potato famine pathogen, *Phytophthora infestans*. *Current genetics*, 49(1):39–46, 2006.

[2] David EL Cooke, Liliana M Cano, Sylvain Raffaele, Ruairidh A Bain, Louise R Cooke, Graham J Etherington, Kenneth L Deahl, Rhys A Farrer, Eleanor M Gilroy, Erica M Goss, et al. Genome analyses of an aggressive and invasive lineage of the irish potato famine pathogen. *PLoS pathogens*, 8(10):e1002940, 2012.

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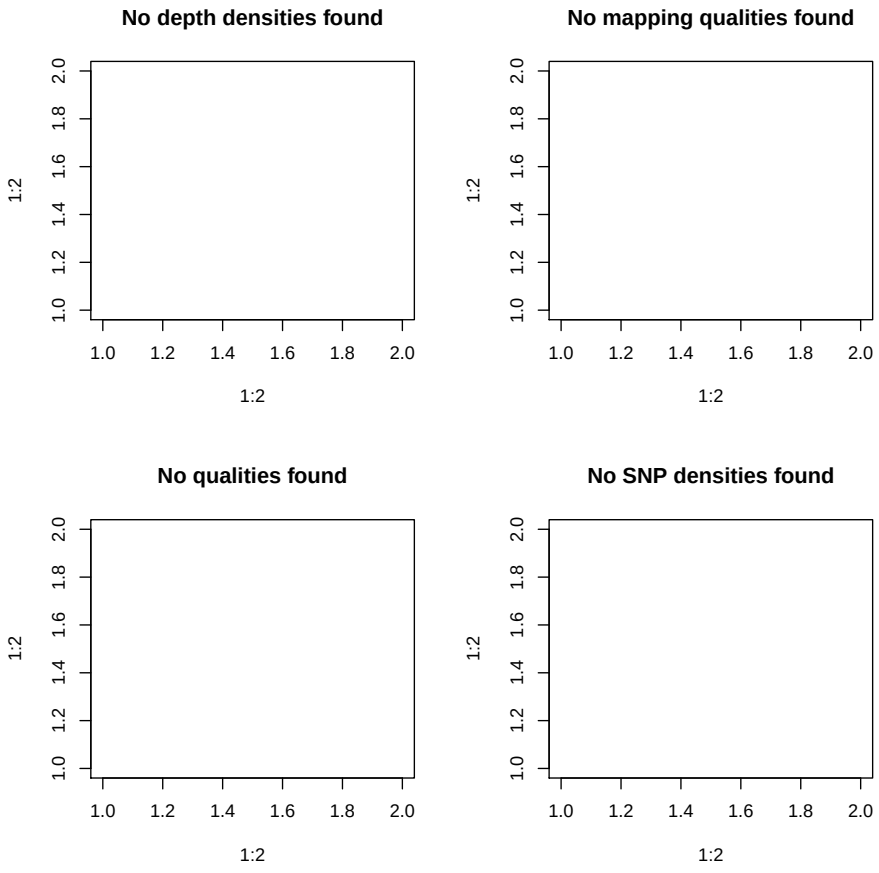

Figure 1: Quality results for the mtDNA SNP calls after filtering.

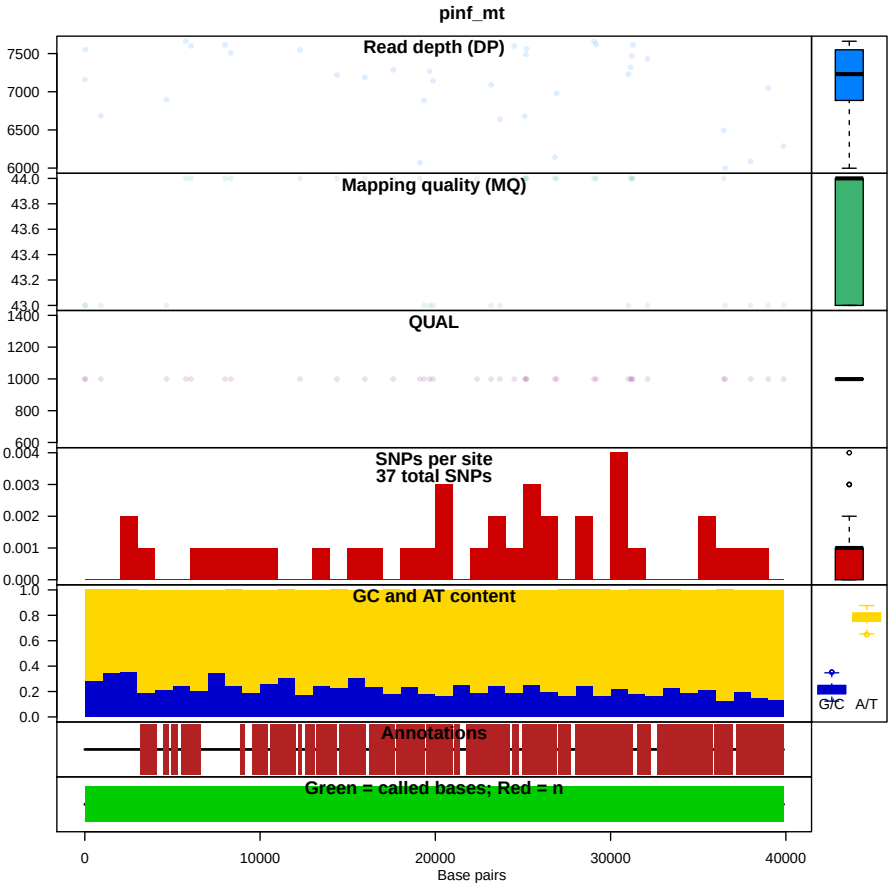

Figure 2: Whole mtDNA genome scan for the P. infestans samples.

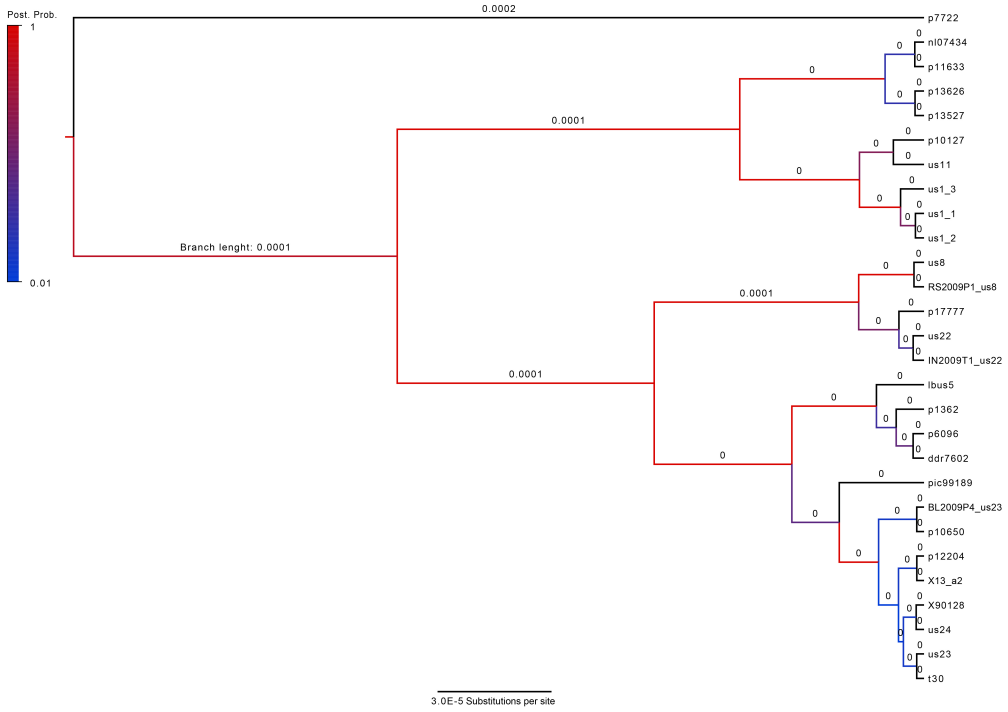

Figure 3: Bayesian coalescent of the whole mtDNA genome of *P.infestans* using `BEAST 1.8.0`. Values above branches represent branch lenghts (Tolopogy is cooncordant to the bifurcating model of a coalescent recosntruction). Branch colors represent posterior probability values (Legend indicates color coding).