

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

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gathered from previous publications as well as data which is not yet available to the public (Judelson, unpublished).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

- The sample ‘T30-4’ was the first sequenced genome and is considered the reference for nuclear work [4]. 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 to resequence this sample using Illumina and 454 technologies.
- Note that both the Yoshida and Martin papers included ancient DNA in their analyses [9, 7]. Here we have omitted those samples and focused on modern samples.
- 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.
- 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 these were different samples and not necessarily the same clone. Therefore differences among these samples may either be due to biological or technical factors.
- The Judelson data 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.

31 We've used the term 'SNP' fairly loosely in this document. The term 'vari-
32 ant' may be more appropriate. Until fairly recently the software tools we've
33 been using could only handle SNPs. They now report short indels as well.

34 2 Read mapping

35 Reads were mapped to the type IIa mitochondrial reference "AY898627.1".
36 Reads were mapped using bowtie2 [5]. Variants were called using SAMtools[6].

37 2.1 Variant filtering

38 To check which of the calls are good calls, the variant files were filtered by
39 quality, read depth and mapping quality (Figures 1,2). For this we used an in-
40 house R package called vcfR. Sequencing depth is cumulative over all sampels.
41 Quality here is for each variant over all samples and ranges from 1-999.

42 The genotype caller in Samtools assumes a diploid, bi-allelic model. Because
43 mitochondrial are assumed to be haploid we tried to filter out heterozygous
44 calls. Samples which included high quality heterozygote calls (p1362, p6096,
45 p10650, p12204, p10127) were mostly from the Yoshida et al. [9] paper and
46 were among the low sequencing depth samples they included. Because these
47 samples are not among the US lineages we're interested in, and because they
48 are apparently of low sequencing depth, we omitted them for now. However, the
49 sample nl07434 was among the high sequencing depth samples from this paper
50 and is perhaps noteworthy. T30-4 was called as a heterozygote for one variant
51 and is perplexinng.

52 The variants remaining after filtering were visualized as a linear chromosome
53 in Figure 3.

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

54 Filtering the variant panel based on quality (QUAL=999), cumulative se-
55 quencing depth (1st quartile >= DP >= 3rd quartile) and mapping quality (1st
56 quartile >= MQ >= 3rd quartile) resulted in 37 variants (Table 1). We have
57 identified a fraction of these as being diagnostic for a small group of samples
58 (Table 2).

59 3 Variant segregation

60 In order to visualize how variants segregated among the samples, a phylogeny
61 was inferred. We then used ancestral state reconstruction to map the characters
62 to the tree. At this time we're not trying to say anything bold about phylogeny

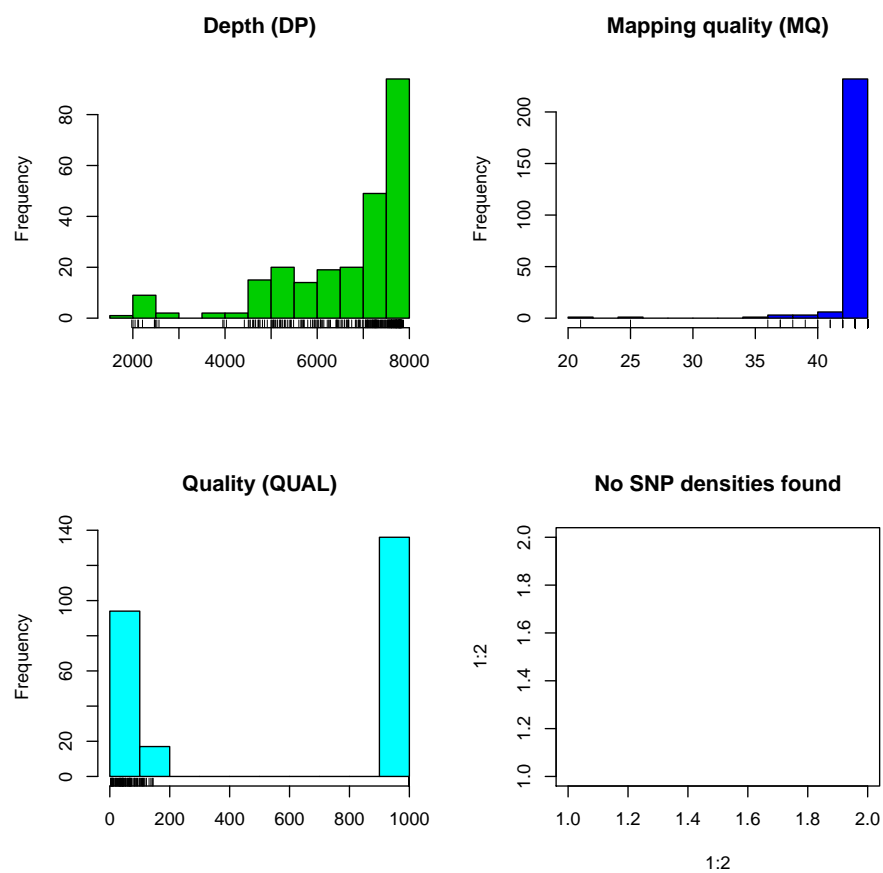


Figure 1: Quality results for the mtDNA SNP calls before filtering.

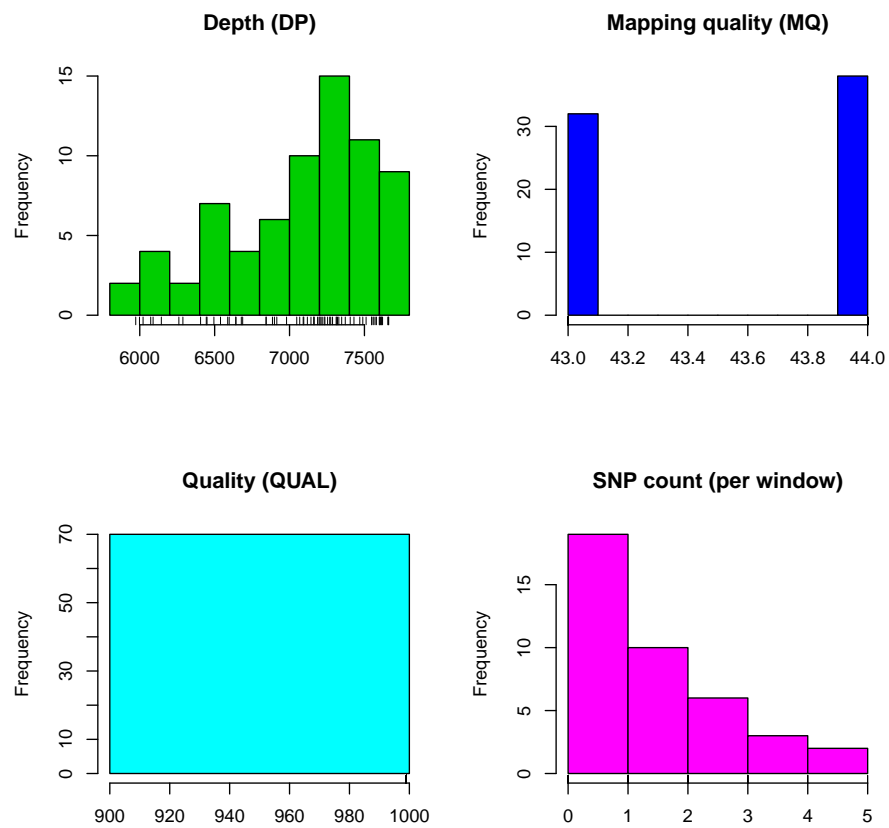


Figure 2: Quality results for the mtDNA SNP calls after filtering and window-izing variants.

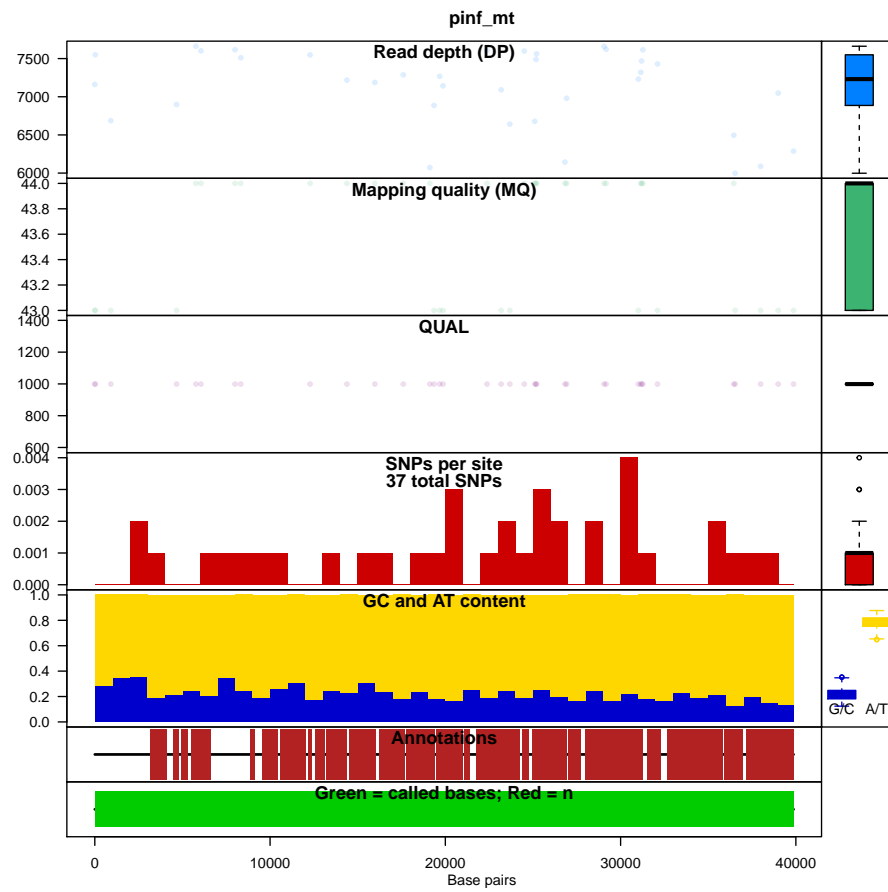


Figure 3: Whole mtDNA genome scan for the *P. infestans* samples.

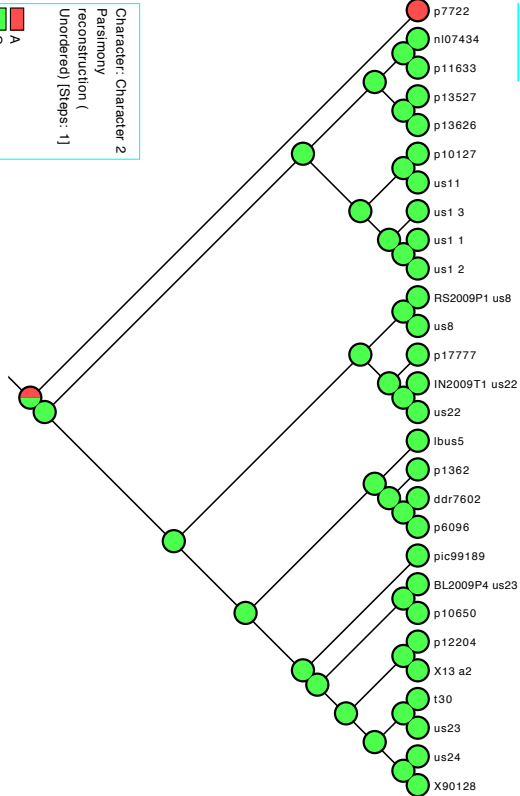
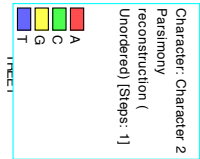
63 or character evolution. We're simply using these tools to visualize how the
64 variants segregate.

65 3.1 Phylogenetic reconstruction

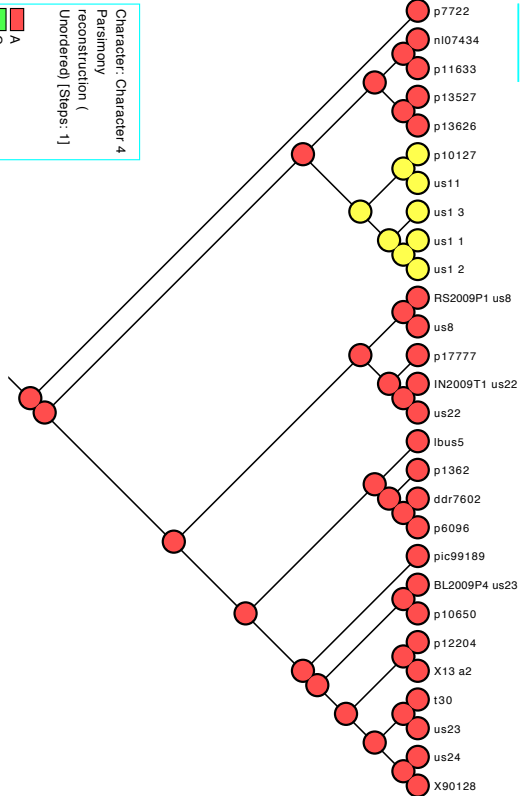
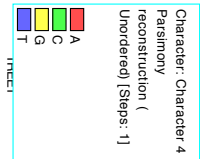
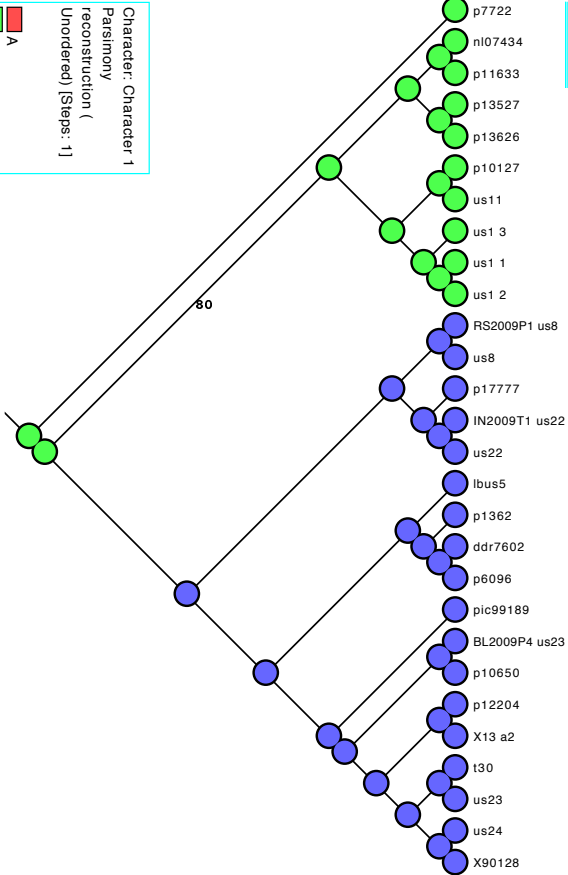
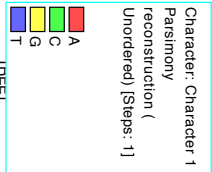
66 Using the whole genome alignment (28 sequences, 39,870 nucleotides) we per-
67 formed a whole-genome phylogeny using maximum likelihood (RAxML) and
68 Bayesian inference (BEAST). We used RAxML using no partitions, 1000 boot-
69 strap replicates, a GTR+I+G model of nucleotide evolution to obtain a biparti-
70 tioned tree with the bootstrap values mapped to the branches. For BEAST,
71 we specified p7722 (*P.mirabilis*) as the outgroup. We used a HKY+G+I model
72 of nucleotide substitutions, a strict molecular clock, a constant population size
73 prior, UPGMA starting tree and 10 million Markov Chains. The best tree is
74 shown in Figure 4.

75 3.2 Mapping the SNP's in the BEAST tree

76 To map the variants found in the mtDNA genome to the coalescent tree, we
77 used Mesquite. We did a removal of invariable regions and ancestral state
78 reconstruction for all 31 SNPs using a parsimony reconstruction state (Figure
79 ??).

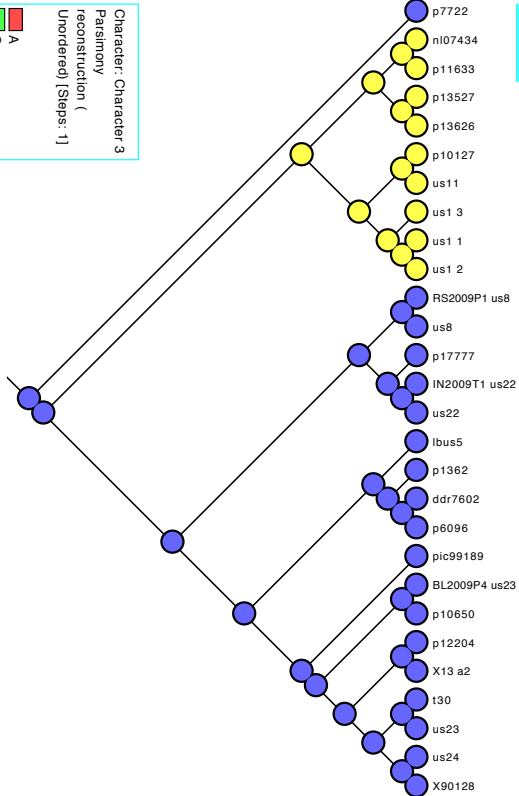
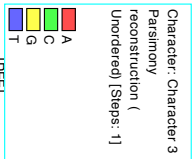


Tree: TREE1



Tree: TREE1

Tree: TREE1



Character: Character 5

Parsimony reconstruction (

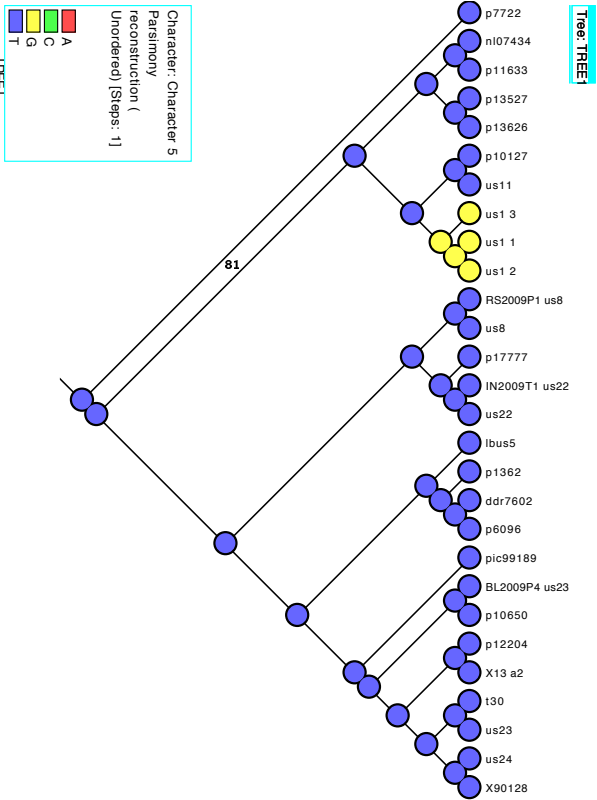
Unordered) (Steps: 1)

A

C

G

T



Character: Character 7

Parsimony reconstruction (

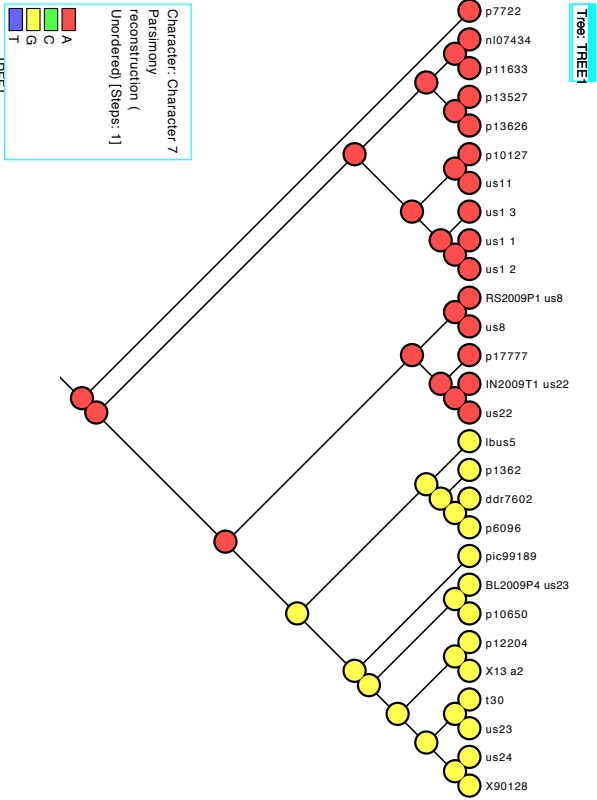
Unordered) (Steps: 1)

A

C

G

T



Character: Character 6

Parsimony reconstruction (

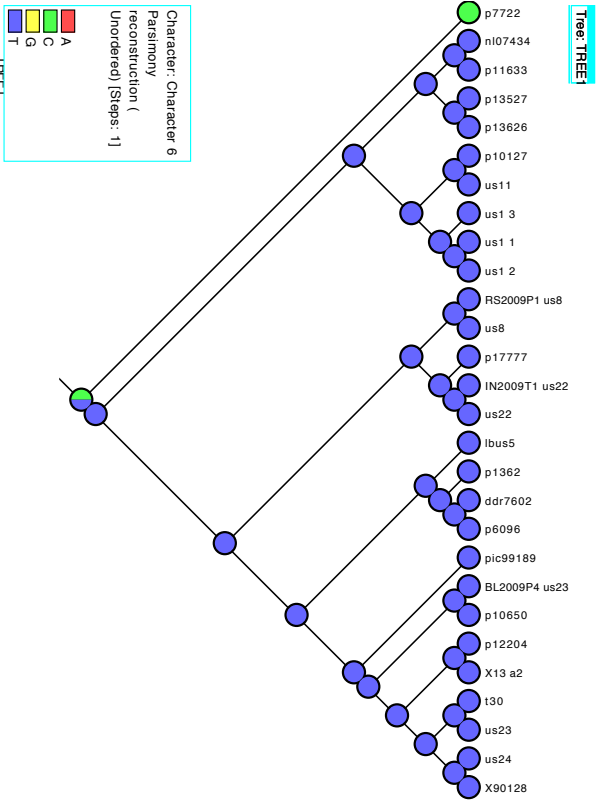
Unordered) (Steps: 1)

A

C

G

T



Character: Character 8

Parsimony reconstruction (

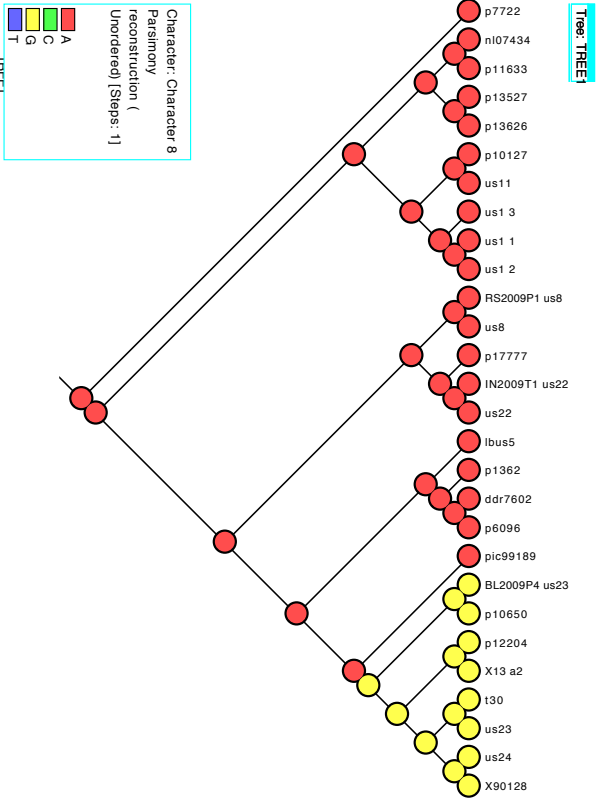
Unordered) (Steps: 1)

A

C

G

T



Character: Character 9

Parsimony reconstruction (

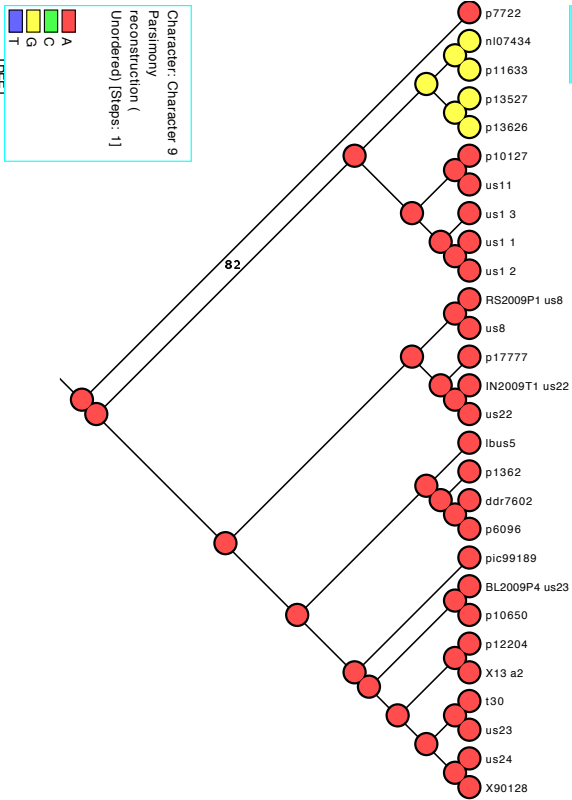
Unordered) [Steps: 1]

A

C

G

T



Character: Character 10

Parsimony reconstruction (

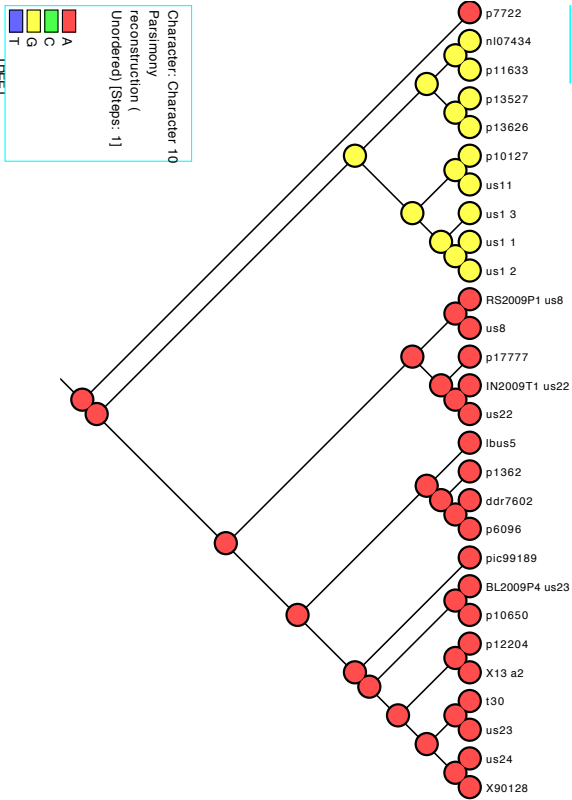
Unordered) [Steps: 1]

A

C

G

T



Character: Character 11

Parsimony reconstruction (

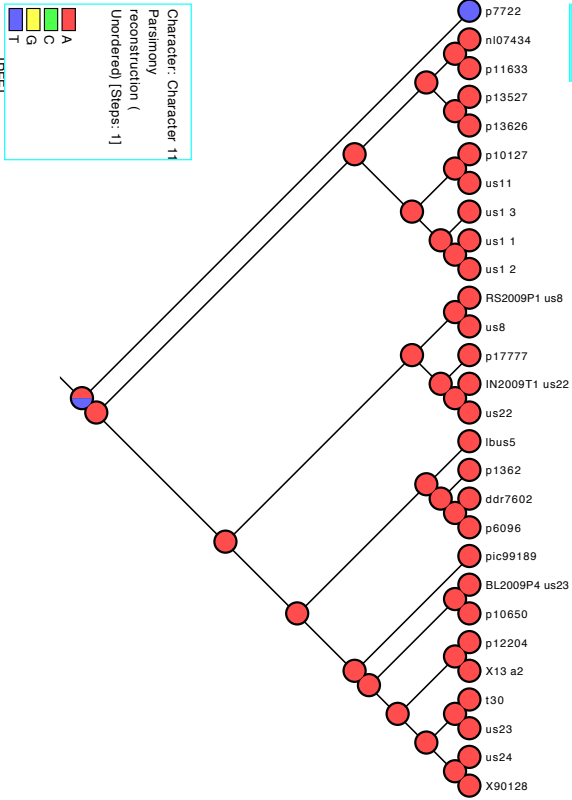
Unordered) [Steps: 1]

A

C

G

T



Character: Character 12

Parsimony reconstruction (

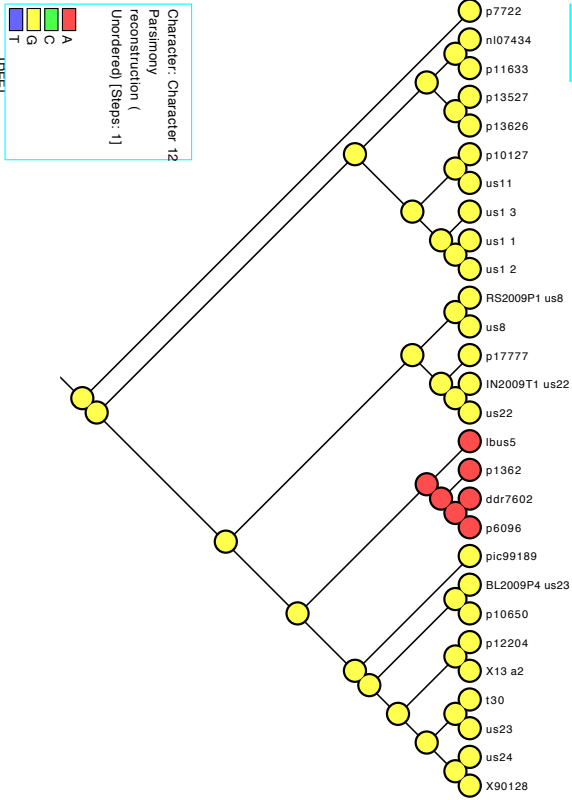
Unordered) [Steps: 1]

A

C

G

T



Character: Character 13

Parsimony reconstruction (

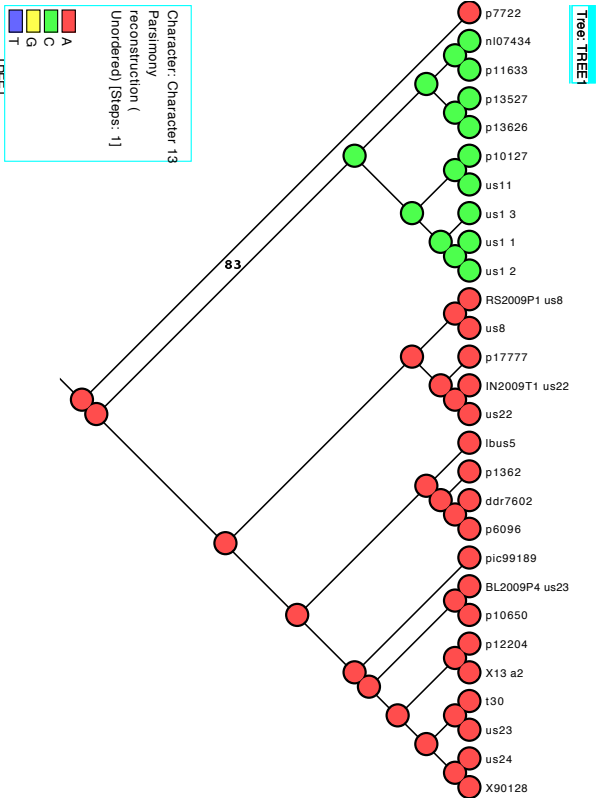
Unordered) (Steps: 1)

A

C

G

T



Character: Character 14

Parsimony reconstruction (

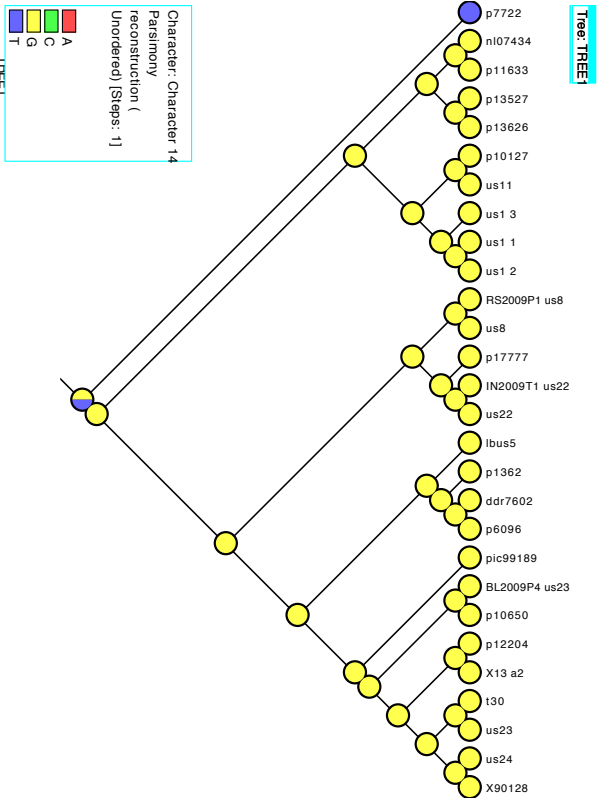
Unordered) (Steps: 1)

A

C

G

T



Character: Character 15

Parsimony reconstruction (

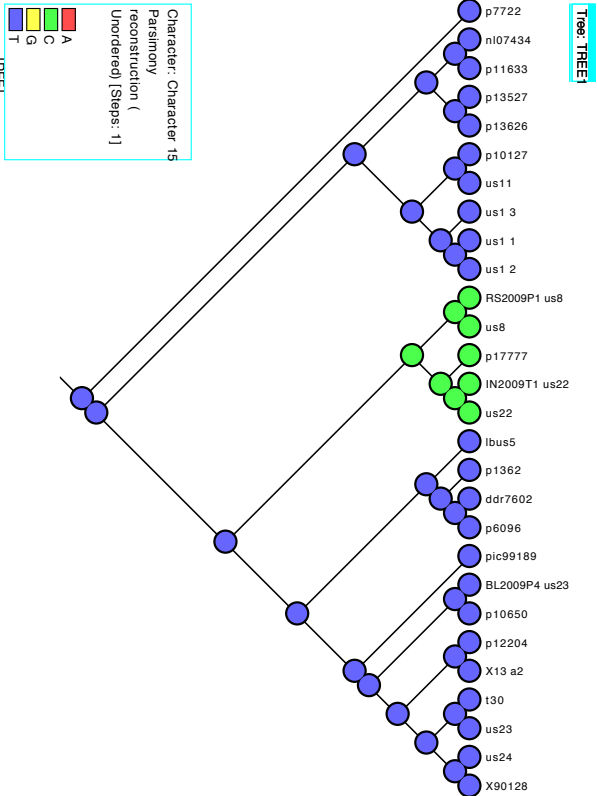
Unordered) (Steps: 1)

A

C

G

T



Character: Character 16

Parsimony reconstruction (

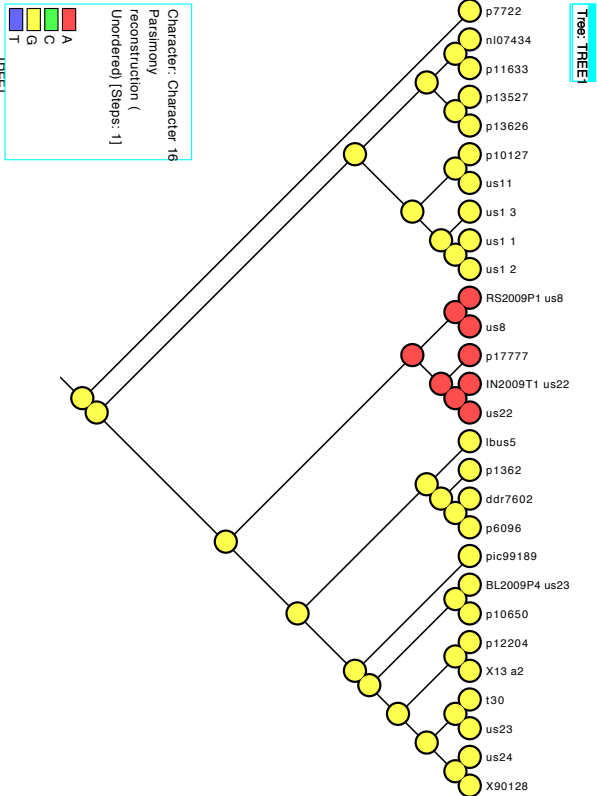
Unordered) (Steps: 1)

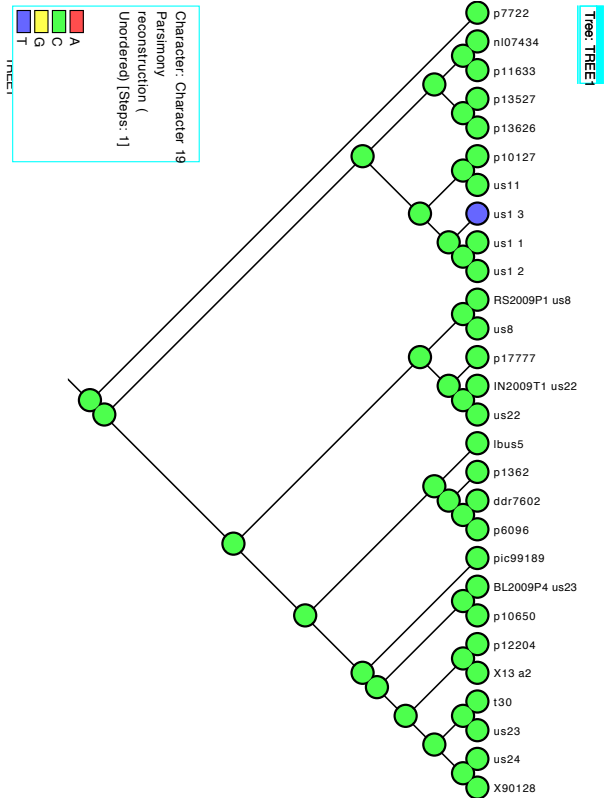
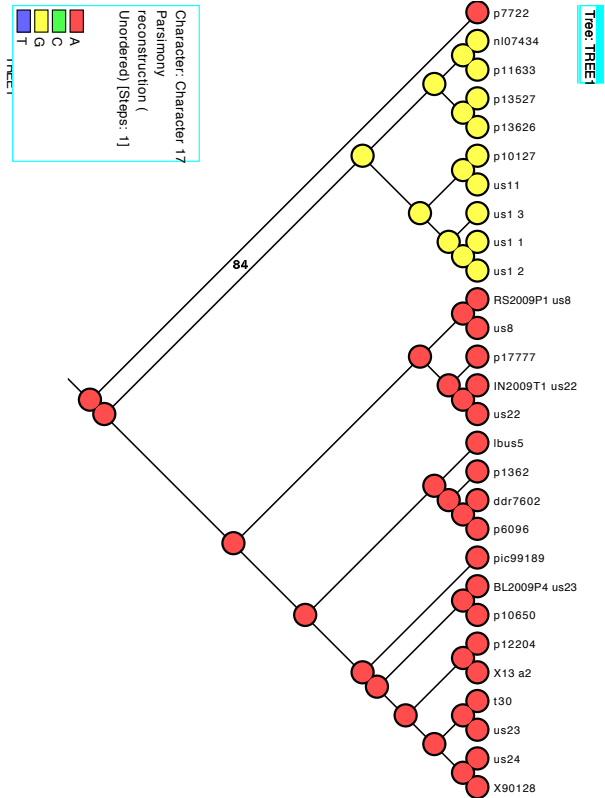
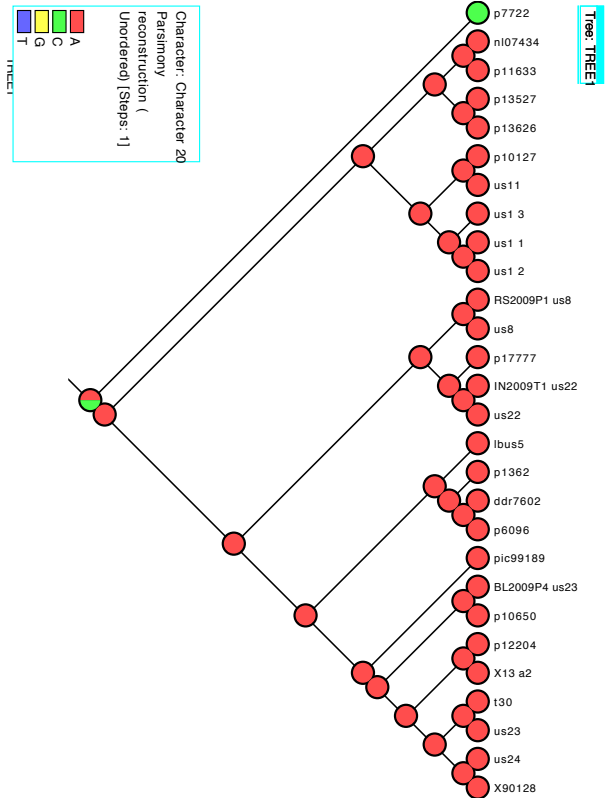
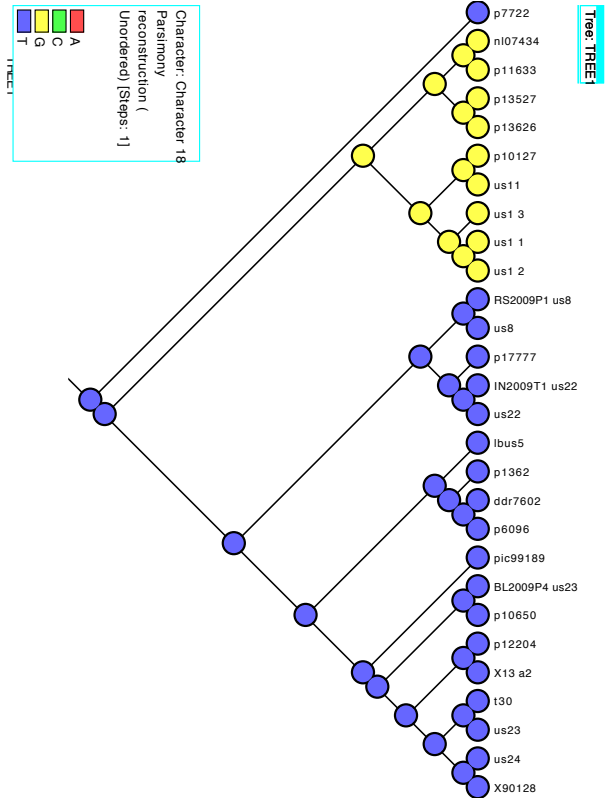
A

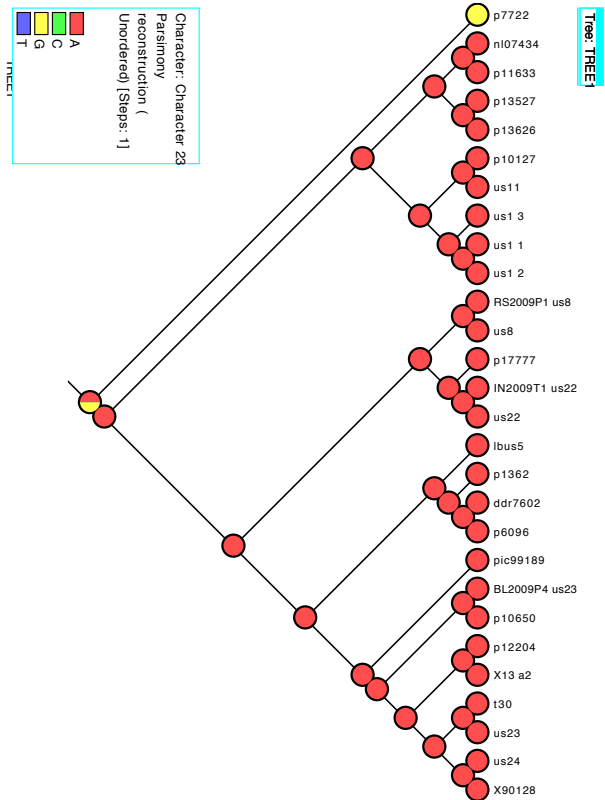
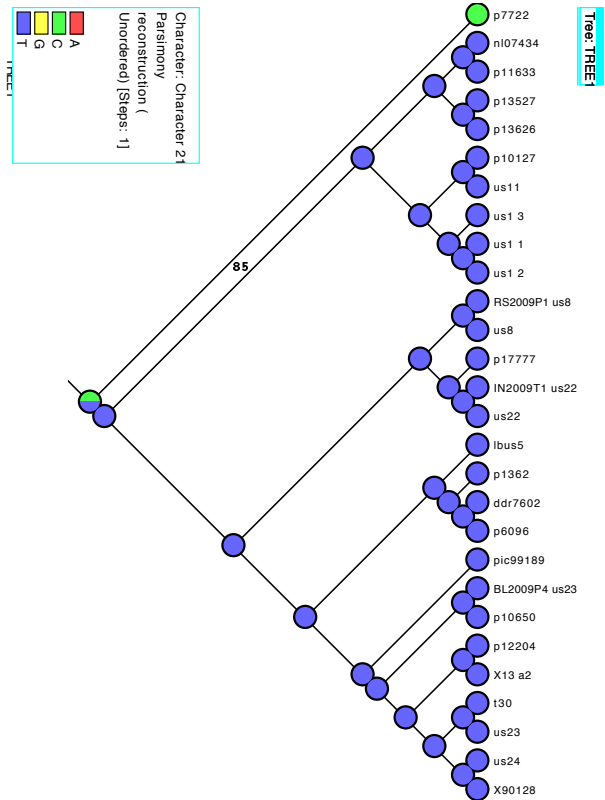
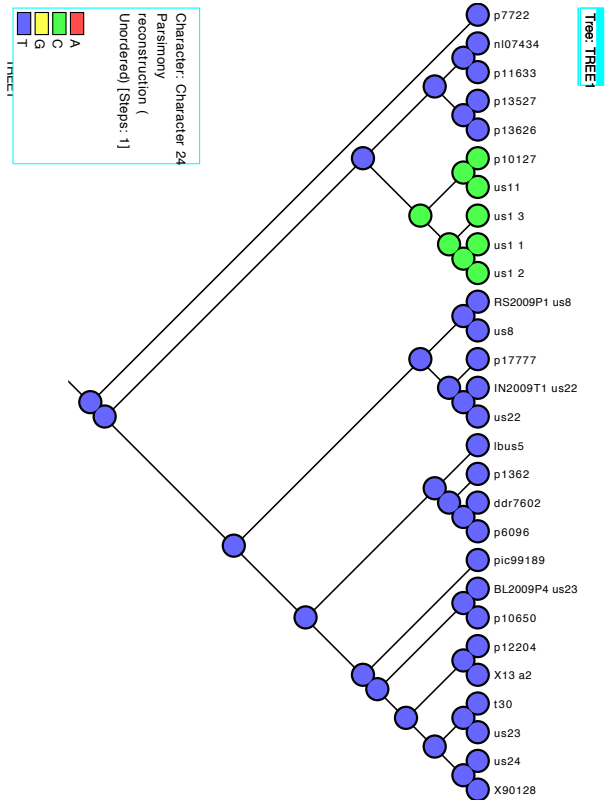
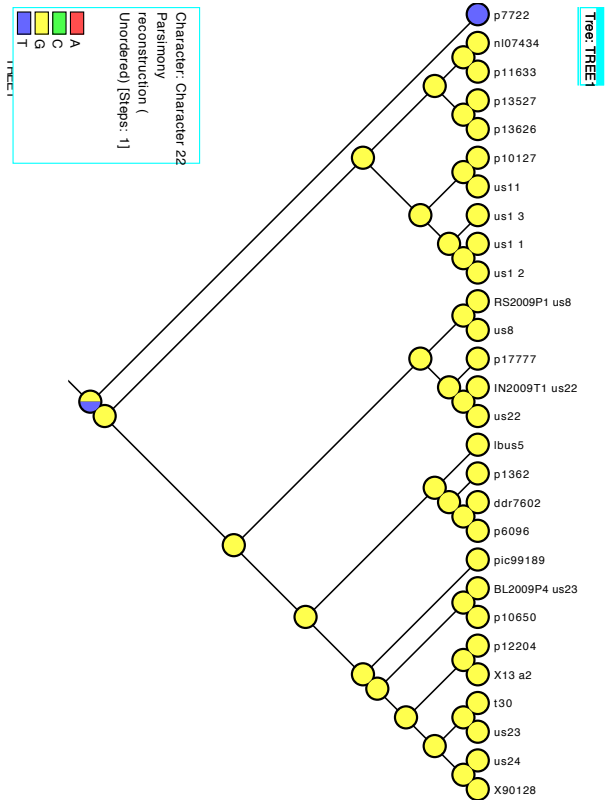
C

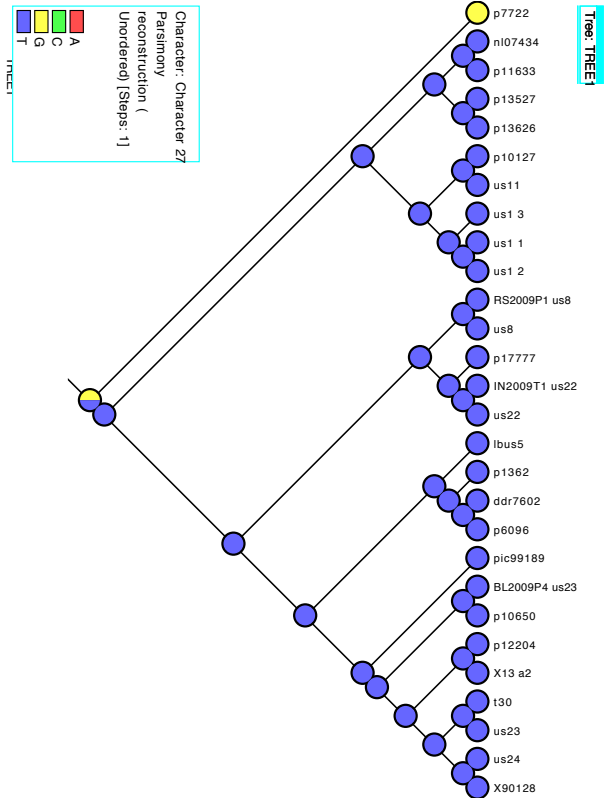
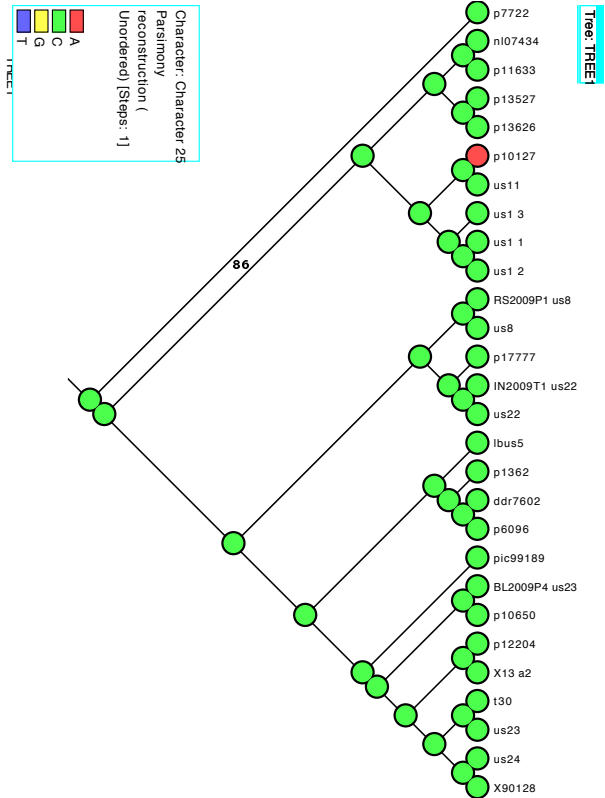
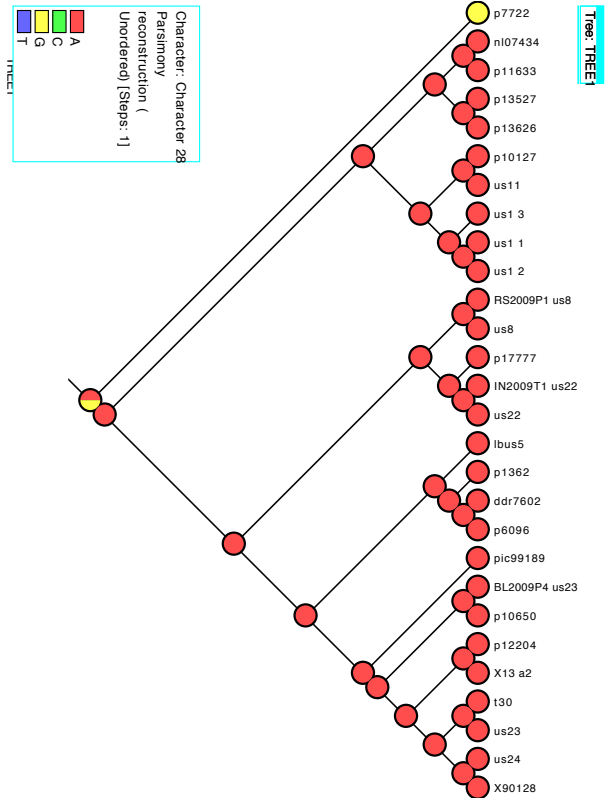
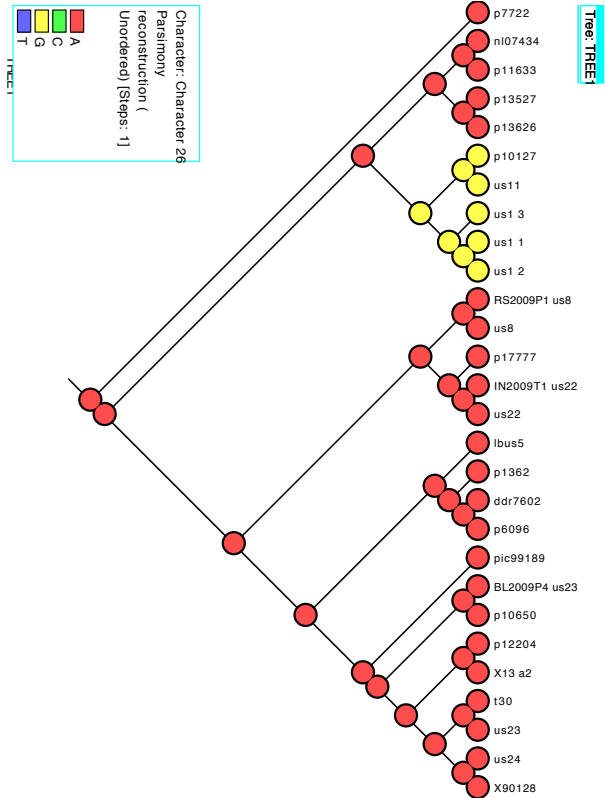
G

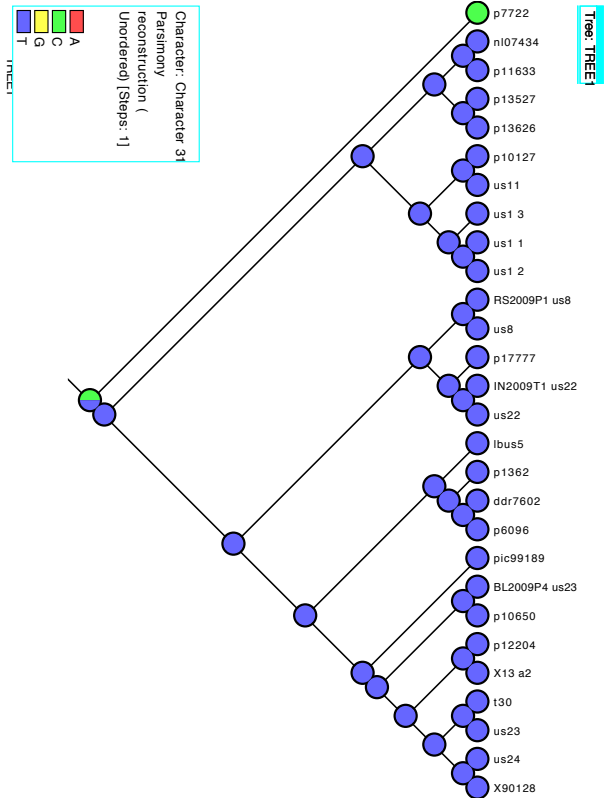
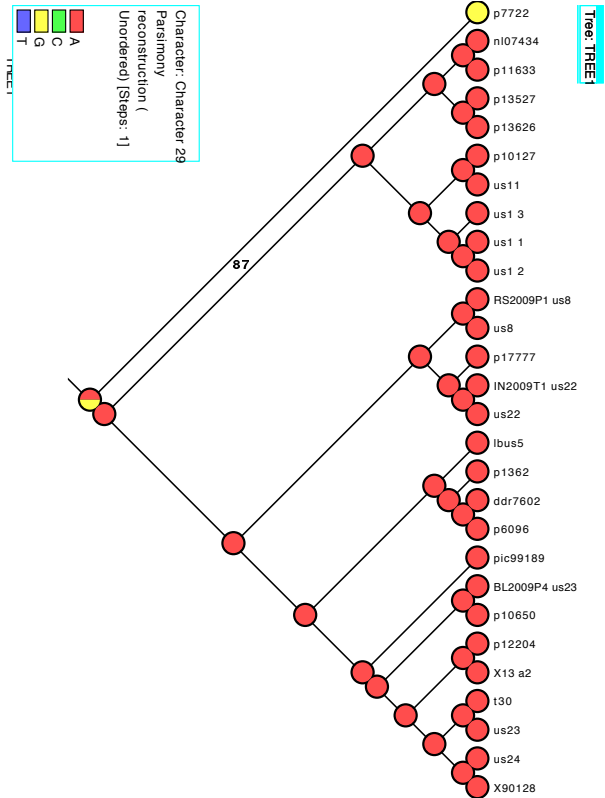
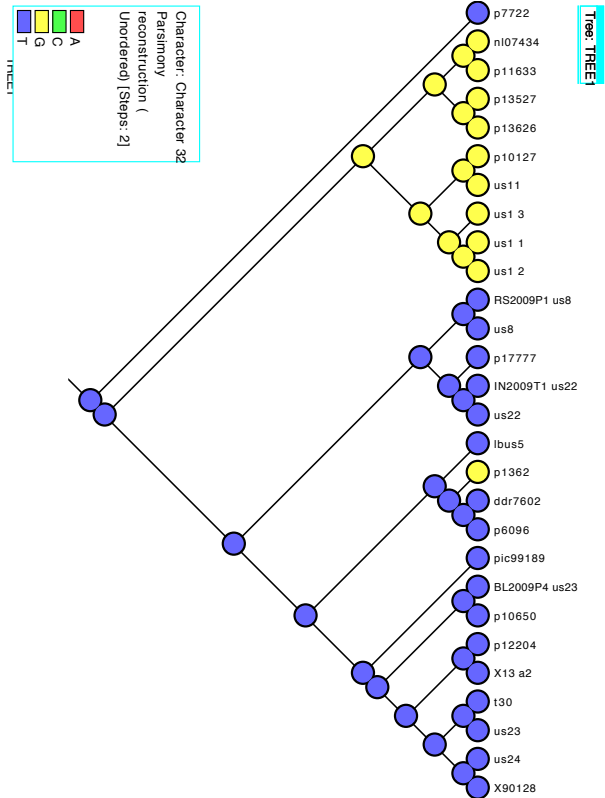
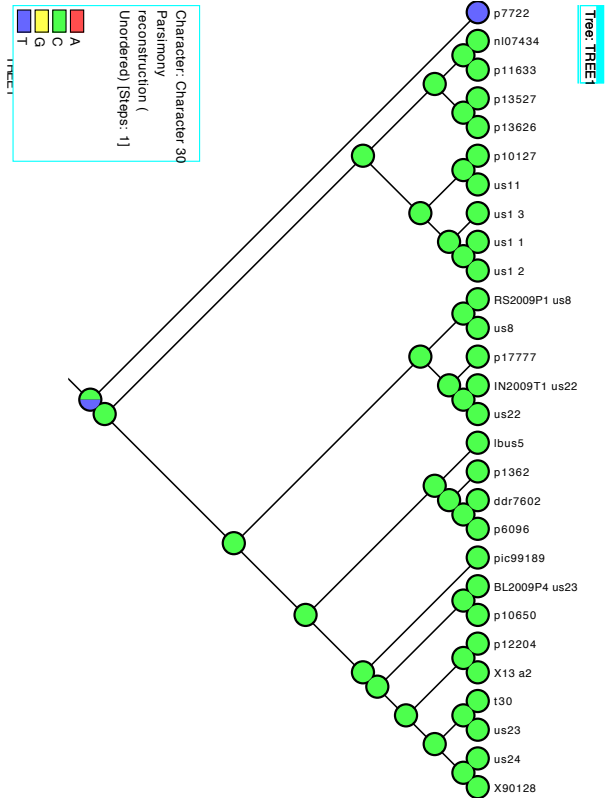
T





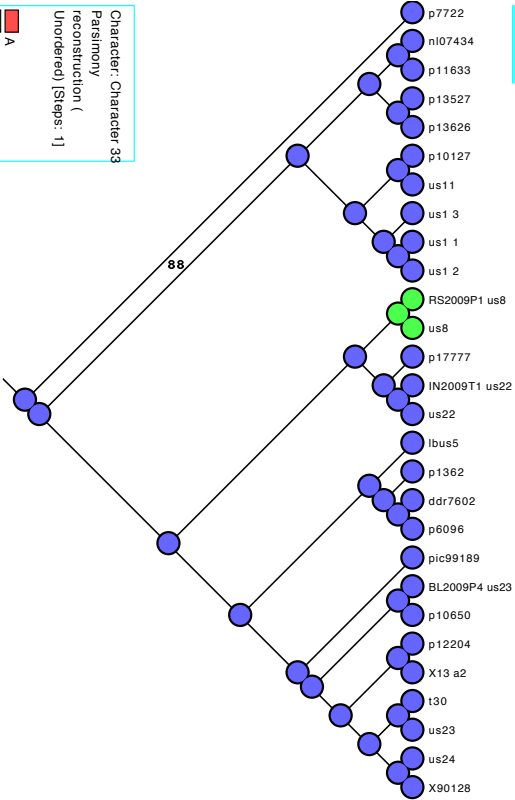






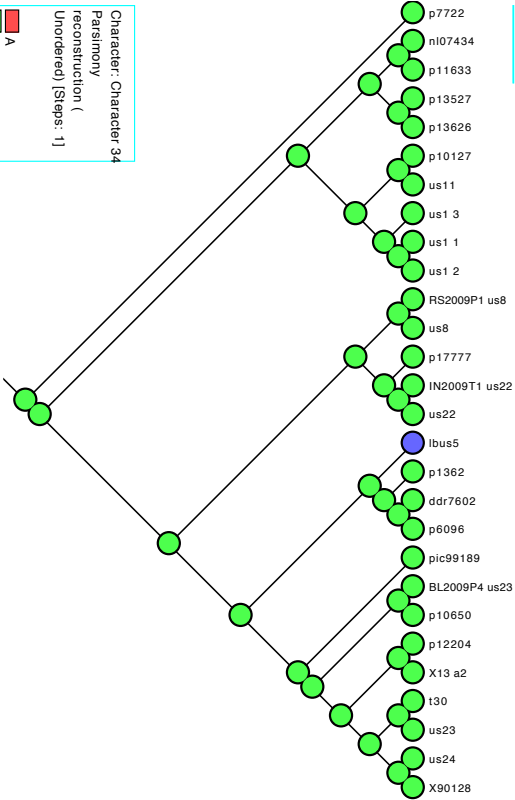
Character: Character 33
Parsimony
reconstruction (Unordered) [Steps: 1]

A
C
G
T



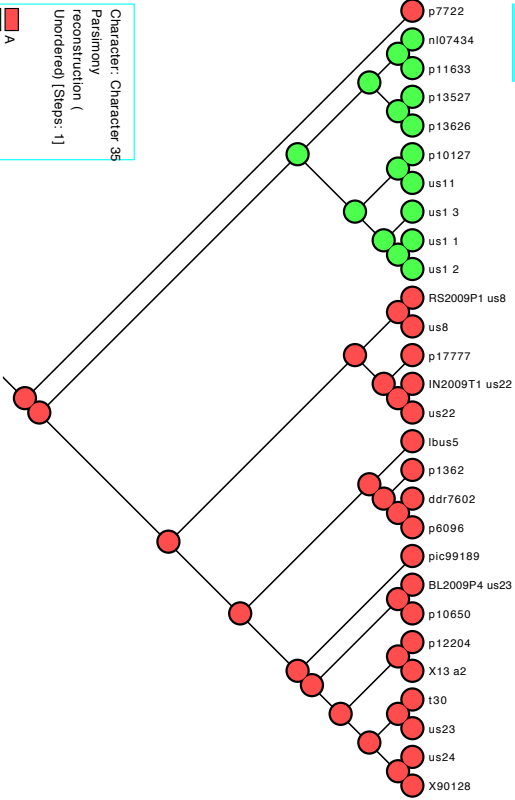
Character: Character 34
Parsimony
reconstruction (Unordered) [Steps: 1]

A
C
G
T



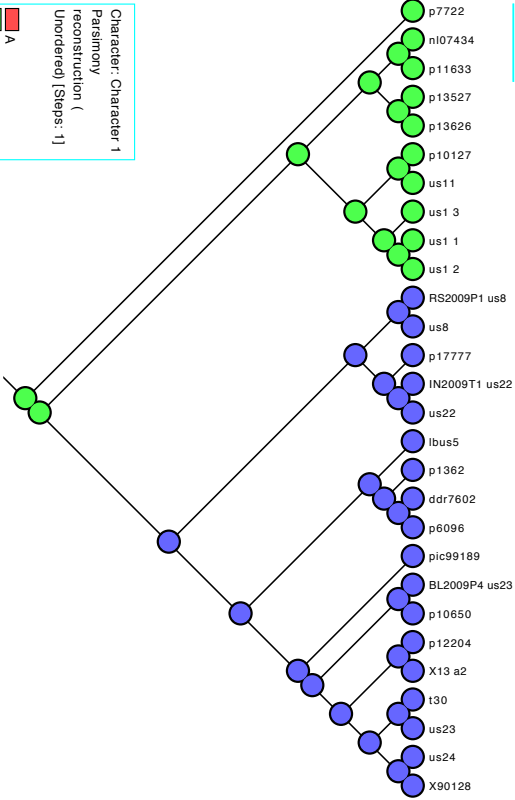
Character: Character 35
Parsimony
reconstruction (Unordered) [Steps: 1]

A
C
G
T



Character: Character 1
Parsimony
reconstruction (Unordered) [Steps: 1]

A
C
G
T



89 4 Session Information

```

sessionInfo()

## R version 3.0.2 (2013-09-25)
## Platform: x86_64-pc-linux-gnu (64-bit)
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
## [1] xtable_1.7-1 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_0.20-24 nlme_3.1-111    stringr_0.6.2   tools_3.0.2

```

90 References

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 115 Orlando, Simon YW Ho, Fred S Dietrich, et al. Reconstructing genome
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 120 ulence factors in the secretome of *Phytophthora infestans*. *BMC genomics*,
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 125 *tophthora infestans* lineage that triggered the Irish potato famine. *eLife*, 2,
 126 2013.

	CHROM	POS	REF	ALT	Tree character
6	Supercontig_1.1	2701	C	T	1
7	Supercontig_1.1	2728	C	A	2
12	Supercontig_1.1	3519	G	T	3
36	Supercontig_1.1	6872	accc	acc	4
42	Supercontig_1.1	7857	A	G	5
44	Supercontig_1.1	8118	T	G	6
59	Supercontig_1.1	9857	T	C	7
62	Supercontig_1.1	10146	A	G	8
85	Supercontig_1.1	13684	A	G	9
106	Supercontig_1.1	15563	G	A	10
114	Supercontig_1.1	16986	G	A	11
119	Supercontig_1.1	18440	A	T	12
123	Supercontig_1.1	19793	G	A	13
124	Supercontig_1.1	20005	C	A	14
125	Supercontig_1.1	20290	G	T	15
128	Supercontig_1.1	20464	T	C	16
141	Supercontig_1.1	22711	G	A	17
147	Supercontig_1.1	23431	G	A	18
150	Supercontig_1.1	23872	G	T	19
154	Supercontig_1.1	24611	C	T	20
159	Supercontig_1.1	25142	A	C	21
160	Supercontig_1.1	25204	T	C	22
161	Supercontig_1.1	25237	G	T	23
169	Supercontig_1.1	26684	A	G	24
171	Supercontig_1.1	26767	T	C	25
186	Supercontig_1.1	28680	C	A	26
187	Supercontig_1.1	28783	A	G	27
192	Supercontig_1.1	30427	T	G	28
193	Supercontig_1.1	30552	A	G	29
194	Supercontig_1.1	30591	A	G	30
195	Supercontig_1.1	30660	C	T	31
201	Supercontig_1.1	31403	T	C	32
221	Supercontig_1.1	35296	G	T	33
222	Supercontig_1.1	35367	taaaaaaaaaaaa	taaaaaaaaaaaa	34
233	Supercontig_1.1	36663	T	C	35
239	Supercontig_1.1	37562	C	T	36
242	Supercontig_1.1	38345	C	A	37

Table 1: Variants remaining after filtering.

Table 2: 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