

Identification of *Phytophthora infestans* US-8 lineage through genetic markers

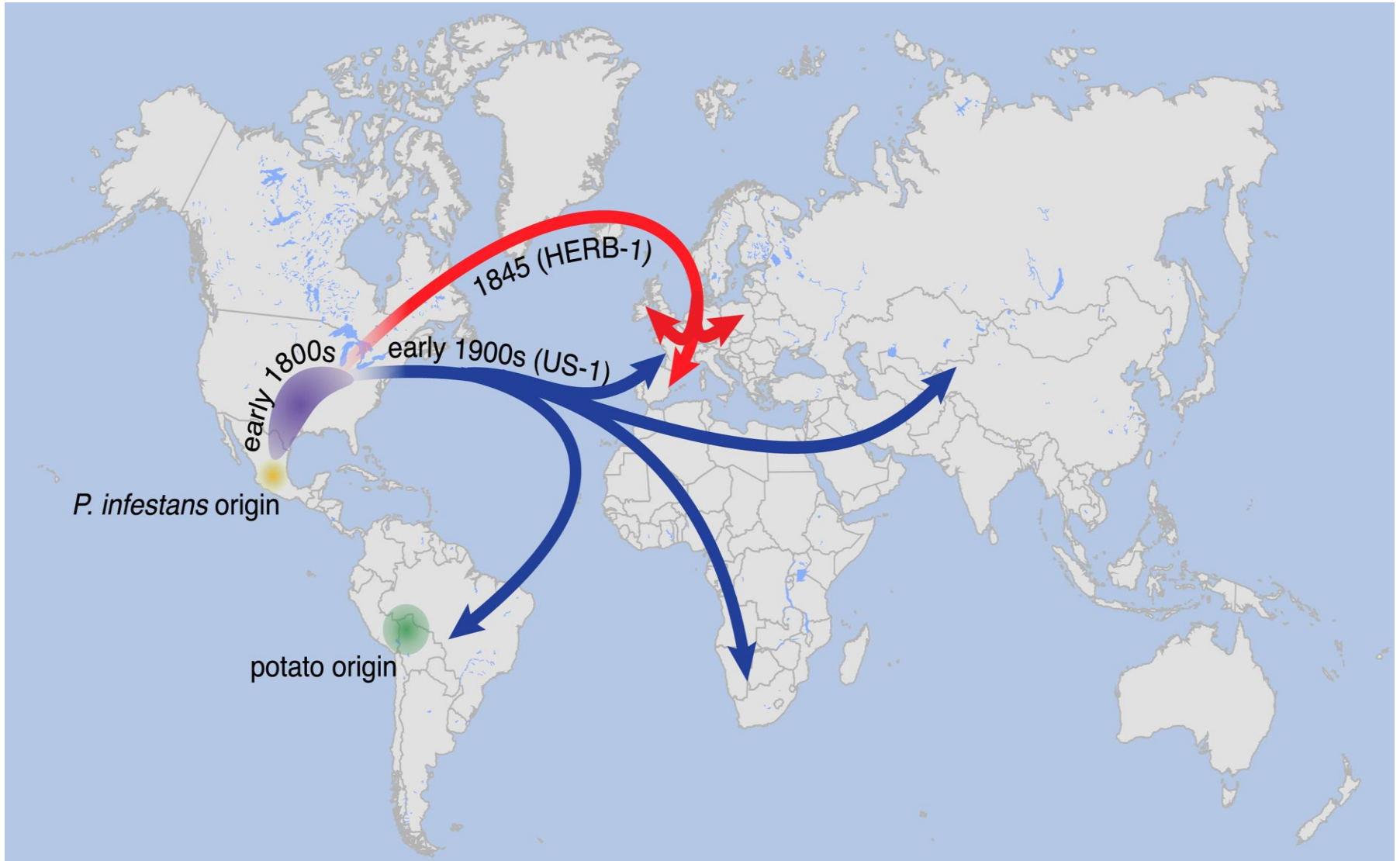


Eric Fritz, Dept. Botany and Plant Pathology, OSU
Dr. Brian Knaus, USDA ARS

Phytophthora infestans: bad spore

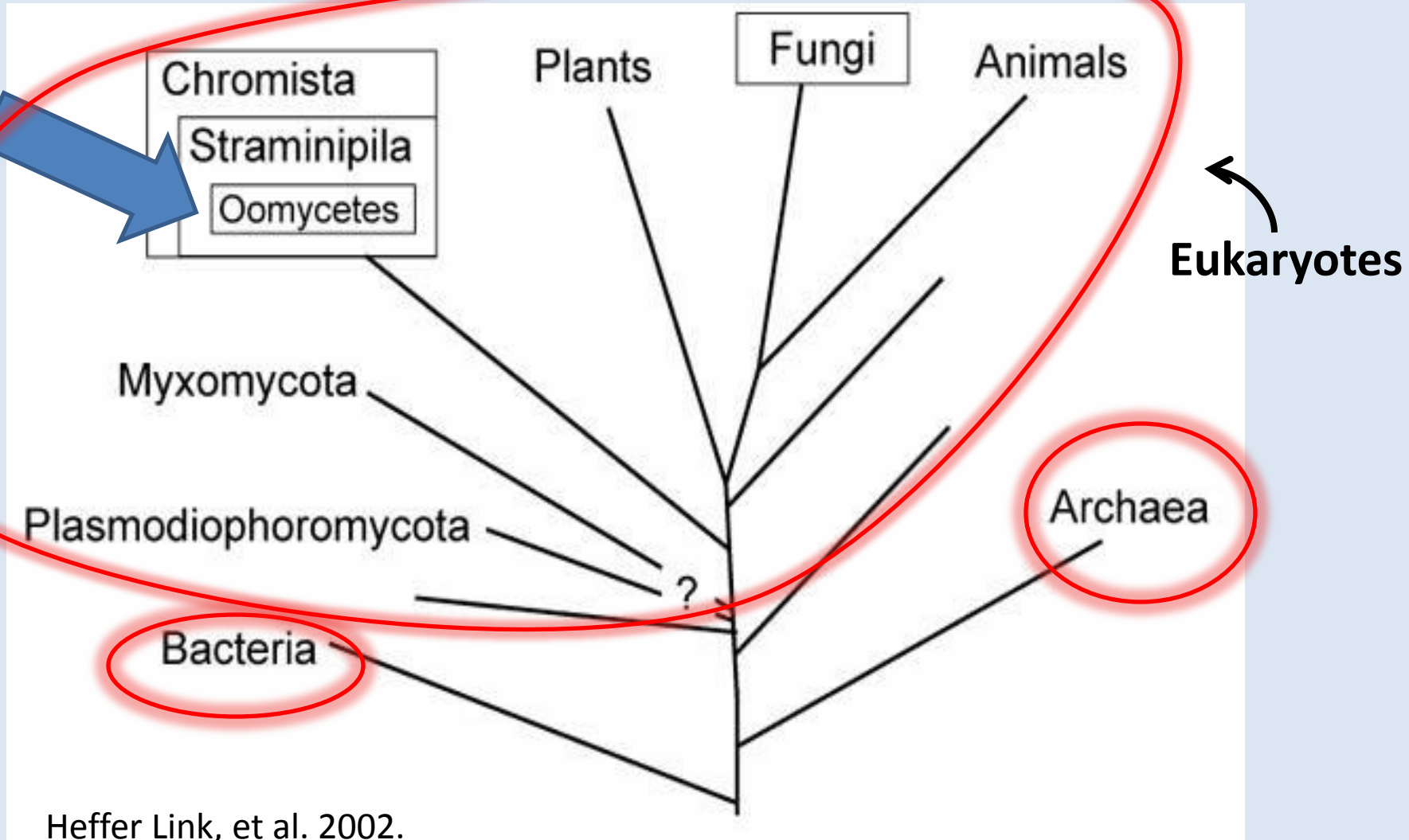
- Results in annual loss of potatoes that would be enough to feed more than 80 million people (Fisher et al., 2012)
- Certain strains, US-8 for example, are known to resist fungicide treatment (Daniels et al., 2013)
- Encysted zoospores and oospores can stay dormant for long periods of time
- 50+ known species of *Phytophthora* affects a wide plant base including: tomatoes, raspberries, cacao, *Rhododendron*, and juniper trees (Kroon, Laurens et al., 2012)

P. infestans global spread

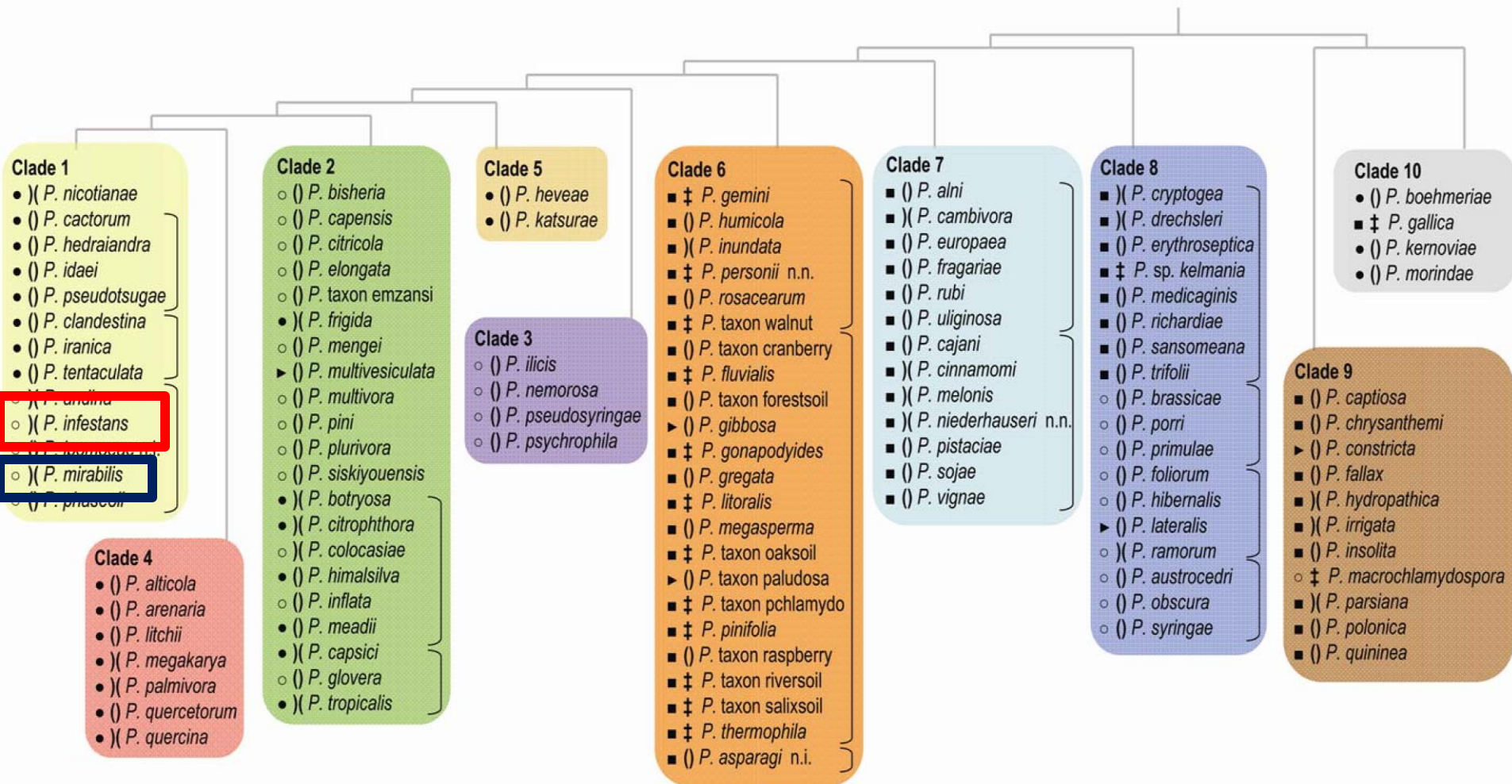


Yoshida, K. et al. 2013.

Where is *Phytophthora* on the Tree of Life? Oomycetes are not 'true' fungi



“Families” of genus *Phytophthora*



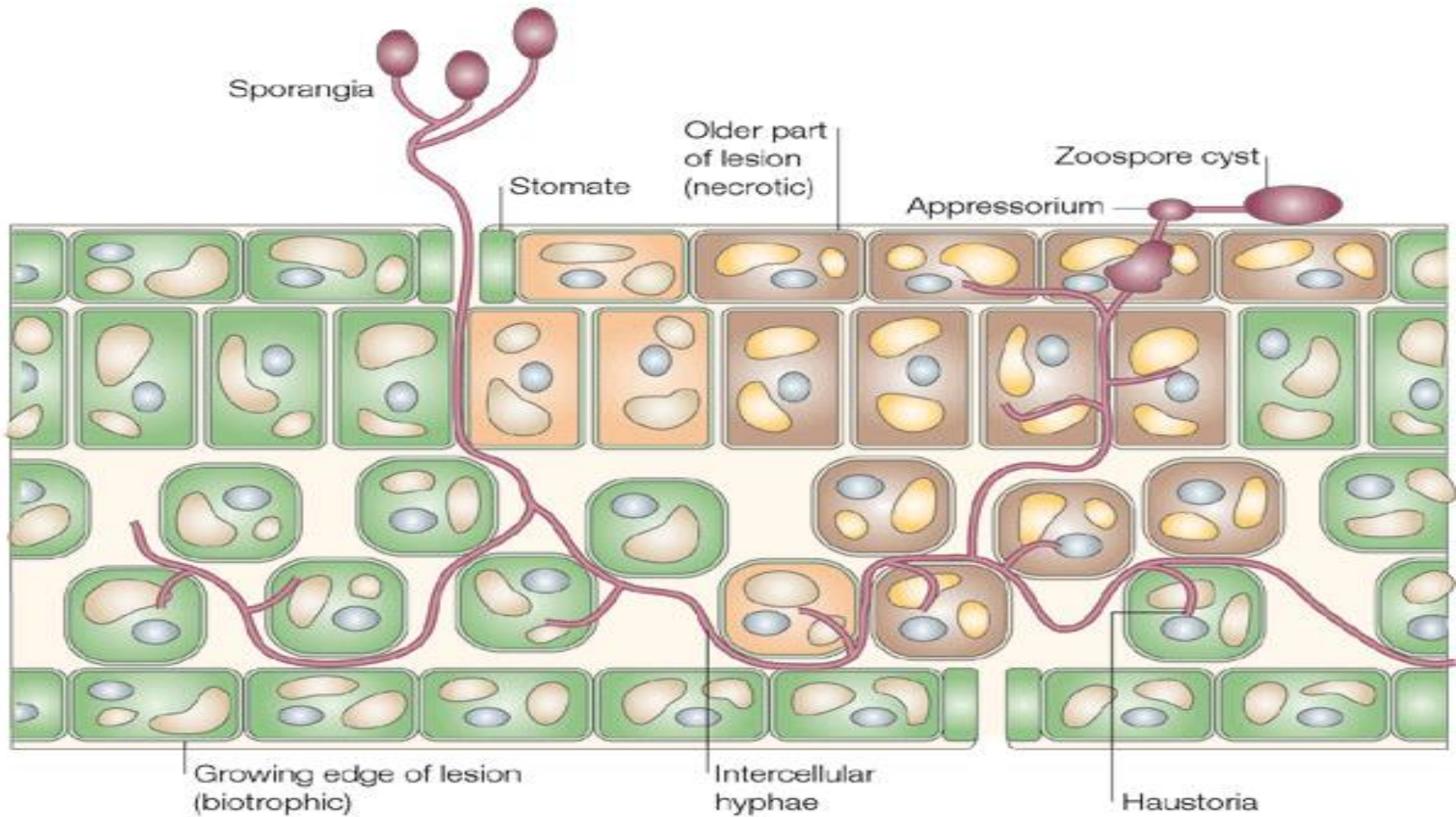
Kroon, et al. 2012.

What is *Phytophthora infestans*?

- Name translates to “plant destroyer”
- Reproduces sexually and asexually



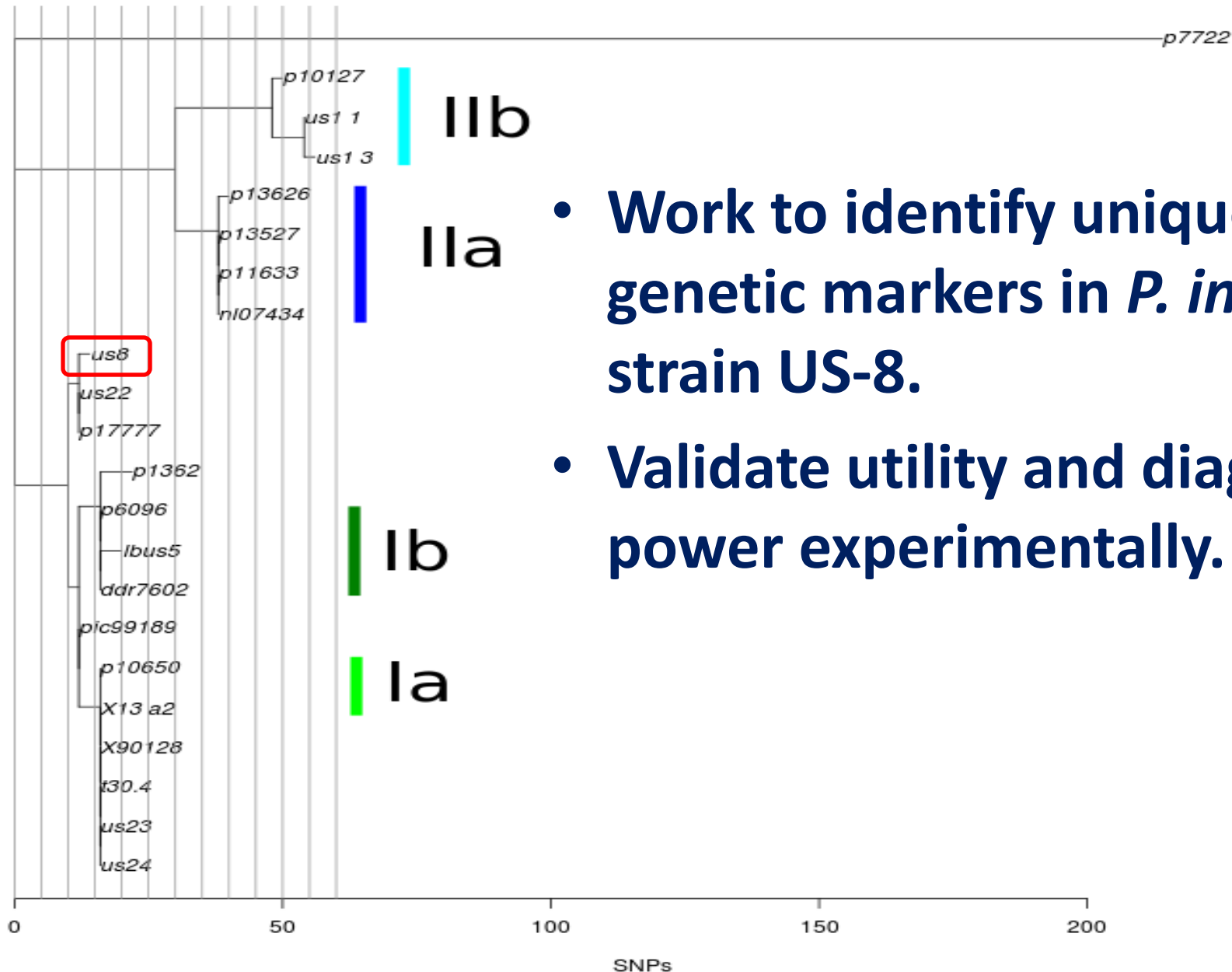
How does *P. Infestans* infect plants?



Nature Reviews | Microbiology

H.S. Judelson & F.A. Blanco. 2005. The spores of *Phytophthora*: weapons of the plant destroyer. *Nature Reviews Microbiology* 3, 47-58 (January 2005)doi:10.1038/nrmicro1064

Goals to accomplish



- Work to identify unique genetic markers in *P. infestans* strain US-8.
- Validate utility and diagnostic power experimentally.

Strategy

Primer Design

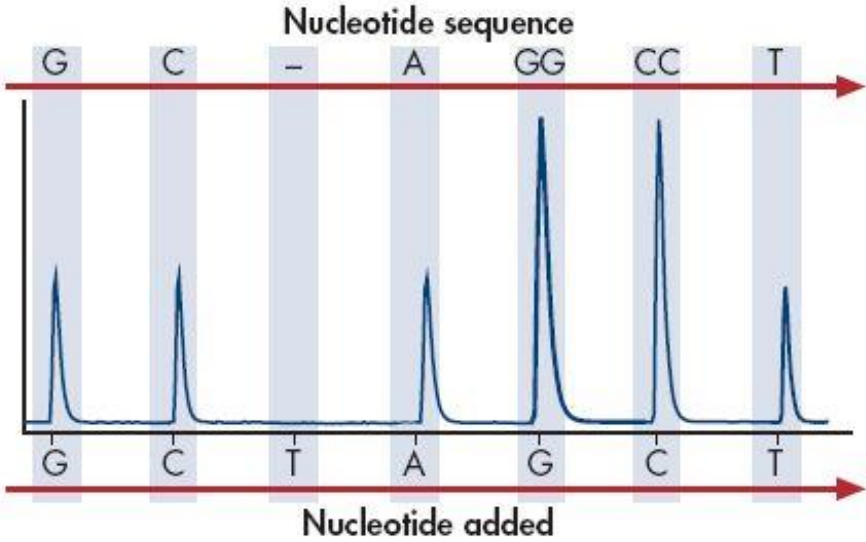
Bioinformatics

Molecular techniques

Pyrosequencing

Amplified DNA product

Analyze Results





Bioinformatics

Bioinformatics:

Cortex¹ assembly or bowtie2²
alignment with SAMtools³
variant discovery.

Global sample of 22 genomes
(Illumina and 454 technologies)



Mitochondrial SNPs

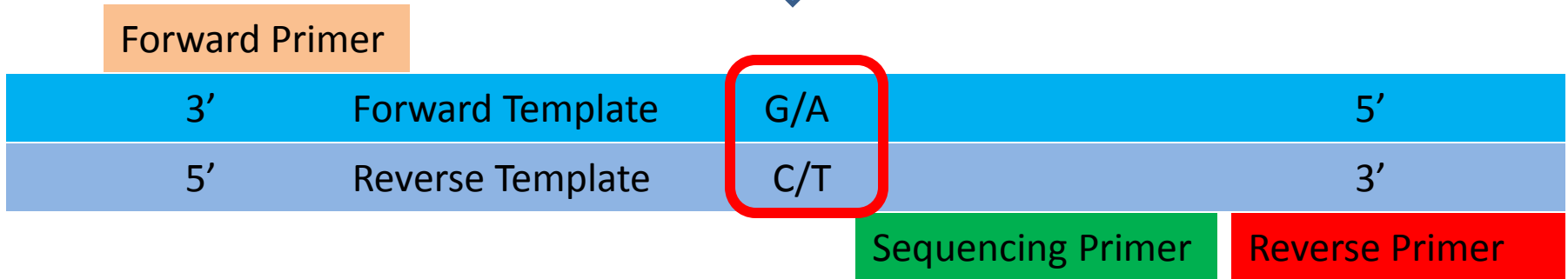
Nuclear SNPs

Design primers around
target area with Batch
Primer3 and Pyromark™
software

¹Iqbal et al. 2012; ²Langmead and Salzberg. 2012; ³Li et al. 2009.

Primer Design for Supercontig 1.1 15563

atacaatataattgattatttgtatcttttttaaatgaggttctctttgattaattatagtatcaatagcaatagaagttttccggtttgt
 ctatctccaataattaattccctttgtcctctaccgataggaattaaactatctad **G/A**gcttttaaacctgtttgtacaggttctttaac
 acttttcttaggcataataaccaggagctttaacttctactctacttttctaatttactattaatttgacctttaccgtcaataggttgtccta
 aagcatctactacttacctaact



Primer Set 1.1 15563			Score: 91 Quality: High		
Primer	Id	Sequence	Nt	Tm, °C	%GC
PCR	FOR	TTTCCGGTTTGTCTATCTCCAAT	23	69.8	39.1
PCR	REV	ATGCTTTAGGACAACCTATTGACG	24	69.5	41.7
Sequencing	SEQ	CTGTACAAACAGGTTTAAAA	20	50.0	30.0

Molecular techniques

1



Suspension of Primers



Biotinylate forward and reverse primers using Qiagen PCR protocol



Run gel electrophoresis to high grade selection process for pyrosequencing



Pyromark assay setup and run



Pyrosequencing

Results

Mitochondrial DNA

- 8 SNPs identified
- Primer sets designed
- PCR & Gel electrophoresis run
- 1 SNP pyrosequenced across 8 *P. infestans* lineages.
- Polymorphism was sequenced successfully, but was identical among the 8 lineages of *P. infestans*.

Nuclear DNA

- 4 SNPs identified
- Primer sets designed
- PCR & Gel electrophoresis run
- 2 SNPs run through pyrosequencer across 7 *P. infestans* lineages
- Samples were sequenced using the Sanger Method.

Assessment and Next Steps

- Validated method to bioinformatically call SNPs through pyrosequencing
- Pyrosequencing first step in validating larger SNP panels throughout the genome of *P. infestans*
- Continued work to identify and validate SNPs from nuclear and mitochondrial DNA of *P. infestans* lineages including US-8

Thanks

- Dr. Brian Knaus and Dr. Sydney Everhart
- Dr. Nik Grünwald and the Grünwald lab
 - Meg Lawson and Val Fieland
- Dr. Kevin Ahern
- Dr. Howard Judelson (UCR)

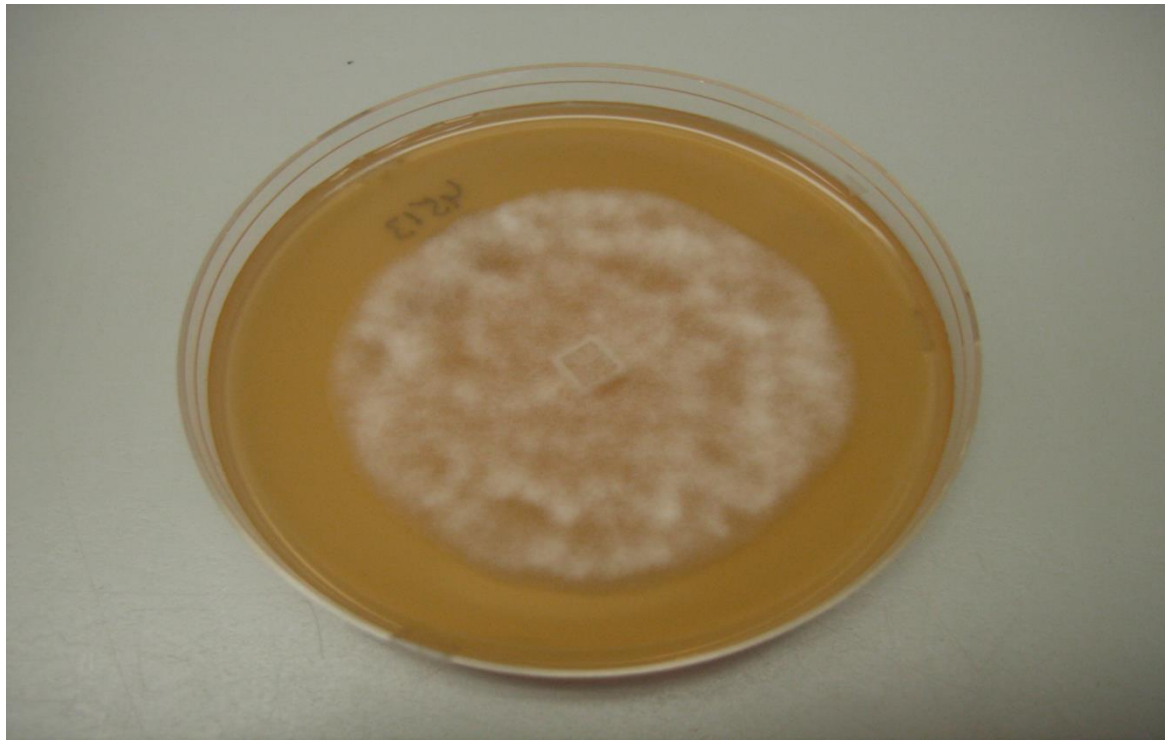


• Class: Oomycetes

• Order: Peronosporales

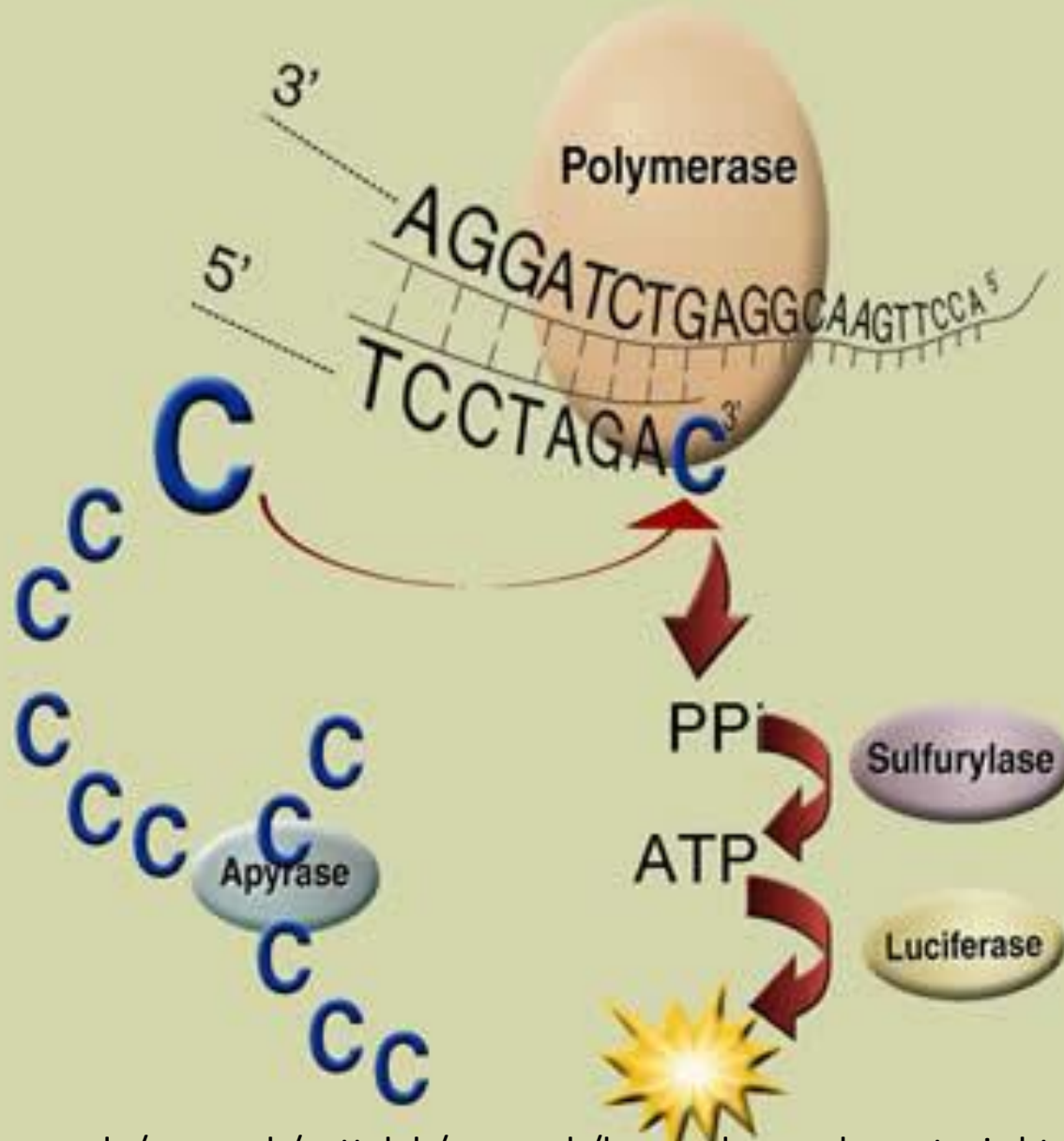
• Family: Pythiaceae

• Genus: *Phytophthora*



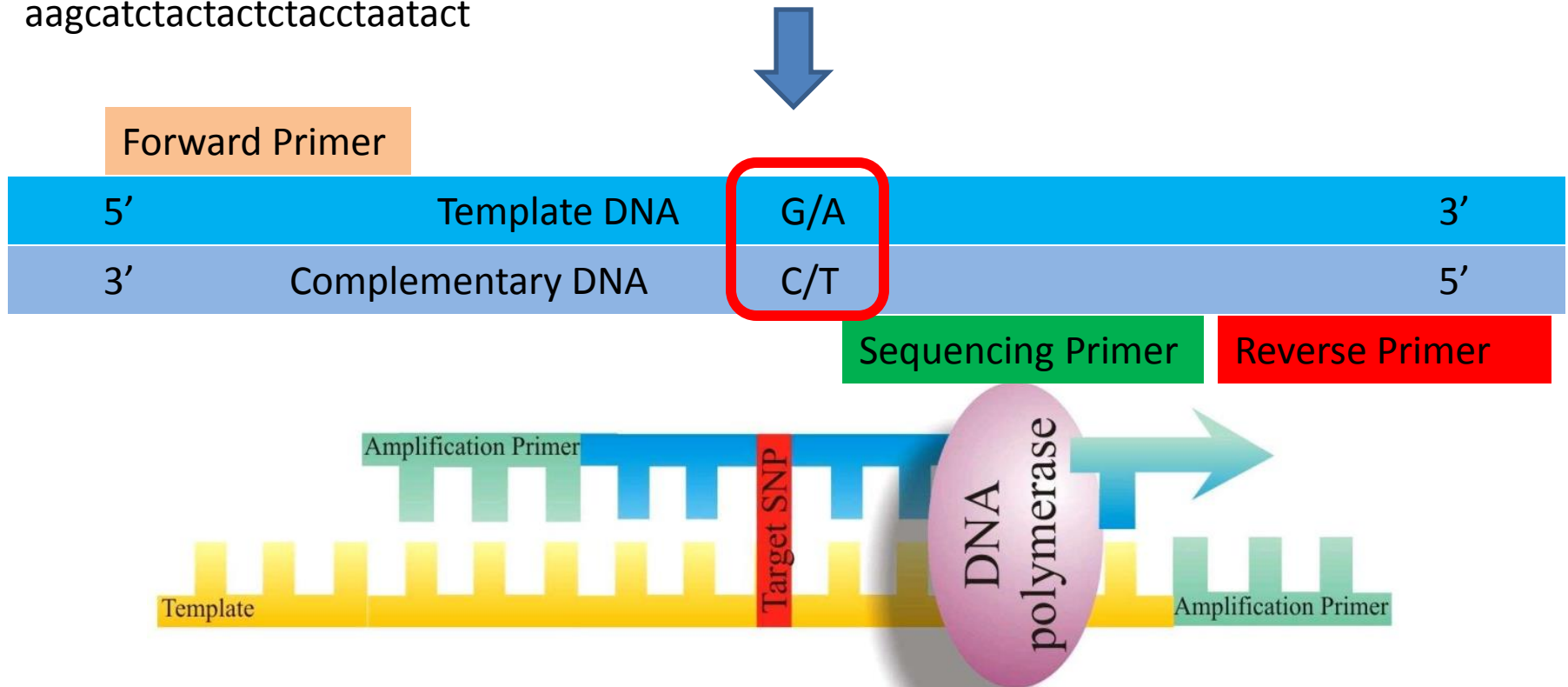
P. plurivora

The Pyrosequencing Method



Primer set-up for Supercontig_1.1_15563

atacaatataattgattatttgtatcttttttaaatgaggttctctttgattaattatagtatcaatagcaatagaagttttccggttgt
ctatctccaataattaattcccttggctctaccgataggaattaaactatctacG/Agcttttaaacctgttgtacaggttctttaac
actttctctaggcataataccaggagctttaacttctactctactttctaatttactattaattgacctttaccgtcaataggttgccta
aagcatctactactacctaact



Amplification of target region

1.43 US-8 nuclear data

A3: GTTARGGGAGTCCTAAGCGAGCGTTCCAAGC

