



Research presentation 1

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Project 1

- *Streptococcus pneumoniae* (pneumococcus)
- Most common bacterial cause of acute respiratory infection and otitis media
- >3 million deaths in children per year from pneumonia, bacteremia or meningitis
- Even more deaths among elderly persons from pneumonia and meningitis
- Increased resistance to antibiotics



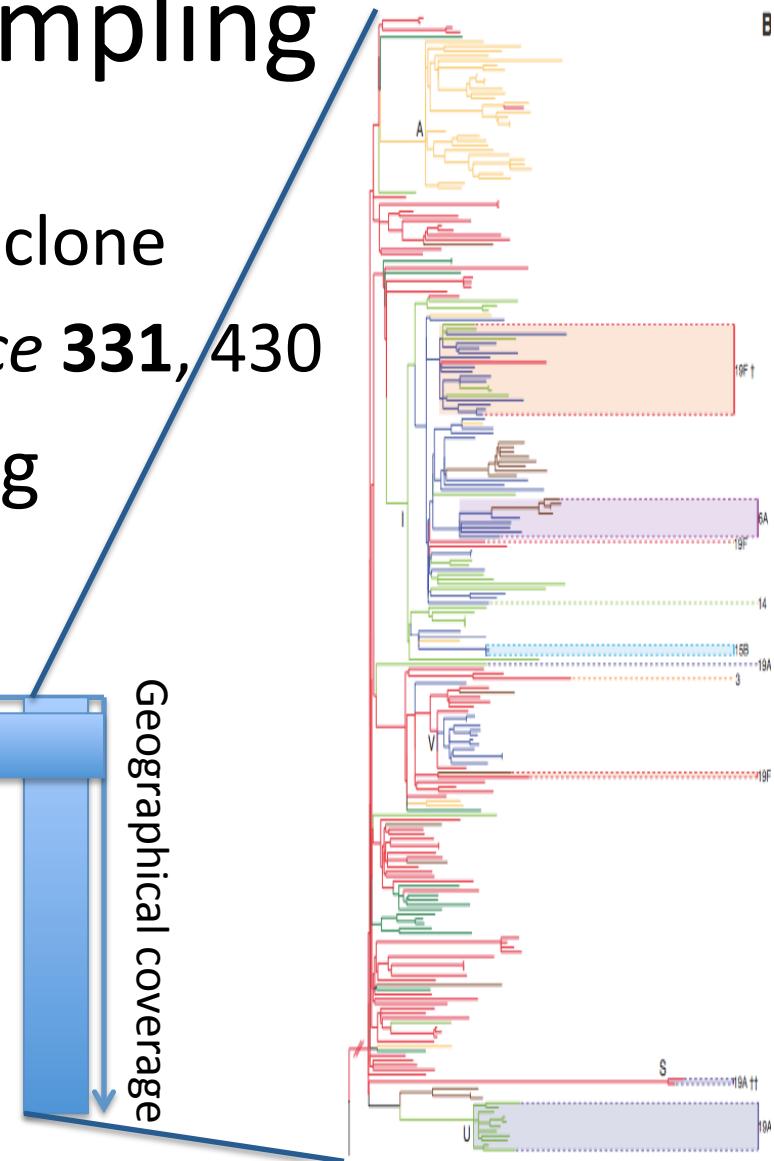
Population sampling

- Worldwide survey
 - PMEN1 multi-drug resistant clone
 - Croucher *et al.* (2011) *Science* **331**, 430
- Local study – Deep sampling
- Time – Third dimension

Genotype diversity

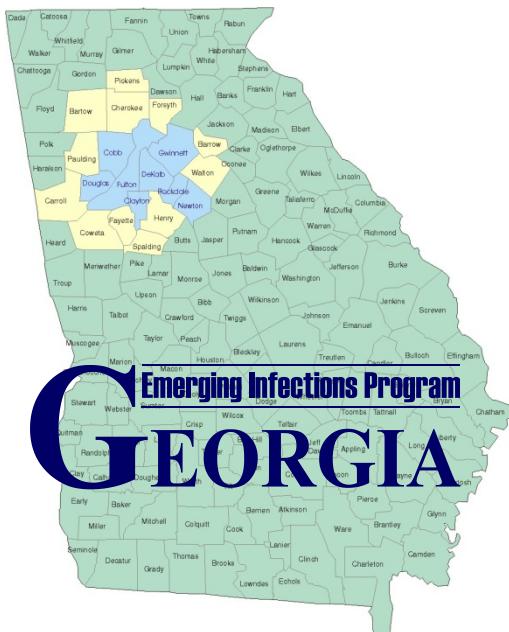


Geographical coverage





Georgia Emerging Infections Program

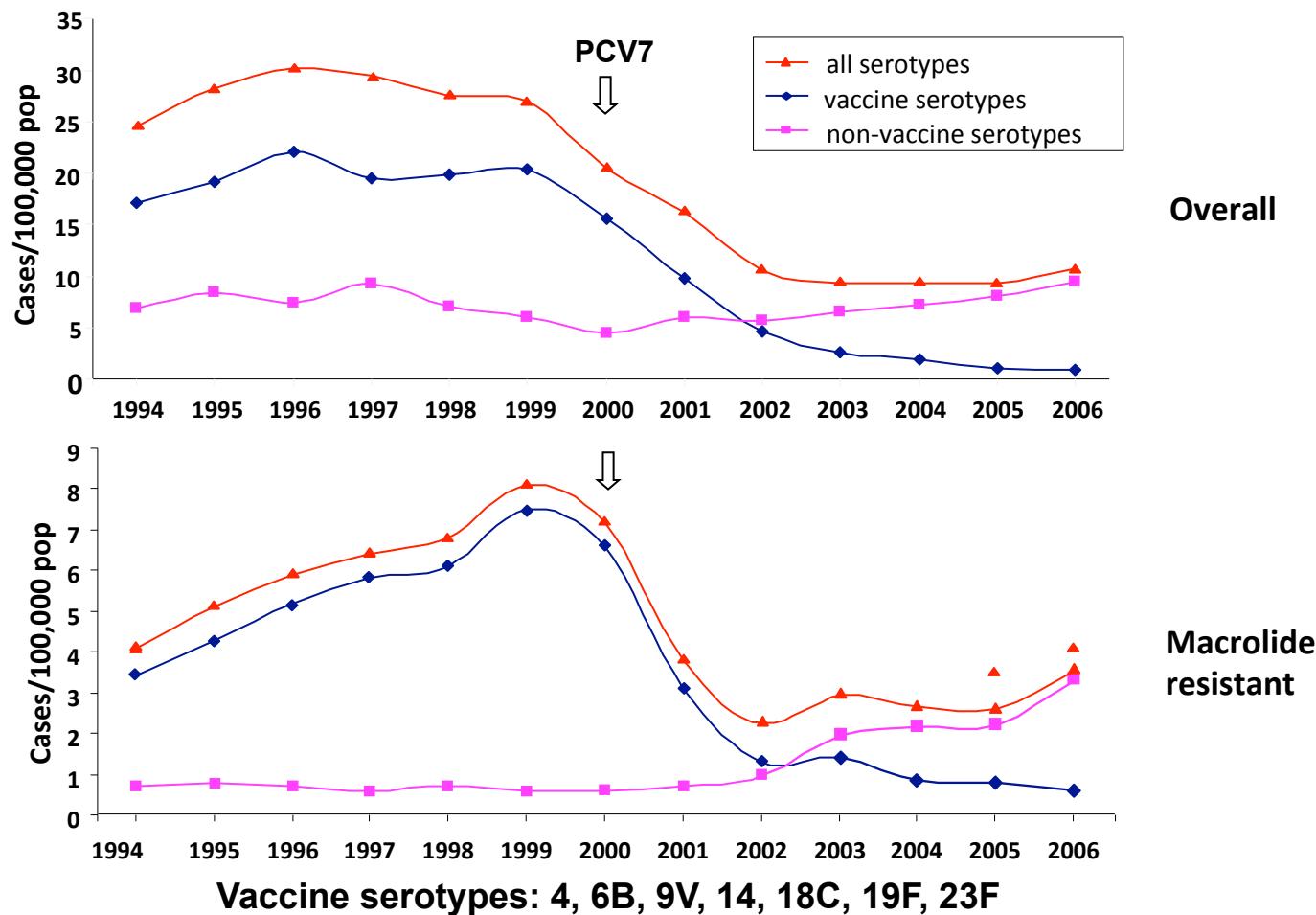


- Active Population-Based Surveillance for Invasive Pneumococcal Disease, 1994-present
- Georgia Health District 3 Atlanta Metropolitan Area
- 7-valent pneumococcal conjugate vaccine PCV7 introduced in Atlanta in 2000
Vaccine serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F
- 13-valent pneumococcal conjugate vaccine PCV13 introduced in Atlanta in 2010
Additional serotypes: 1, 3, 5, 6A, 7F, 19A

Goal: Monitor the incidence of macrolide resistance in invasive pneumococcal isolates and determine the effect of PCV7 and PCV13 on the distribution of macrolide resistance determinants



Incidence of vaccine and non-vaccine serotypes



Project overview

- 142 strains from the Georgia program
- 10 worldwide strains
- Samples encompass
 - 23 serotypes
 - 82 MLST types
 - Resistance to 13 antibiotics
 - 11 disease outcomes
- 454 sequencing approach – Two libraries
 - High quality draft genomes, few gaps

Analyses

- Loaded all genomes into the IGS Sybil comparative genomics software package
 - Added 14 closed reference genomes
 - Added 3 plasmids
- Characteristics of clonal complexes associated with disease
- Phylogeny of Atlanta isolates
- Evolution of resistance

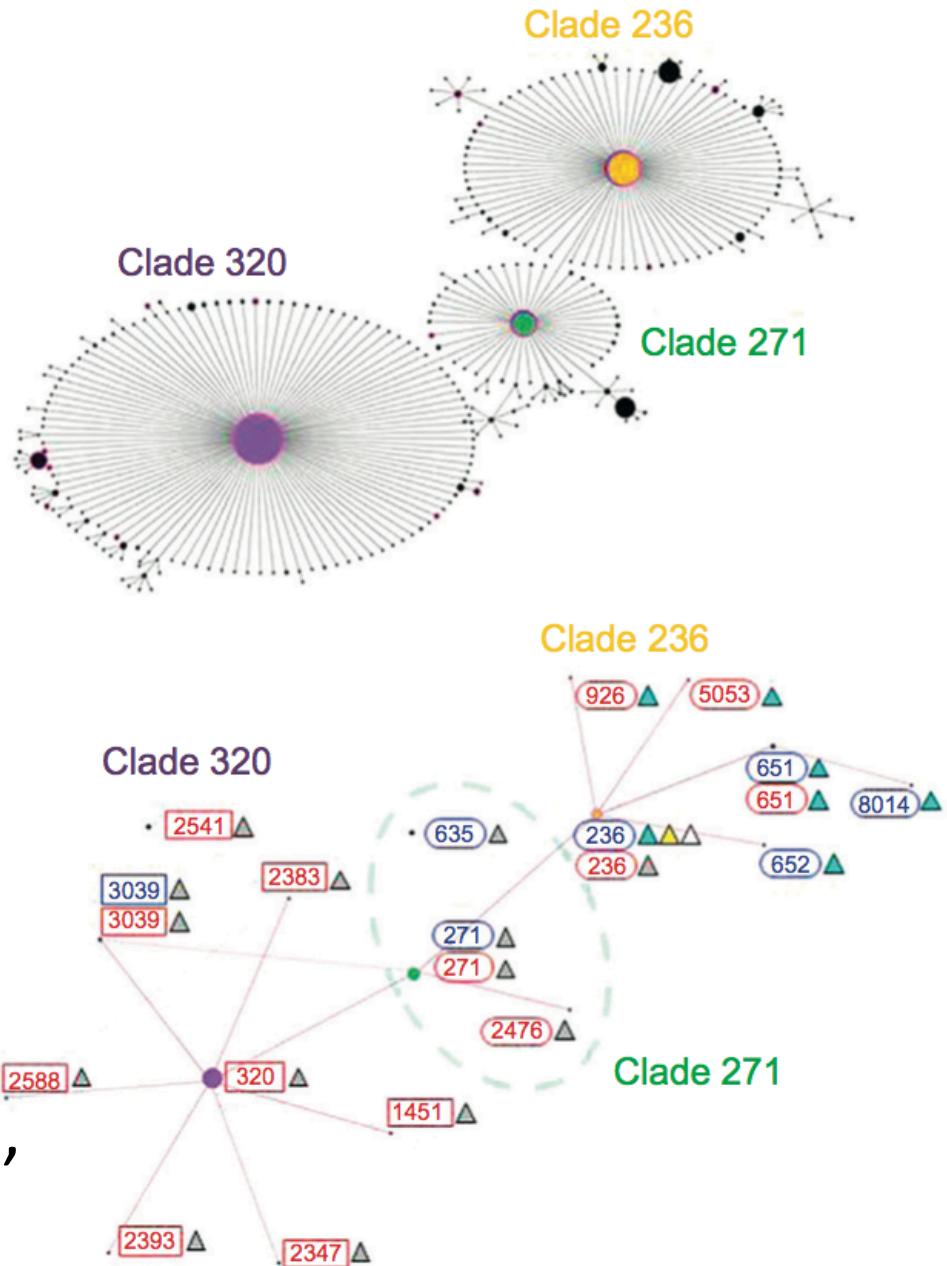
Clonal complexes

- Multi-Locus Sequence Typing – MLST
 - 7 housekeeping genes
 - Sequence types – ST
 - Clonal complexes – CC
- CC320 highly prevalent, multi-drug resistant
 - Serotypes 19A and 19F
- 32 strains of CC320 sequenced
 - Whole genome alignment – Mugsy



CC320 eBURST

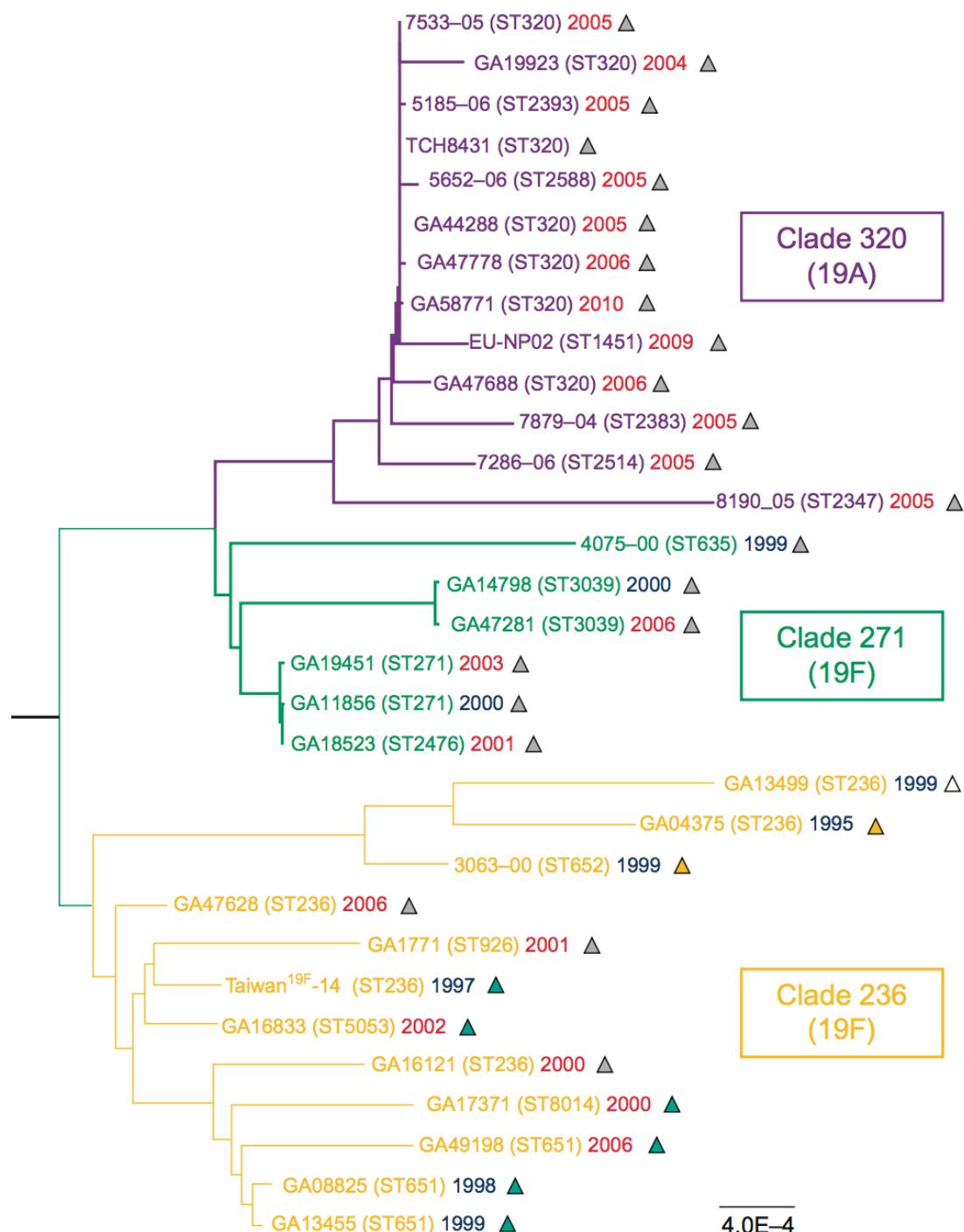
- Top: mlst.net
- Bottom: Atlanta
 - Box: 19A
 - Oval: 19F
 - Blue: pre-PCV7
 - Red: post-PCV7
 - Triangles: mobile elements with macrolide resistance, grey Tn2010





Core genome

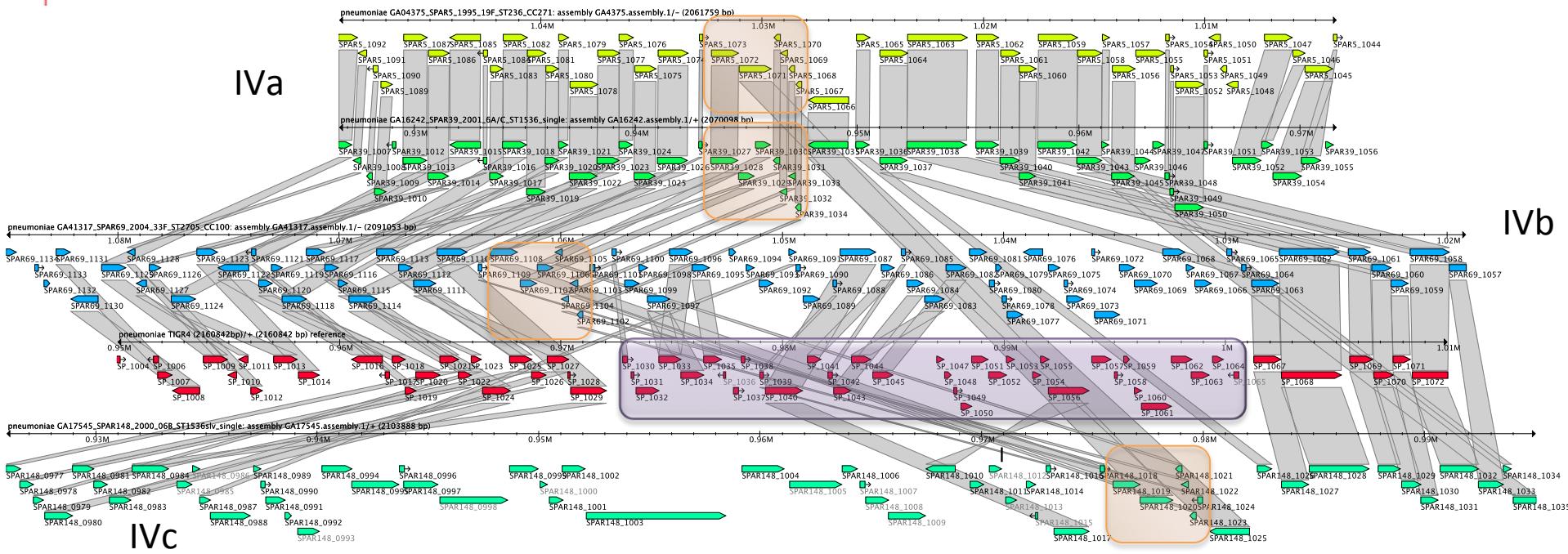
- Same colors
- Mugsy MAF file
- Phylomark core SNPs
- Similar clades





MEGA Class IV insertion site

MEGA: Macrolide Efflux Genetic Assembly
Pneumococcal pathogenicity island 1 (PPI-1)



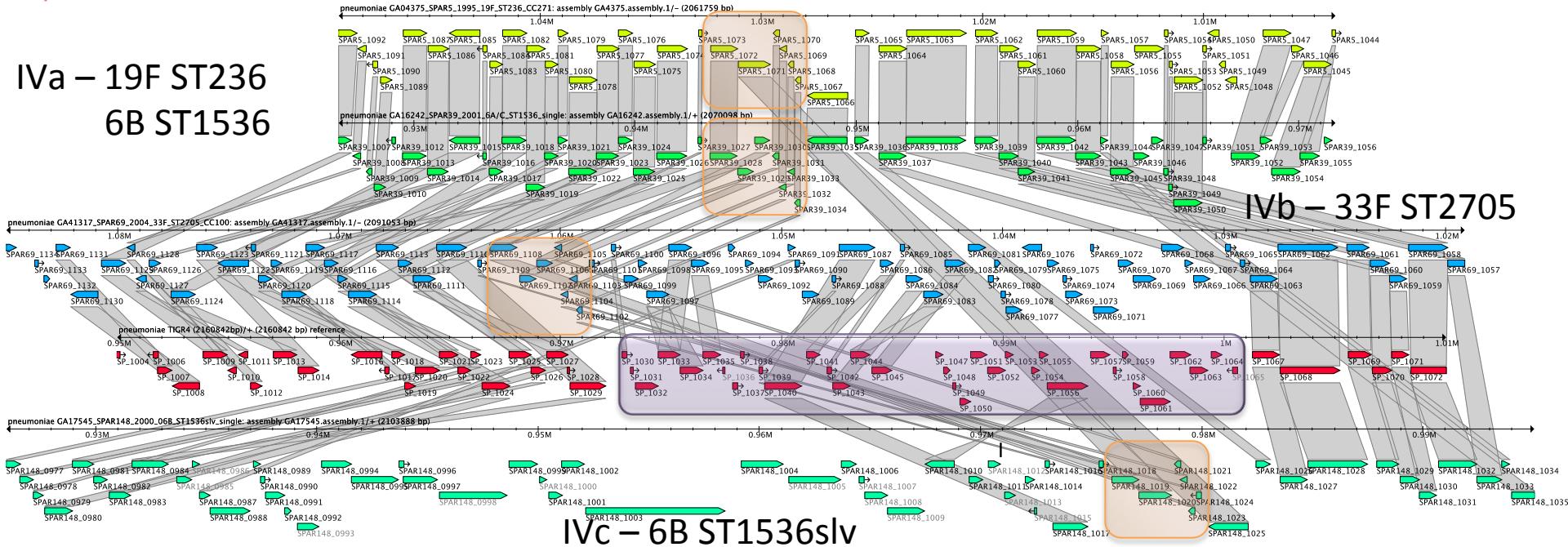


MEGA Class IV insertion site

MEGA: Macrolide Efflux Genetic Assembly
Pneumococcal pathogenicity island 1 (PPI-1)

IVa – 19F ST236
6B ST1536

IVb – 33F ST2705



Project 2

- Comparative genomics of clinical isolates of *Mycobacterium abscessus*
- “Rapid grower”
- Mycobacterial pulmonary infections mostly, but not exclusively, in patients with underlying lung conditions
- 6 patients – Serial bacterial isolates
- Focusing on *M. massiliense* - Emerging

Mycobacterium massiliense

- *M. massiliense* responsible for a series of epidemics post-surgical infections
- 3 patients
- Reference *M. massiliense* type strain (ATCC)
- 454 + Illumina sequencing approach
 - One 3 Kb paired-end 454 library
 - One 100 nt paired-end library on HiSeq 2000



Patient 1S

Year/Origin	Species ID/Isolate	Genome size (bp)	Contigs	GC content, (%)	ORF	Percent coding	tRNA	rRNA	Proteins w/ assigned function (%)	Hypothetical proteins (%)
3/2004 (BAL)	15-4 <i>M. massiliense</i>	4,896,163	5	64.3	4,935	98.9	47	3	72.2	27.8
9/2004 (sputum)	15-3 <i>M. massiliense</i>	4,894,992	13	64.3	4,942	98.9	47	3	72.1	27.9
9/2007 (BAL)	15-2 <i>M. massiliense</i>	4,897,774	2	64.3	4,934	98.9	47	3	72.3	27.7
9/2008 (BAL)	15-1 <i>M. massiliense</i>	4,895,449	6	64.3	4,931	98.9	47	3	72.2	27.8

Patient 2B

Year/Origin	Species ID	Genome size (bp)	Contigs	GC content, (%)	Genes	Percent coding	tRNA	rRNA	Proteins w/ assigned function (%)	Hypothetical proteins (%)
6/2000 (BAL)	2B_0626 <i>M. massiliense</i>	4,811,120	6	64.2	4,813	98.9	46	3	73.1	26.9
12/2002 (BAL)	2B_1231 <i>M. massiliense</i>	4,811,624	9	64.2	4,818	98.9	46	3	73.1	26.9
9/2005 (sputum)	2B_0912_R <i>M. massiliense</i> (R)*	4,810,354	9	64.2	4,819	98.9	46	3	73.1	26.9
9/2005 (sputum)	2B_0912_S <i>M. massiliense</i> (S)*	4,809,779	12	64.2	4,822	98.9	46	3	73	27
3/2007 (sputum)	2B_0307 <i>M. massiliense</i>	4,786,898	9	64.2	4,759	98.9	46	3	73.8	26.2
1/2008 (sputum)	2B_0107 <i>M. massiliense</i>	4,811,657	7	64.2	4,813	98.9	46	3	73.2	26.8



Patient 5S

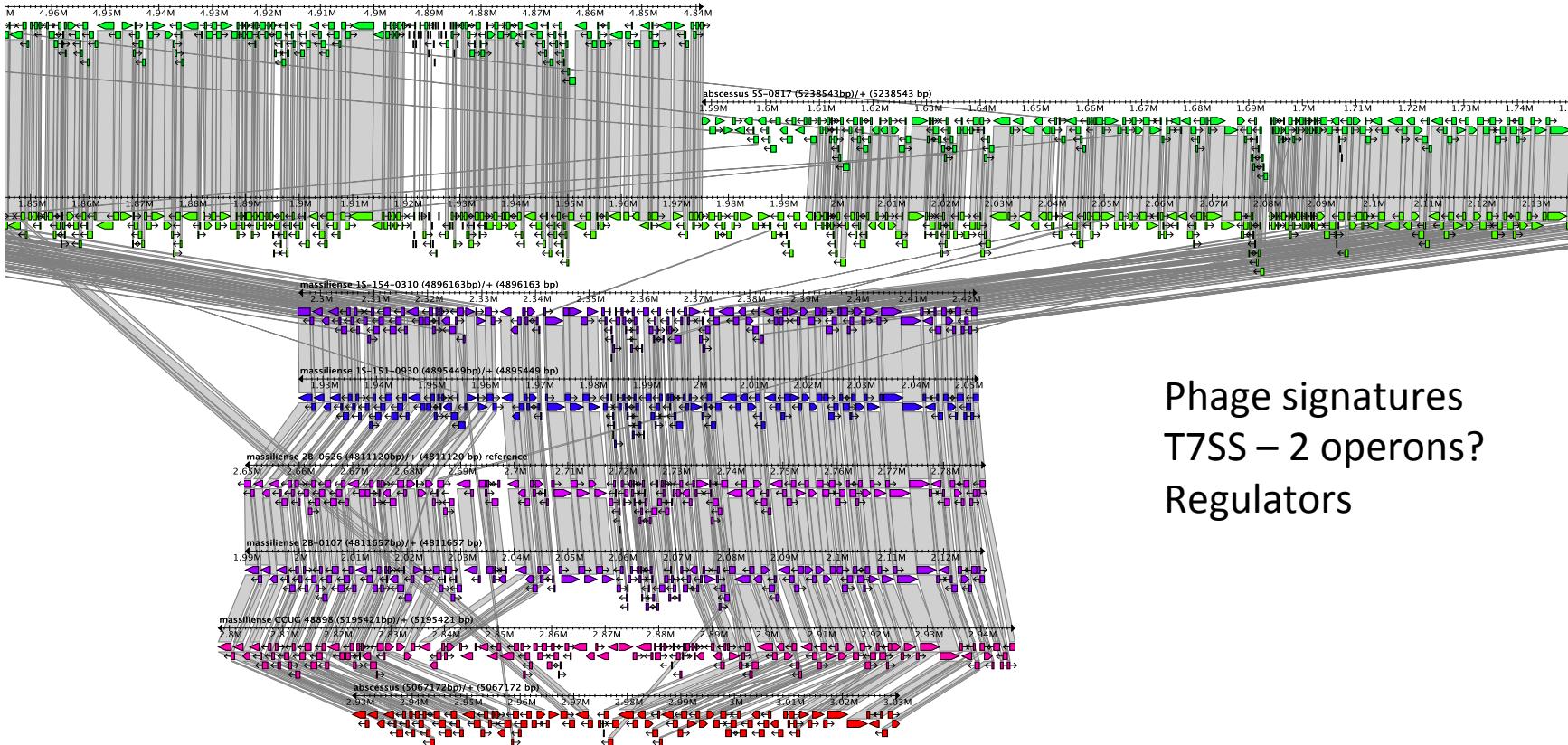
Year/Origin	Species ID	Genome size (bp)	Contigs	GC content, (%)	Genes	Percent coding	tRNA	rRNA	Proteins w/ assigned function (%)	Hypothetical proteins (%)
3/1998 (sputum)	5S_0304 <i>M. massiliense</i>	5,253,369	7	64	5,355	98.4	82	3	68.3	31.7
4/2000 (sputum)	5S_0421 <i>M. massiliense</i>	5,233,858	6	64	5,438	98.4	82	3	68.2	31.8
7/2002 (sputum)	5S_0708 <i>M. massiliense</i>	5,253,504	6	64	5,355	98.4	82	3	68.3	31.7
4/2004 (sputum)	5S_0422 <i>M. massiliense</i>	5,305,910	13	64	5,416	98.4	82	3	68.2	31.8
9/2006 (node bx)	M5S_0921 <i>M. massiliense</i>	5,255,116	12	64	5,373	98.4	82	3	68.3	31.7
12/2006 (sputum)	5S_1212 <i>M. massiliense</i>	5,238,864	7	64	5,349	98.4	82	3	68.2	31.8
12/2008 (sputum)	5S_1215 <i>M. massiliense</i>	5,205,410	5	64	5,314	98.4	81	3	68.1	31.9
8/2009 (autopsy)	5S_0817 <i>M. massiliense</i>	5,238,543	12	64	5,348	98.4	82	3	68.3	31.7

Type strains

Year/Origin	Species ID	Genome size (bp)	Contigs	GC content, (%)	Genes	Percent coding	tRNA	rRNA	Proteins w/ assigned function (%)	Hypothetical proteins (%)
2012	<i>M. massiliense</i> CCUG 48898	5,195,421	5	64.1	5,243	99.04	47	3	70	30
2009	<i>M. abscessus</i> ATCC 19977	5,067,172	1	64.1	4,991	98.9	47	3	60.5**	39.5**



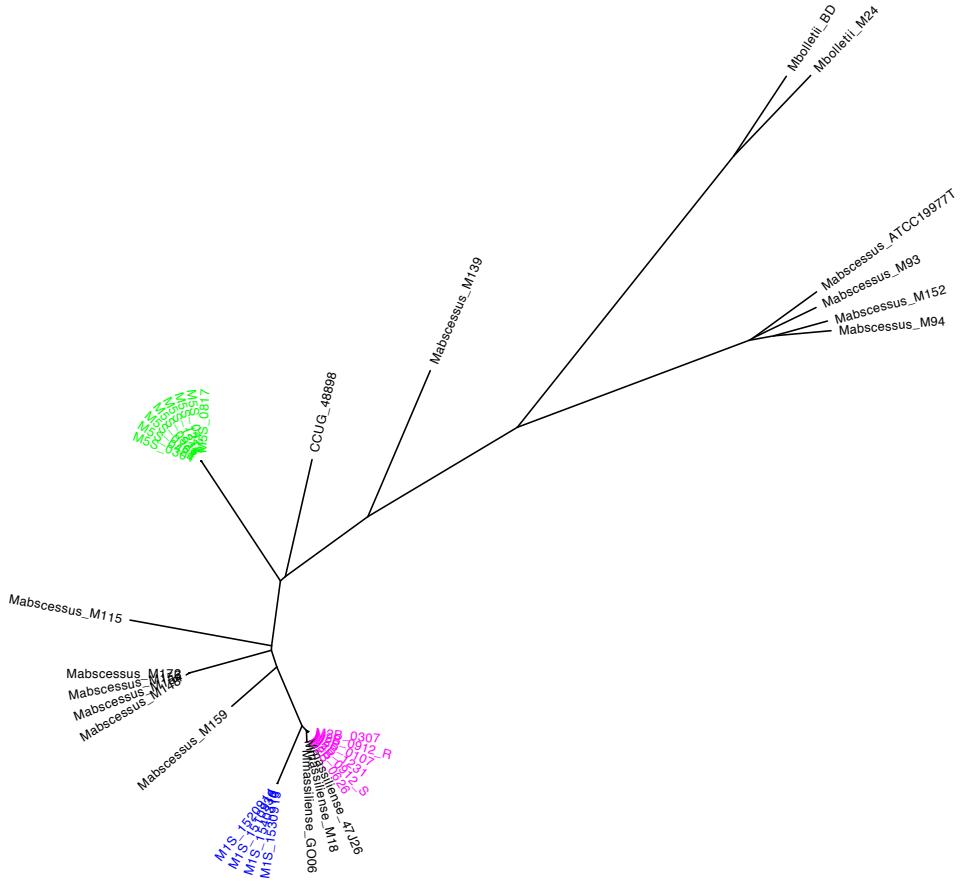
Patient 5S – Large insertion

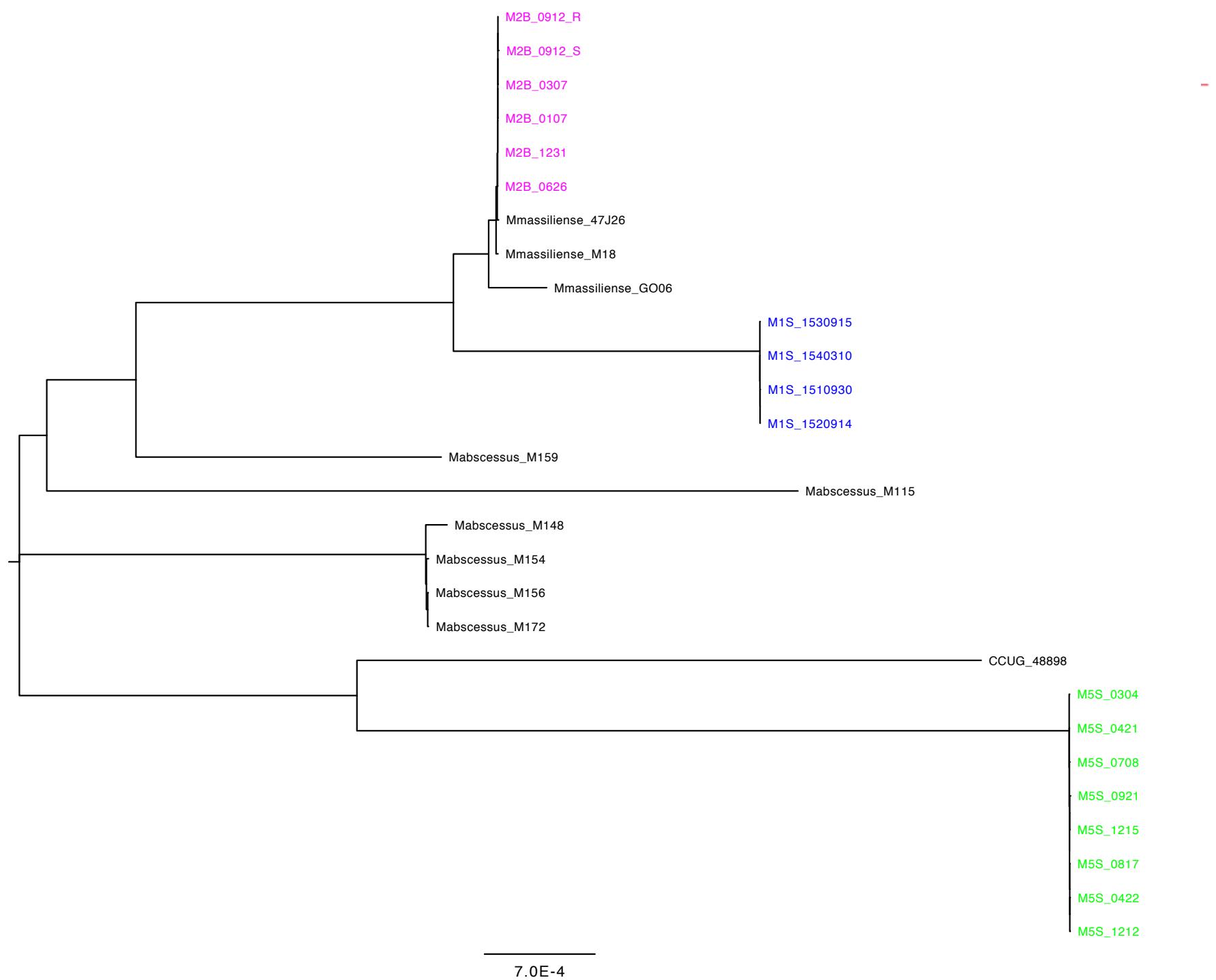


Phage signatures
T7SS – 2 operons?
Regulators



Mugsy-based NJ tree





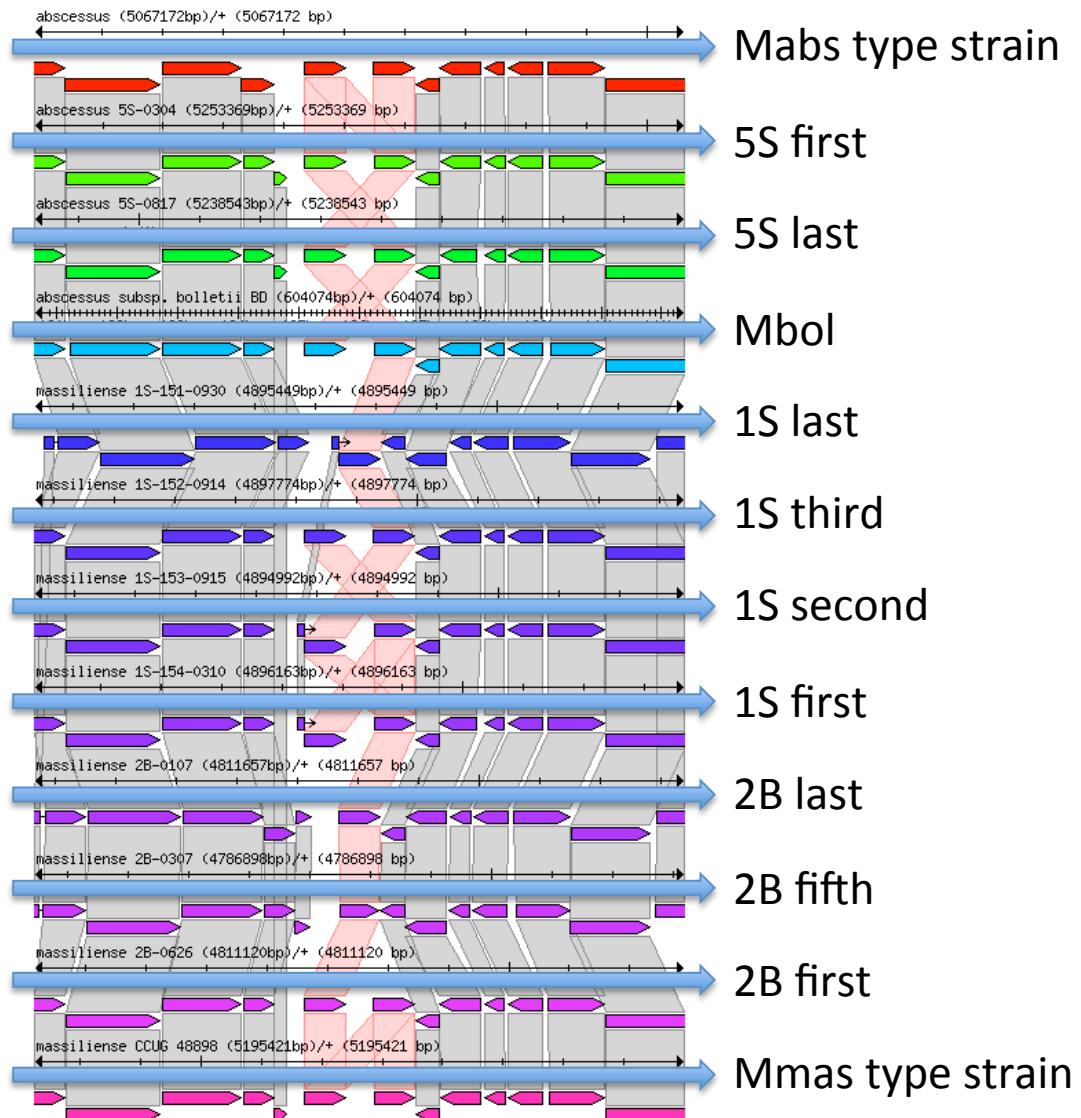


Mugsy clusters

Cluster	Isolate 1 (first)	#	Isolate 2	#	Isolate 3	#	Isolate 4 (last)	#
C4406	M1S_154_0310	0	M1S_153_0915	0	M1S_152_0914	1	M1S_151_0930	1
C4738	M1S_154_0310	0	M1S_153_0915	1	M1S_152_0914	1	M1S_151_0930	1
C4784	M1S_154_0310	0	M1S_153_0915	0	M1S_152_0914	1	M1S_151_0930	1
C4807	M1S_154_0310	0	M1S_153_0915	0	M1S_152_0914	1	M1S_151_0930	1
C4869	M1S_154_0310	0	M1S_153_0915	0	M1S_152_0914	1	M1S_151_0930	1
C4887	M1S_154_0310	0	M1S_153_0915	0	M1S_152_0914	1	M1S_151_0930	1
C4938	M1S_154_0310	0	M1S_153_0915	1	M1S_152_0914	0	M1S_151_0930	1
C4951	M1S_154_0310	0	M1S_153_0915	0	M1S_152_0914	1	M1S_151_0930	1
C3014	M1S_154_0310	1	M1S_153_0915	1	M1S_152_0914	0	M1S_151_0930	0
C3701	M1S_154_0310	1	M1S_153_0915	1	M1S_152_0914	0	M1S_151_0930	0
C4447	M1S_154_0310	1	M1S_153_0915	1	M1S_152_0914	0	M1S_151_0930	0
C4572	M1S_154_0310	1	M1S_153_0915	1	M1S_152_0914	0	M1S_151_0930	0

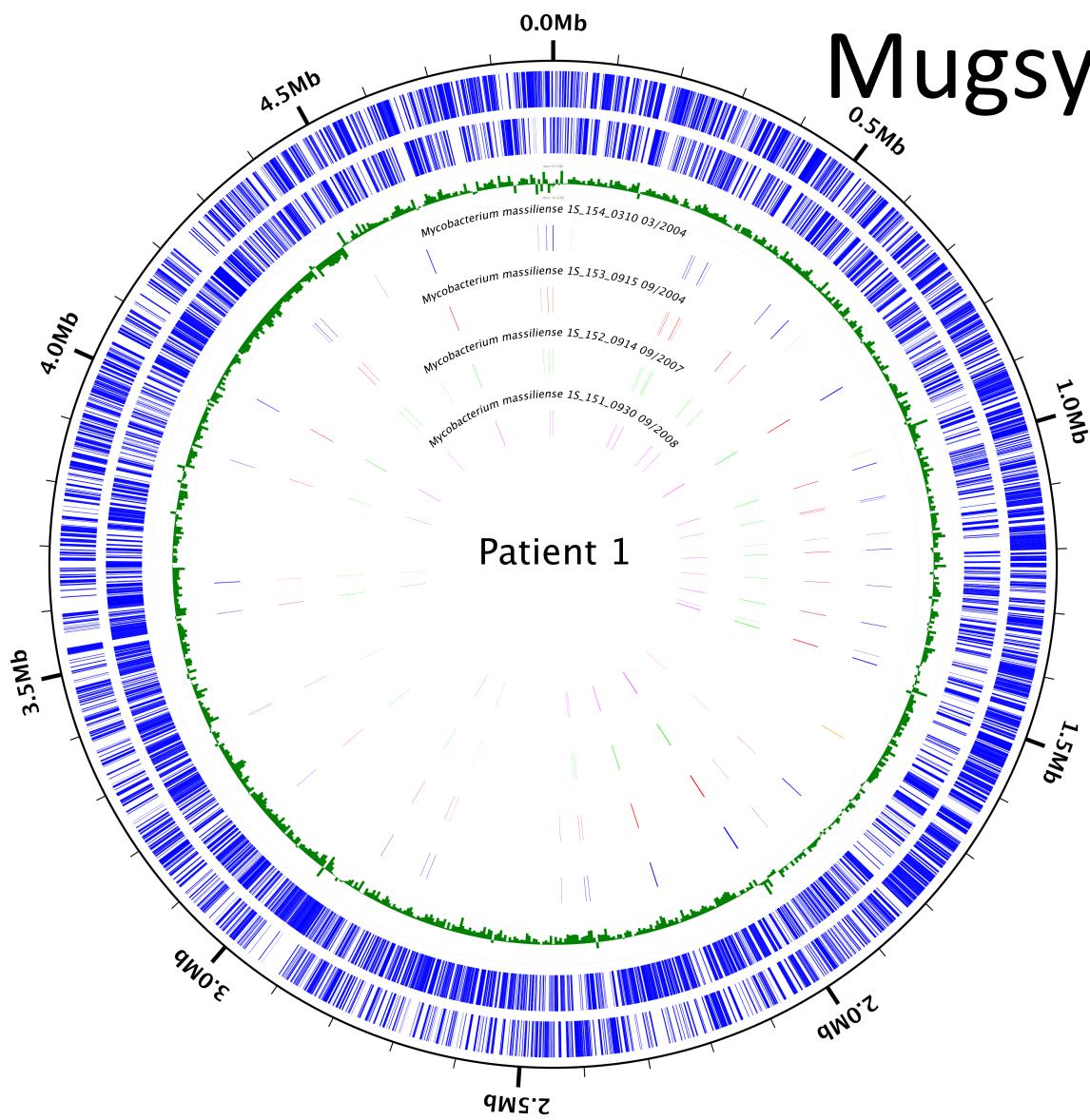


Mugsy clusters



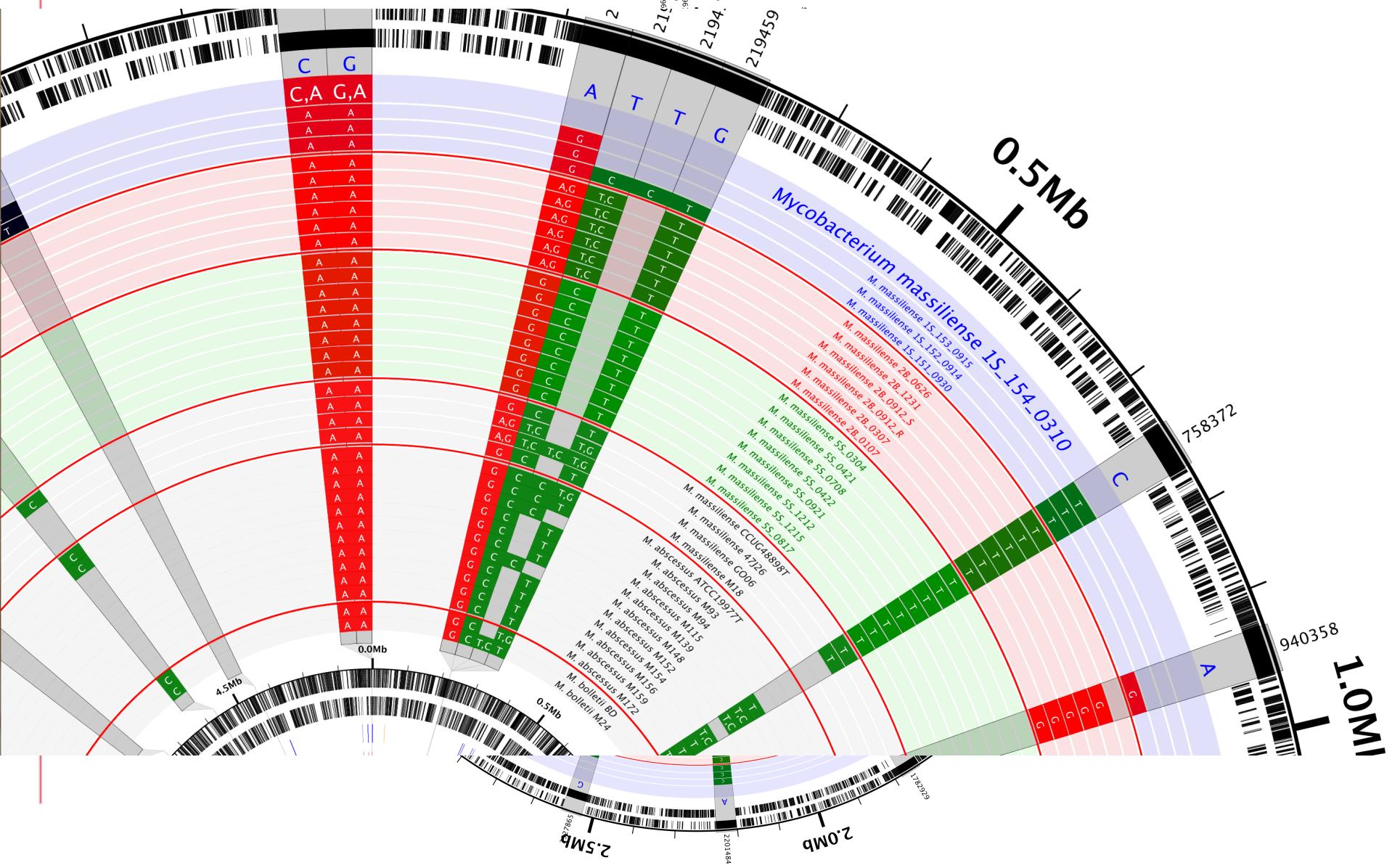


Circular view Mugsy clusters





Circular view – SNPs





erm(41) gene

CLUSTERED PROTEINS [hide]						
Order	Organism	Protein	Alt. Protein Name	Length	# exons	Gene Product
1	add/remove <i>[M. abscessus]</i>	<i>abscessus:MAB_2297</i>		173 aa	1 exon(s)	Probable methyltransferase
2	add/remove <i>[M. abscessus 3A-0119-R]</i>	<i>3A-0119-R:M3A_0119_R_2332</i>		138 aa	1 exon(s)	<i>erm</i>
3	add/remove <i>[M. abscessus 3A-0122-R]</i>	<i>3A-0122-R:M3A_0122_R_2387</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 3A-0122-S]</i>	<i>3A-0122-S:M3A_0122_S_1941</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 3A-0731]</i>	<i>3A-0731:M3A_0731_2289</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 3A-0810-R]</i>	<i>3A-0810-R:m3a0810r_2420</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 3A-0930-R]</i>	<i>3A-0930-R:M3A_0930_R_2425</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 3A-0930-S]</i>	<i>3A-0930-S:M3A_0930_S_1976</i>		138 aa	1 exon(s)	<i>erm</i>
4	add/remove <i>[M. abscessus 4S-0116-R]</i>	<i>4S-0116-R:M4S_0116_R_236</i>		173 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 4S-0116-S]</i>	<i>4S-0116-S:M4S_0116_S_1136</i>		138 aa	1 exon(s)	ribosome methyltransferase
5	add/remove <i>[M. abscessus 4S-0206]</i>	<i>4S-0206:m4s0206_1597</i>		118 aa	1 exon(s)	ribosome methyltransferase
6	add/remove <i>[M. abscessus 4S-0303]</i>	<i>4S-0303:M4S_0303_139</i>		138 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 4S-0726-RA]</i>	<i>4S-0726-RA:M4S_0726_RA_2001</i>		138 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 4S-0726-RB]</i>	<i>4S-0726-RB:M4S_0726_RB_1588</i>		138 aa	1 exon(s)	ribosome methyltransferase
8	add/remove <i>[M. abscessus 5S-0304]</i>	<i>5S-0304:M5S_0304_1150</i>		138 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 5S-0421]</i>	<i>5S-0421:M5S_0421_1430</i>		138 aa	1 exon(s)	ribosome methyltransferase
9	add/remove <i>[M. abscessus 5S-0422]</i>	<i>5S-0422:M5S_0422_2165</i>		173 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 5S-0708]</i>	<i>5S-0708:M5S_0708_1655</i>		173 aa	1 exon(s)	ribosome methyltransferase
7	add/remove <i>[M. abscessus 5S-0817]</i>	<i>5S-0817:M5S_0817_1209</i>		138 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 5S-0921]</i>	<i>5S-0921:m5s0921_1908</i>		173 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 5S-1212]</i>	<i>5S-1212:M5S_1212_1598</i>		173 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 5S-1215]</i>	<i>5S-1215:M5S_1215_912</i>		138 aa	1 exon(s)	ribosome methyltransferase
-	add/remove <i>[M. abscessus 6G-0125-R]</i>	<i>6G-0125-R:M6G_0125_R_1400</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 6G-0125-S]</i>	<i>6G-0125-S:M6G_0125_S_2441</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 6G-0212]</i>	<i>6G-0212:m6g0212_2427</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 6G-0728-R]</i>	<i>6G-0728-R:m6g0728r_2367</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 6G-0728-S]</i>	<i>6G-0728-S:M6G_0728_S_1493</i>		138 aa	1 exon(s)	<i>erm</i>
-	add/remove <i>[M. abscessus 6G-1108]</i>	<i>6G-1108:M6G_1108_2367</i>		138 aa	1 exon(s)	<i>erm</i>
10	add/remove <i>[M. abscessus subsp. <i>bolletii</i> BD]</i>	<i>subsp. <i>bolletii</i> BD:MBOL_21130</i>		173 aa	1 exon(s)	methyltransferase
11	add/remove <i>[M. africanum GM041182]</i>	<i>GM041182:MAF_19990</i>		179 aa	1 exon(s)	putative methyltransferase
12	add/remove <i>[M. tuberculosis KZN 1435]</i>	<i>KZN_1435:TBMG_02000</i>		179 aa	1 exon(s)	methyltransferase

hide protein list





Erm(41) protein

CLUSTAL ALIGNMENT [hide]

[Regenerate AA Alignment](#)

[Regenerate NUC Alignment](#)

- Note: If an alignment contains only X's, click on 'Regenerate AA Alignment' to align the sequences.

abscessus_MAB_2297
3A-0119-R_M3A_0119_R_2332
3A-0122-R_M3A_0122_R_2387
4S-0116-R_M4S_0116_R_236
4S-0206_m4s0206_1597
4S-0303_M4S_0303_139
5S-0817_M5S_0817_1209
5S-0304_M5S_0304_1150
5S-0422_M5S_0422_2165
subsp. *bolletii* BD MBOL_21130
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KZN_1435_TBMG_02000

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MSALGRSRRRA WGWHRLHDEW AARVVSAAAV RPGELVFDIG AGEGALTAAHL VRAGARVVAV ELHPRRVGVL RERFP--GIT VVHADAASIR LPGRPFRVVA
MSALGRSRRRA WGWHRLHDEW AARVVSAAAV RPGELVFDIG AGEGALTAAHL VRAGARVVAV ELHPRRVGVL RERFP--GIT VVHADAASIR LPGRPFRVVA
*:*** ***** . * .**. : . . .** ;: * * *****

abscessus_MAB_2297
3A-0119-R_M3A_0119_R_2332
3A-0122-R_M3A_0122_R_2387
4S-0116-R_M4S_0116_R_236
4S-0206_m4s0206_1597
4S-0303_M4S_0303_139
5S-0817_M5S_0817_1209
5S-0304_M5S_0304_1150
5S-0422_M5S_0422_2165
subsp. *bolletii* BD MBOL_21130
GM041182_MAF_19990
KZN_1435_TBMG_02000

SPPYQVTSAL IRSLLTPESR LLAADLVLQRA GAVHKHAKRA PVRHWTLRAG ITLPRSAFHH PPQVDSSVILV IRRR-----
SPPYHVTSAI IRSLLTPESR LLAADLVLQRA GAVHKHAKRA PVRNWTLRAG ITLPRSAFHH PPQVDSSVILV IRRR-----
NPPYGISSRL LRTLLAPNSG LVAADLVLQRA ALVCKFASRN -ARRFTLTGV LMLPRRRAFLP PPHVDSAVLV VRRRKCGDWQ GR
NPPYGISSRL LRTLLAPNSG LVAADLVLQRA ALVCKFASRN -ARRFTLTGV LMLPRRRAFLP PPHVDSAVLV VRRRKCGDWQ GR
.*** :* * :*;***:*** *;***** . * .**. : . . .** ;: *** **. ***:***:*** ;***

1812/2184 (83.0%) of the characters in the alignment match the consensus sequence (includes gaps)