Characterization of Island-3 and its Relatives in Mycobacteriophage Cluster I

Presented by: Lianne B. Cohen

for

The NGRI Team at Carnegie Mellon University

Turi A. Alcoser, Lisa M. Alexander, Ian M. Bayles, Keshav Budwal, Ian W. Campbell, Lianne B. Cohen, Belle E.V. English, Laura Z. Filliger, Tyler M. Fox, Stephanie L. Guerra, Siping He, Kaitlin E. Healy, Paul G. Jasinto, Andrew J. Medenbach, Rachel E. Pferdehirt, Michael J. Reiss, Judith Savitskaya, Madhav K. Shroff, Jasper Thompson, Hannah S. Wirtshafter, David A. Zaidins, Kathryn E. Sheldon, Jonathan W. Jarvik, A. Javier Lopez

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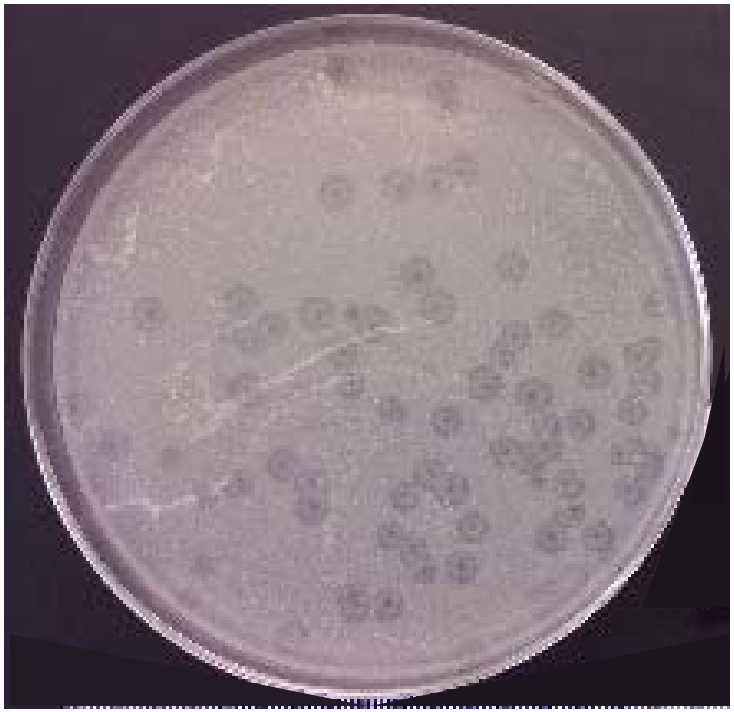
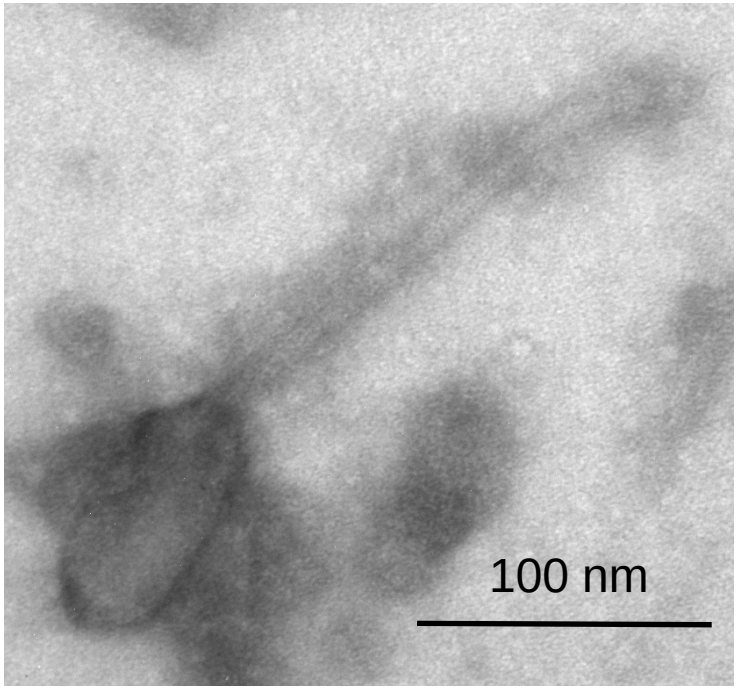
Isolation of Island-3

Overnight enrichment in
M. smegmatis liquid culture

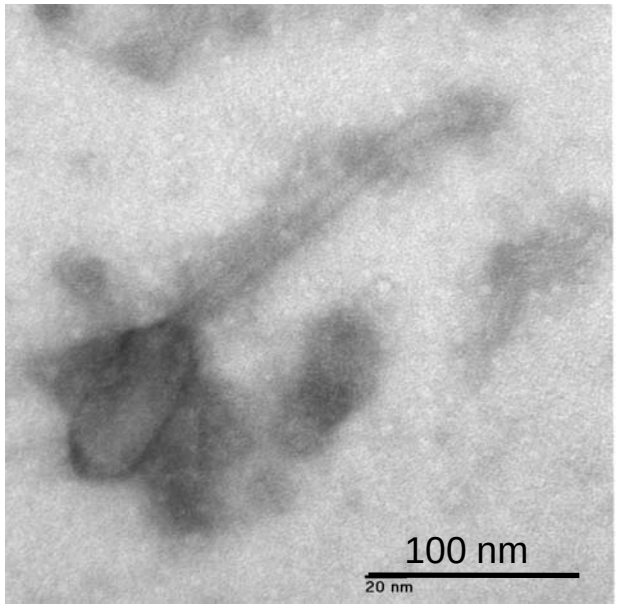
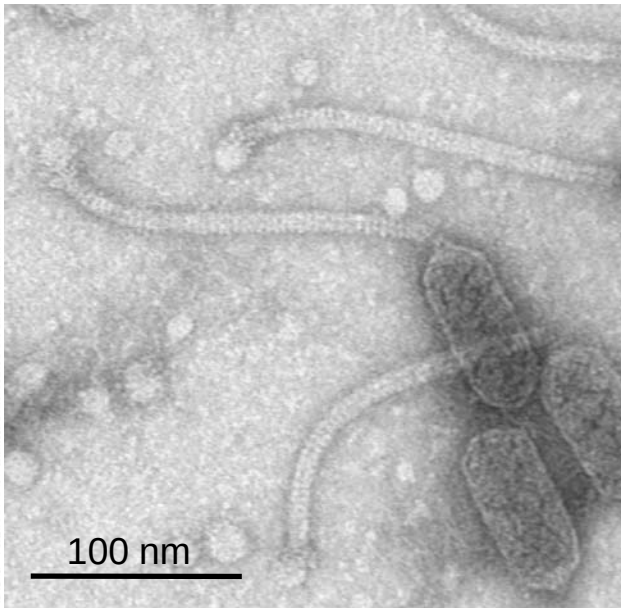
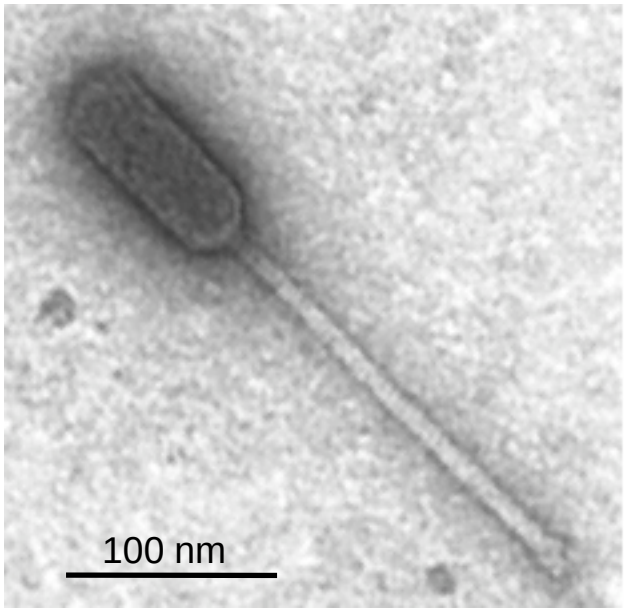


Turbid: 1 mm center, 4-5 mm halo



Non-contractile tail: ~179 nm
Prolate ellipsoid Head: ~ 86 nm x 39 nm
Estimated genome size: 45-50 kb

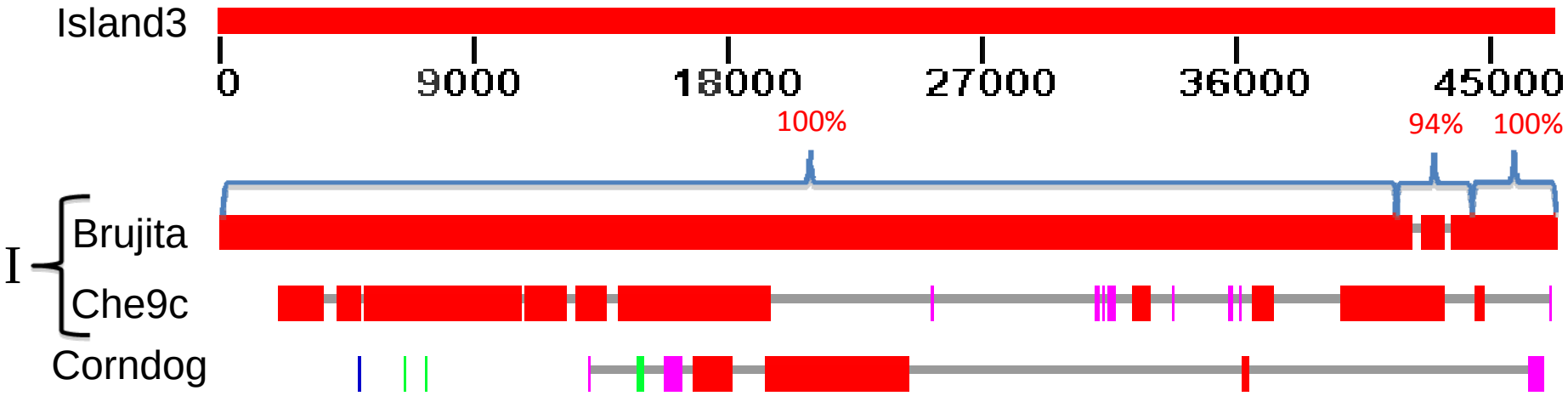
Similarity to Group I Mycobacteriophages

Island-3	Brujita	Che9c
		
Pennsylvania	Virginia	Chenai, India
Head: 86 x 39 nm	85 x 35 nm	110 x 40 nm
Tail: 180 nm	180 nm	226 nm
Genome: 47,287 bp	47,057 bp	57,050 bp
Plaques*: bull's eye	bull's eye	bull's eye

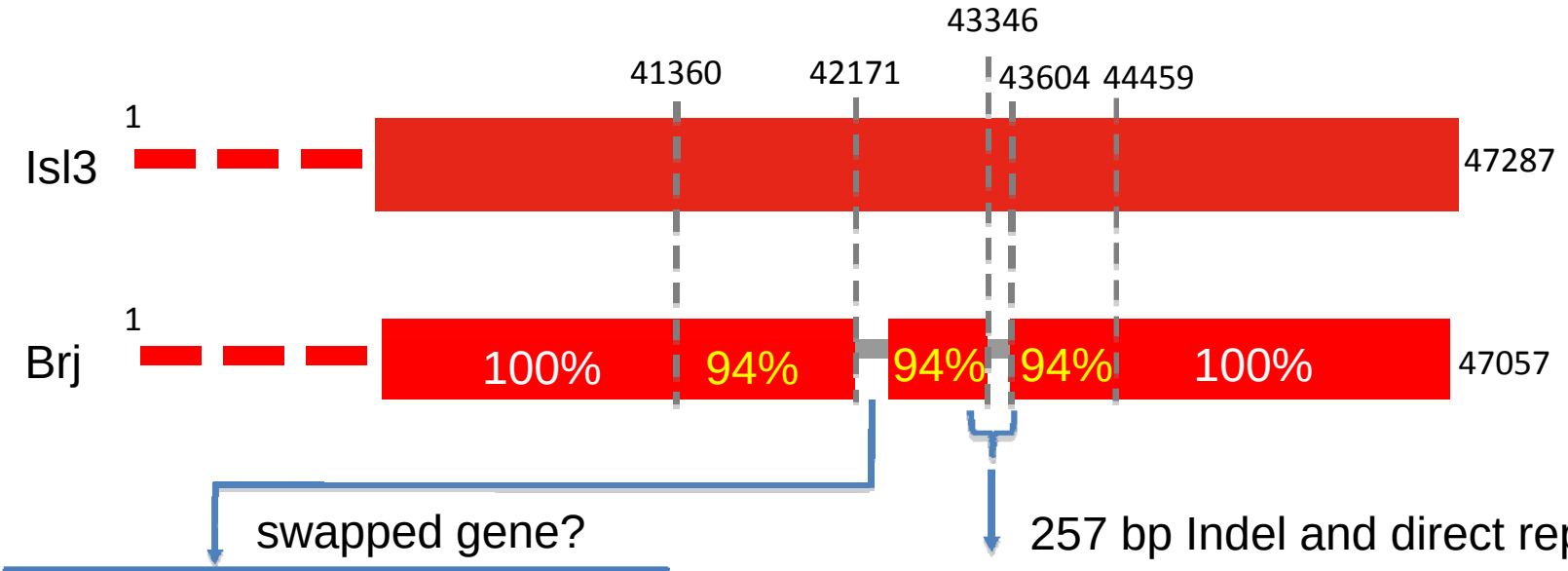
*tested on same batch of medium and same culture of *M. smegmatis*

BLAST Confirms that Island-3 Sequence is Most Closely Related to the Group I Mycobacteriophages and is almost identical to Brujita

Three best matches by BLAST:



Details of Divergence Region From Brujita



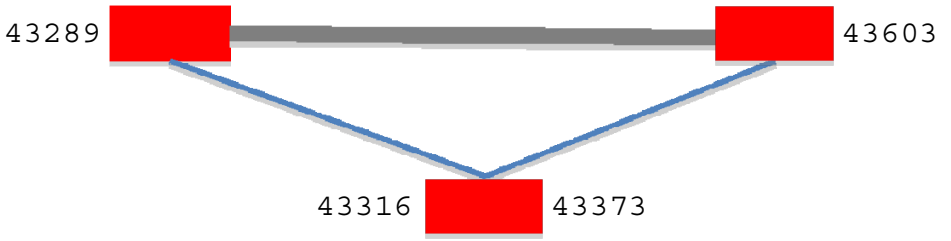
swapped gene?

273 bp Isl-3
300 bp Brj

257 bp Indel and direct repeat

end of Isl3 gene 66

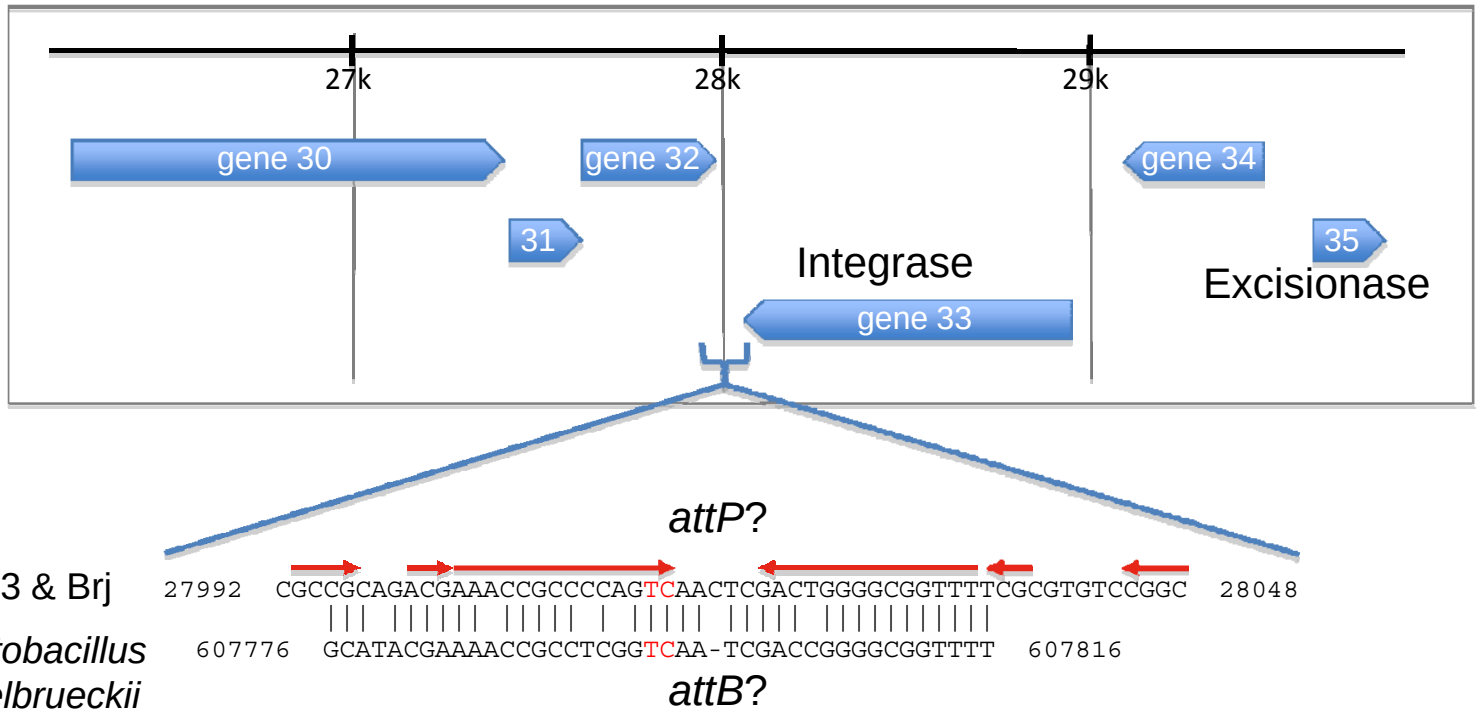
CCGCTATCAGCTGGCGCACGTGCGCGAGGTCGCCGGCCGCAAGAGGCGGCGCCGATGA
TGGCGCGGGCATGGCGCGTAATGCCGCGCACCGACGCGCTCGGAAATCAGCTGTGGTC
GCTGCAATACGAGACGATCTGGCGCGACGGCAGCCGTTACGGGCTGTGGCGCGAGGAA
AGCGTGTTTCGCGATGAGGGGCGGGCGCTCGCCGAGCTGGCGCGGCGCCGGACGCCCT
GCCCGCATGAGCACGAGCACGAGGGCCGGTACCAGTTGGCGCACGTGCGCGACGTCGC
CGGCCGCAAGAGTCGGCGCCGATGA



CCGCTACCAGCTGGCGCACGTGCGCGAGGTCGCCGGCCGCAAGAGGCGGCGCAGATGA

end of Brj gene 66

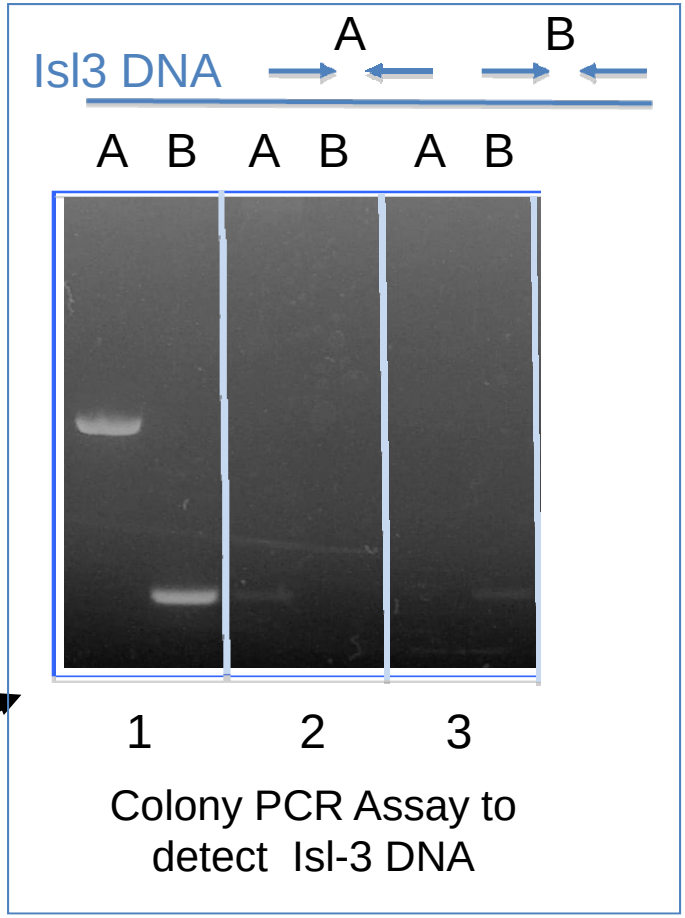
Presence of an integration cassette suggests lysogenic capability and integration into bacterial genome by site-specific recombination.



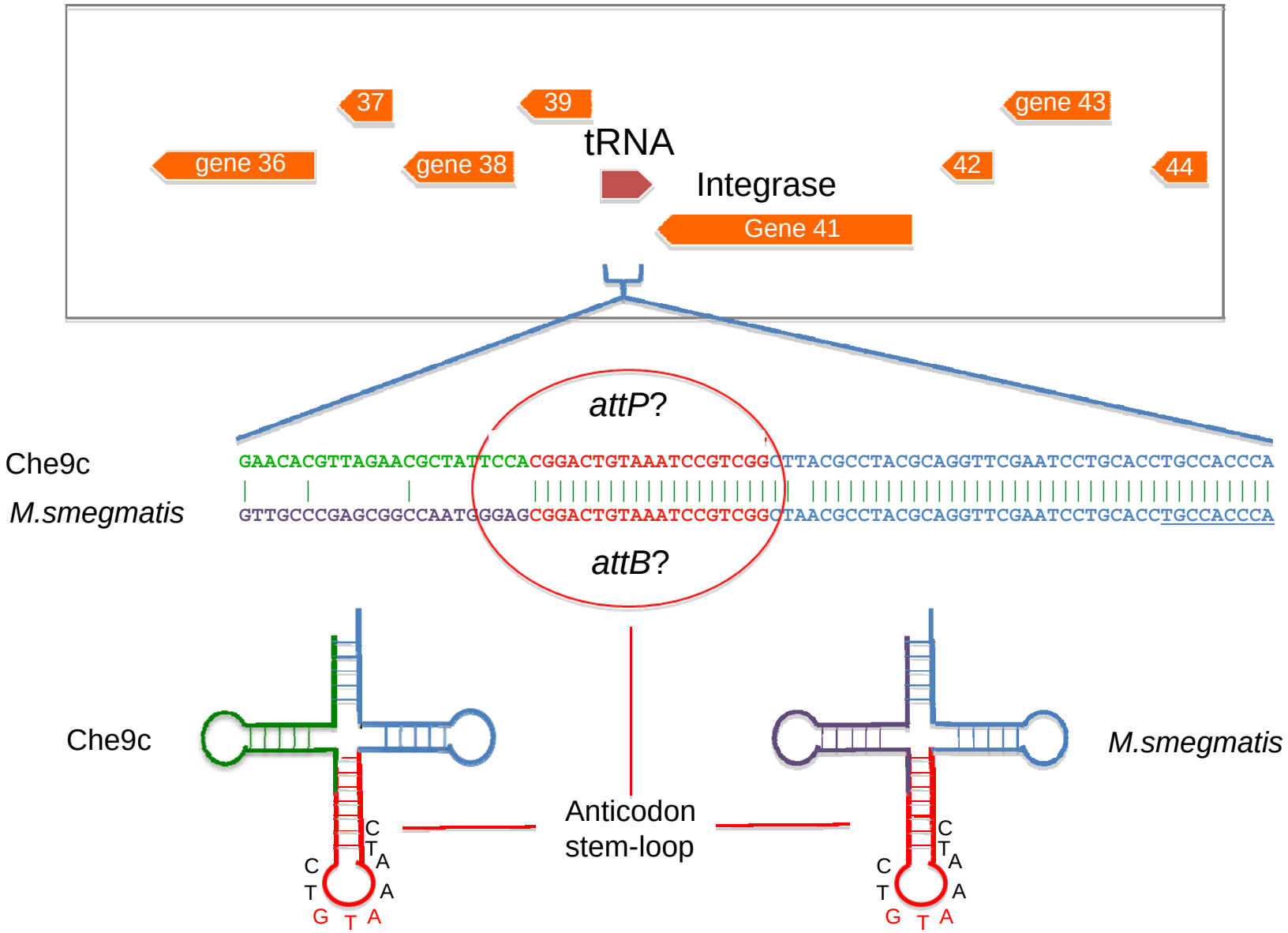
Island 3 Lysogenizes *M. smegmatis*

Isolation strategy: - Infect at high multiplicity of infection
- Restreak surviving colonies 3X
- 3 Tests for Lysogeny

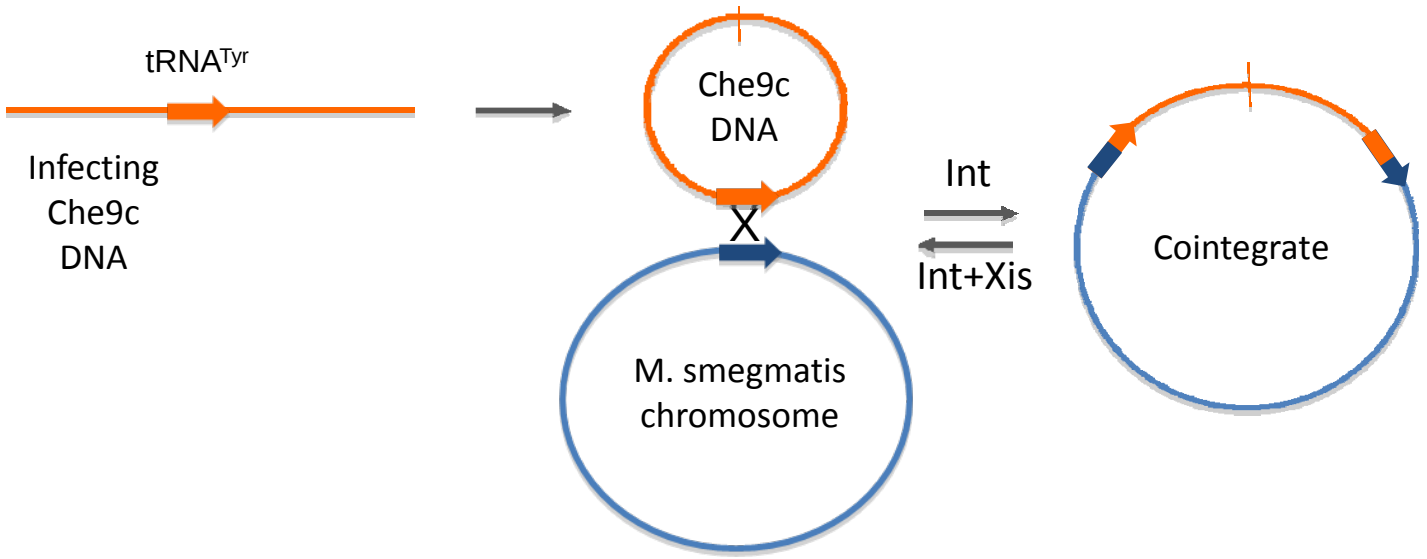
Candidate Isl3 Lysogens:	1	2	3	4	5	6	7
1. Resistance to Isl-3:	+	-	-	+	+	+	+
2. Cells behave as infectious centers:	+	-	-	+	+	+	+
3. Phage DNA in cells:	+	-	-	+	+	+	+



The putative *attP* site of Che9c lies within a predicted tRNA-Tyr gene



Hypothesis: Che9c integrates via recombination with $tRNA^{Tyr}$ of *M. smegmatis*

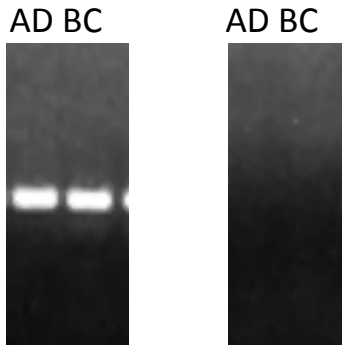


Che9c Integrates via tRNA Sequence

Isolate candidate lysogens: Test for lysogeny and integration

Candidate:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Resistance to Che9c	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-
PCR A+D (left junction)	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-
PCR B+C (right junction)	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-

Representative Colony-PCR Results



Complex Immunity Relationships

Candidate Isl3 Lysogen:	1	2	3	4	5	6	7
Resistance to Isl3	+	-	-	+	+	+	+
Resistance to Brj	+	-	-	+	+	+	+
Resistance to Che9c	-	-	-	-	-	-	-

Candidate Che9c Lysogen:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Resistance to Isl3	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-
Resistance to Brj	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-
Resistance to Che9c	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-

Despite lower similarity, Che9 confers resistance to all three Group I phage

*Resistance: <0.5% of the plaque forming efficiency on control *M. smegmatis mc² 155*
*Sensitivity: 100% of the plaque forming efficiency on control *M. smegmatis mc² 155*

Comparison with L5 Integration

Che9c *attP*?
GAACACGTTAGAACGCTATTCCACGGACTGTAAATCCGTCGGCTTACGCCTACGCAGGTTCGAATCCTGCACCTGCCACCCA

M.smeg *attB*?
GTTGCCCGAGCGGCCAATGGGAGCGGACTGTAAATCCGTCGGCTAACGCCTACGCAGGTTCGAATCCTGCACCTGCCACCCA

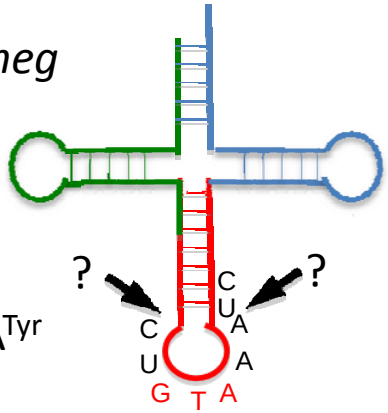
L5 *attP*

-20 0 +20
TGTGGTCGGCAGACACCCATTCTTCCAAACTAGCTACGCGGGTTCGATTCCCGTCGCCCGCTCCGCTGGT
ACACCAGCCGTCTGTGGGTAAGAAGGTTTGATCGATGCGCCCAAGCTAAGGGCAGCGGGCGAGGCGACCA

M.smeg *attB*

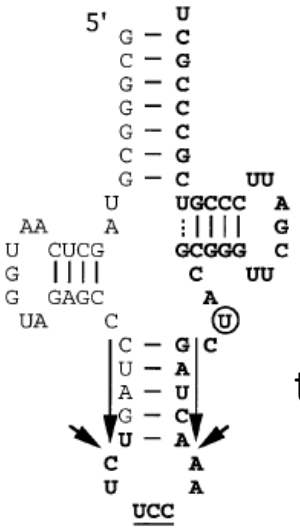
-20 0 +20 tRNA-gly 3'
GCTCAATGGTAGAGCCCTAGTCTTCCAAACTAGCTACGCGGGTTCGATTCCCGTCGCCCGCTCGGTAGGG
CGAGTTACCATCTCGGGATCAGAAGGTTTGATCGATGCGCCCAAGCTAAGGGCAGCGGGCGAGCCATCCC

Che9c/*M.smeg*



tRNA^{Tyr}

L5/*M.smeg*



tRNA^{Gly}

(L5 from Peña et al 1996)

Conclusions

- Island-3 is a member of Group I and is almost identical to Brujita

- Island-3 Lysogenizes M. smegmatis.
  - Don’t know whether it integrates into the bacterial chromosome

- Che9c Lysogenizes M. smegmatis and integrates into the bacterial chromosome at the $tRNA^{Tyr}$ gene.

- Complex immunity relationships within Cluster I:
  - Island-3 lysogens resistant only to Island-3 and Brujita
  - Che9c lysogens resistant to all three members of Cluster I.

The CMU NGRI Team 2008-2009