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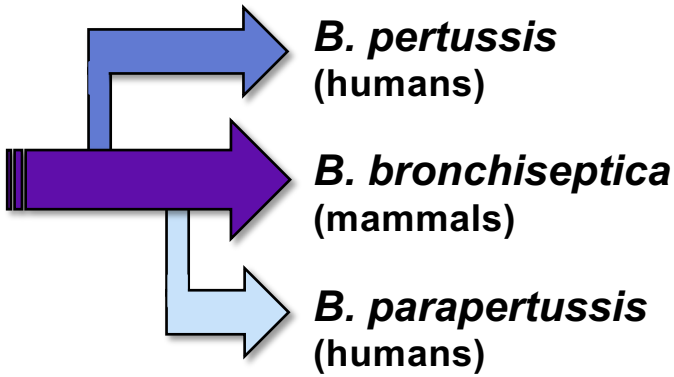
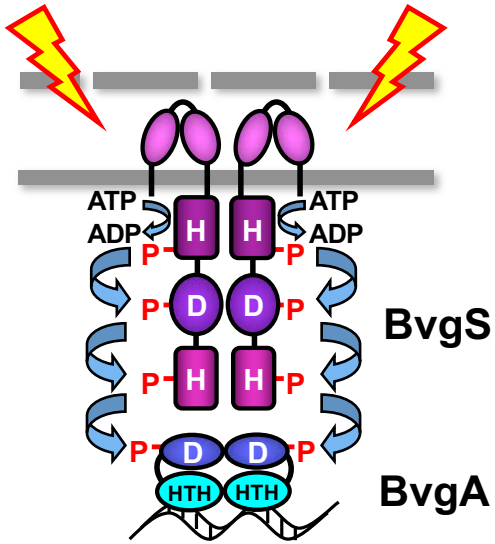
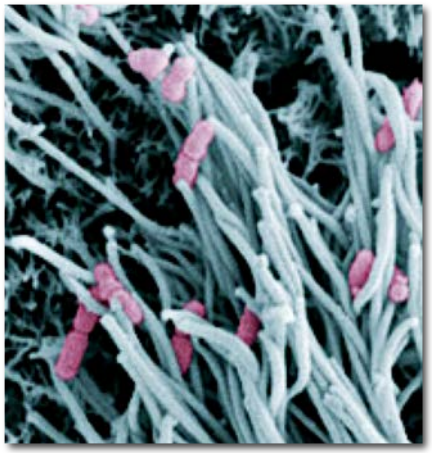
Accelerated Evolution by Diversity-Generating Retroelements

Jeff F. Miller *et al.*
California NanoSystems Institute
-and-
Microbiology, Immunology and Molecular Genetics
University of California, Los Angeles

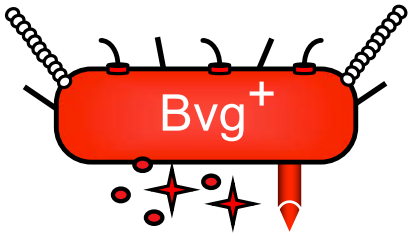
Bordetella







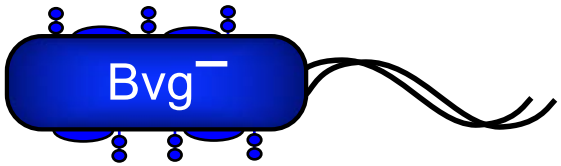
ciliated respiratory epithelium



Respiratory tract
(toxins, adhesins,T3SS, etc.)

BvgAS, etc…

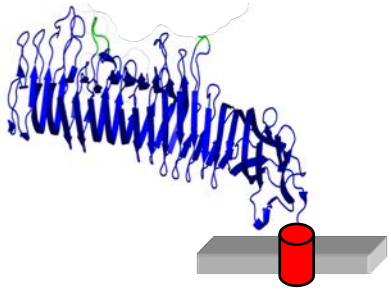
B. bronchiseptica



Ex vivo

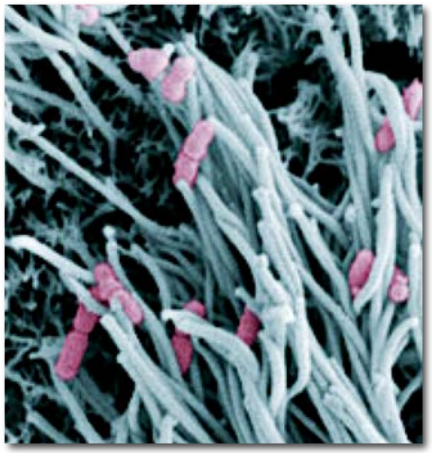
Melvin, J.A. *et al., 2014, Nature Rev. Microbiol. 12: 274-288*

Bordetella phage



Pertactin (Prn)

- *Outer membrane*
- *Bvg-regulated*
- *Acellular vaccine (DTaP) component*



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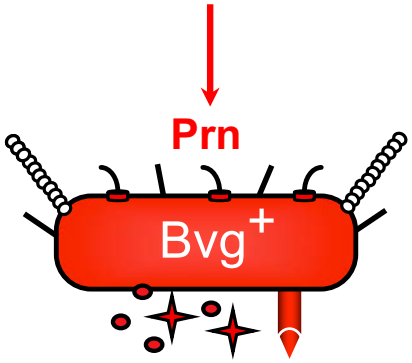

Respiratory tract
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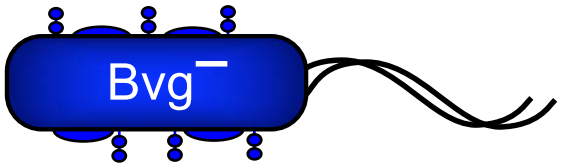
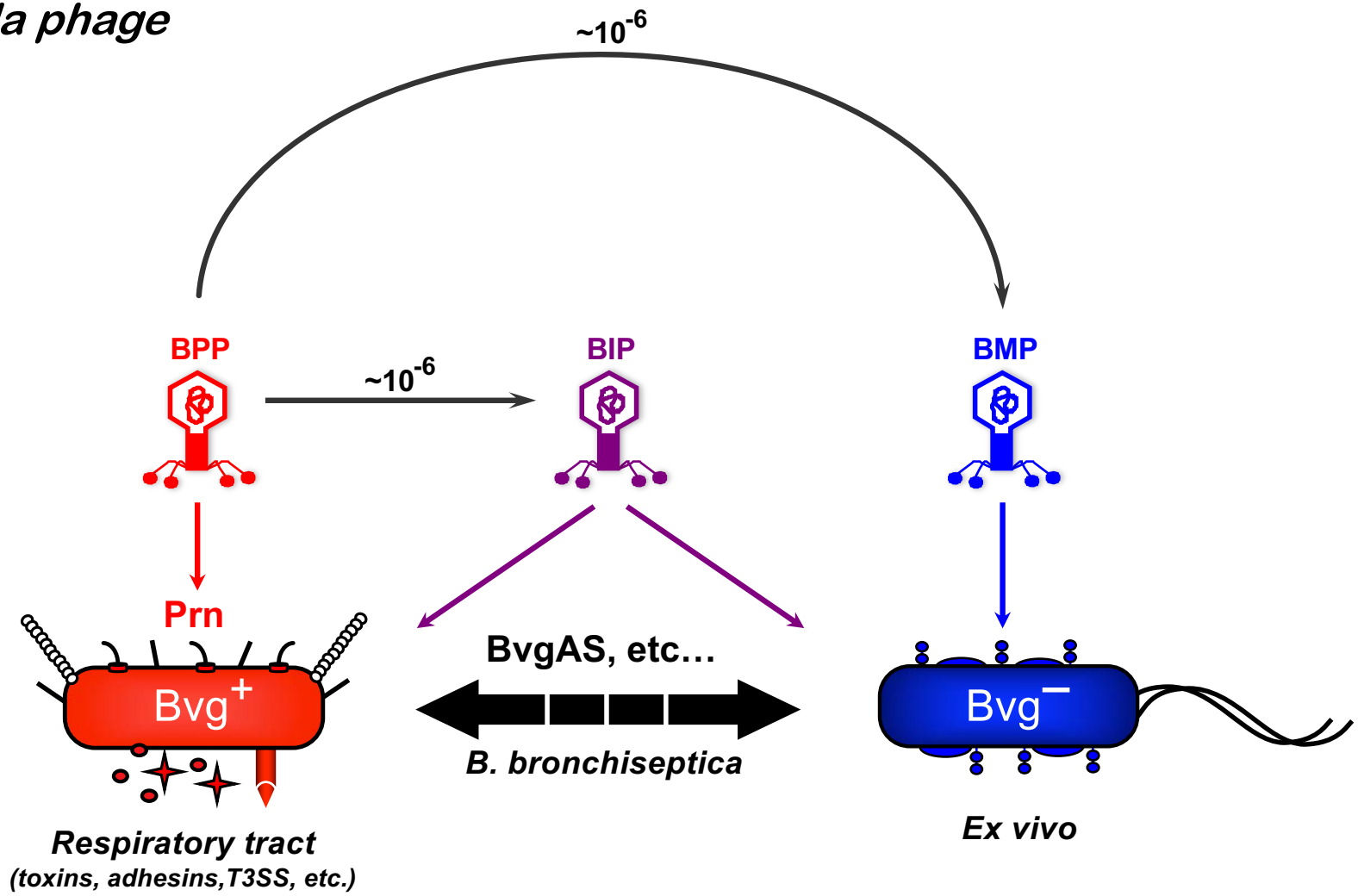


Ex vivo

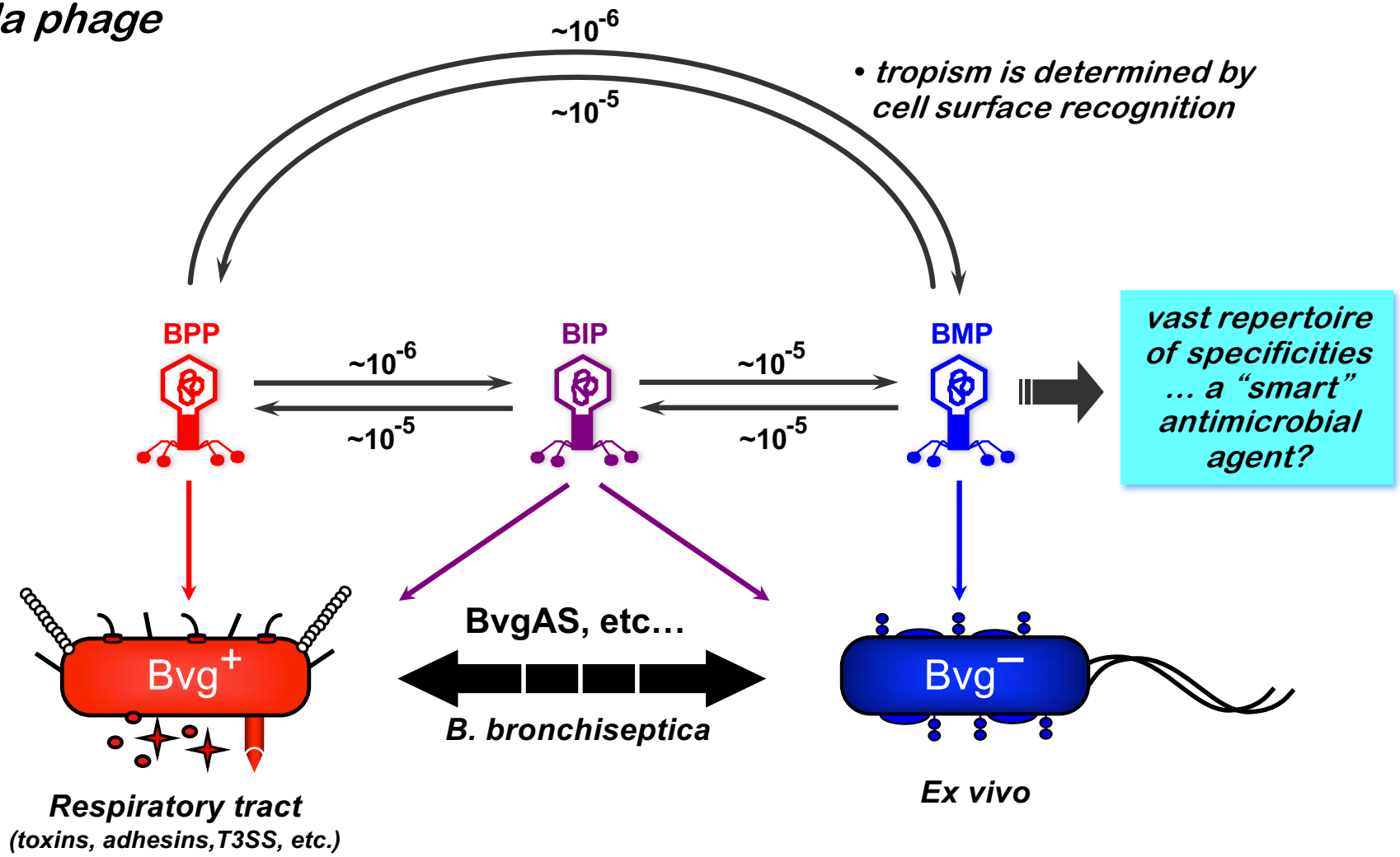
Ming Liu *et al., 2002, Science*

Bordetella phage



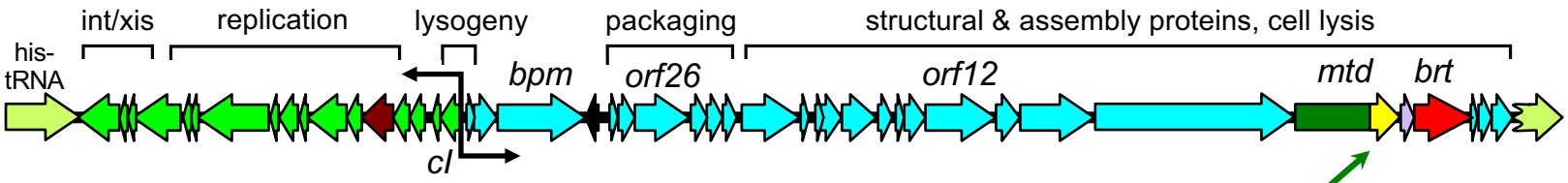
Ming Liu *et al., 2002, Science*

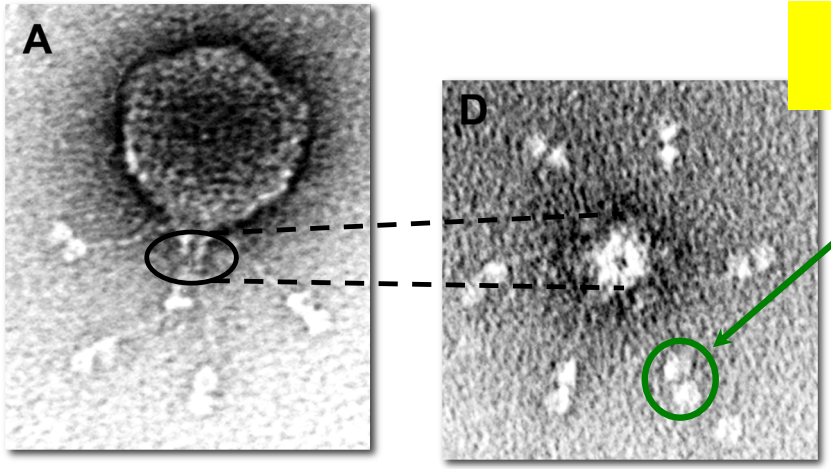
Bordetella phage



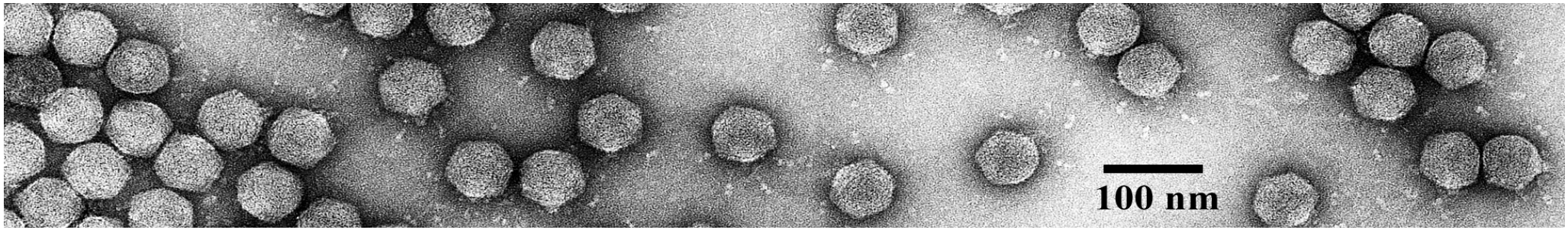
Ming Liu *et al., 2002, Science*

Bordetella phage

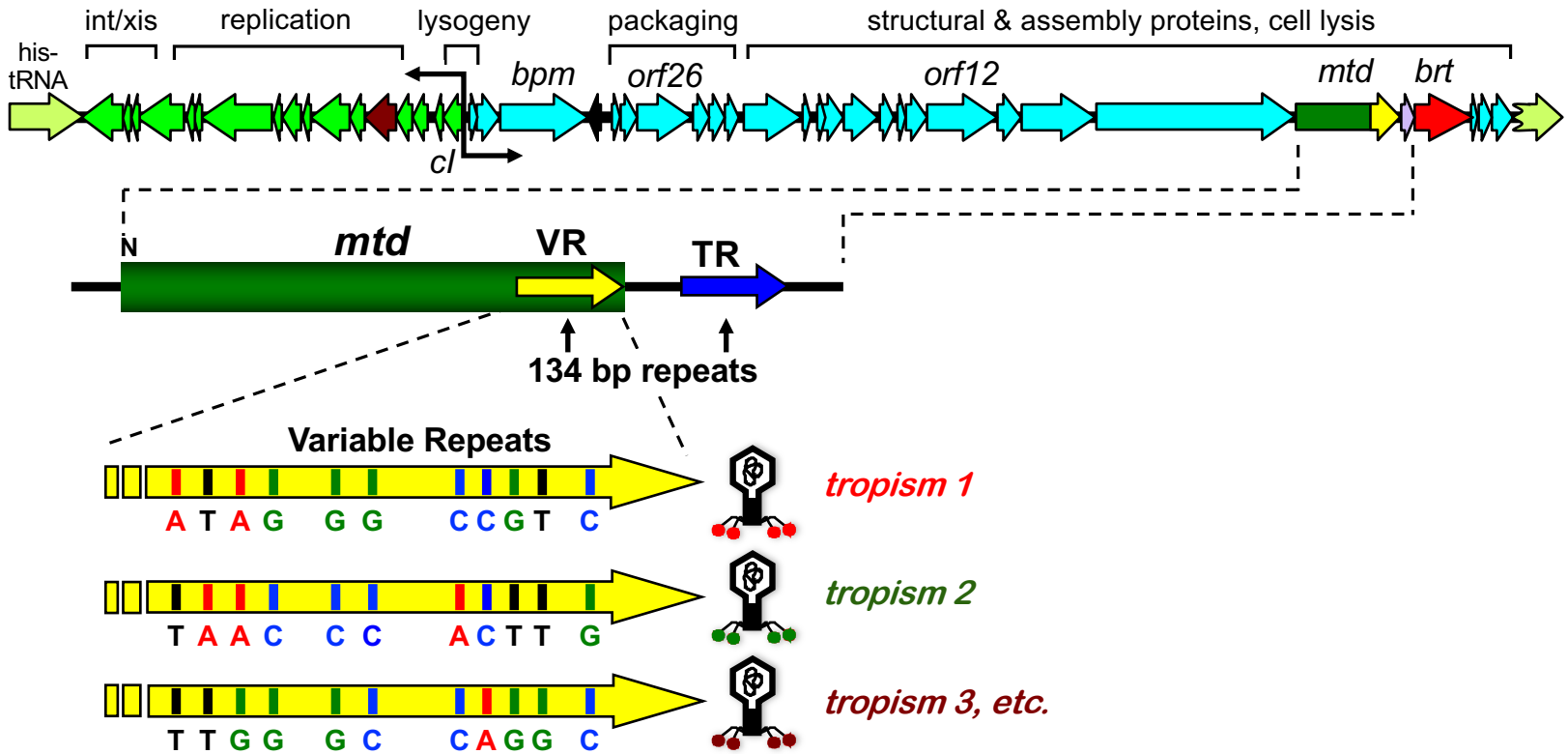




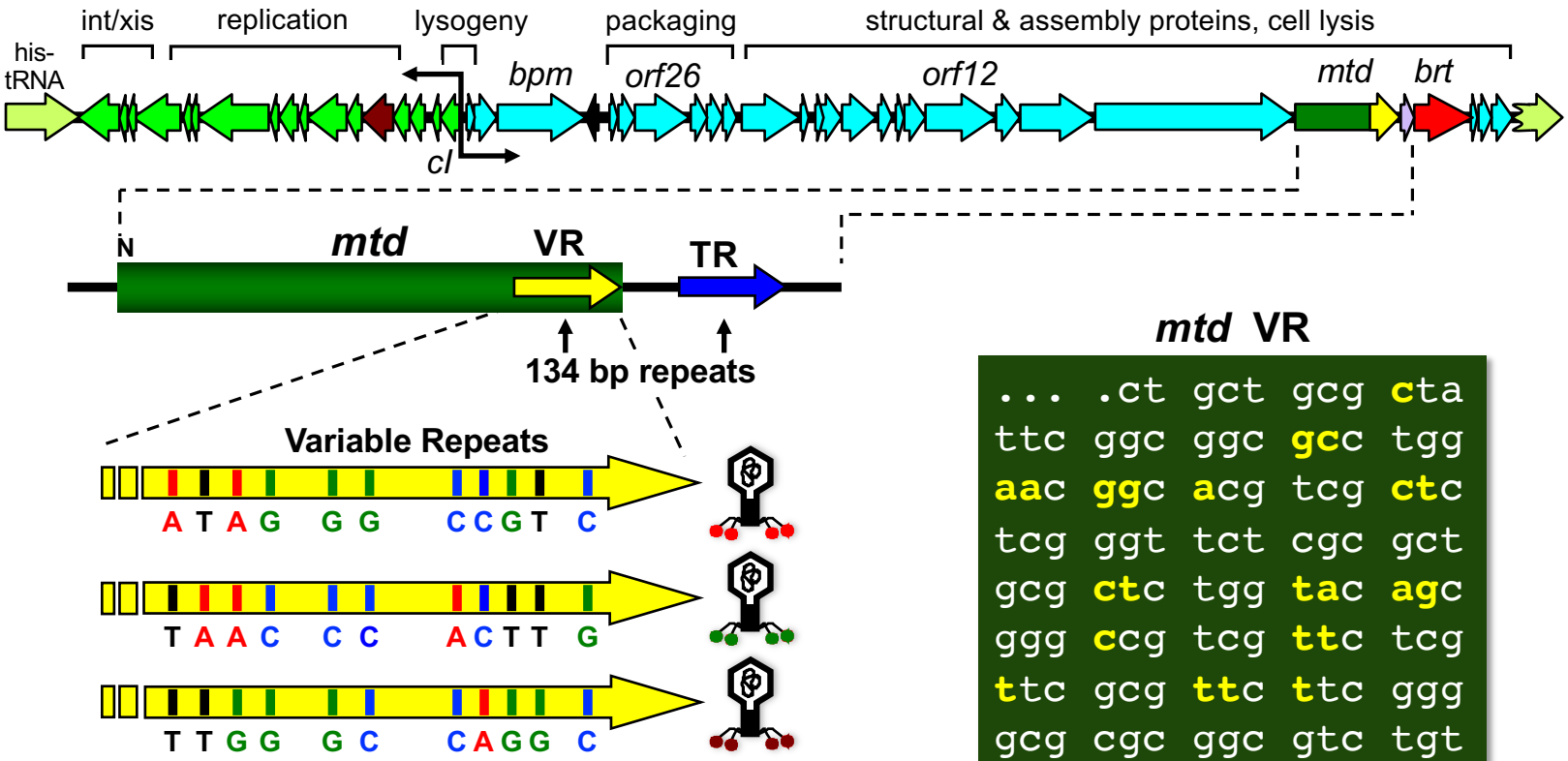
Variable Region **(VR)**
receptor binding gene *mtd*



Bordetella phage



Bordetella phage

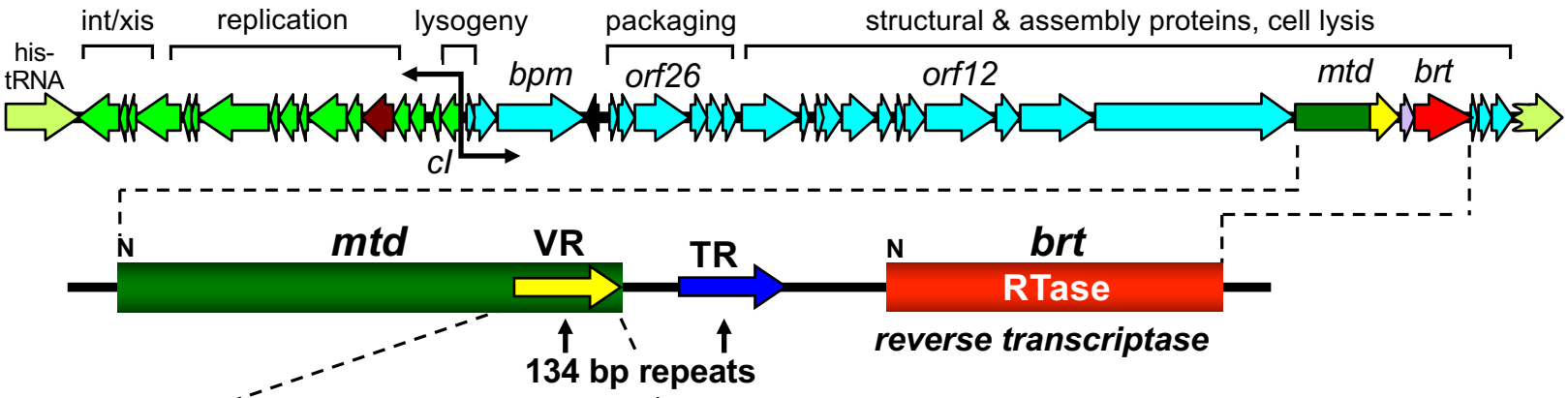


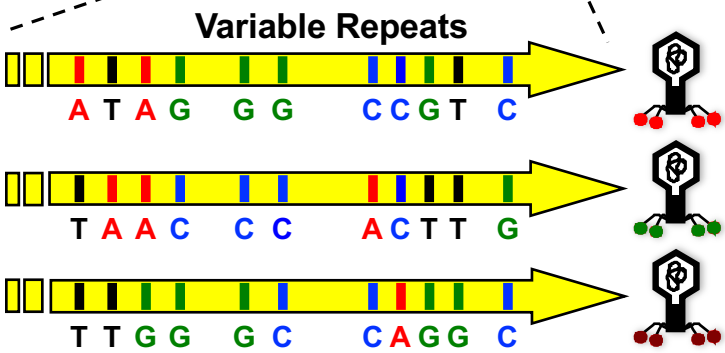
- 23 variable positions
- DNA diversity = $4^{23} \approx 10^{14}$
- Protein diversity $\approx 10^{13}$

***mtd* VR**

```
... .ct gct gcg cta
ttc ggc ggc gcc tgg
aac ggc acg tcg ctc
tcg ggt tct cgc gct
gcg ctc tgg tac agc
ggg ccg tcg ttc tcg
ttc gcg ttc ttc ggg
gcg cgc ggc gtc tgt
gac cac ctg att ctt
gag stop
```

Bordetella phage





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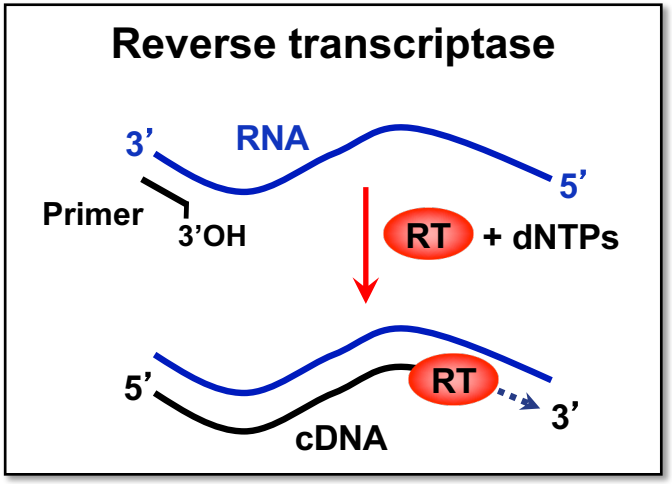
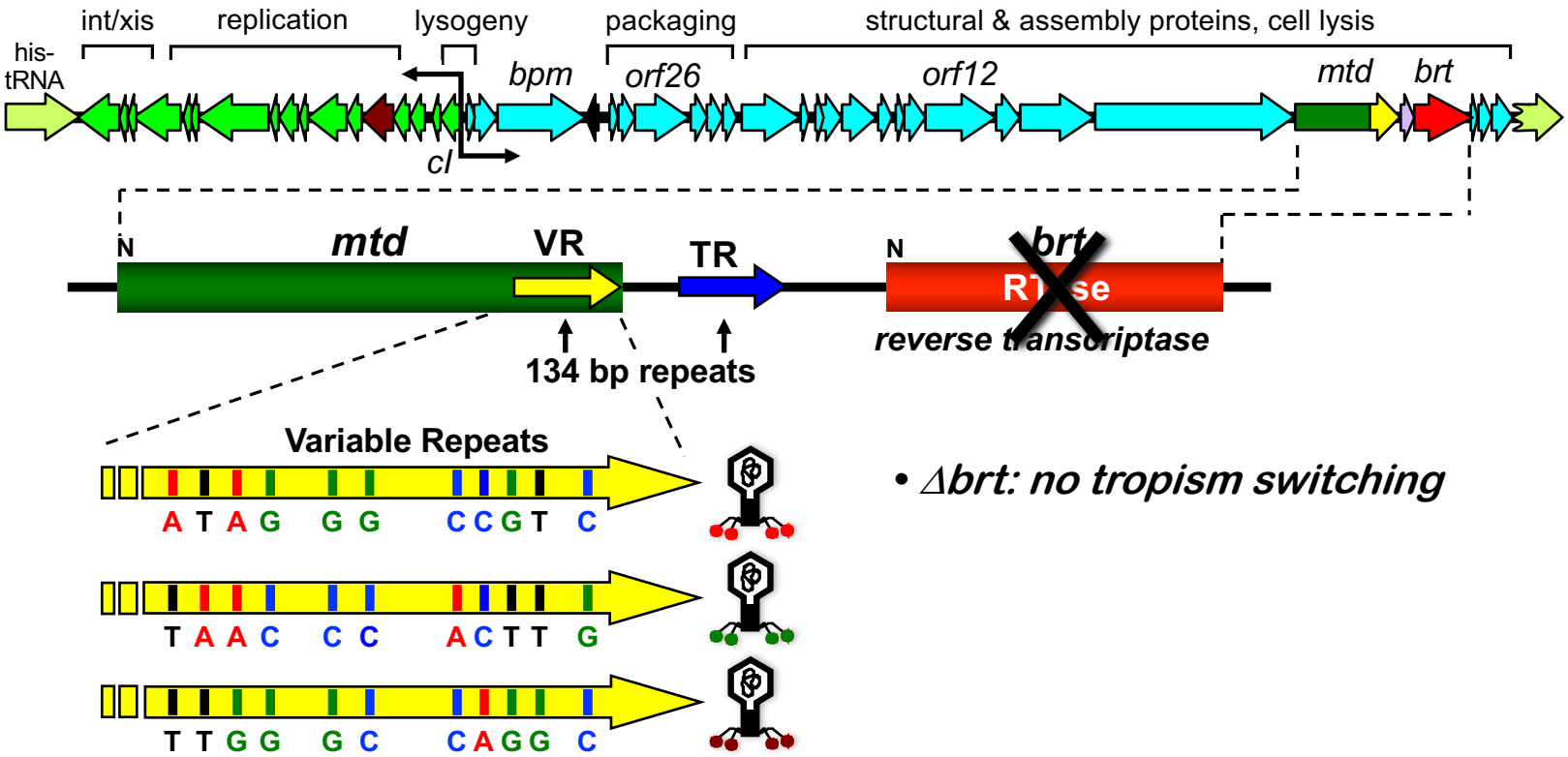
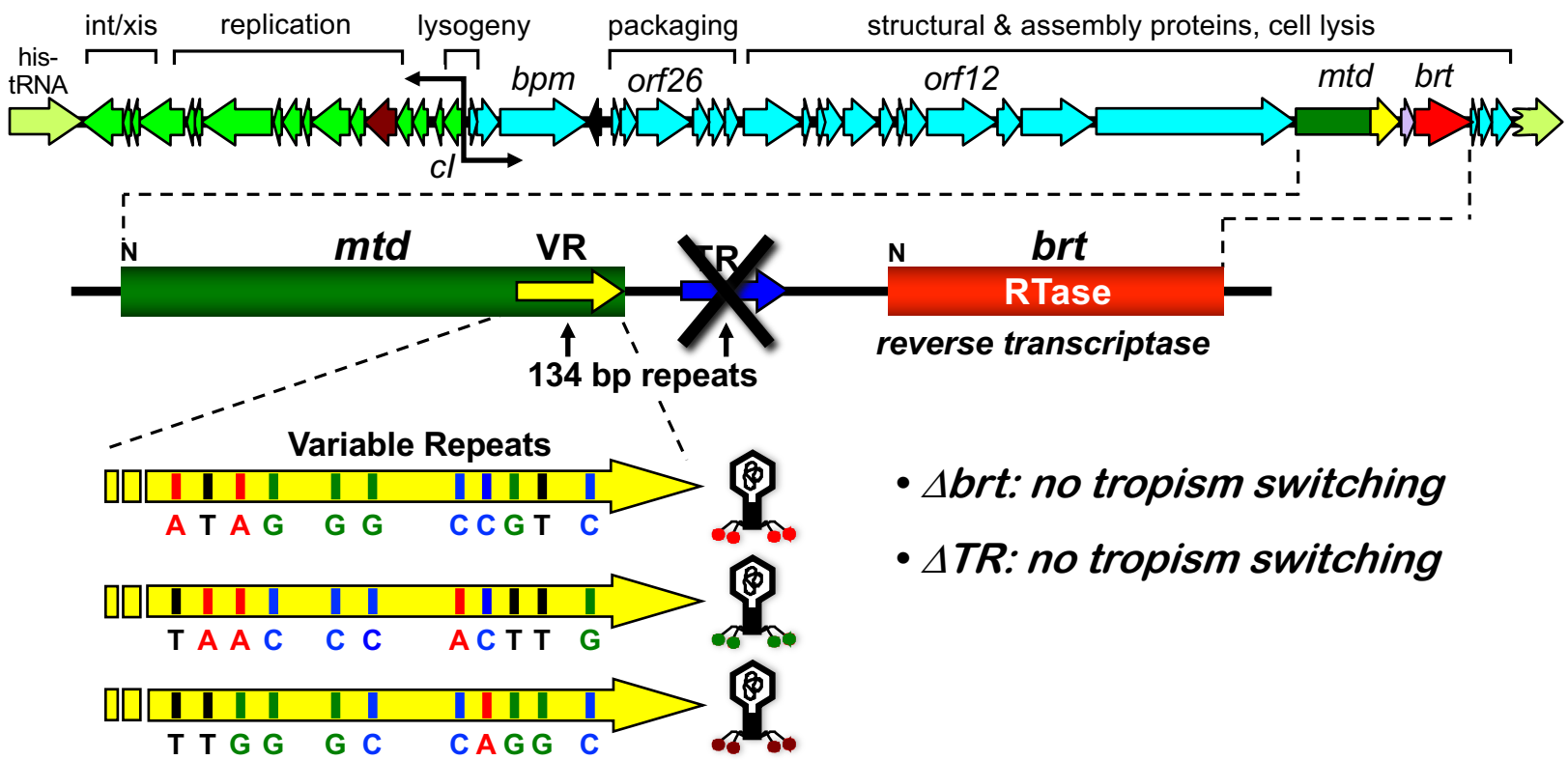


Bordetella phage



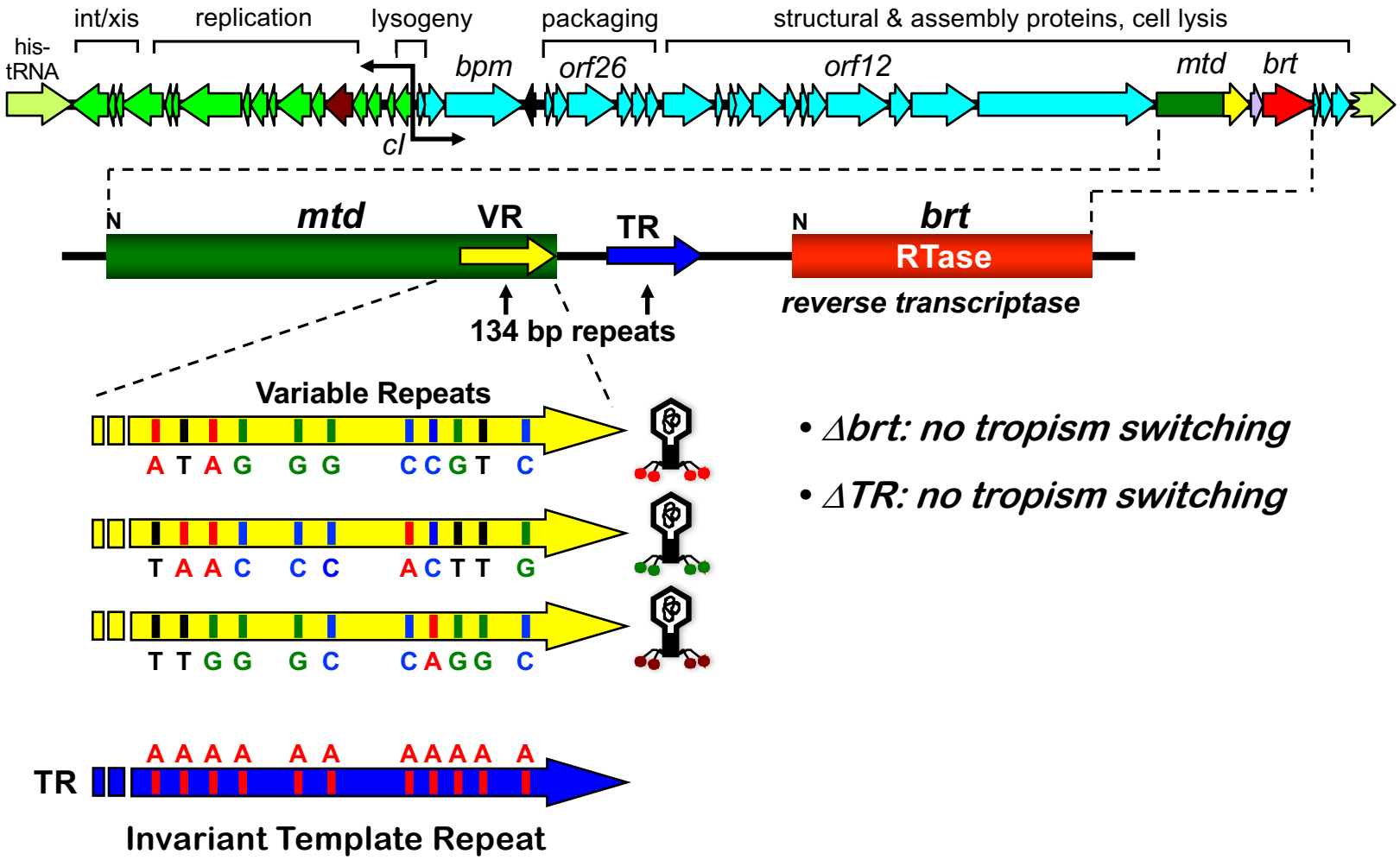
- *Δbrt: no tropism switching*

Bordetella phage



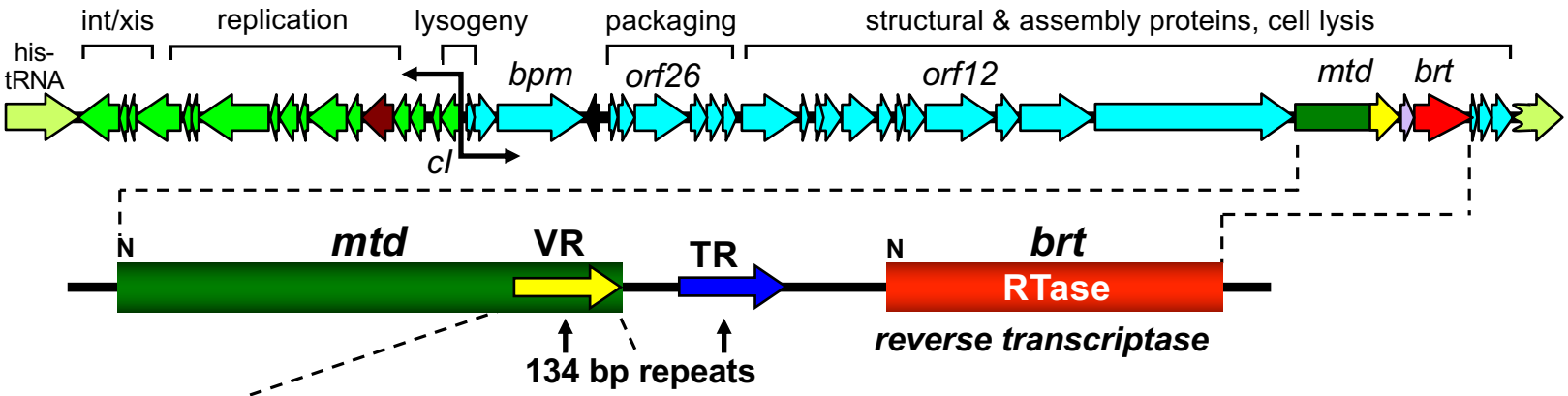
- *Δbrt: no tropism switching*
- *ΔTR: no tropism switching*

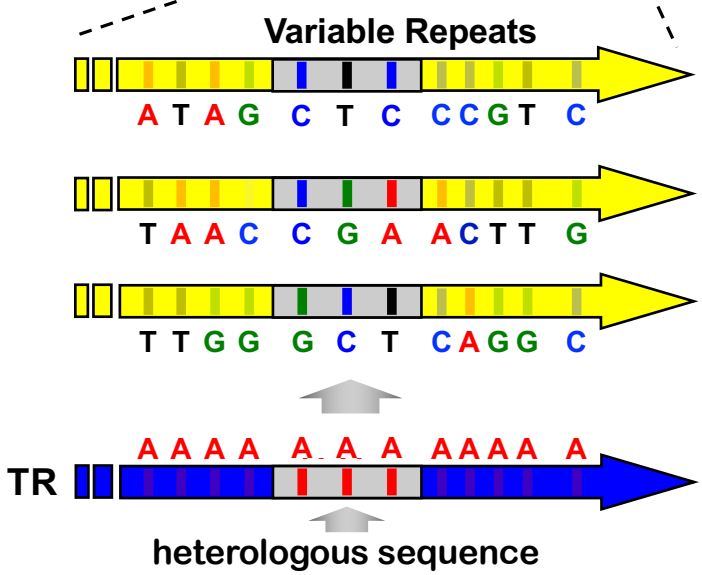
Bordetella phage



- *Δbrt: no tropism switching*
- *ΔTR: no tropism switching*

Bordetella phage



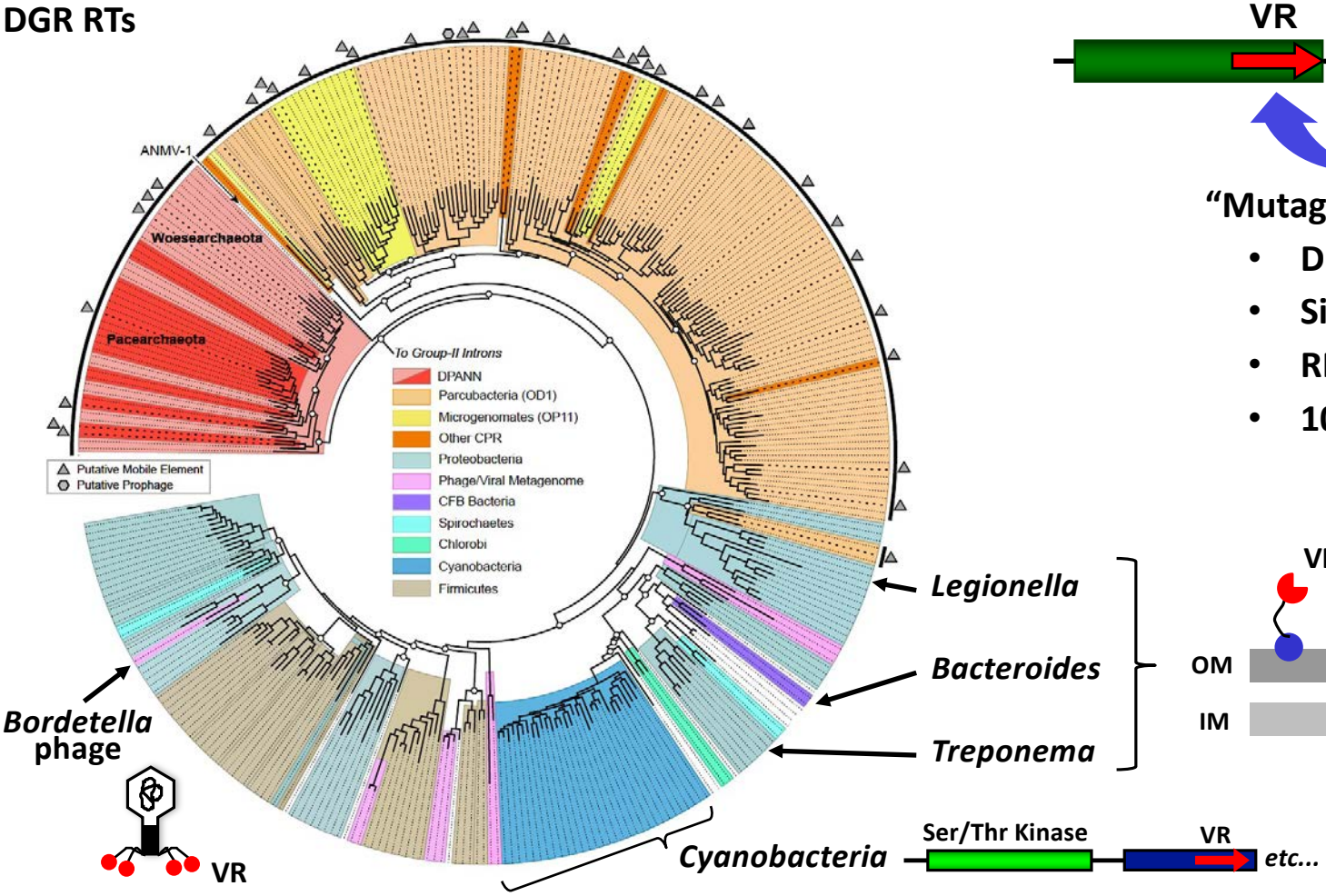


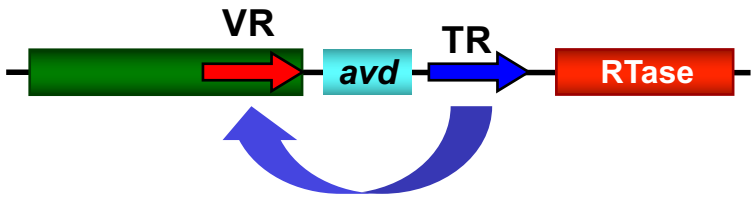
- *Δbrt: no tropism switching*
- *ΔTR: no tropism switching*
- *sequence info is "transferred" from TR to VR…*
- *…and is accompanied by A-specific mutagenesis*

Sergei Doulatov, Ming Liu *et al.*, 2004, *Nature*
Huatao Guo *et al.*, 2008, *Molecular Cell*

Diversity-generating retroelement (DGR)

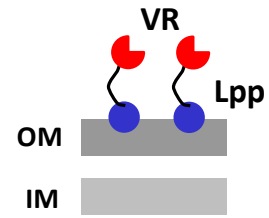
DGR RTs





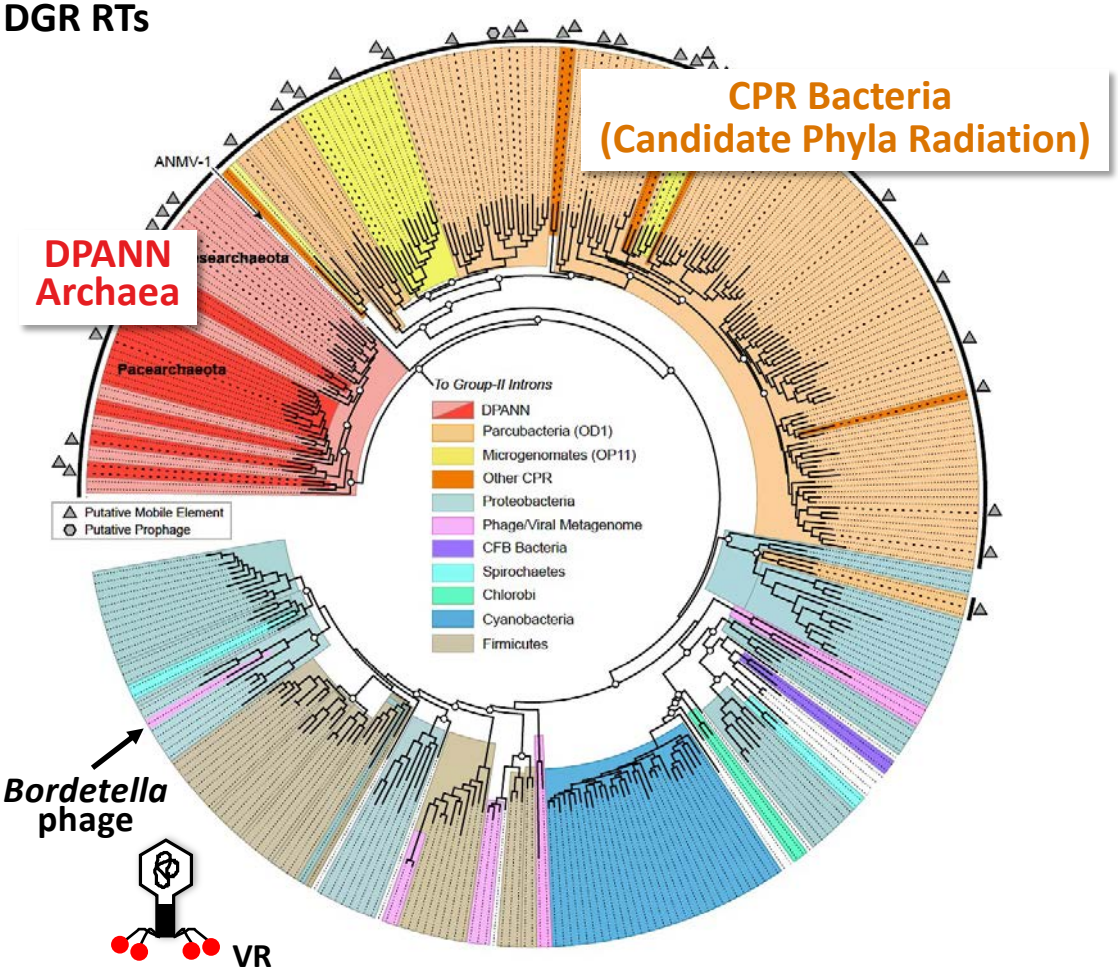
"Mutagenic homing"

- **Directional**
- **Site specific, A-mutagenesis**
- **RNA intermediate**
- **10^{14}-10^{26} variants!**



Diversity-generating retroelement (DGR)

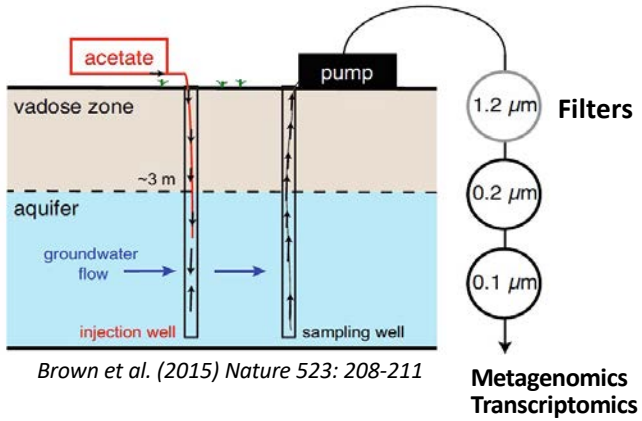
DGR RTs



Blair Paul, D. Valentine (UCSB)
D. Burstein, C. Castelle, B. Thomas, J. Banfield (UCB)
S. Handa, P. Ghosh (UCSD)
D. Arambula, E. Czorynj, J. Miller (UCLA)

Rifle, CO

39° 31′ 45″ N
107° 46′ 20″ W



Brown et al. (2015) Nature 523: 208-211

- **1,136 new DGRs**
- **majority "chromosomal"**
- **actively diversifying...**

Blair Paul *et al.*, 2017, *Nature Microbiol.*

Diversity-generating retroelement (DGR)

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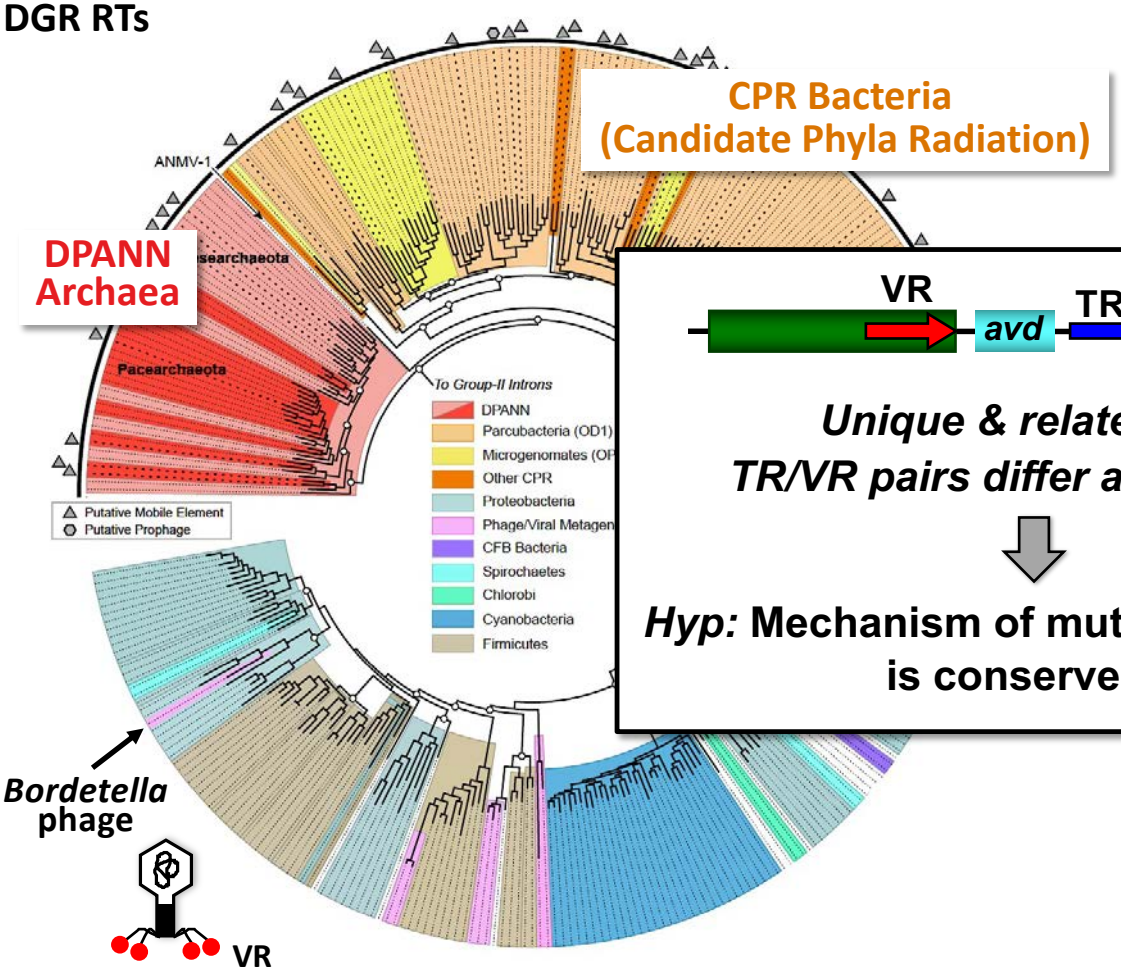
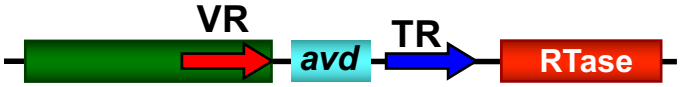
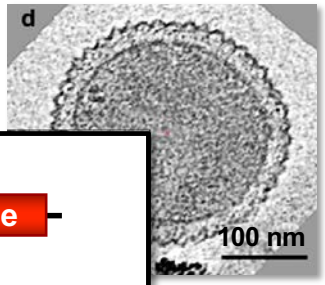
DGR RTs

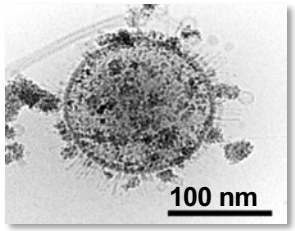




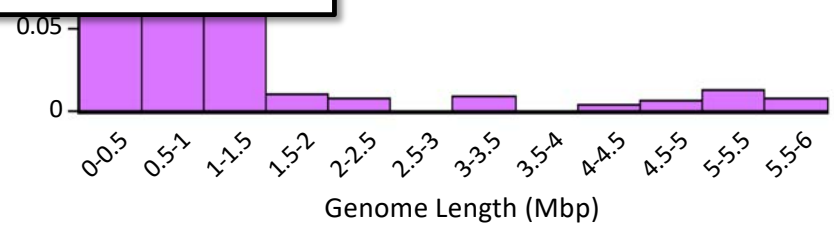
Unique & related RTs
TR/VR pairs differ at Adenines

***Hyp:* Mechanism of mutagenic homing is conserved...**



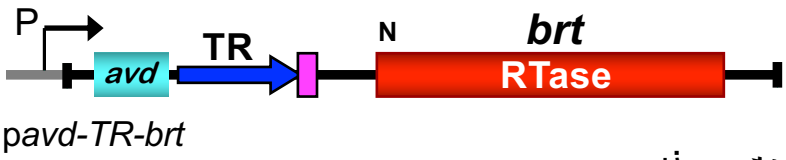


Luef B. *et al. Nat. Commun.* 6:6372, 2015

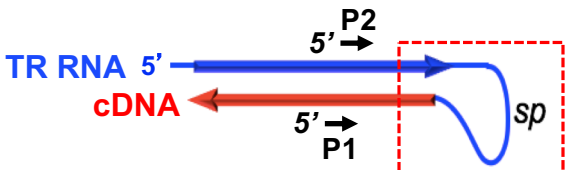


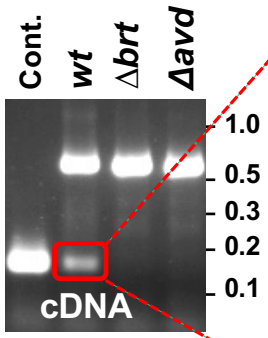
Blair Paul *et al.*, 2017, *Nature Microbiol.*

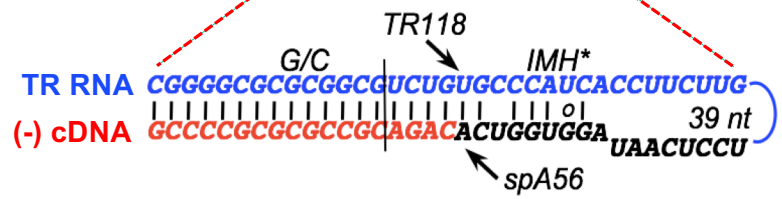
What primes reverse transcription?



pavd-TR-brt







cDNAs mutagenized at adenines:

```
     TR GTCGACCTCGAACGCGAACATCGGGGCGC
cDNA 1 GTCGACCTCGCACGCGTACATCGGGGCGC
cDNA 2 GTCGCCCTCGATCGCGTACATCGGGGCGC
cDNA 3 GTCGCCCTCGACCGCGTACATCGGGGCGC
cDNA 4 GTCGACCTCGTTCGCGAACGTCGGGGCGC
cDNA 5 GTCGCCCTCGAGCGCGCACATCGGGGCGC
cDNA 6 GTCGACCTCGTACGCGGGCGTCGGGGCGC
cDNA 7 GTCGCCCTCGAACGCGGACATCGGGGCGC
cDNA 8 GTCGACCTCGTACGCGAGCGTCGGGGCGC
       ****  *****   ****   *  *********
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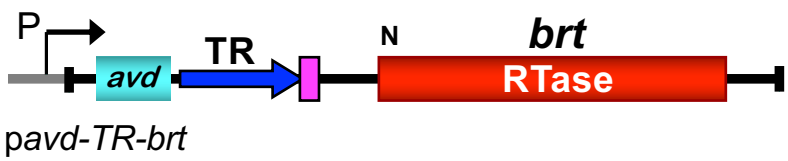
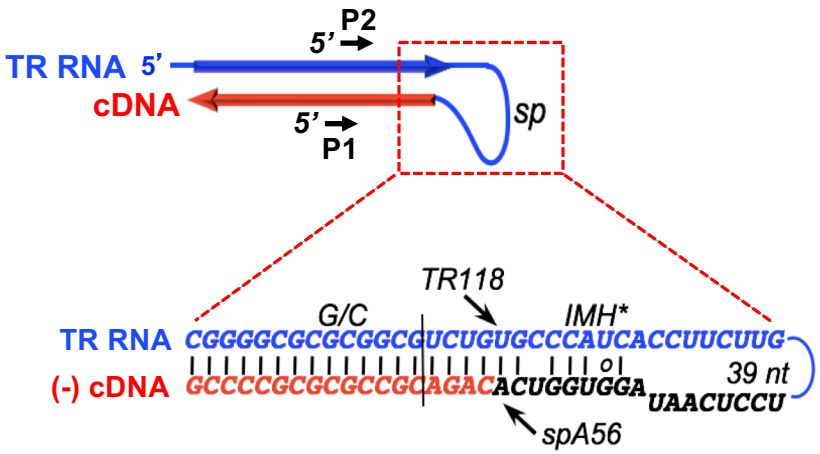
Santa Naorem, Huatao Guo *et al.*, 2017, *PNAS*

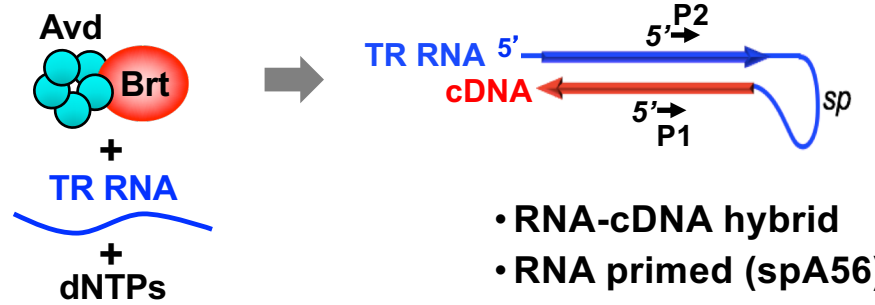
What primes reverse transcription?



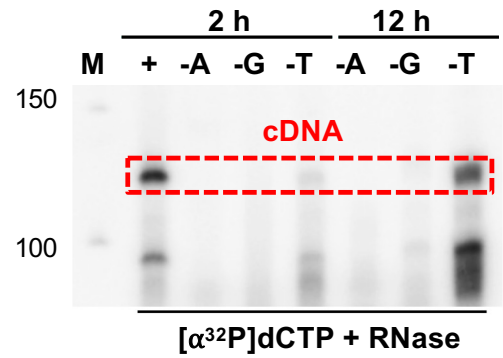


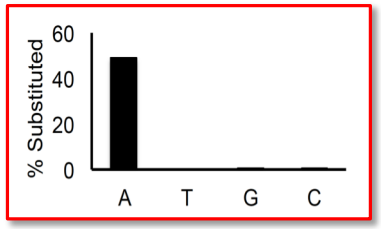
How does A-mutagenesis occur?

in vitro reconstitution:



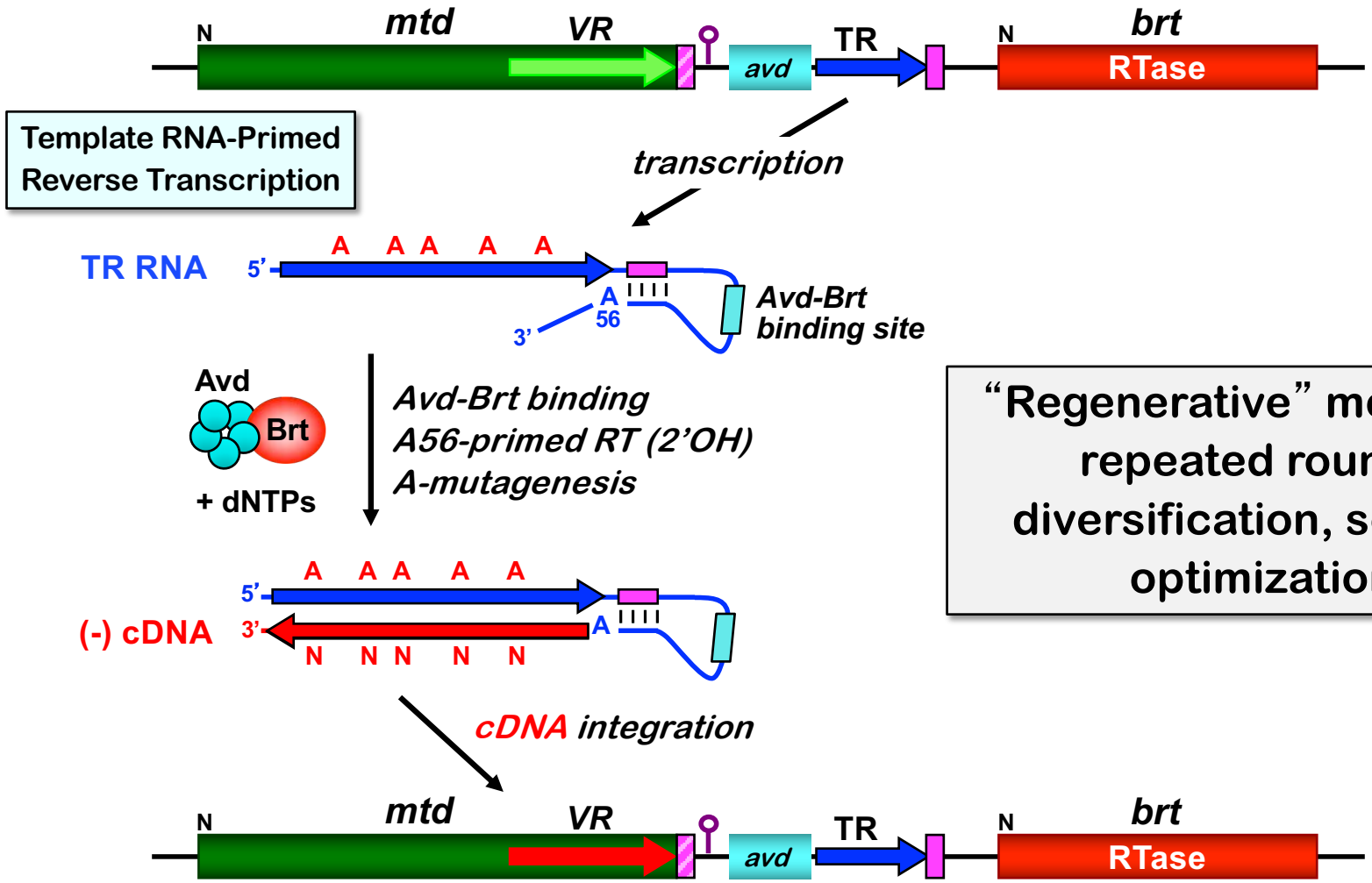
- RNA-cDNA hybrid
- RNA primed (spA56)
- A-mutagenesis





Santa Naorem, Huatao Guo *et al.*, 2017, *PNAS*

Sumit Handa, Partho Ghosh *et al.*, 2018, NAR In press

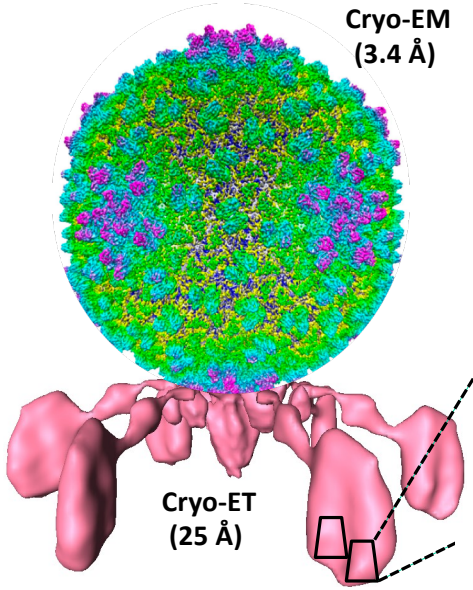


"Regenerative" mechanism: repeated rounds of diversification, selection, optimization…

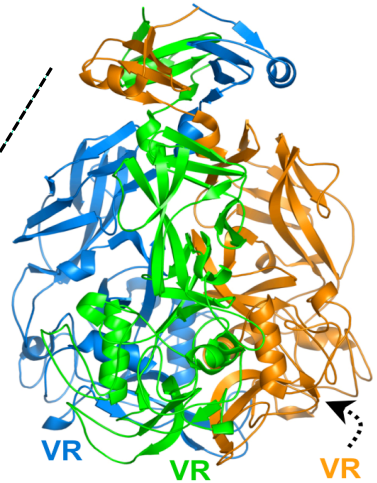
Santa Naorem, Huatao Guo *et al.*, 2017, *PNAS*

Sumit Handa, Partho Ghosh *et al.*, 2018, NAR In press

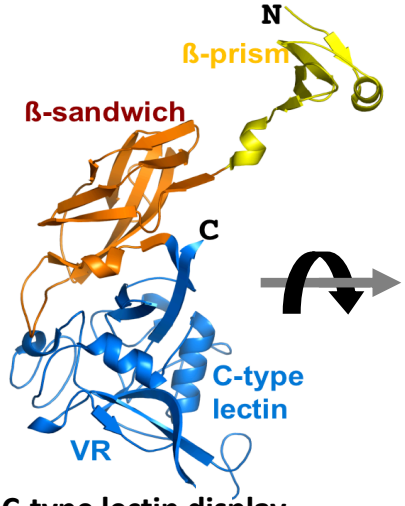
BPP-1



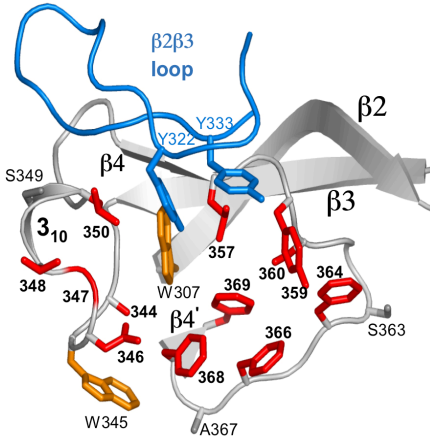
2 Mtd trimers/tail fiber



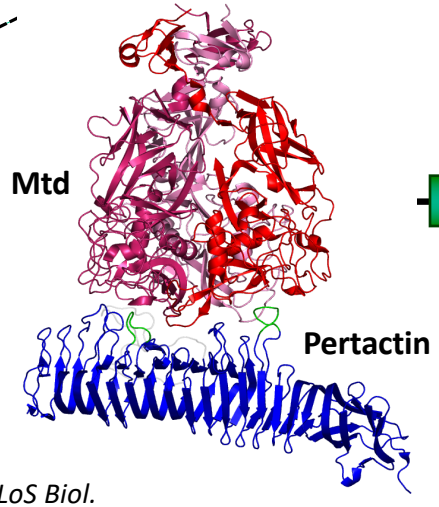
3 VRs/Mtd trimer

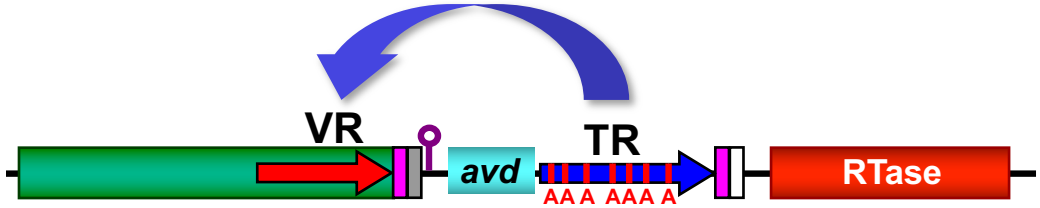


C-type lectin display



Variable residues

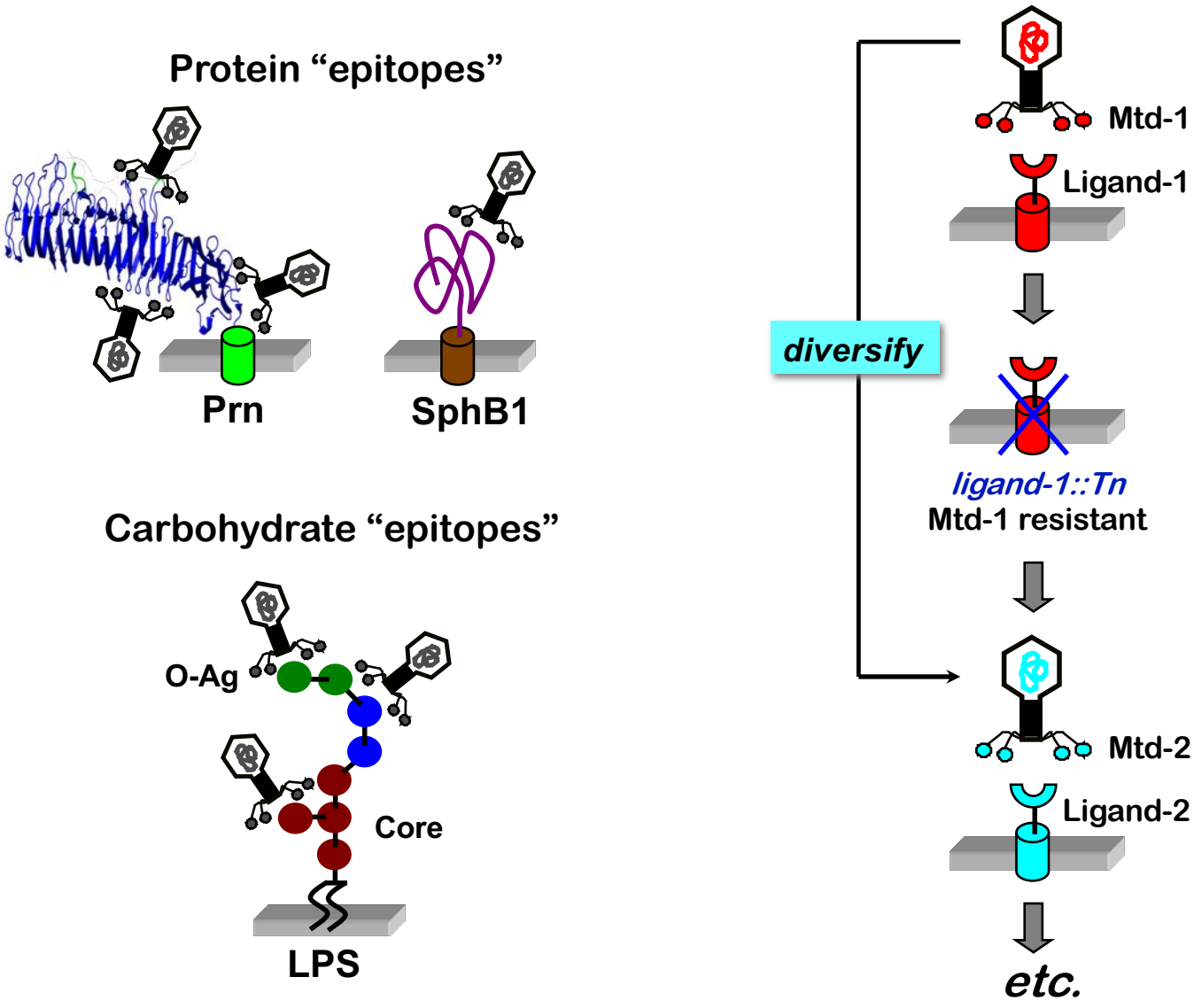




TR adenines are positioned to diversify ligand binding residues in VR

Xing Zhang, Hong Zhou *et al.*, 2013, *eLife*

Jason Miller, Partho Ghosh *et al.*, 2008, *PLoS Biol.*

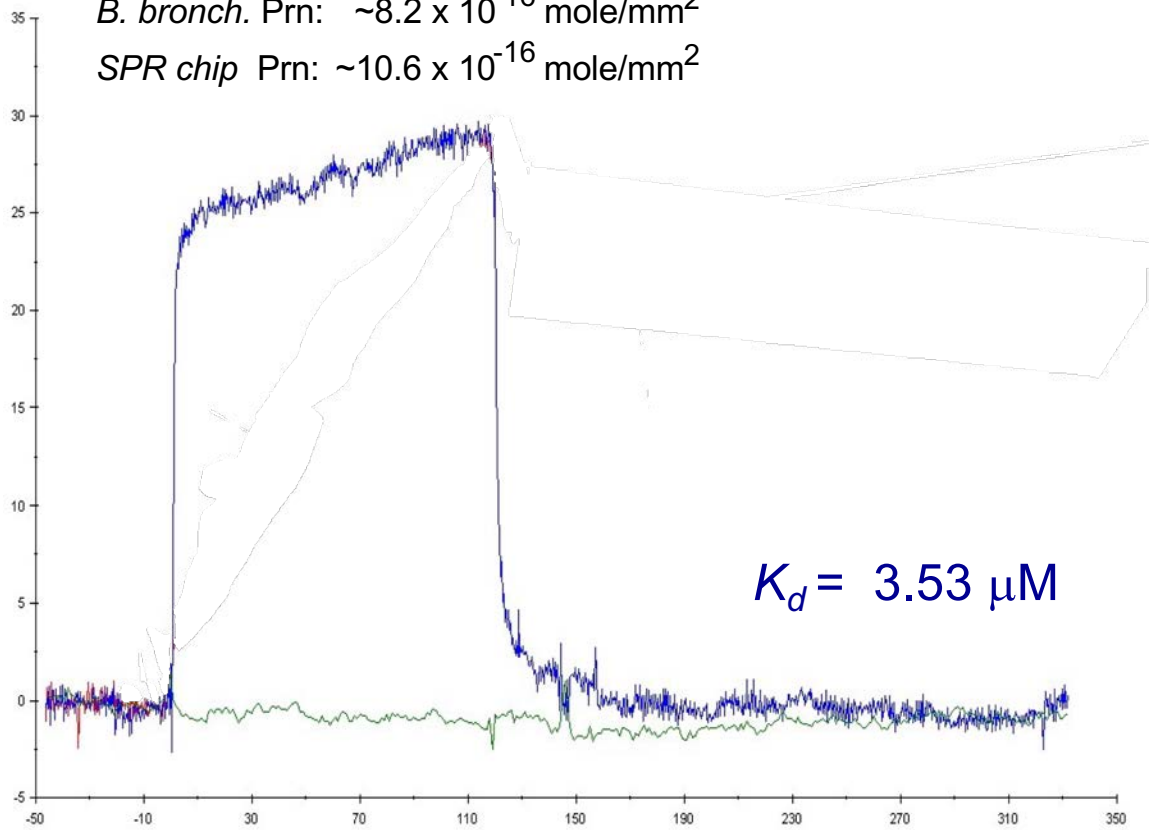

Protein "epitopes"
Prn
SphB1
Carbohydrate "epitopes"
O-Ag
Core
LPS
diversify
Mtd-1
Ligand-1
ligand-1::Tn
Mtd-1 resistant
Mtd-2
Ligand-2
etc.

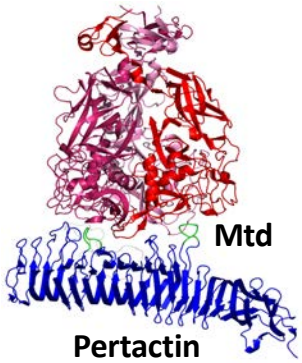
Surface plasmon resonance (SPR)

(biotinylated pertactin immobilized on streptavidin chip)

B. bronch. Prn: ~8.2 x 10^{-16} mole/mm^2

SPR chip Prn: ~10.6 x 10^{-16} mole/mm^2





Jason Miller (UCSD), Asher Hodes (UCLA)

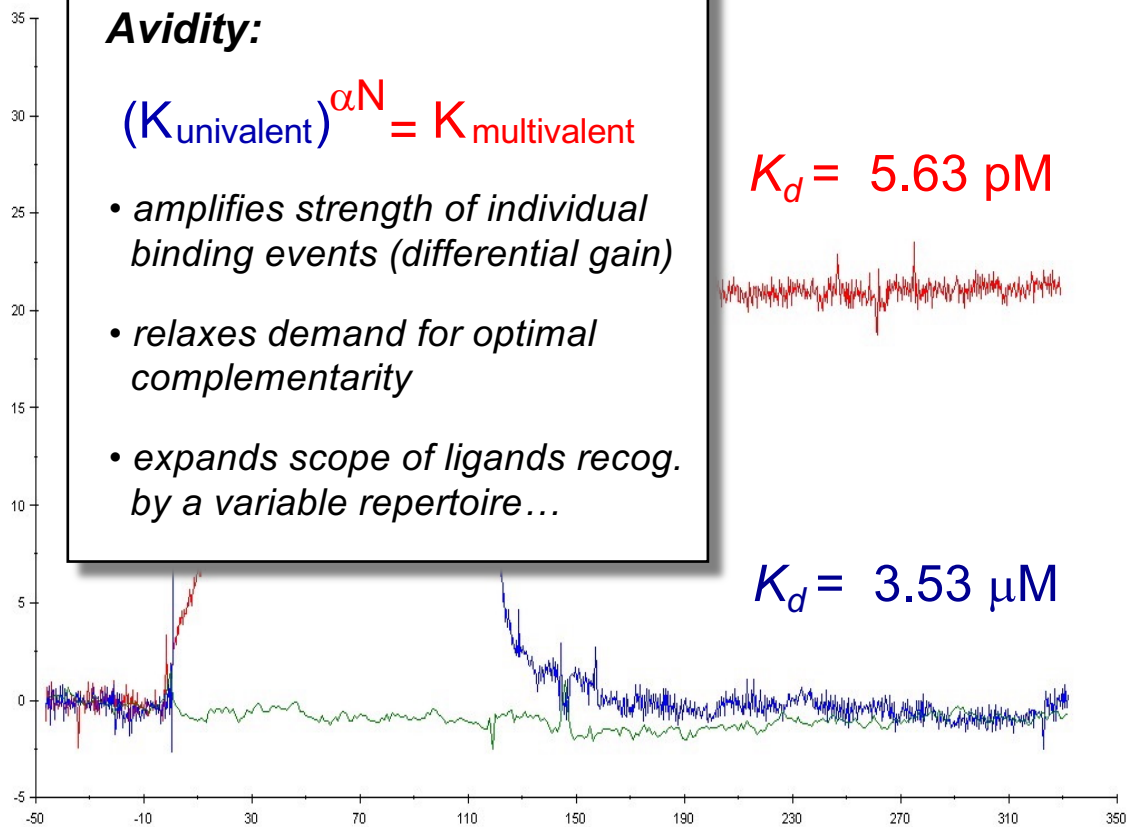
Surface plasmon resonance (SPR)

(biotinylated pertactin immobilized on streptavidin chip)

Avidity:

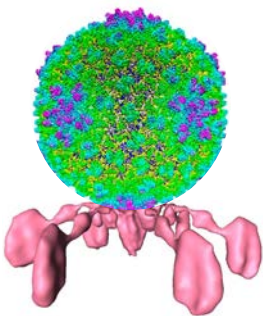
$$(K_{univalent})^{\alpha N} = K_{multivalent}$$

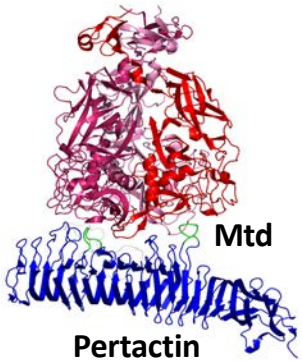
- *amplifies strength of individual binding events (differential gain)*
- *relaxes demand for optimal complementarity*
- *expands scope of ligands recog. by a variable repertoire…*



K_d = 5.63 pM

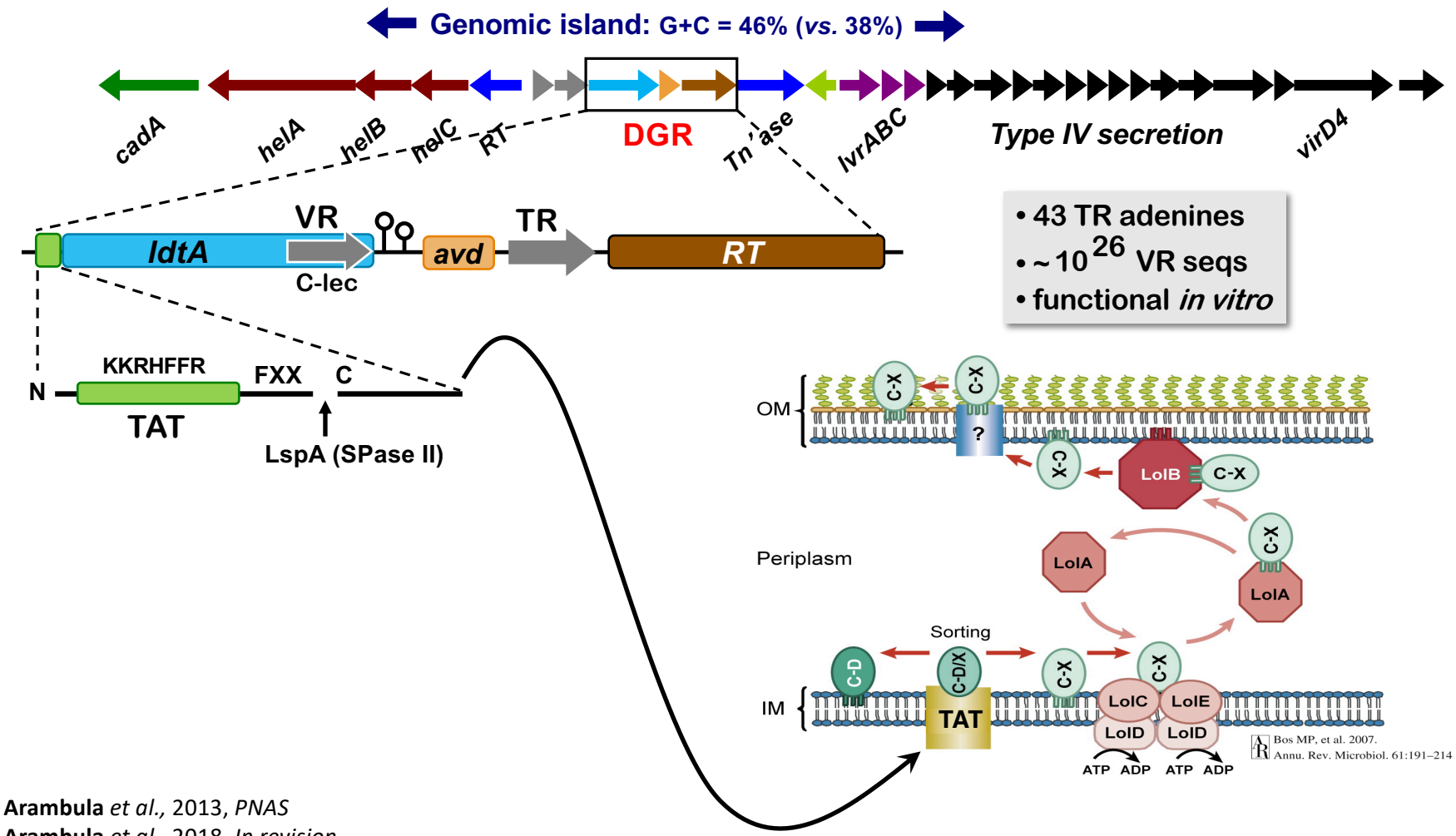
K_d = 3.53 μM





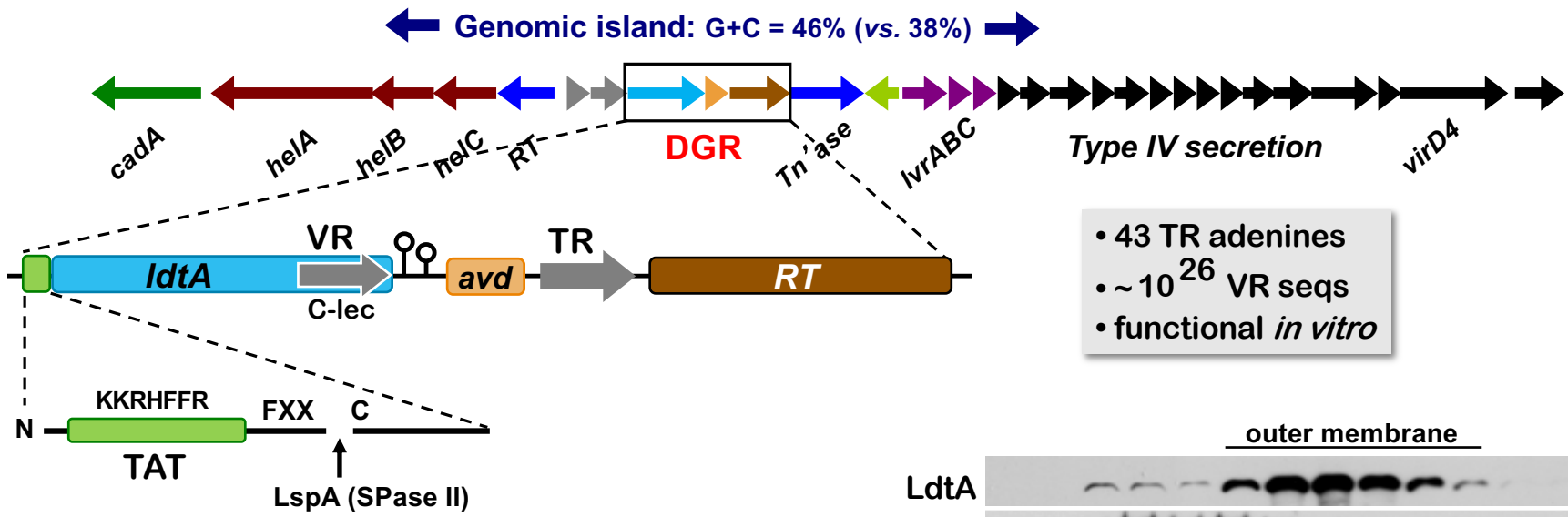
Jason Miller (UCSD), Asher Hodes (UCLA)

Legionella pneumophila Corby ("Legionnaire's disease")

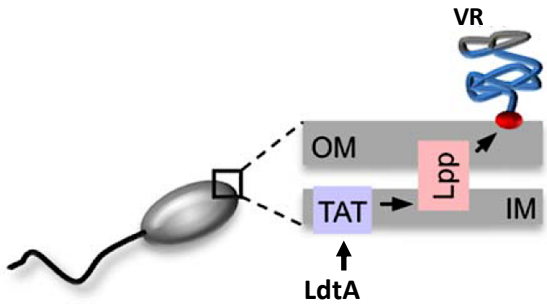


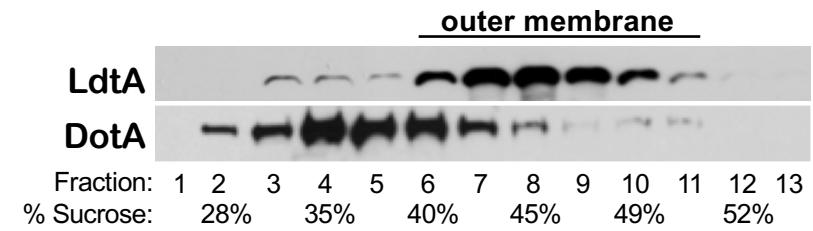
Diego Arambula *et al.*, 2013, *PNAS*
Diego Arambula *et al.*, 2018, *In revision*

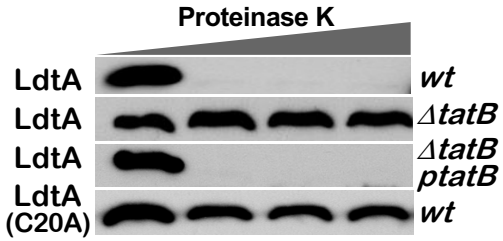
Legionella pneumophila Corby ("Legionnaire's disease")

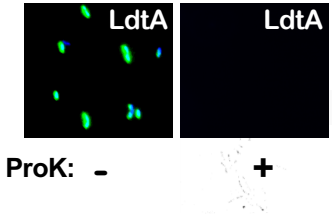


- 43 TR adenines
- ~ 10^{26} VR seqs
- functional *in vitro*





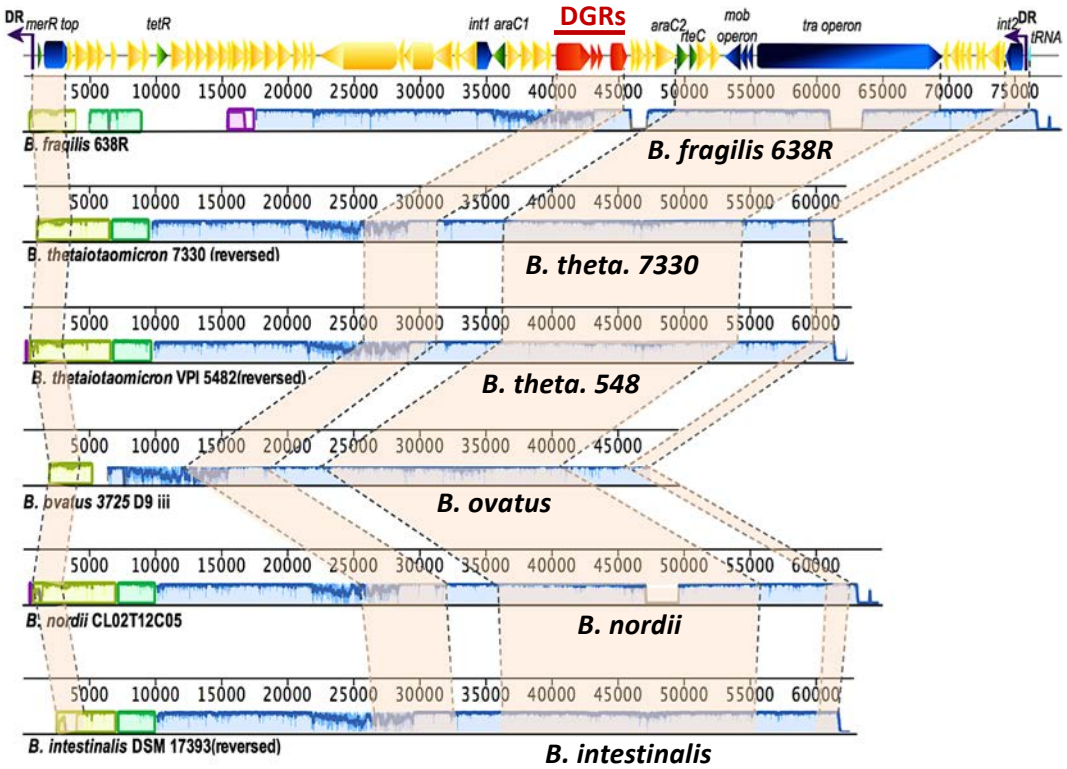




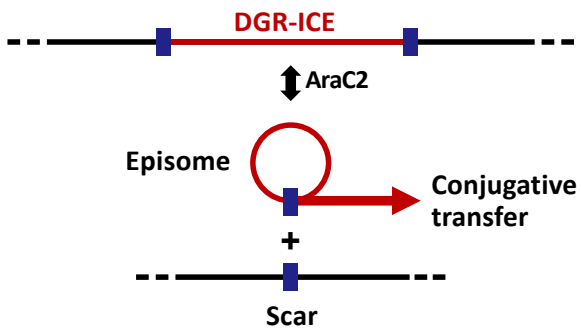
Diego Arambula *et al.*, 2013, *PNAS*
Diego Arambula *et al.*, 2018, *In revision*

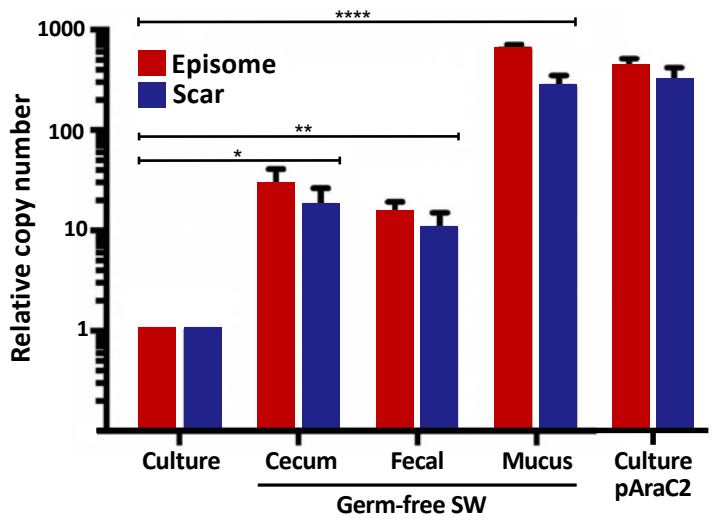
Bacteroides DGRs

Integrative and Conjugative Elements (ICEs):



B. fragilis 638R

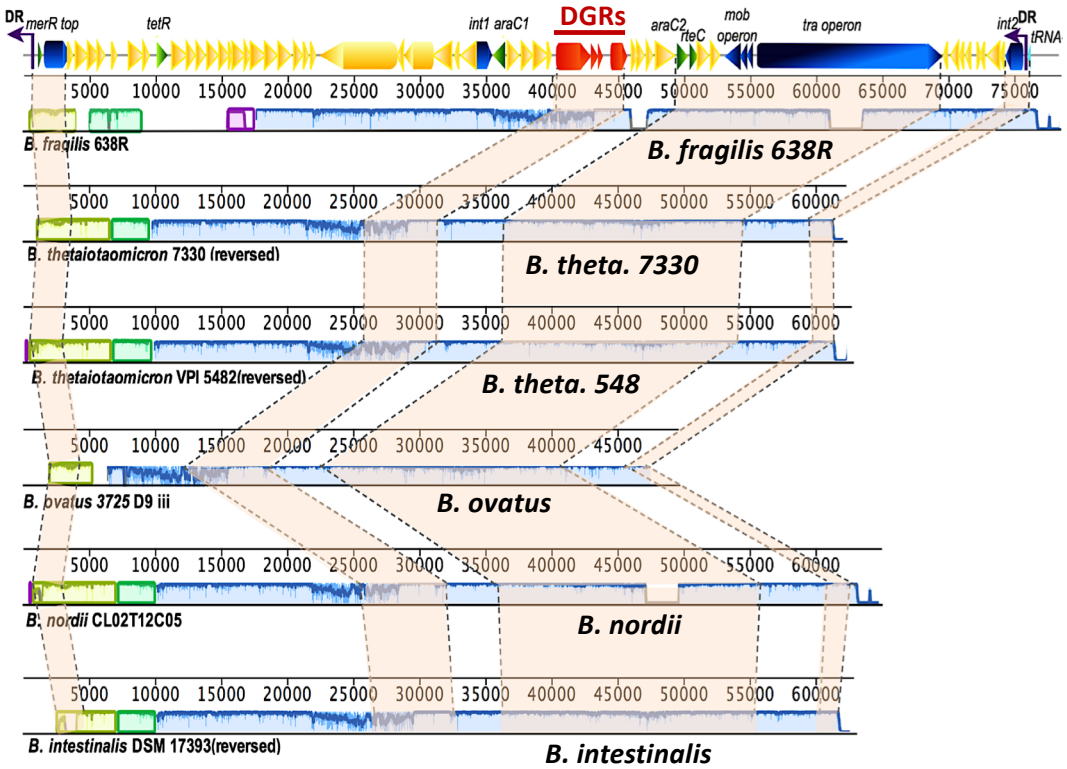




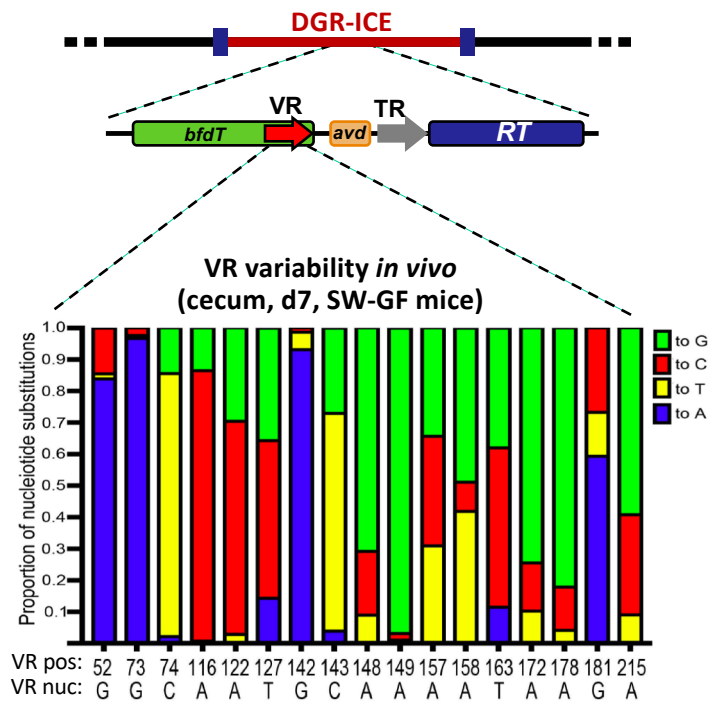
Yanling Wang & Diego Arambula (UCLA)
Blair Paul (UCSB)
Sarkis Mazmanian (Caltech)

Bacteroides DGRs

Integrative and Conjugative Elements (ICEs):



B. fragilis 638R



- 41 TR Adenines, $5x10^{24}$ variants
- Frequency of variation ~10^{-3}
- 17/41 positions "hotspots"

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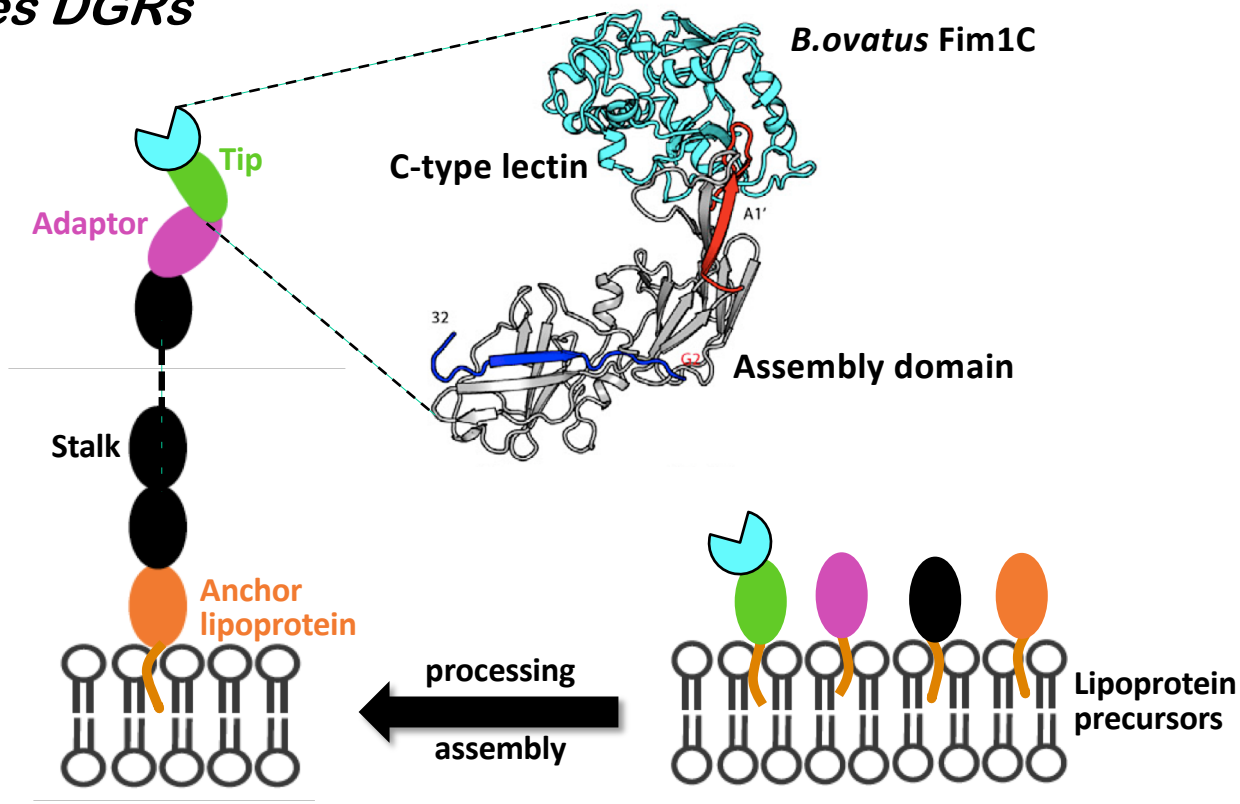
Bacteroides DGRs

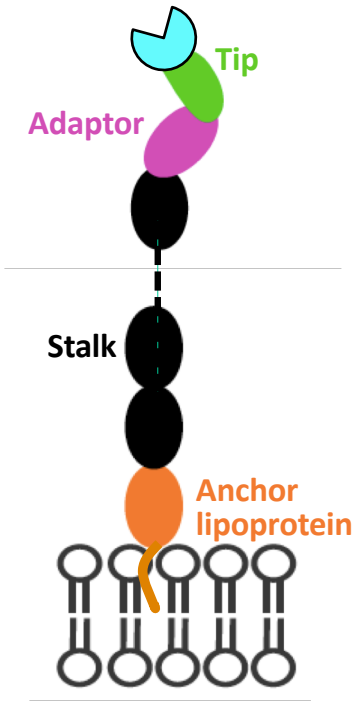


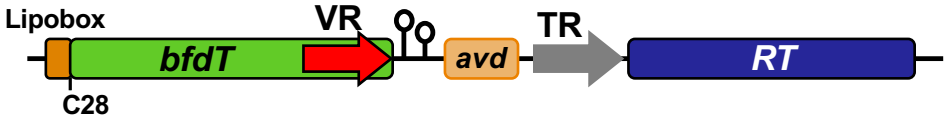
A Distinct Type of Pilus from the Human Microbiome

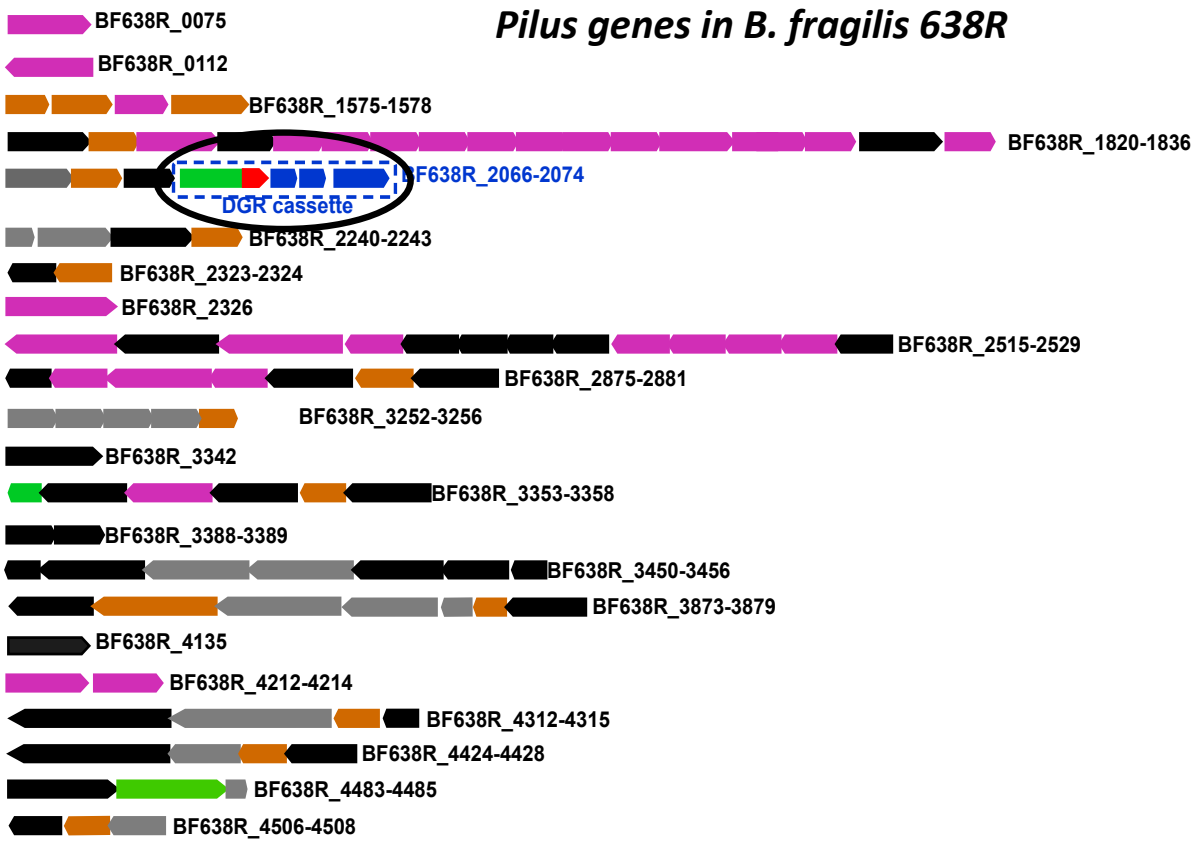
Qingping Xu,[1,2,9] Mikio Shoji,[3,9] Satoshi Shibata,[3] Mariko Naito,[3] Keiko Sato,[3] Marc-André Elsliger,[1,4] Joanna C. Grant,[1,5] Herbert L. Axelrod,[1,2] Hsiu-Ju Chiu,[1,2] Carol L. Farr,[1,5] Lukasz Jaroszewski,[1,6,7] Mark W. Knuth,[1,5] Ashley M. Deacon,[1,2] Adam Godzik,[1,6,7] Scott A. Lesley,[1,4,5] Michael A. Curtis,[8] Koji Nakayama,[3,*] and Ian A. Wilson[1,4,*]

Cell *165*, 690–703, April 21, 2016

Bacteroides DGRs







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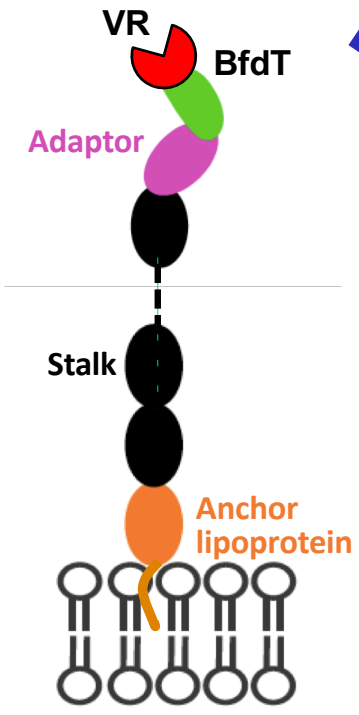
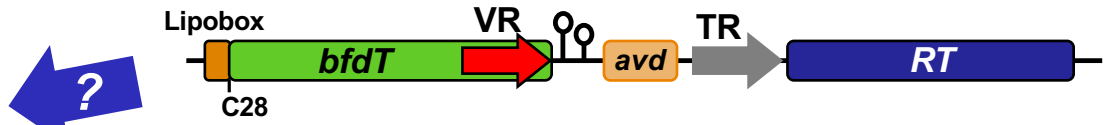
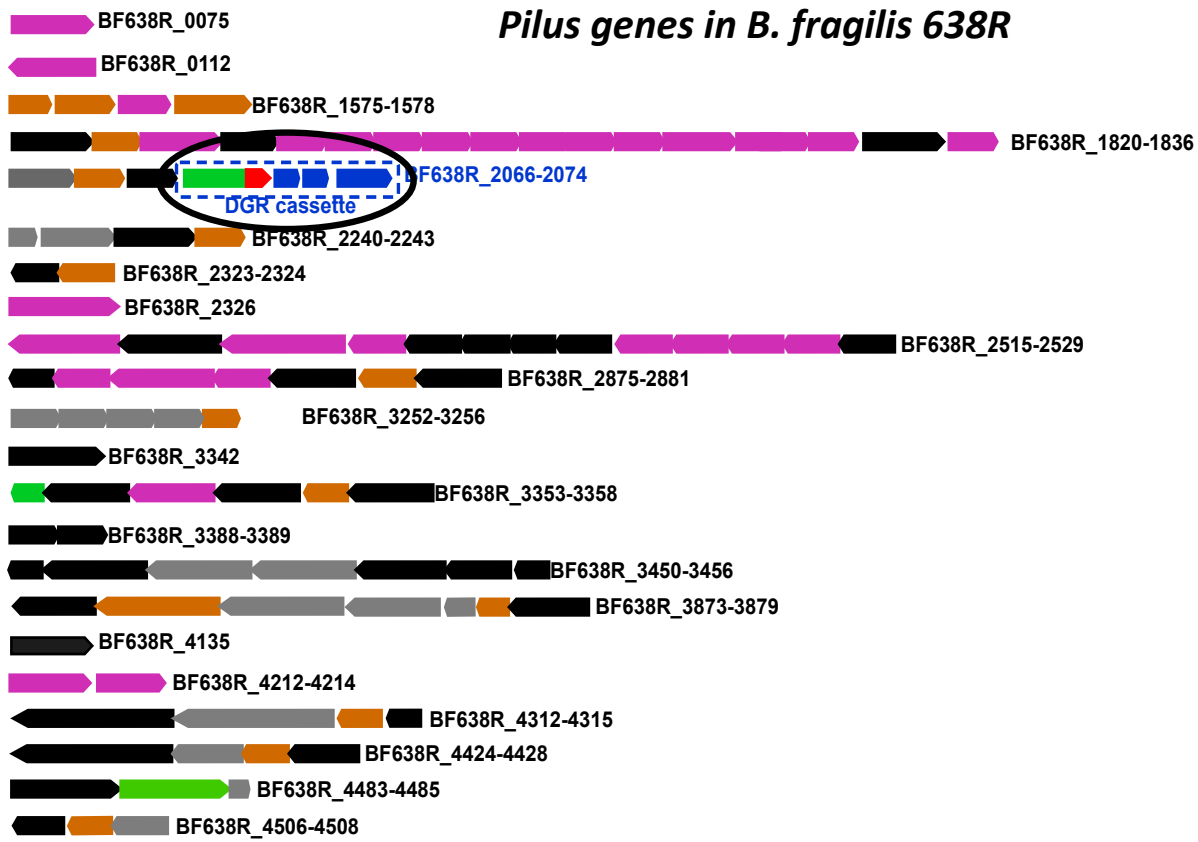
Bacteroides DGRs







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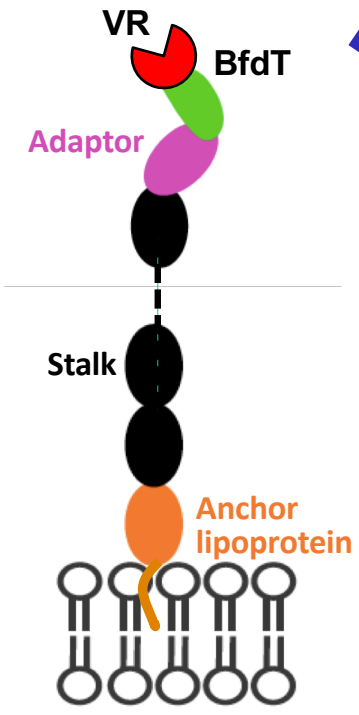
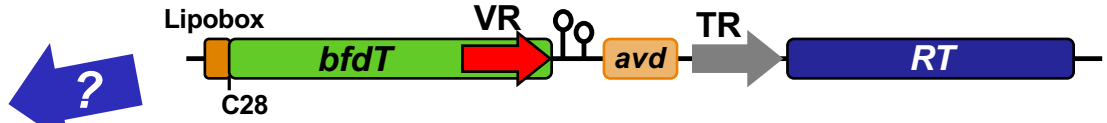
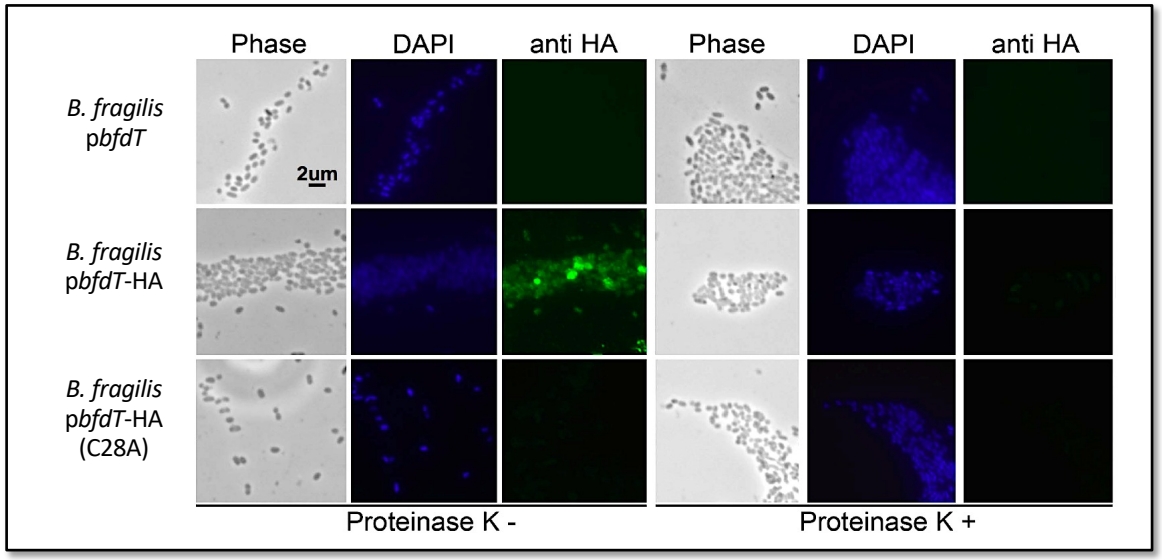
Bacteroides DGRs







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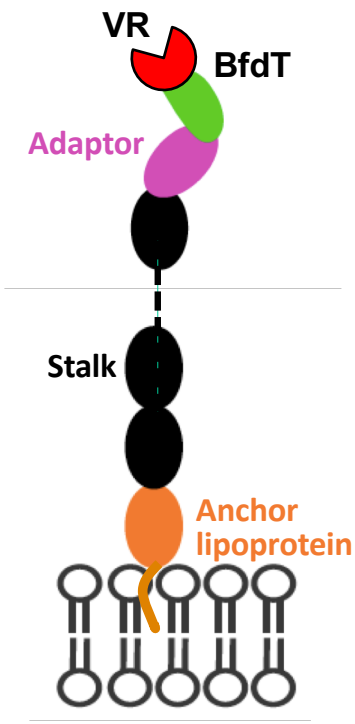
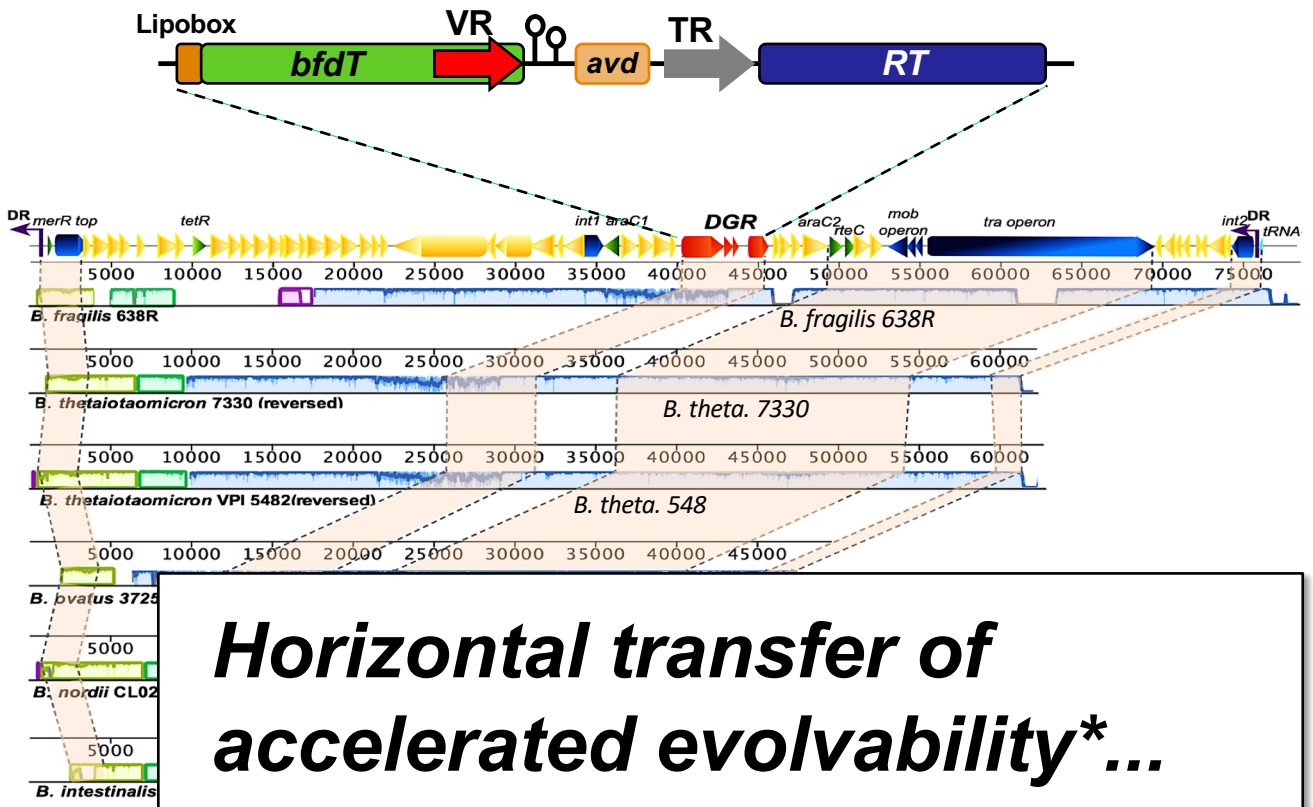
Bacteroides DGRs





Horizontal transfer of accelerated evolvability...*

***capacity of a system for adaptive evolution**

Yanling Wang & Diego Arambula (UCLA)
Blair Paul (UCSB)
Sarkis Mazmanian (Caltech)

*THANK YOU!**

UCLA Past

Sergei Doulatov (U. Washington)
Huatao Guo (U. Missouri)
Ming Liu (Los Angeles VA)
Bob Medhekar (Stanford)
Yanling Wang (Milennium)

UCLA Present

Umesh Ahuja
Diego Arambula
Liz Czornyj
Stephanie Orchanian
Blair Paul

Collaborators

David Burnstein, Cindy Castelle, Jill Banfield (UC Berkeley)
Sumit Handa, Yong Jiang, Partho Ghosh (UCSD)
Santa Naorem, Jin Han, Huatao Guo (U. Missouri)
Blair Paul, David Valentine (UCSB)
Steve Zimmerly (U. Calgary)

**JFM is Co-founder, equity holder, and SAB Chair, Pylum Bioscience Inc.*

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