SHORT READ MAPPING USING SNIPPY

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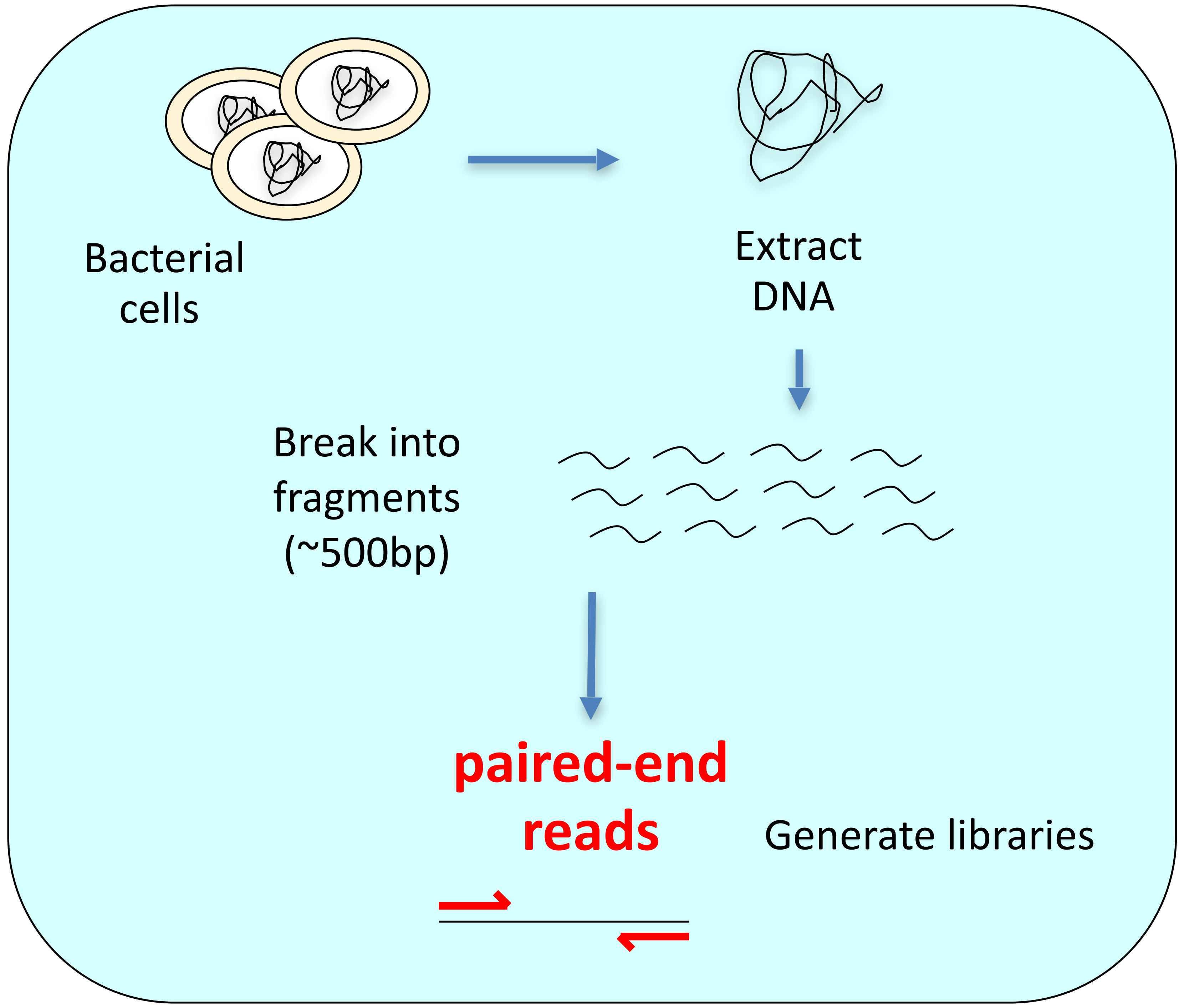
Workflow: generating sequencing reads and *in silico* analysis

Wet Lab



Bacterial cells

Extract DNA

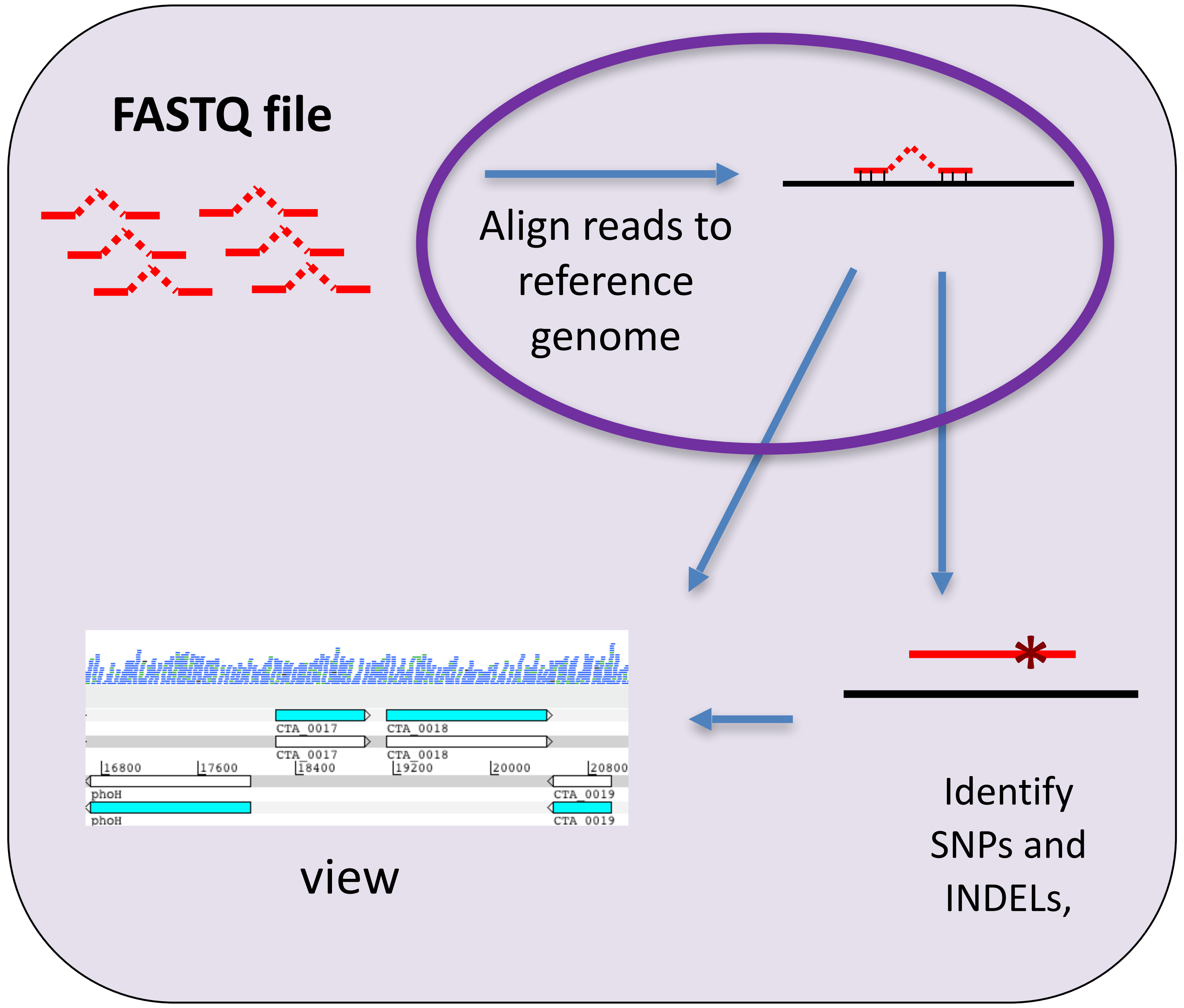
Break into fragments (~500bp)

paired-end reads

Generate libraries

DNA extraction and DNA library generation

in silico analysis



FASTQ file

Align reads to reference genome

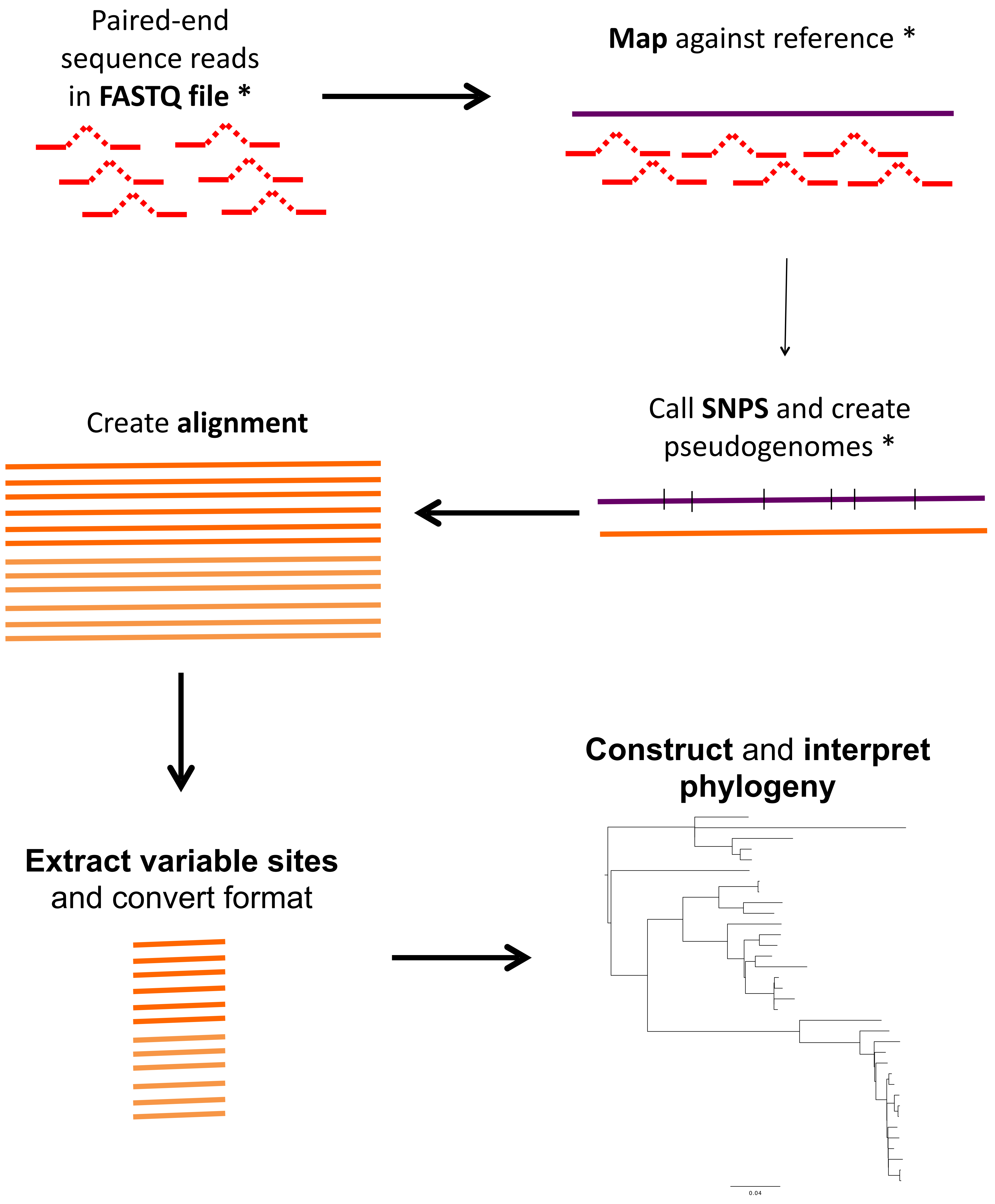
view

Identify SNPs and INDELs,

Mapping is **aligning a sequence to a known reference to determine genetic differences**

Why do we map reads to a reference?

- Identify variation:
  - Single Nucleotide Polymorphisms (SNPs),
  - insertions and deletions (indels)
  - Copy Number Variants (CNVs) between variants of the same bacteria.
  - Presence / absence of genes (AMR)



Paired-end sequence reads in **FASTQ file** *

Map against reference *

Call **SNPS** and create pseudogenomes *

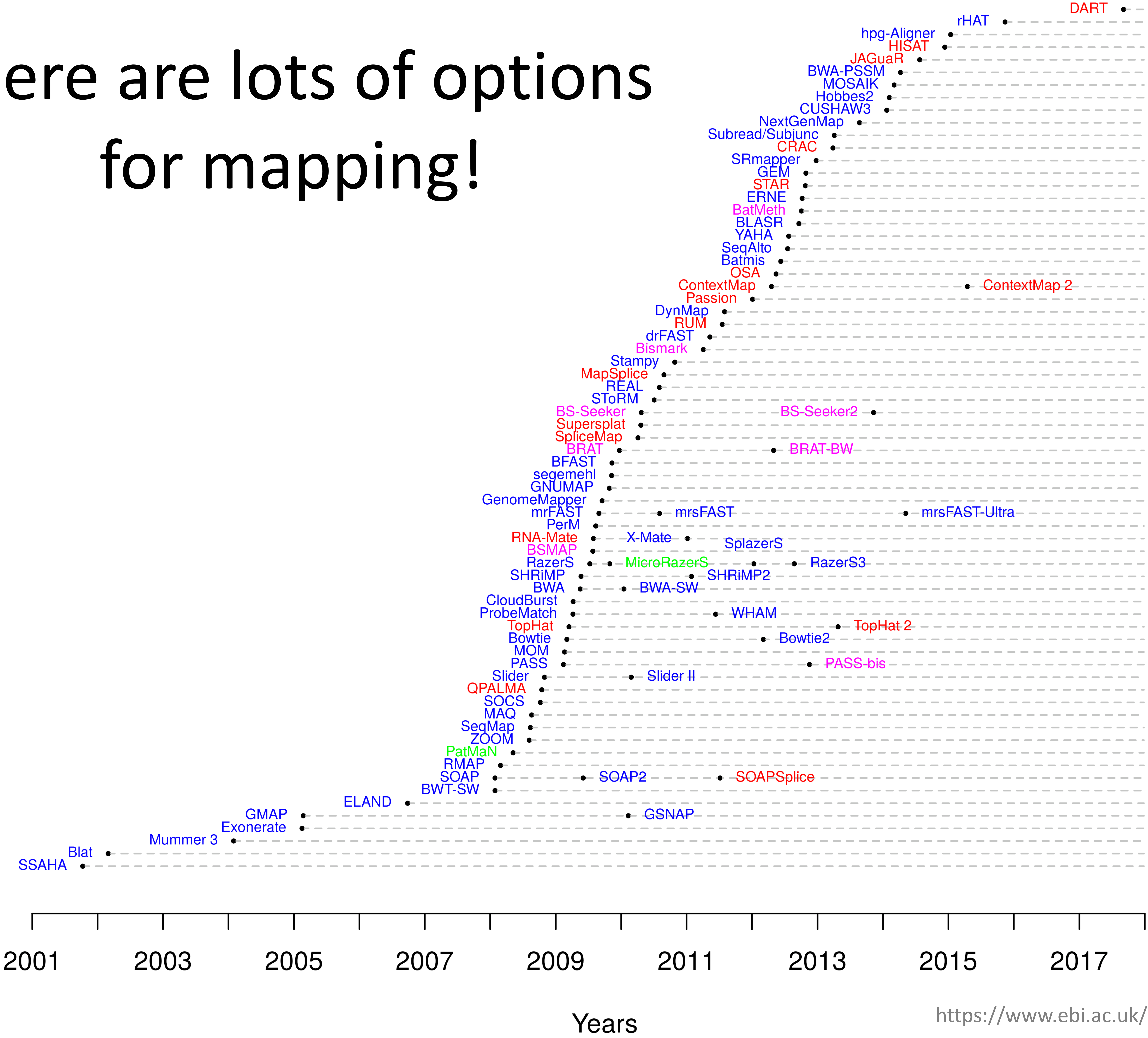
Create **alignment**

Extract variable sites and convert format

Construct and **interpret phylogeny**

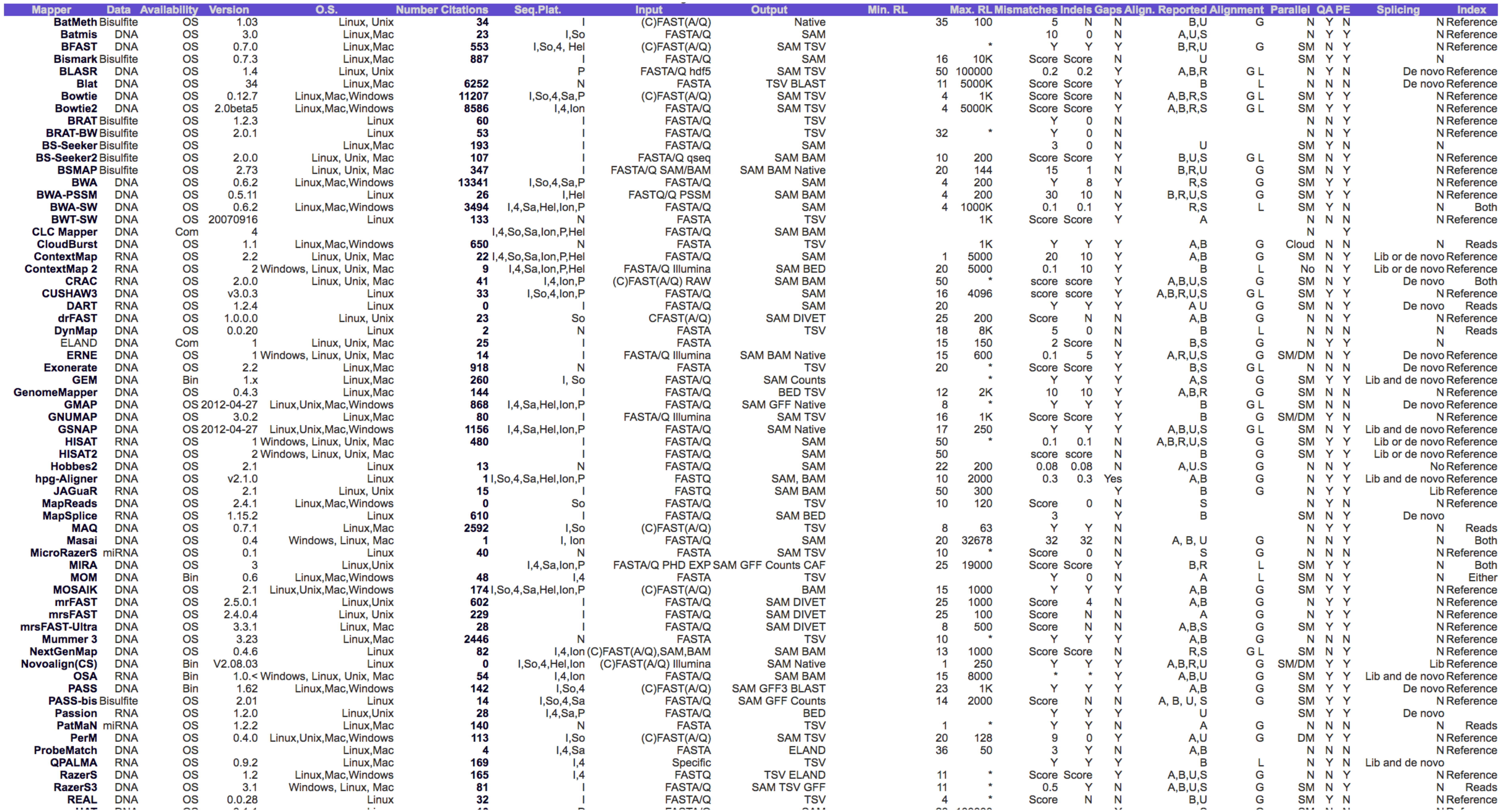
*do for each isolate

There are lots of options for mapping!



https://www.ebi.ac.uk/~nf/hts_mappers

Comparison of different mappers



Mapper	Data	Availability	Version	O.S.	Number Citations	Seq.Plat.	Input	Output	Min. RL	Max. RL	Mismatches	Indels	Gaps	Align.	Reported Alignment	Parallel	QA	PE	Splicing	Index
BatMeth	Bisulfite	OS	1.03	Linux, Unix	**34**	I	(C)FAST(A/Q)	Native	35	100	5	N	N	B,U	G	N	Y	N	N	Reference
Batmis	DNA	OS	3.0	Linux,Mac	**23**	I,So	FASTA/Q	SAM			10	0	N	A,U,S		N	Y	Y	N	Reference
BFAST	DNA	OS	0.7.0	Linux,Mac	**553**	I,So,4, Hel	(C)FAST(A/Q)	SAM TSV		*	Y	Y	Y	B,R,U	G	SM	N	Y	N	Reference
Bismark	Bisulfite	OS	0.7.3	Linux,Mac	**887**	I	FASTA/Q	SAM	16	10K	Score	Score	N	U		SM	Y	Y	N	
BLASR	DNA	OS	1.4	Linux, Unix		P	FASTA/Q hdf5	SAM TSV	50	100000	0.2	0.2	Y	A,B,R	G L	N	Y	N	De novo	Reference
Blat	DNA	OS	34	Linux,Mac	**6252**	N	FASTA	TSV BLAST	11	5000K	Score	Score	Y	B	L	N	N	N	De novo	Reference
Bowtie	DNA	OS	0.12.7	Linux,Mac,Windows	**11207**	I,So,4,Sa,P	(C)FAST(A/Q)	SAM TSV	4	1K	Score	Score	N	A,B,R,S	G L	SM	Y	Y	N	Reference
Bowtie2	DNA	OS	2.0beta5	Linux,Mac,Windows	**8586**	I,4,Ion	FASTA/Q	SAM TSV	4	5000K	Score	Score	Y	A,B,R,S	G L	SM	Y	Y	N	Reference
BRAT	Bisulfite	OS	1.2.3	Linux	**60**	I	FASTA/Q	TSV			Y	0	N			N	N	Y	N	Reference
BRAT-BW	Bisulfite	OS	2.0.1	Linux	**53**	I	FASTA/Q	TSV	32	*	Y	0	N			N	N	Y	N	Reference
BS-Seeker	Bisulfite	OS		Linux,Mac	**193**	I	FASTA/Q	SAM			3	0	N	U		SM	Y	N	N	
BS-Seeker2	Bisulfite	OS	2.0.0	Linux, Unix, Mac	**107**	I	FASTA/Q qseq	SAM BAM	10	200	Score	Score	Y	B,U,S	G L	SM	N	Y	N	Reference
BSMAP	Bisulfite	OS	2.73	Linux, Unix, Mac	**347**	I	FASTA/Q SAM/BAM	SAM BAM Native	20	144	15	1	N	B,R,U	G	SM	N	Y	N	Reference
BWA	DNA	OS	0.6.2	Linux,Mac,Windows	**13341**	I,So,4,Sa,P	FASTA/Q	SAM	4	200	Y	8	Y	R,S	G	SM	Y	Y	N	Reference
BWA-PSSM	DNA	OS	0.5.11	Linux	**26**	I,Hel	FASTQ/Q PSSM	SAM BAM	4	200	30	10	N	B,R,U,S	G	SM	Y	Y	N	Reference
BWA-SW	DNA	OS	0.6.2	Linux,Mac,Windows	**3494**	I,4,Sa,Hel,Ion,P	FASTA/Q	SAM	4	1000K	0.1	0.1	Y	R,S	L	SM	Y	N	N	Both
BWT-SW	DNA	OS	20070916	Linux	**133**	N	FASTA	TSV		1K	Score	Score	Y	A		N	N	N	N	Reference
CLC Mapper	DNA	Com	4			I,4,So,Sa,Ion,P,Hel	FASTA/Q	SAM BAM								N		Y		
CloudBurst	DNA	OS	1.1	Linux,Mac,Windows	**650**	N	FASTA	TSV		1K	Y	Y	Y	A,B	G	Cloud	N	N	N	Reads
ContextMap	RNA	OS	2.2	Linux, Unix, Mac	**22**	I,4,So,Sa,Ion,P,Hel	FASTA/Q	SAM	1	5000	20	10	Y	A,B	G	SM	N	Y	Lib or de novo	Reference
ContextMap 2	RNA	OS	2	Windows, Linux, Unix, Mac	**9**	I,4,Sa,Ion,P,Hel	FASTA/Q Illumina	SAM BED	20	5000	0.1	10	Y	B	L	No	N	Y	Lib or de novo	Reference
CRAC	RNA	OS	2.0.0	Linux, Unix, Mac	**41**	I,4,Ion,P	(C)FAST(A/Q) RAW	SAM BAM	50	*	score	score	Y	A,B,U,S	G	SM	N	Y	De novo	Both
CUSHAW3	DNA	OS	v3.0.3	Linux	**33**	I,So,4,Ion,P	FASTA/Q	SAM	16	4096	score	score	Y	A,B,R,U,S	G L	SM	Y	Y	N	Reference
DART	RNA	OS	1.2.4	Linux	**0**	I	FASTA/Q	SAM	20		Y	Y	Y	A U	G	SM	N	Y	De novo	Reads
drFAST	DNA	OS	1.0.0.0	Linux, Unix	**23**	So	CFAST(A/Q)	SAM DIVET	25	200	Score	N	N	A,B	G	N	N	Y	N	Reference
DynMap	DNA	OS	0.0.20	Linux	**2**	N	FASTA	TSV	18	8K	5	0	N	B	L	N	N	N	N	Reads
ELAND	DNA	Com	1	Linux, Unix, Mac	**25**	I	FASTA		15	150	2	Score	N	B,S	G	N	Y	Y	N	
ERNE	DNA	OS	1	Windows, Linux, Unix, Mac	**14**	I	FASTA/Q Illumina	SAM BAM Native	15	600	0.1	5	Y	A,R,U,S	G	SM/DM	N	Y	De novo	Reference
Exonerate	DNA	OS	2.2	Linux,Mac	**918**	N	FASTA	TSV	20	*	Score	Score	Y	B,S	G L	N	N	N	De novo	Reference
GEM	DNA	Bin	1.x	Linux,Mac	**260**	I, So	FASTA/Q	SAM Counts		*	Y	Y	Y	A,S	G	SM	Y	Y	Lib and de novo	Reference
GenomeMapper	DNA	OS	0.4.3	Linux,Mac	**144**	I	FASTA/Q	BED TSV	12	2K	10	10	Y	A,B,R	G	SM	N	N	N	Reference
GMAP	DNA	OS	2012-04-27	Linux,Unix,Mac,Windows	**868**	I,4,Sa,Hel,Ion,P	FASTA/Q	SAM GFF Native	8	*	Y	Y	Y	B	G L	SM	N	N	De novo	Reference
GNUMAP	DNA	OS	3.0.2	Linux,Mac	**80**	I	FASTA/Q Illumina	SAM TSV	16	1K	Score	Score	Y	B	G	SM/DM	Y	N	N	Reference
GSNAP	DNA	OS	2012-04-27	Linux,Unix,Mac,Windows	**1156**	I,4,Sa,Hel,Ion,P	FASTA/Q	SAM Native	17	250	Y	Y	Y	A,B,U,S	G L	SM	N	Y	Lib and de novo	Reference
HISAT	RNA	OS	1	Windows, Linux, Unix, Mac	**480**	I	FASTA/Q	SAM	50	*	0.1	0.1	N	A,B,R,U,S	G	SM	Y	Y	Lib or de novo	Reference
HISAT2	DNA	OS	2	Windows, Linux, Unix, Mac		I	FASTA/Q	SAM	50		score	score	N	B	G	SM	Y	Y	Lib or de novo	Reference
Hobbes2	DNA	OS	2.1	Linux	**13**	N	FASTA/Q	SAM	22	200	0.08	0.08	N	A,U.S	G	N	N	Y	No	Reference
hpg-Aligner	DNA	OS	v2.1.0	Linux	**1**	I,So,4,Sa,Hel,Ion,P	FASTQ	SAM, BAM	10	2000	0.3	0.3	Yes	A,B	G	N	Y	Y	Lib and de novo	Reference
JAGuaR	RNA	OS	2.1	Linux, Unix	**15**	I	FASTQ	SAM BAM	50	300			Y	B	G	N	Y	Y	Lib	Reference
MapReads	DNA	OS	2.4.1	Linux,Mac,Windows	**0**	So	FASTA/Q	TSV	10	120	Score	0	N	S		N	Y	N	N	Reference
MapSplice	RNA	OS	1.15.2	Linux	**610**	I	FASTA/Q	SAM BED			3		Y	B		SM	N	Y	De novo	
MAQ	DNA	OS	0.7.1	Linux,Mac	**2592**	I,So	(C)FAST(A/Q)	TSV	8	63	Y	Y	N			N	Y	Y	N	Reads
Masai	DNA	OS	0.4	Windows, Linux, Mac	**1**	I, Ion	FASTA/Q	SAM	20	32678	32	32	N	A, B, U	G	N	N	Y	N	Both
MicroRazerS	miRNA	OS	0.1	Linux	**40**	N	FASTA	SAM TSV	10	*	Score	0	N	S	G	N	N	N	N	Reference
MIRA	DNA	OS	3	Linux,Unix		I,4,Sa,Ion,P	FASTA/Q PHD EXP	SAM GFF Counts CAF	25	19000	Score	Score	Y	B,R	L	SM	Y	Y	N	Both
MOM	DNA	Bin	0.6	Linux,Mac,Windows	**48**	I,4	FASTA	TSV			Y	0	N	A	L	SM	N	Y	N	Either
MOSAIK	DNA	OS	2.1	Linux,Unix,Mac,Windows	**174**	I,So,4,Sa,Hel,Ion,P	(C)FAST(A/Q)	BAM	15	1000	Y	Y	Y	A,B	G	SM	Y	Y	N	Reference
mrFAST	DNA	OS	2.5.0.1	Linux,Unix	**602**	I	FASTA/Q	SAM DIVET	25	1000	Score	4	N	A,B	G	N	Y	Y	N	Reference
mrsFAST	DNA	OS	2.4.0.4	Linux, Unix	**229**	I	FASTA/Q	SAM DIVET	25	100	Score	N	N	A	G	N	Y	Y	N	Reference
mrsFAST-Ultra	DNA	OS	3.3.1	Linux, Mac	**28**	I	FASTA/Q	SAM DIVET	8	500	Score	N	N	A,B,S	G	SM	Y	Y	N	Reference
Mummer 3	DNA	OS	3.23	Linux,Mac	**2446**	N	FASTA	TSV	10	*	Y	Y	Y	A,B	G	N	N	N	N	Reference
NextGenMap	DNA	OS	0.4.6	Linux	**82**	I,4,Ion	(C)FAST(A/Q),SAM,BAM	SAM BAM	13	1000	Score	Score	N	R,S	G L	SM	N	Y	N	Reference
Novoalign(CS)	DNA	Bin	V2.08.03	Linux	**0**	I,So,4,Hel,Ion	(C)FAST(A/Q) Illumina	SAM Native	1	250	Y	Y	Y	A,B,R,U	G	SM/DM	Y	Y	Lib	Reference
OSA	RNA	Bin	1.0.<	Windows, Linux, Unix, Mac	**54**	I,4,Ion	FASTA/Q	SAM BAM	15	8000	*	*	Y	A,B,U	G	SM	Y	Y	Lib and de novo	Reference
PASS	DNA	Bin	1.62	Linux,Mac,Windows	**142**	I,So,4	(C)FAST(A/Q)	SAM GFF3 BLAST	23	1K	Y	Y	Y	A,B	G	SM	Y	Y	De novo	Reference
PASS-bis	Bisulfite	OS	2.01	Linux	**14**	I,So,4,Sa	FASTA/Q	SAM GFF Counts	14	2000	Score	N	N	A, B, U, S	G	SM	Y	Y	N	Reference
Passion	RNA	OS	1.2.0	Linux,Unix	**28**	I,4,Sa,P	FASTA/Q	BED			Y	Y	Y	U		SM	Y	Y	De novo	
PatMaN	miRNA	OS	1.2.2	Linux,Mac	**140**	N	FASTA	TSV	1	*	Y	Y	N	A	G	N	N	N	N	Reads
PerM	DNA	OS	0.4.0	Linux,Unix,Mac,Windows	**113**	I,So	(C)FAST(A/Q)	SAM TSV	20	128	9	0	Y	A,U	G	DM	Y	Y	N	Reference
ProbeMatch	DNA	OS		Linux,Mac	**4**	I,4,Sa	FASTA	ELAND	36	50	3	Y	N	A,B		N	N	N	N	Reference
QPALMA	RNA	OS	0.9.2	Linux,Mac	**169**	I,4	Specific	TSV			Y	Y	Y	B	L	N	Y	N	Lib and de novo	
RazerS	DNA	OS	1.2	Linux,Mac,Windows	**165**	I,4	FASTQ	TSV ELAND	11	*	Score	Score	Y	A,B,U,S	G	N	N	Y	N	Reference
RazerS3	DNA	OS	3.1	Windows, Linux, Mac	**81**	I	FASTA/Q	SAM TSV GFF	11	*	0.5	Y	N	A,B,U,S	G	SM	N	Y	N	Reads
REAL	DNA	OS	0.0.28	Linux	**32**	I	FASTA/Q	TSV	4	*	Score	N	N	B,U	G	SM	Y	N	N	Reference

https://www.ebi.ac.uk/~nf/hts_mappers

Good general aligners

bwa

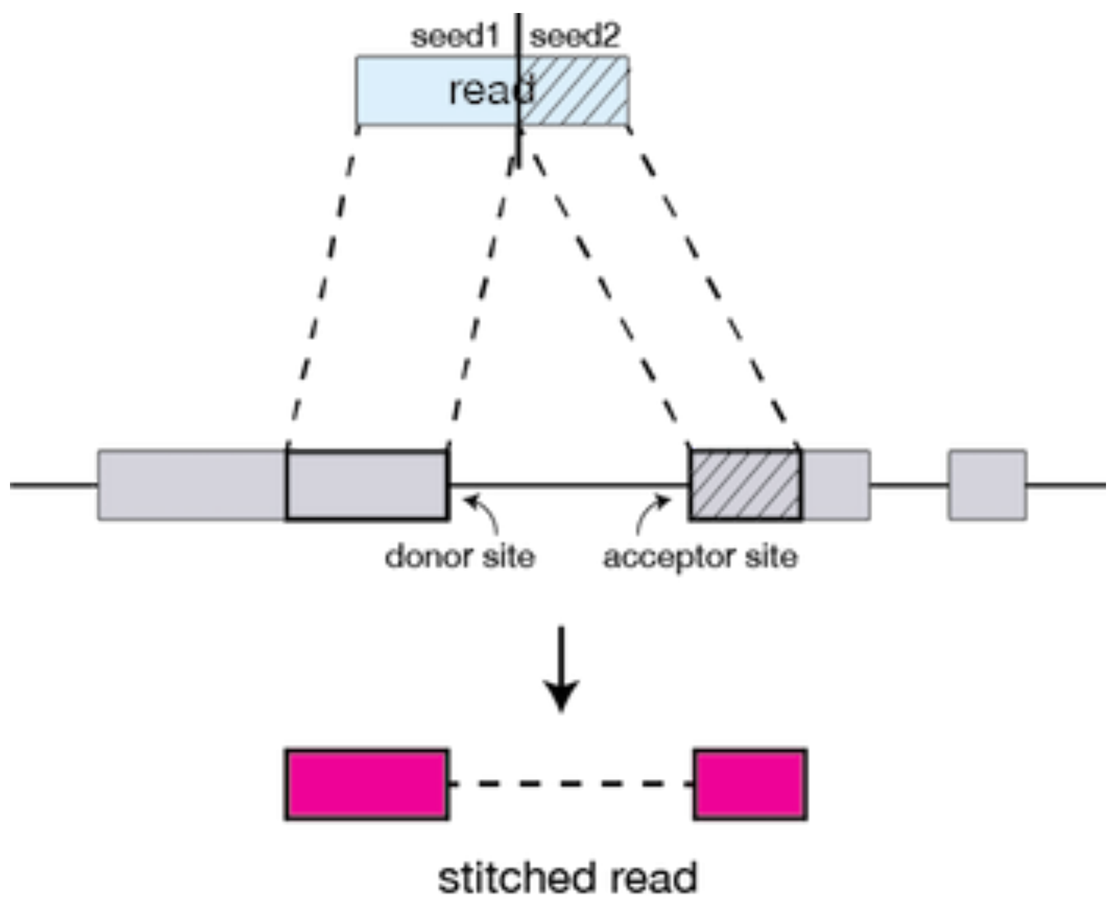
bowtie2

→ Fast, sensitive and easy to use!

Splice-aware aligners for RNA-seq

STAR

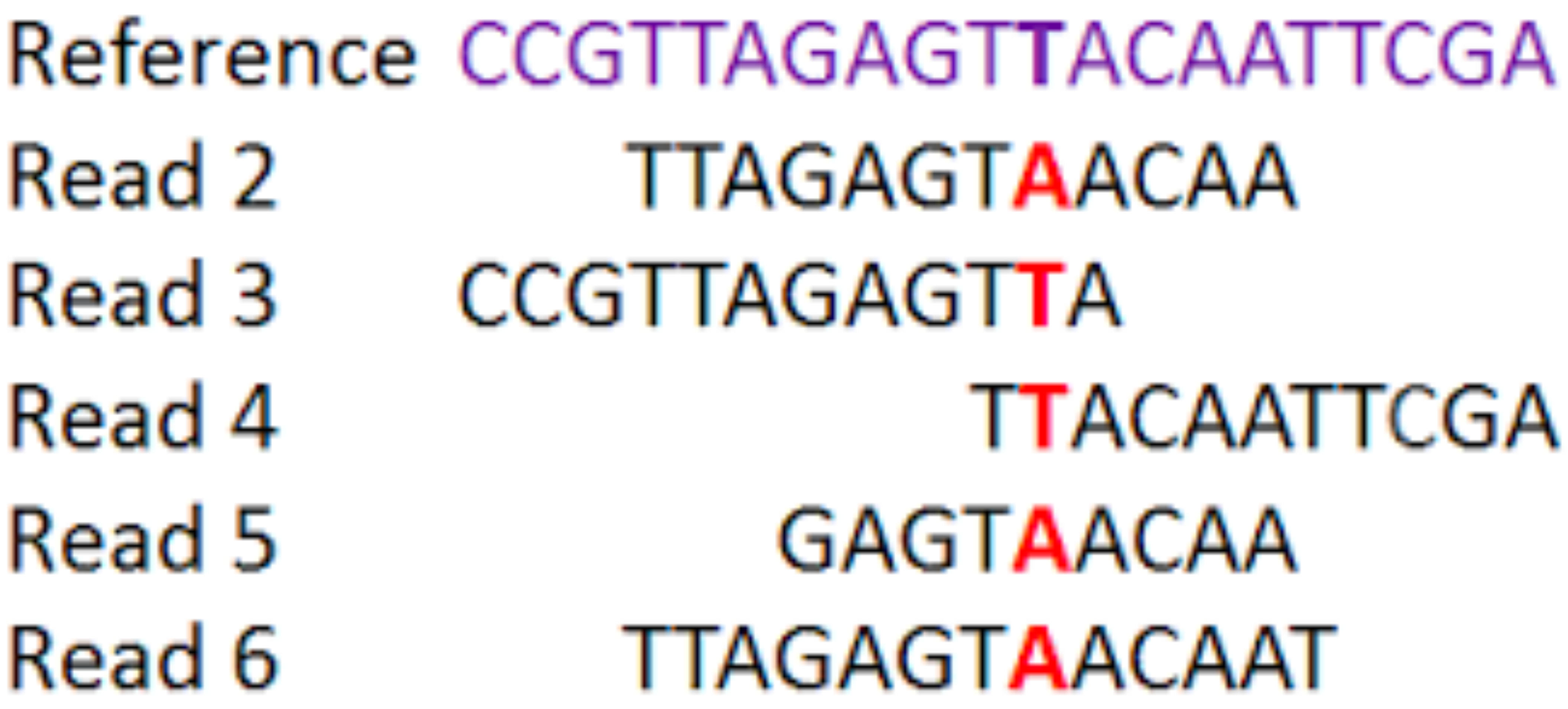
HISAT2



Why do we map to a reference?

- Identify variation:
  - Single Nucleotide Polymorphisms (SNPs),
  - insertions and deletions (indels)
  - Copy Number Variants (CNVs) between variants of the same bacteria.
  - Presence / absence of genes (AMR)

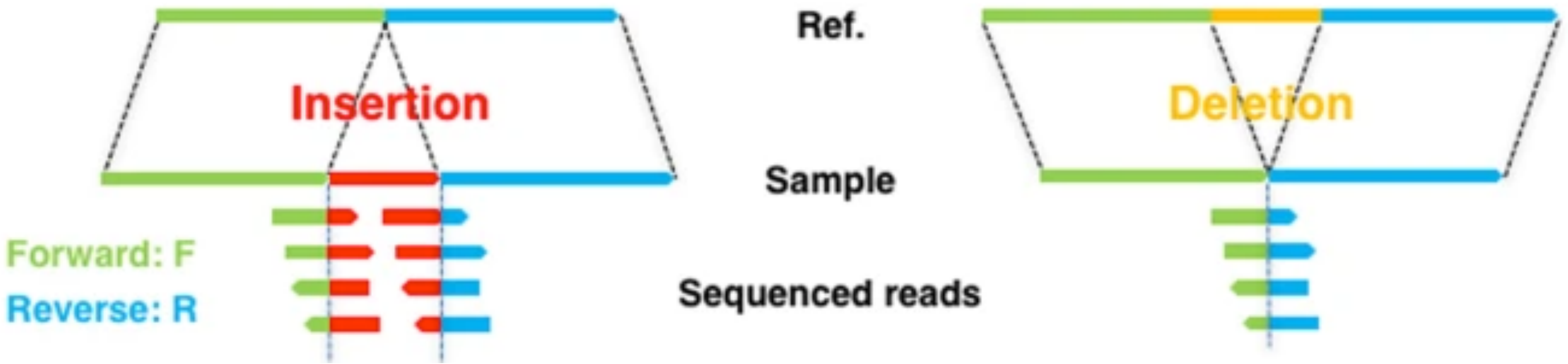
Single Nucleotide Polymorphisms (SNPs)



```
Reference  CCGTTAGAGTTACAATTCGA
Read 2        TTAGAGTAACAA
Read 3     CCGTTAGAGTTA
Read 4              TTACAATTCGA
Read 5           GAGTAACAA
Read 6        TTAGAGTAACAAT
```

https://aschuerch.github.io/MolecularEpidemiology_AnalysisWGS/09-SNPphylo/index.html

INDELS

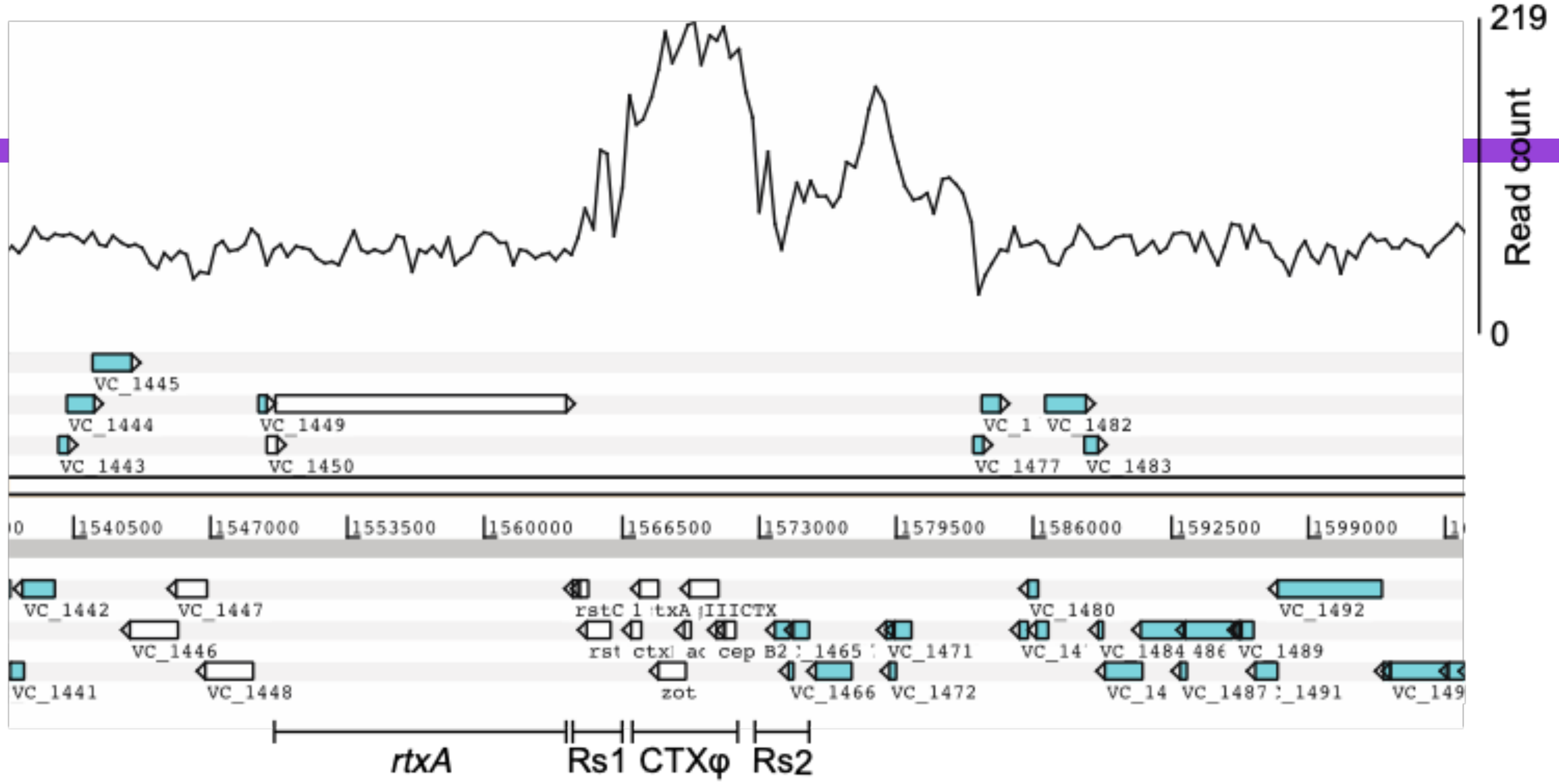


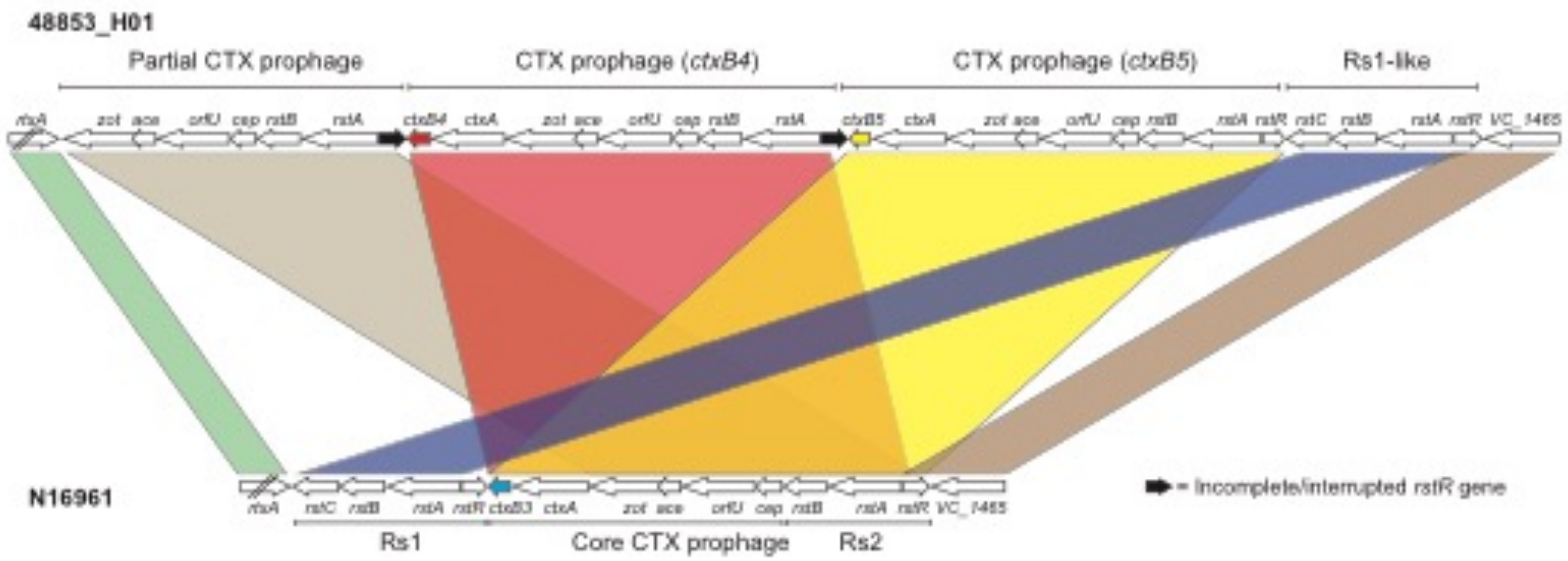
https://www.nature.com/articles/s41598-018-23978-z

Why do we map to a reference?

- Identify variation:
  - Single Nucleotide Polymorphisms (SNPs),
  - insertions and deletions (indels)
  - Copy Number Variants (CNVs) between variants of the same bacteria.
  - Presence / absence of genes (AMR)

Copy number variation

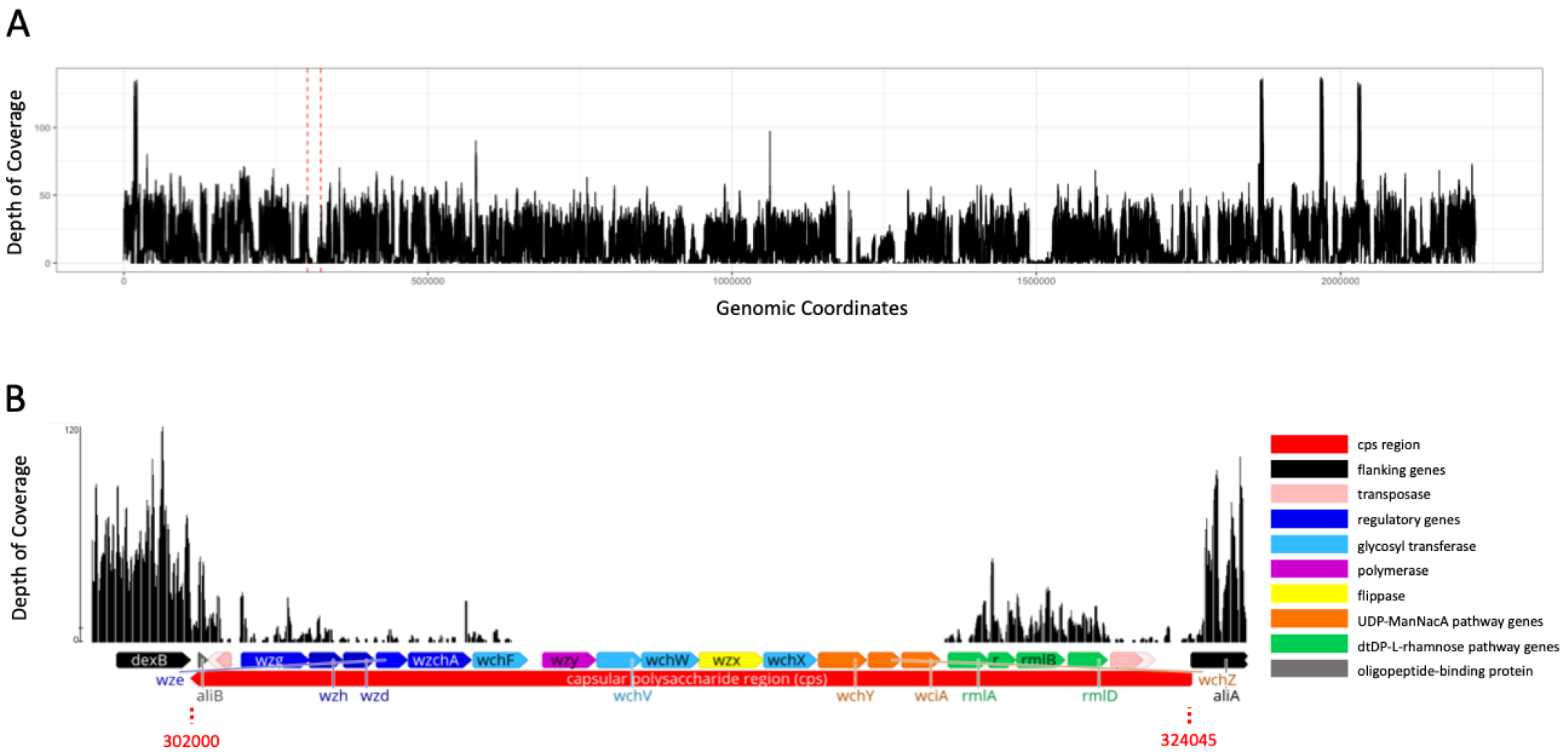




Gene presence/absence: AMR

- Absence/Deletions is easier to spot
- *To identify insertions is a little tricky.

Deletion:

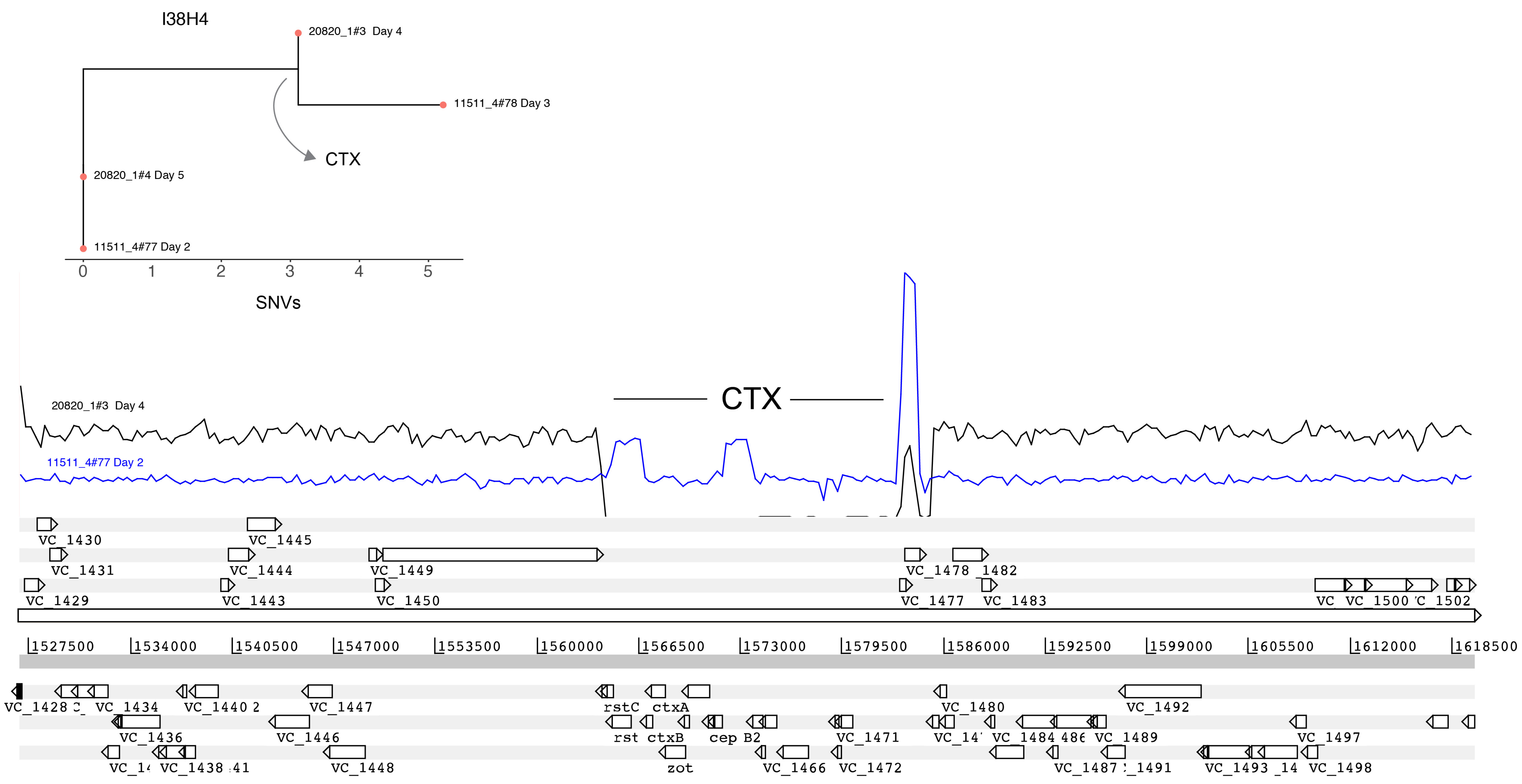


Today’s Agenda

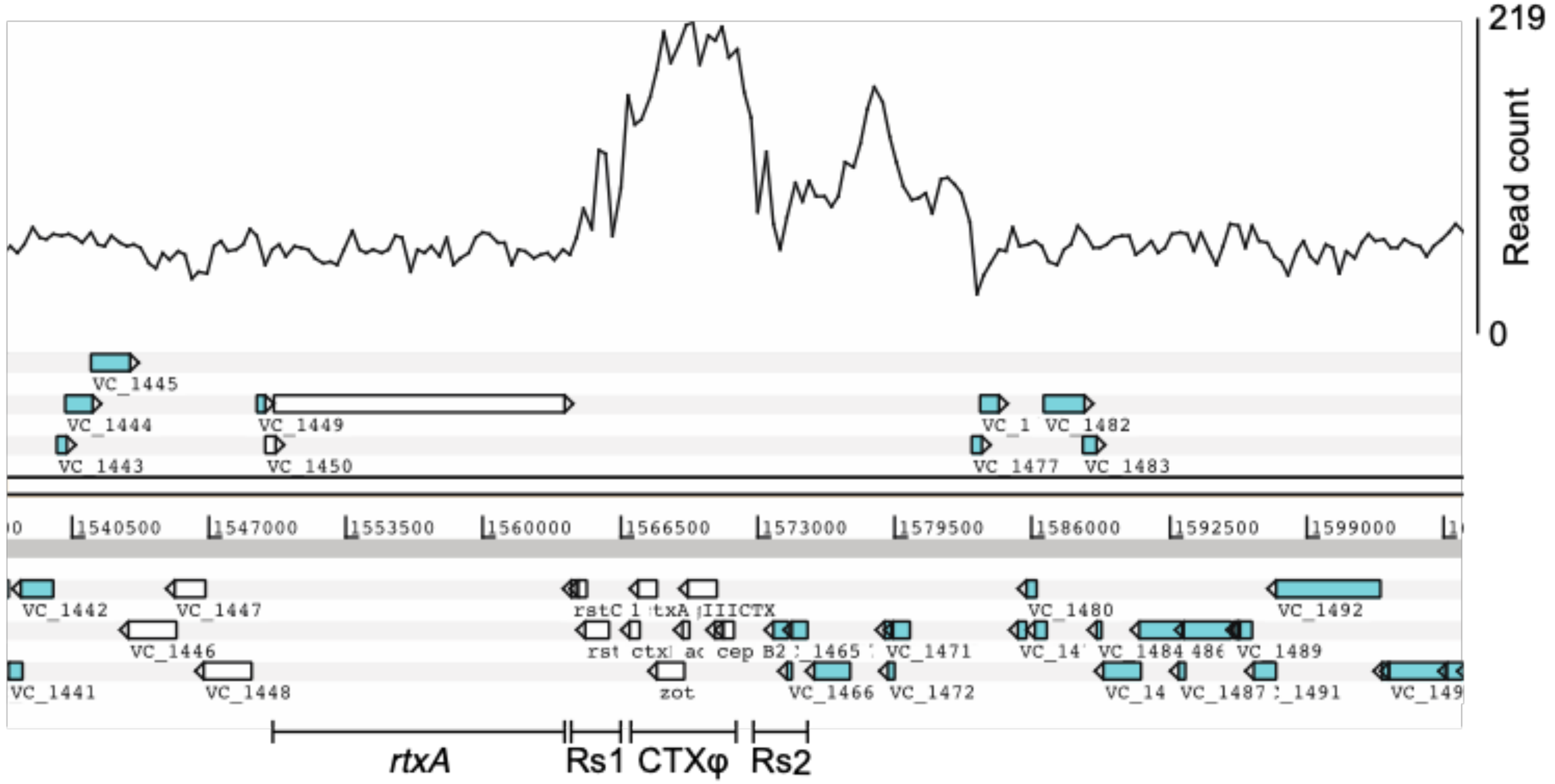
- ✓ Look at two *M. tuberculosis* datasets
- ✓ Use conda to install new tools
- ✓ Use snippy to map and call variants
- ✓ Look at resistance mutations

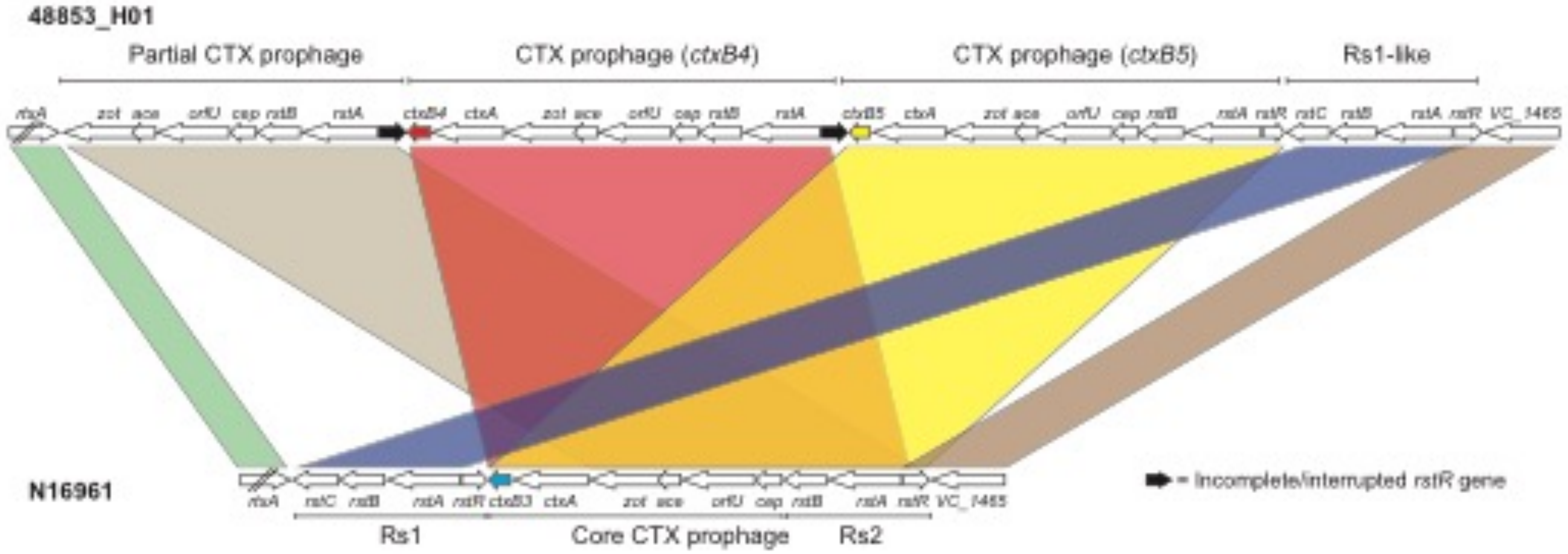
Questions?

Gene presence / absence



Copy number variation


Read count
VC_1445
VC_1444
VC_1449
VC_1
VC_1482
VC_1443
VC_1450
VC_1477
VC_1483
VC_1442
VC_1447
rstC
ctxA
IIICTX
VC_1480
VC_1492
VC_1446
rst
ctx
ac
cep
B2
VC_1471
VC_14
VC_1484
VC_1489
VC_1441
VC_1448
zot
VC_1466
VC_1472
VC_14
VC_1487
VC_149
rtxA
Rs1
CTXφ
Rs2


48853_H01
Partial CTX prophage
CTX prophage (ctxB4)
CTX prophage (ctxB5)
Rs1-like
rtxA
zot
ace
orfU
cep
rstB
rstA
ctxB4
ctxA
zot
ace
orfU
cep
rstB
rstA
ctxB5
ctxA
zot
ace
orfU
cep
rstB
rstA
rstR
rstC
rstB
rstA
rstR
VC_1465
N16961
rtxA
rstC
rstB
rstA
rstR
ctxB3
ctxA
zot
ace
orfU
cep
rstB
rstA
rstR
VC_1465
Rs1
Core CTX prophage
Rs2
= Incomplete/interrupted rstR gene