

RIFAQUIN Whole Genome Study

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RIFAQUIN

High-Dose Rifapentine with Moxifloxacin for Pulmonary Tuberculosis

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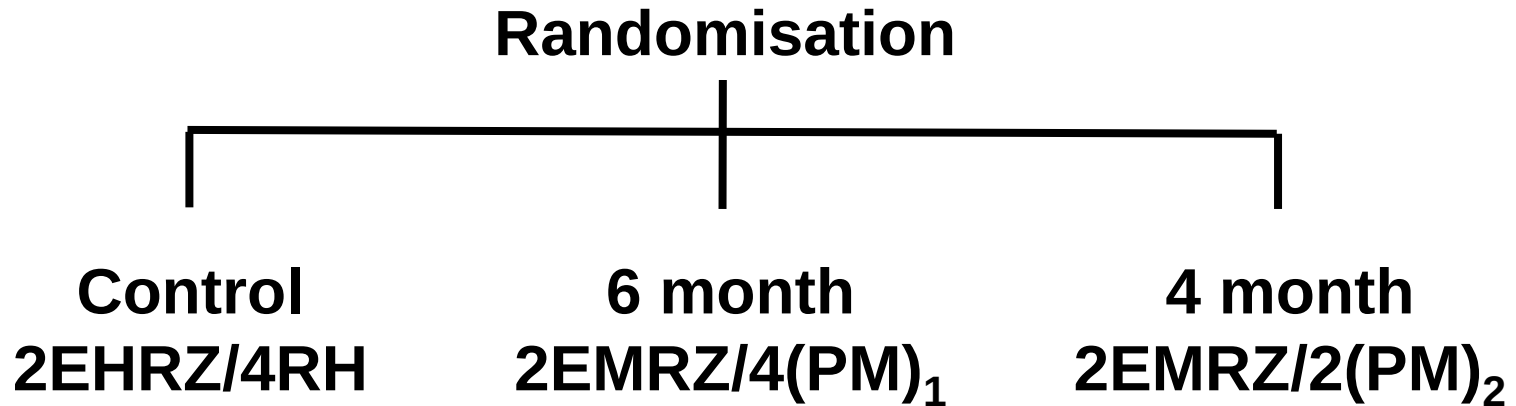
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<http://www.nejm.org/doi/full/10.1056/NEJMoa1314210>

RIFAQUIN trial

- **High dose Rifapentine with Moxifloxacin for pulmonary Tuberculosis**
- **827 patients. Control, 4 month and 6 month regimens**

RIFAQUIN trial design



Rpe 15 mg/kg + Moxifloxacin 500 mg (Twice weekly)

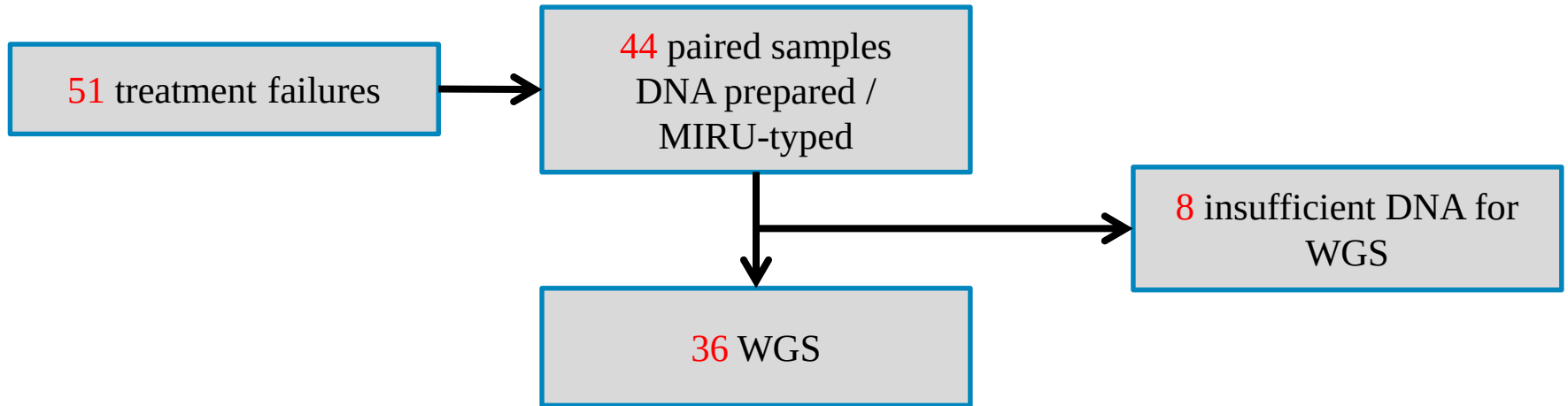
Rpe 20/kg mg + Moxifloxacin 500 mg (Once weekly)

Rifampicin 600 mg + INH 300 mg

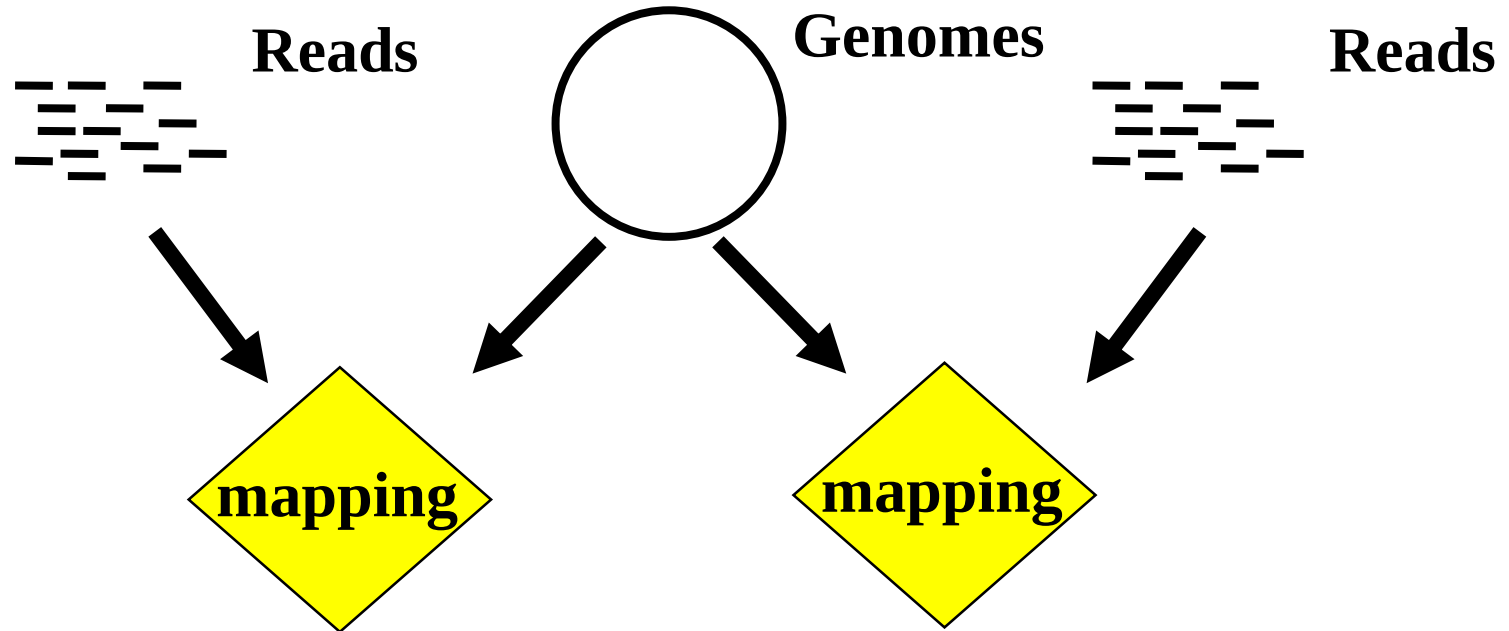
RIFAQUIN trial design

- High dose Rifapentine with Moxifloxacin for pulmonary Tuberculosis
- 827 patients. Control, 4 month and 6 month regimens
- Primary endpoint was treatment failure and relapse.
- But was post-treatment infection:
 - *relapse due to drug failure*
 - *reinfection*
- Pre and post treatment isolates the same or different?
- Traditional methods e.g. VNTR, Spoligotyping
- Can Whole Genome Sequencing (WGS) help?

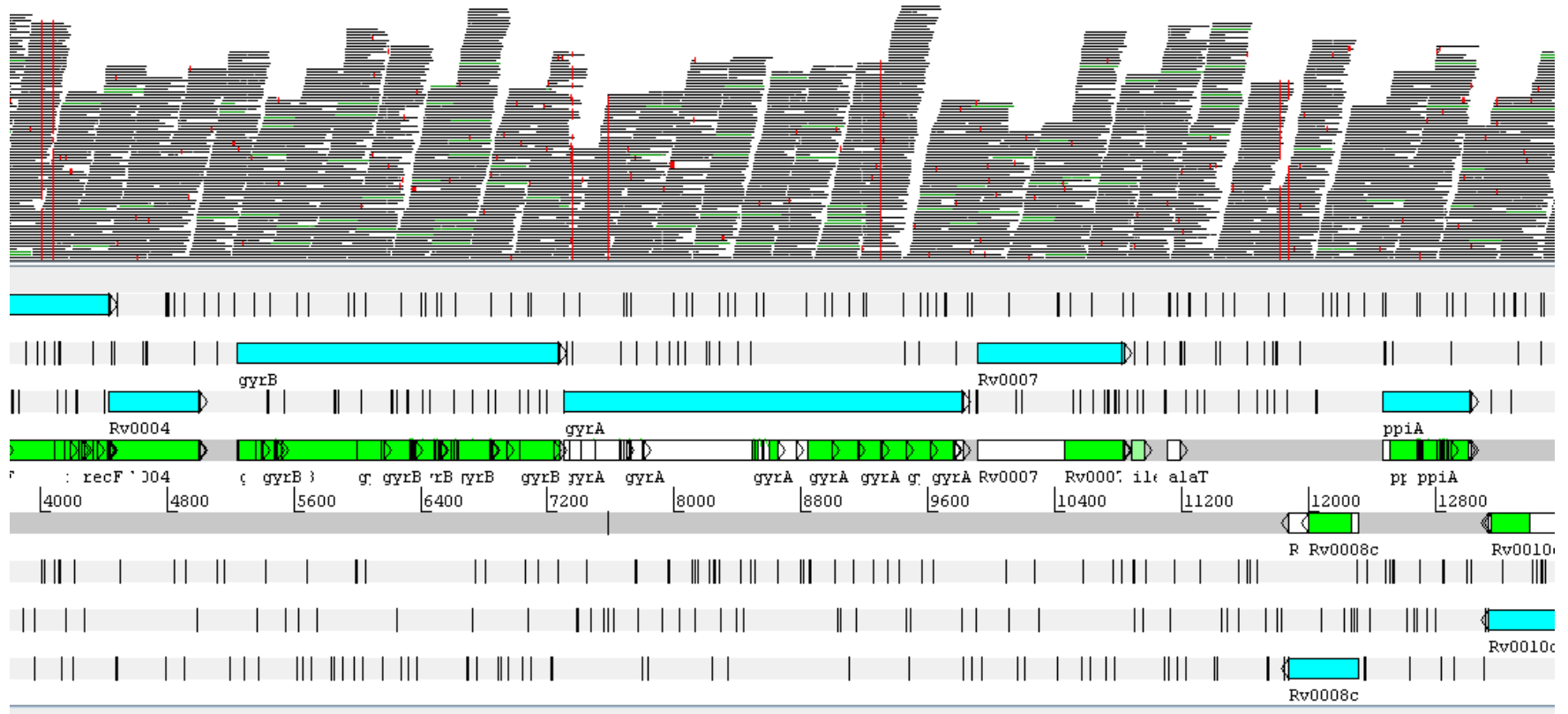
RIFAQUIN – treatment failures?



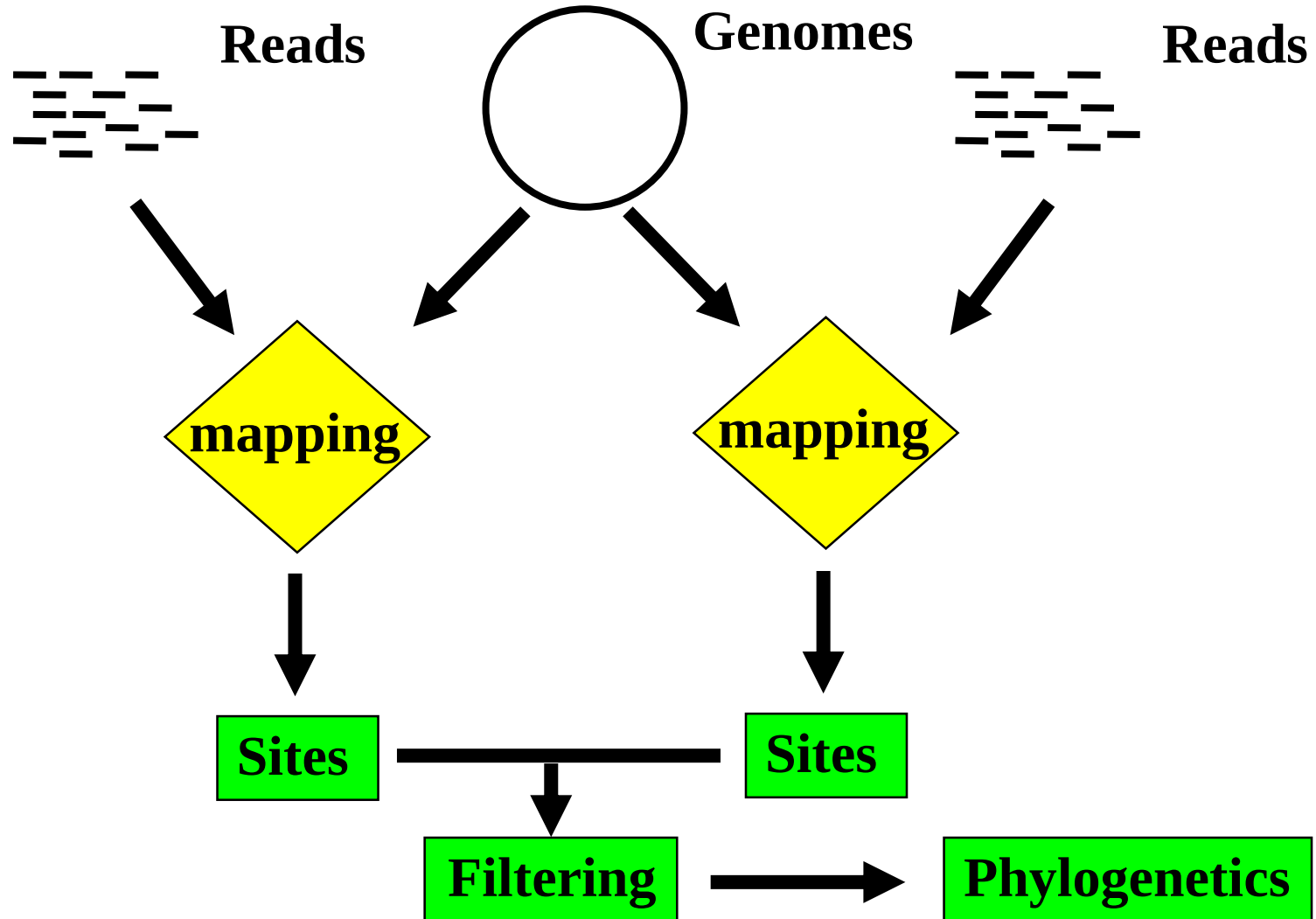
Analysis workflow – phylogenetics



Analysis Workflow - Mapping



Analysis workflow – phylogenetics

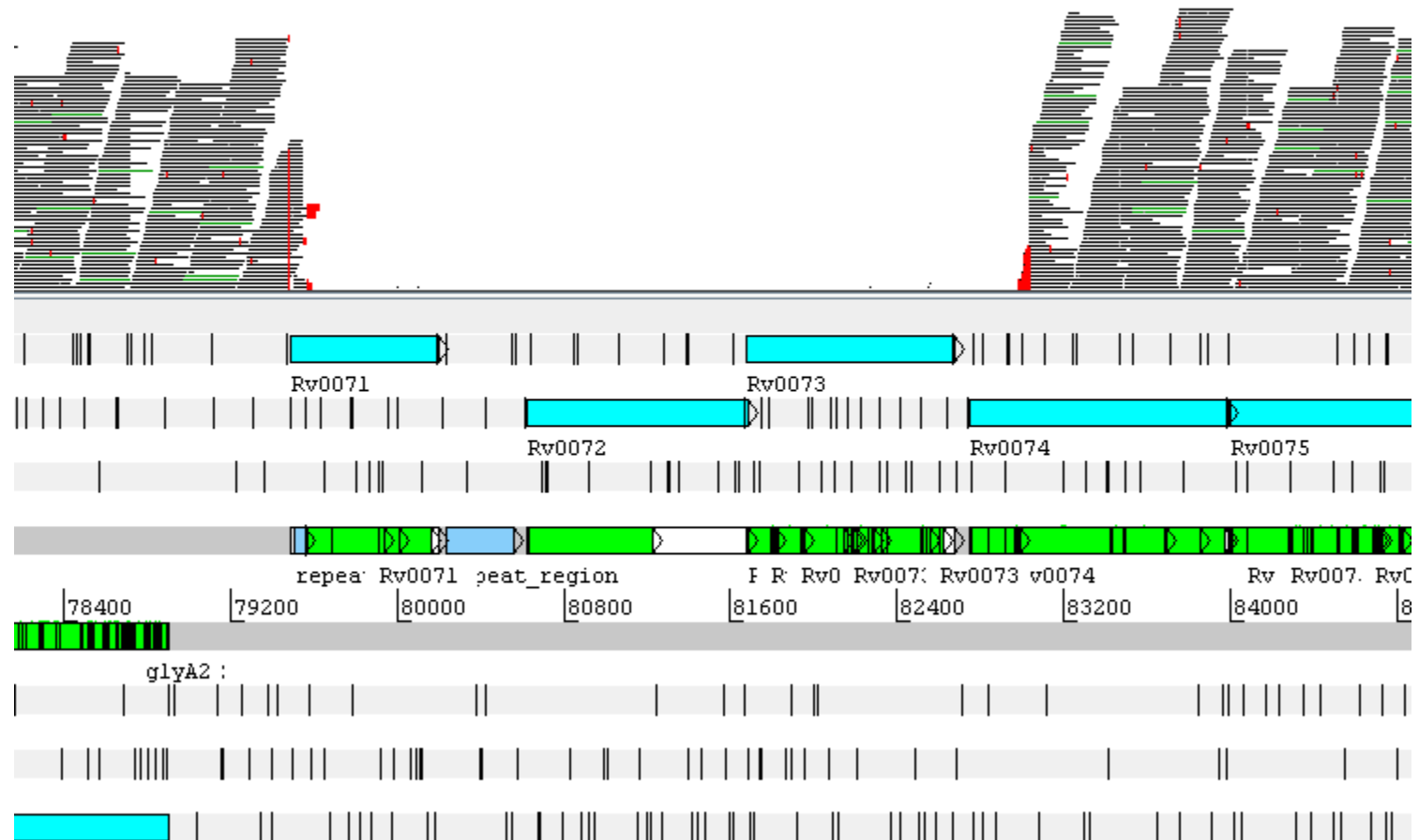


Site calling – phylogenetics

- Build consensus for all sites for each isolate
- Filter sites
 - Depth of coverage

A	GATGGTA
B	GATCGTC
C	GATCATA
D	GCTCGTA
R	GATCGTA

Site calling - depth filtering

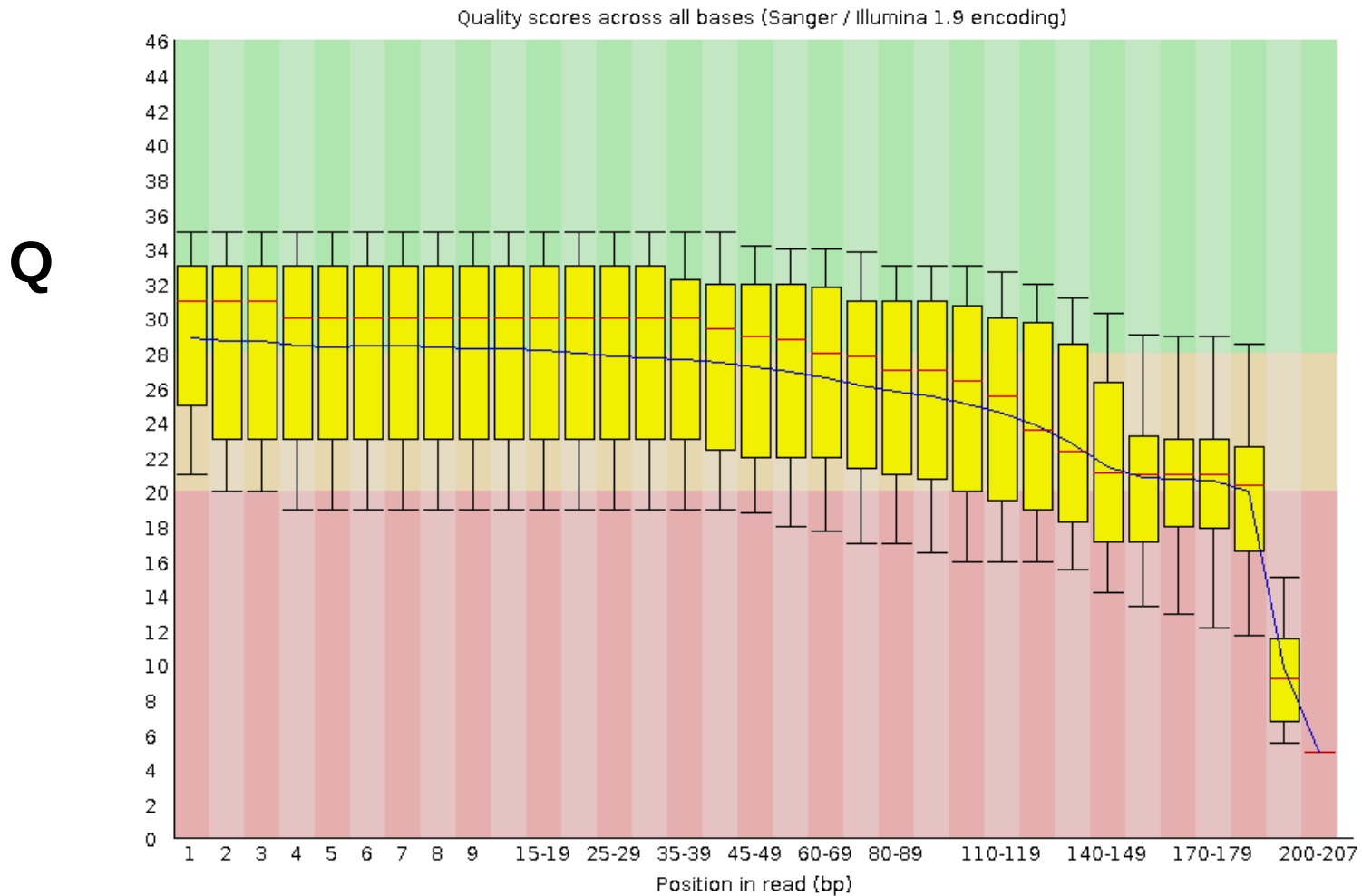


Site calling - phylogenetics

- Build consensus for all sites for each isolate
- Filter sites
 - Depth of coverage
 - Base quality score

A	GATGGTA
B	GATCGTC
C	GATCATA
D	GCTCGTA
R	GATCGTA

Site calling - quality filtering



Site calling - phylogenetics

- Build consensus for all sites for each isolate
- Filter sites
 - Depth of coverage
 - Base quality score
 - Heterogenous sites

A	GATCGTA
B	GATCGTC
C	GATCATA
D	GCTCGTA
R	GATCGTA



Site calling - phylogenetics

- Build consensus for all sites for each isolate
- Filter sites
 - Depth of coverage
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 - Heterogenous sites

A	GATCGTA
B	GATCGTC
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Site calling - phylogenetics

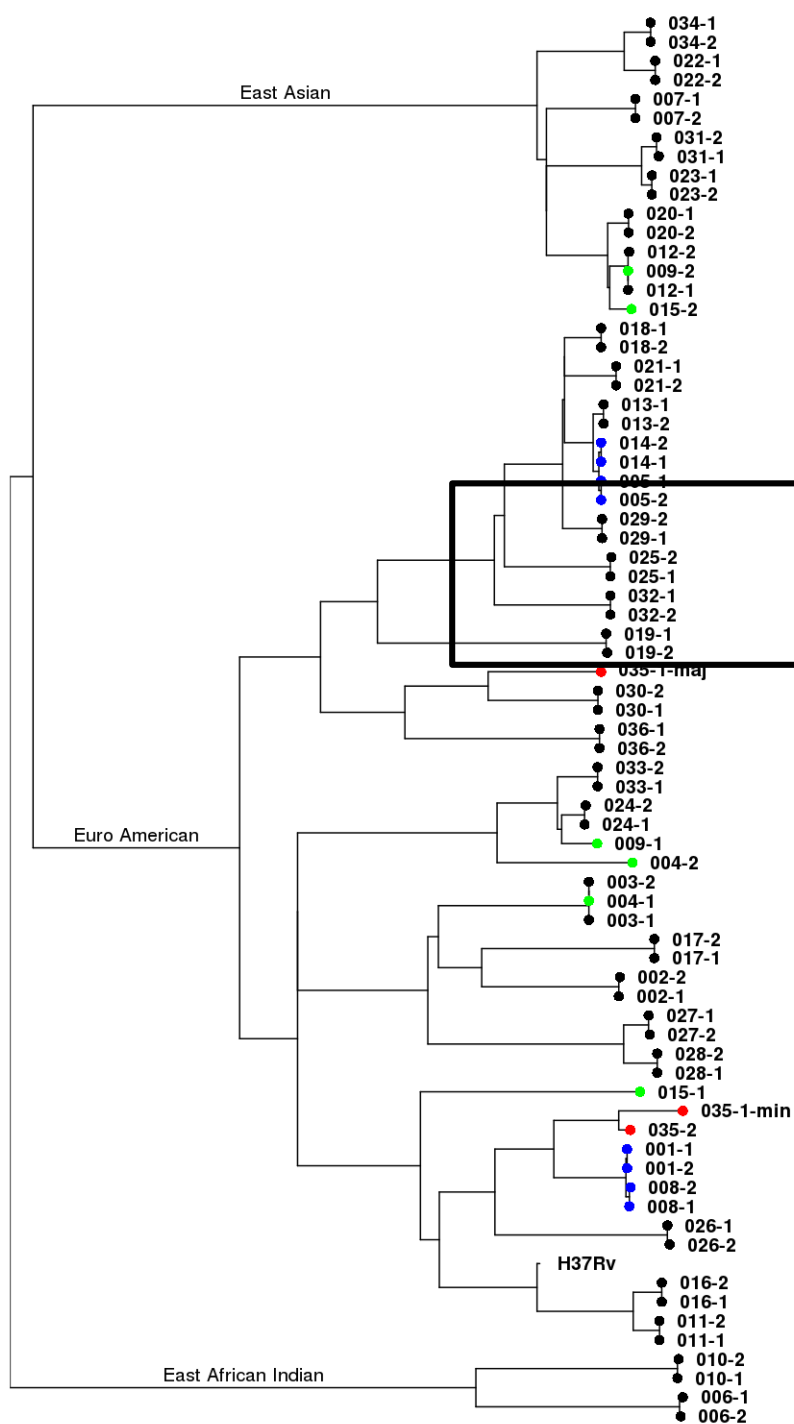
- Build consensus for all sites for each isolate
- Filter sites
 - Depth of coverage
 - Base quality score
 - Heterogenous sites
- Remove filtered sites from all isolates
- Remove non-variant sites
- Concatenate remaining variant sites

A	GATGGTA
B	GATCGTC
C	GATCATA
D	GCTCGTA
R	GATCGTA

Site calling - phylogenetics

- Build consensus for all sites for each isolate
- Filter sites
 - Depth of coverage
 - Base quality score
 - Heterogenous sites
- Remove filtered sites from all isolates
- Remove non-variant sites
- Concatenate remaining variant sites
- Maximum Likelihood estimation (RAxML)

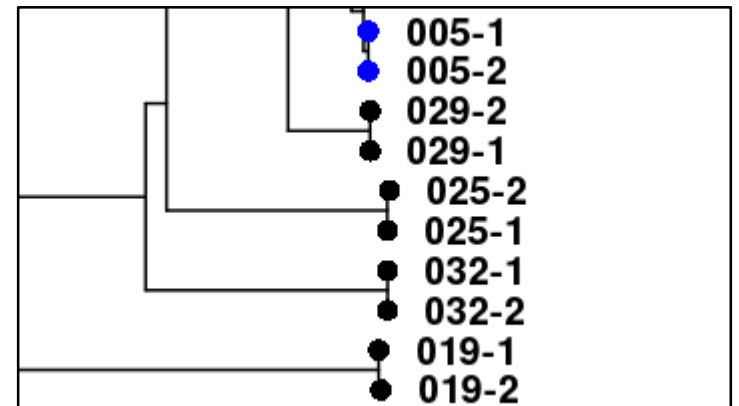
A	GA
B	GC
C	AA
D	GA
R	GA



5123 sites

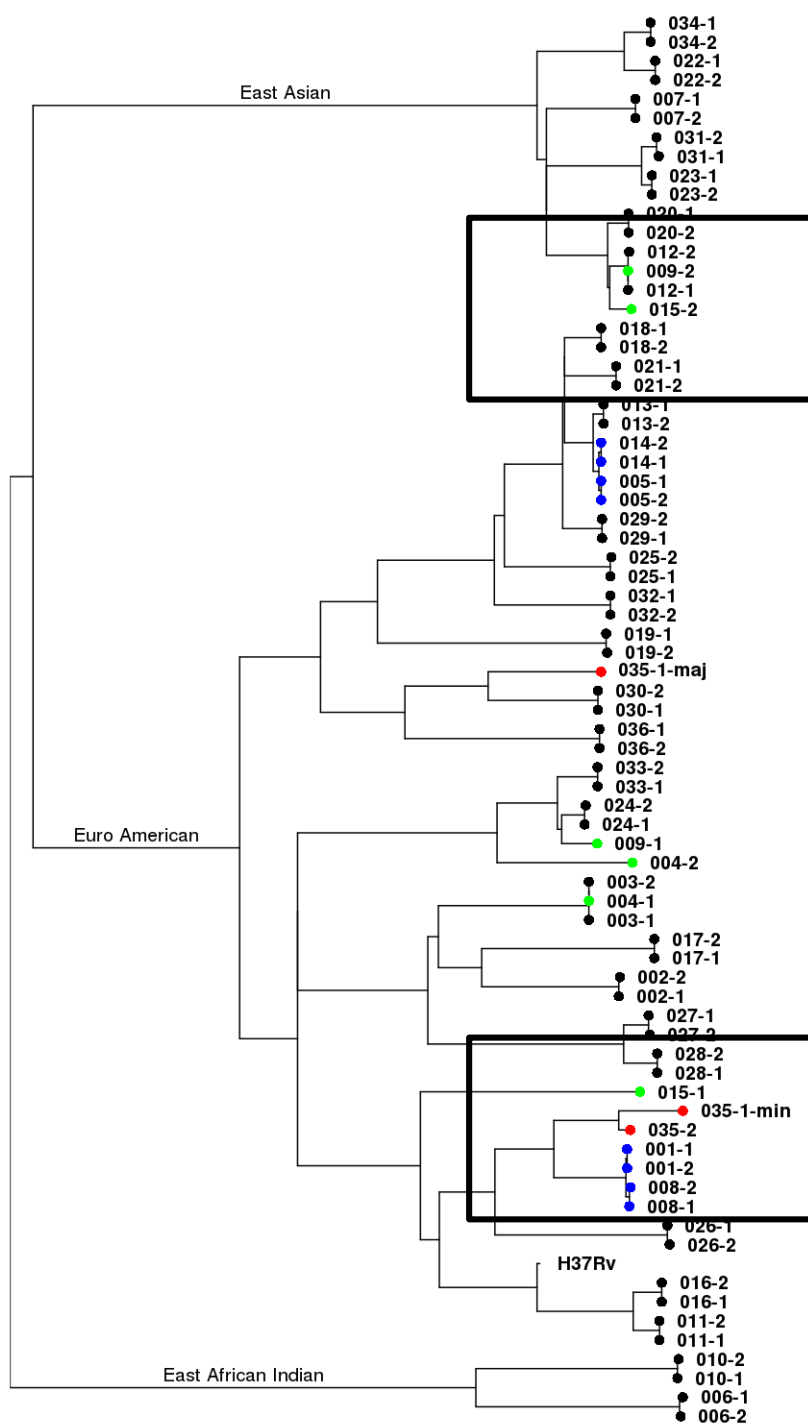
xxx-1 baseline

xxx-2 post treatment



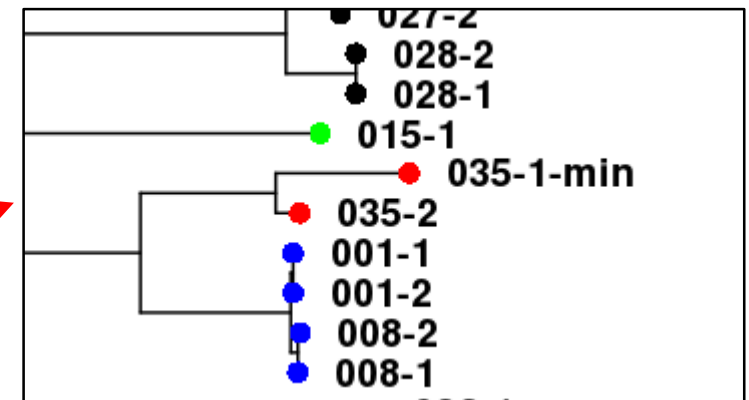
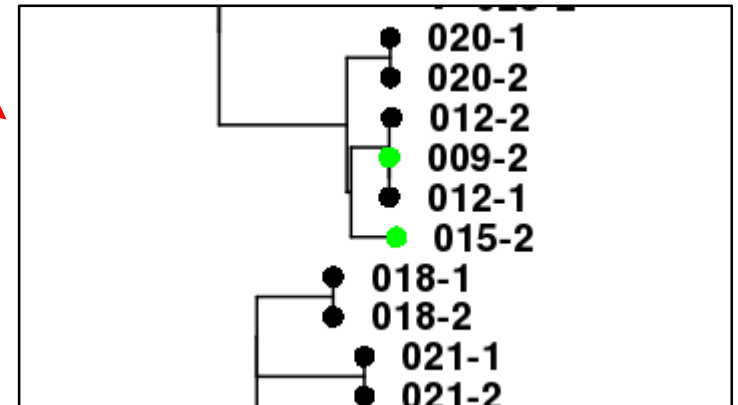
relapse

= treatment failure



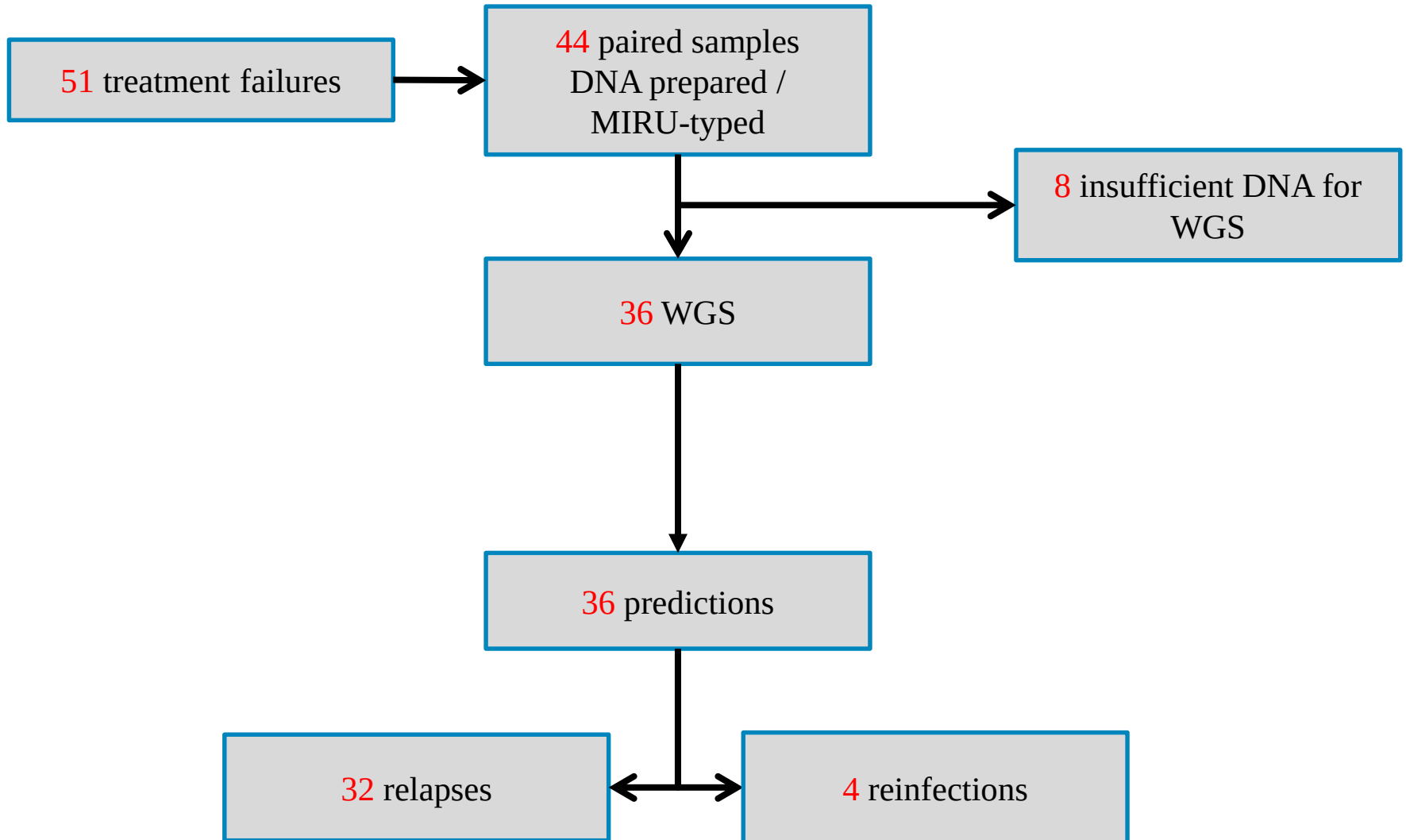
5123 sites

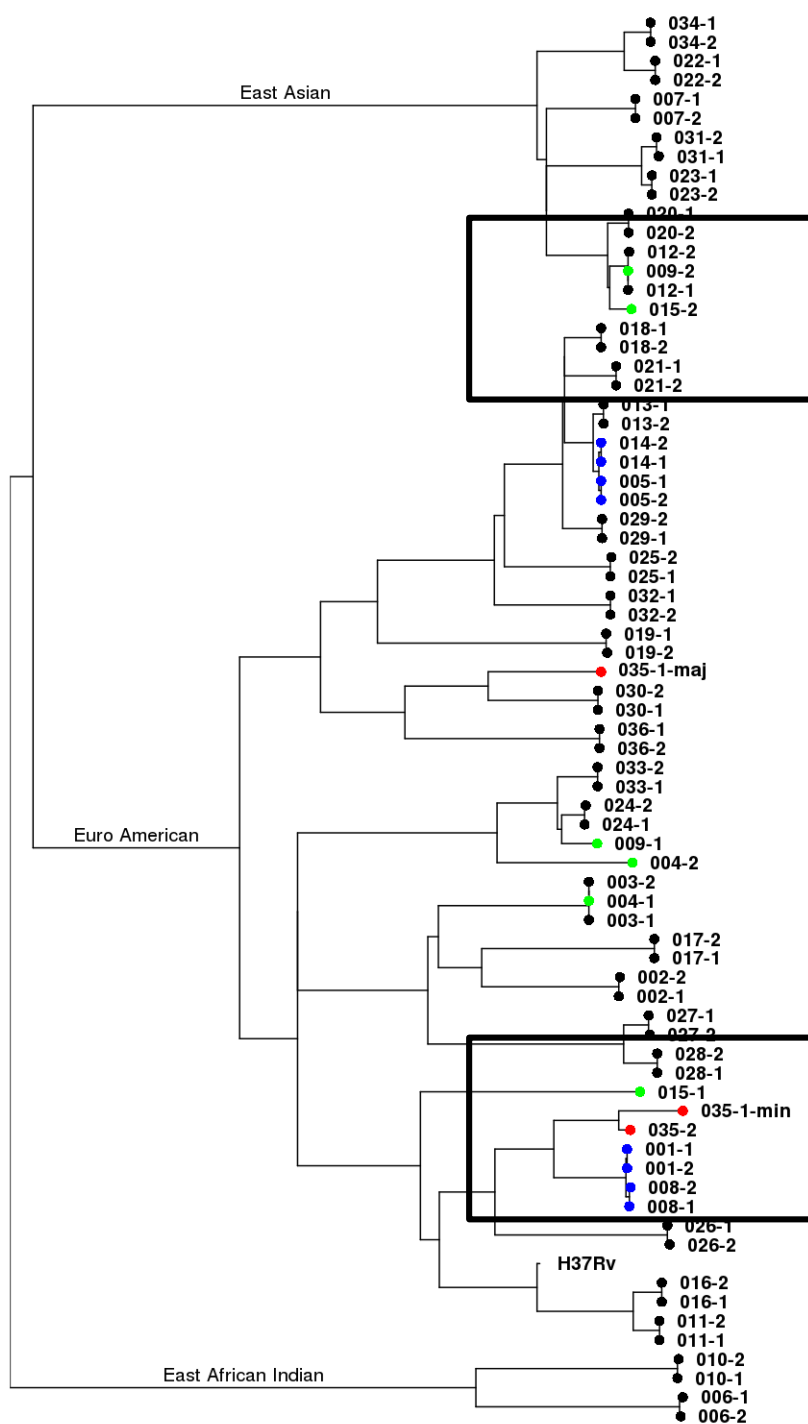
xxx-1 baseline
xxx-2 post treatment



reinfection

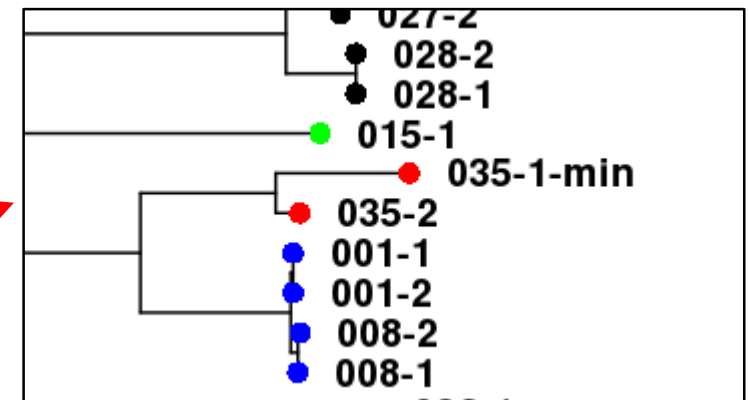
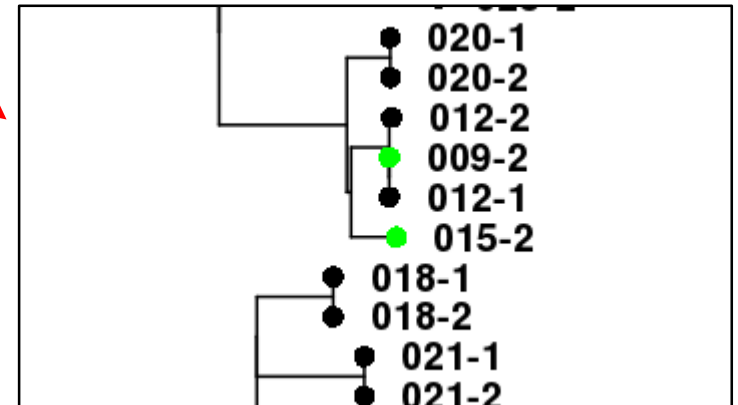
Treatment failures





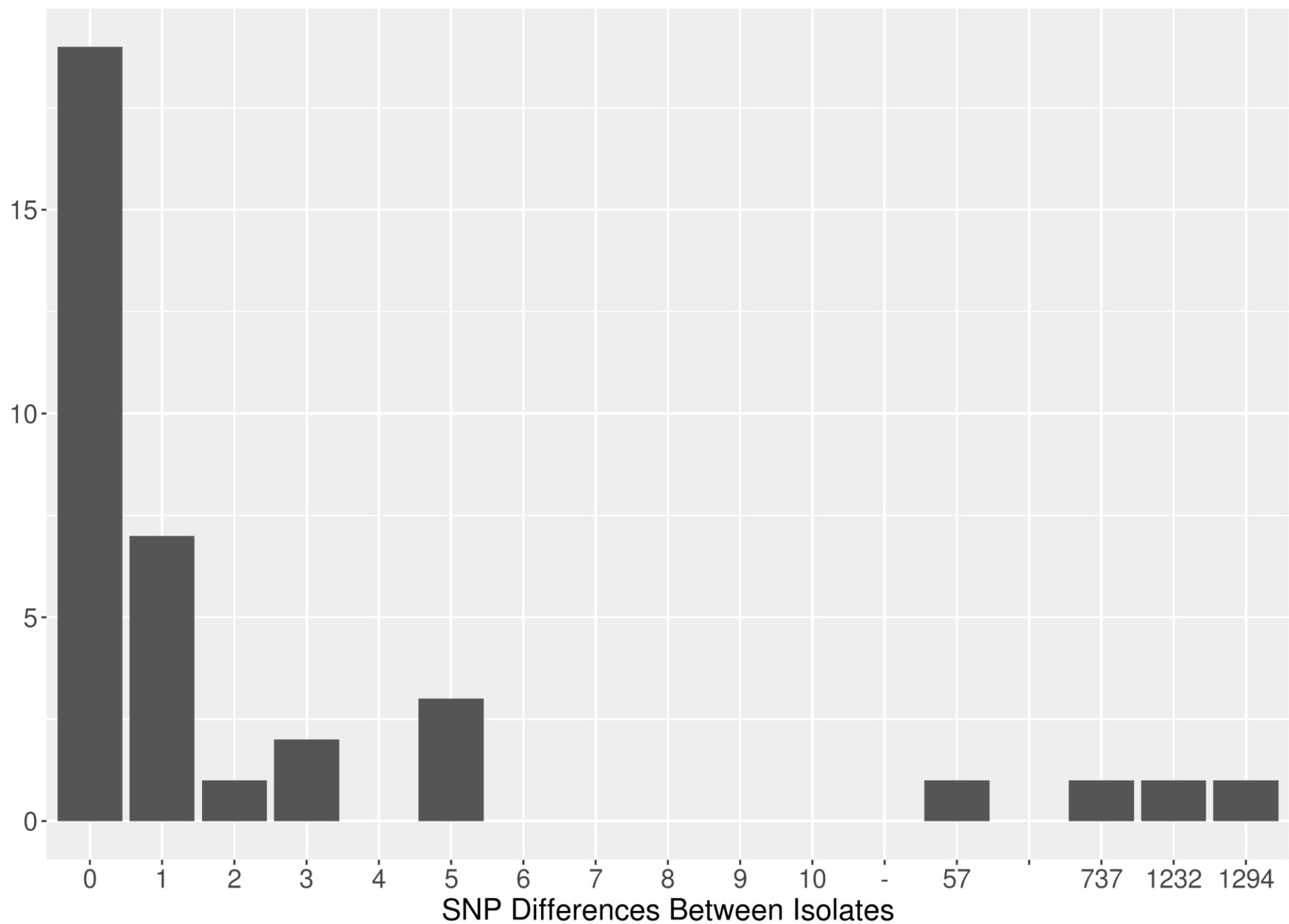
5123 sites

xxx-1 baseline
xxx-2 post treatment



reinfection

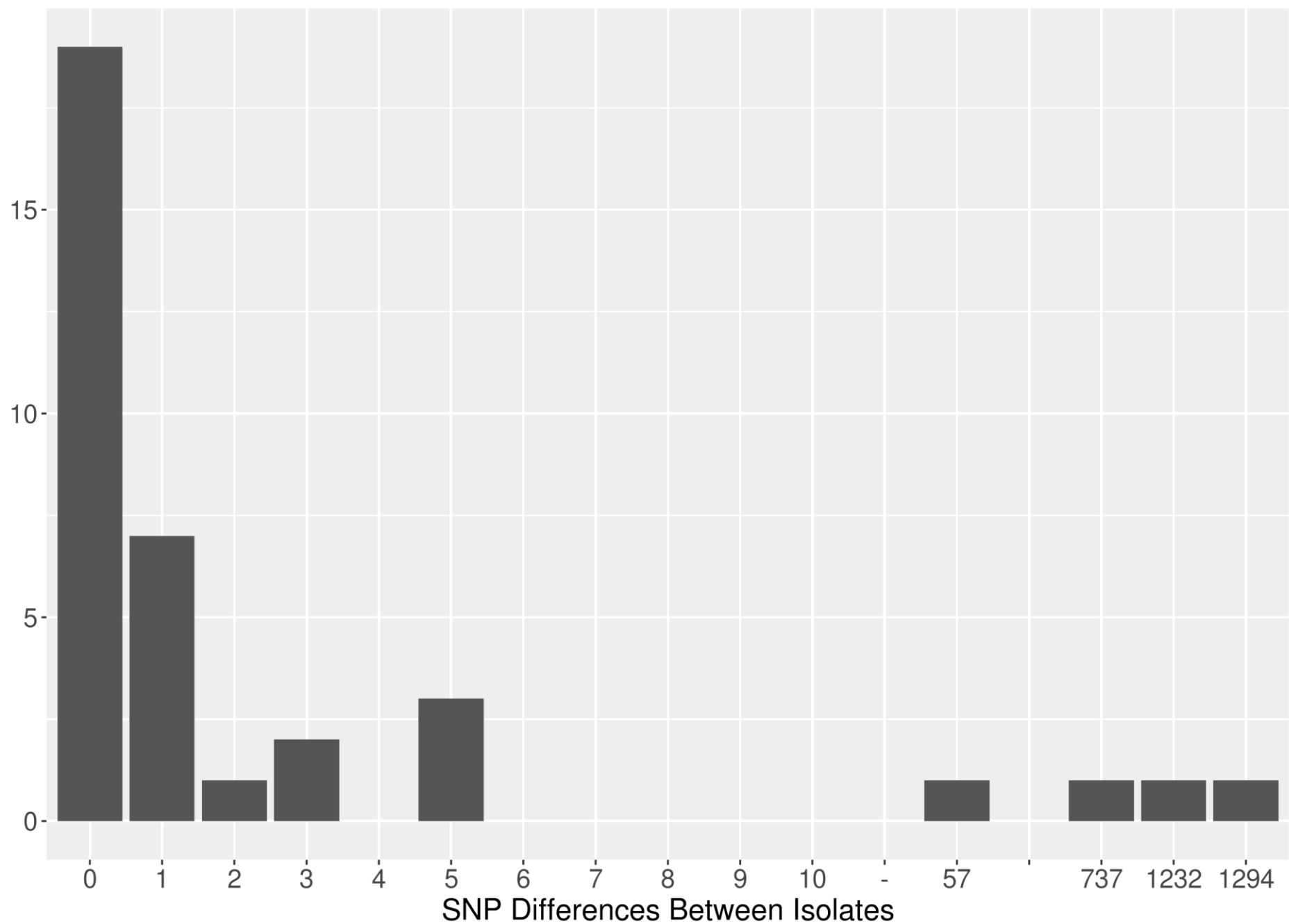
Isolate Pairs Count

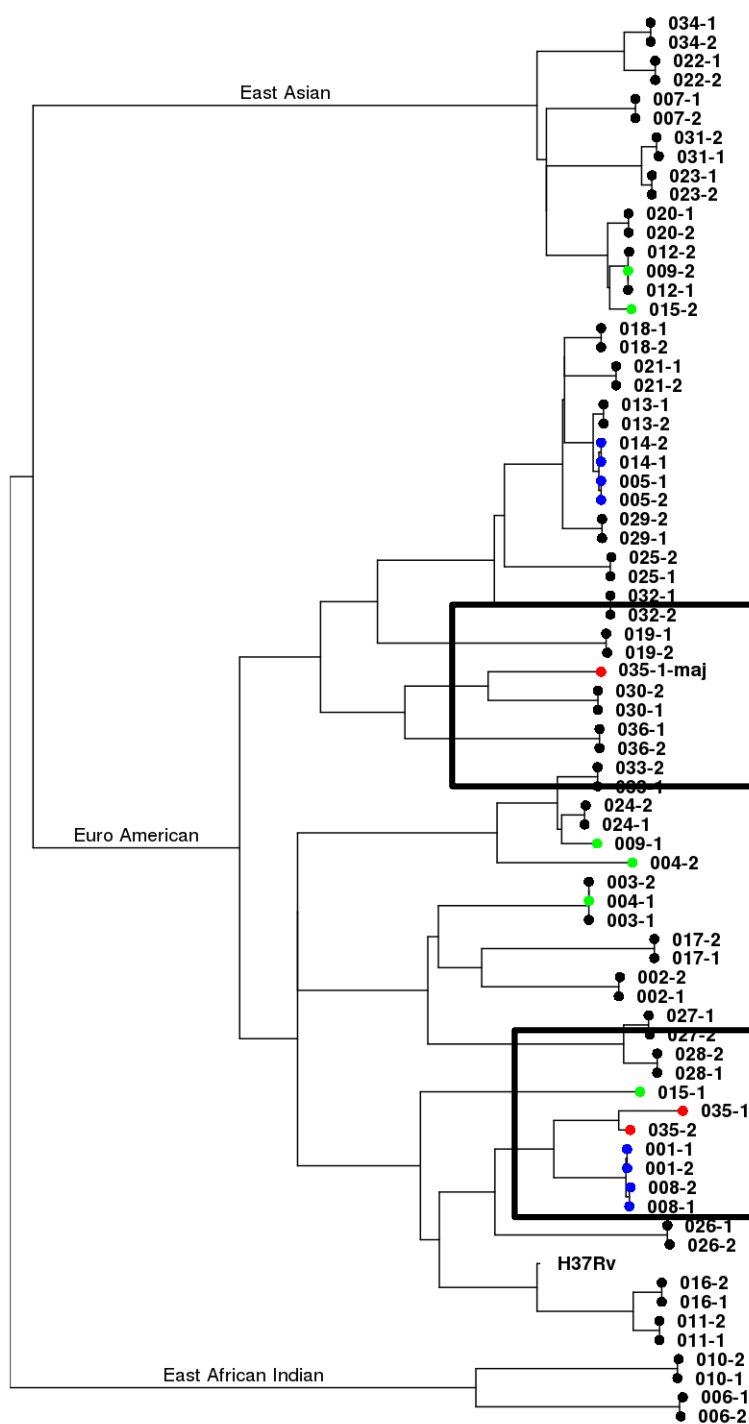


Relapse / reinfection SNP distances

Relapse group	Reinfection group	Maximum length of follow up	Study
0-6	>1300	18 m	Bryant <i>et al</i>
0-8	>100	>12 y	Guerra-Assuncao <i>et al</i>

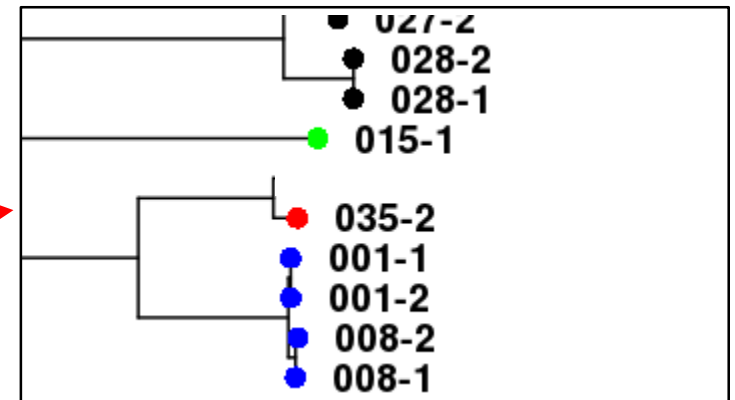
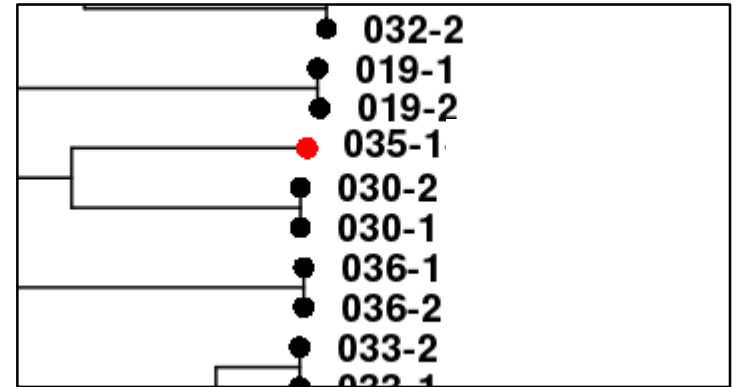
Isolate Pairs Count





5123 sites

xxx-1 baseline
xxx-2 post treatment



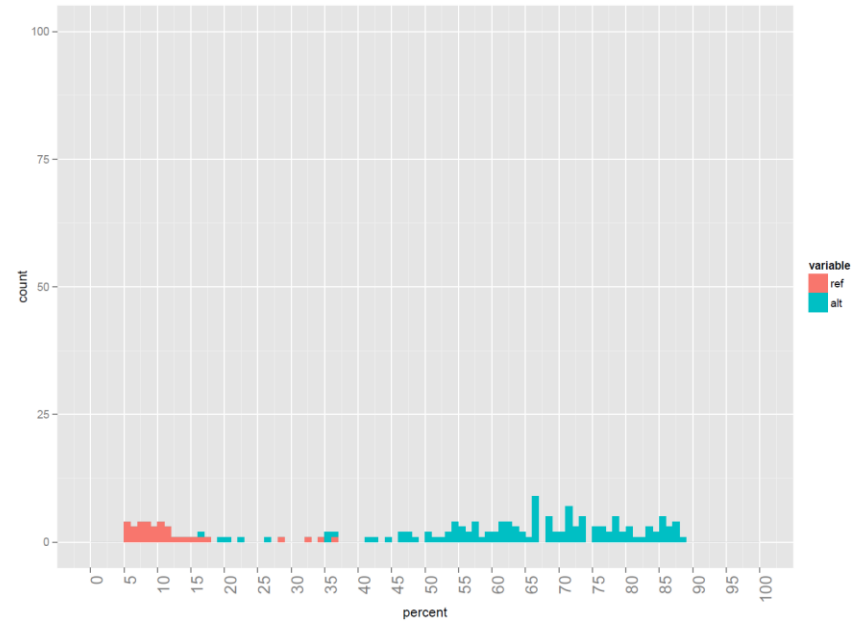
reinfection?



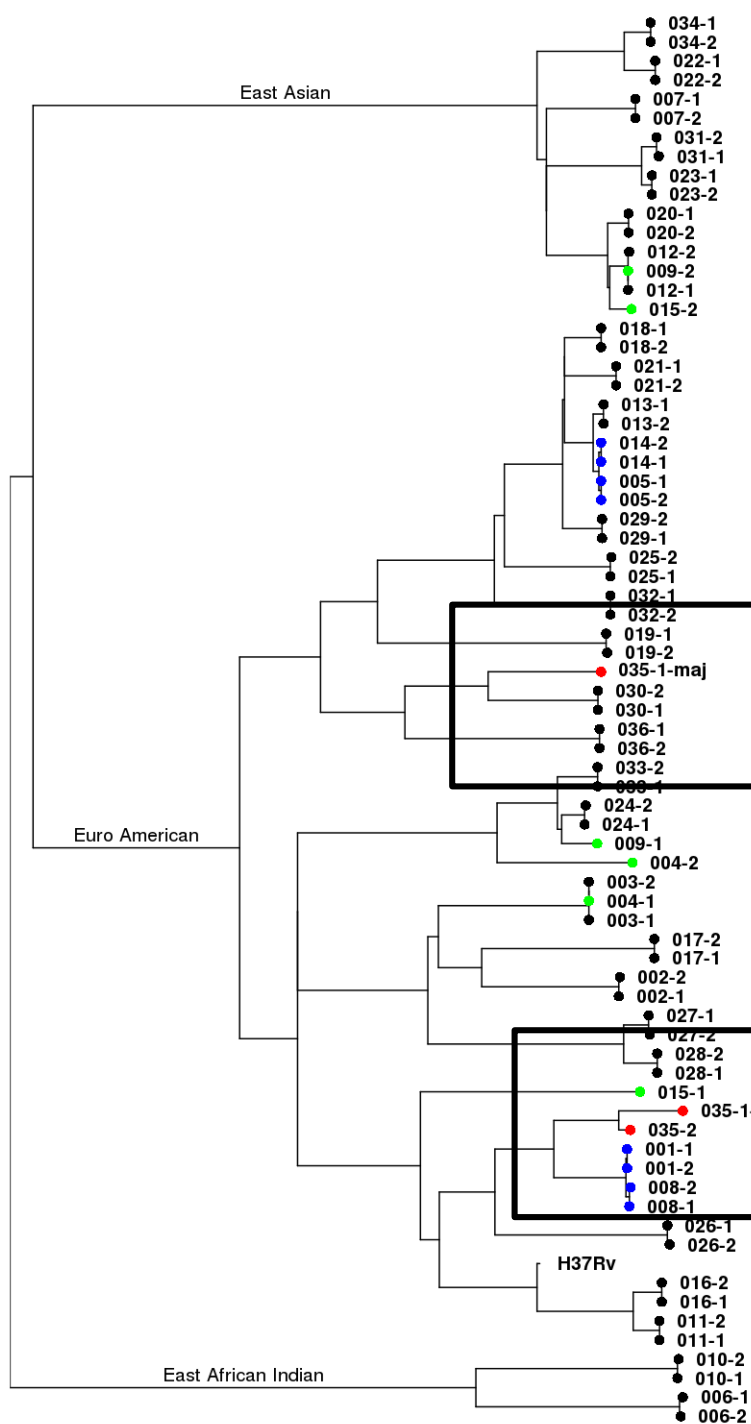
Mixed sites – 035-1



1449 sites

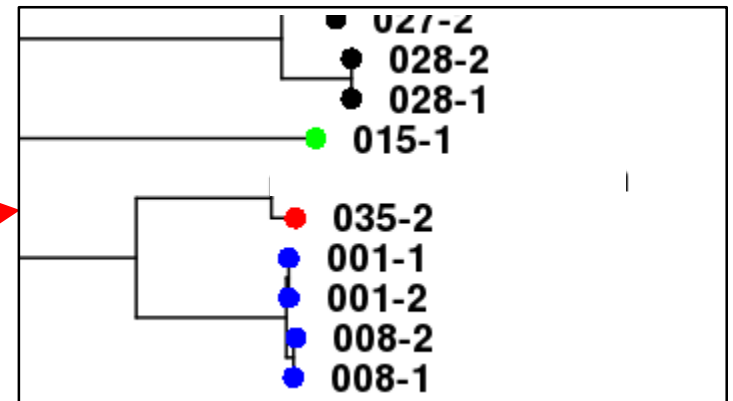
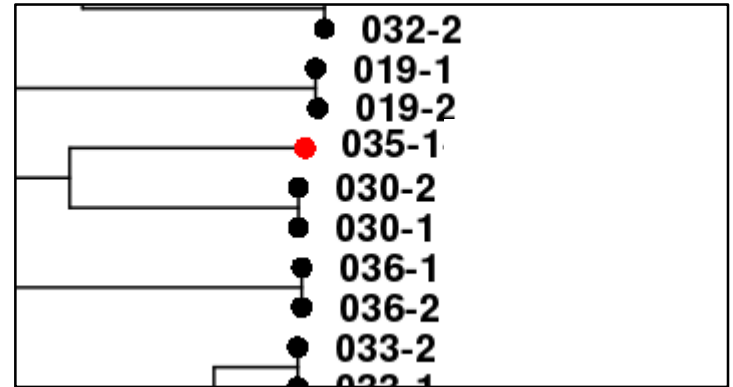


126 sites

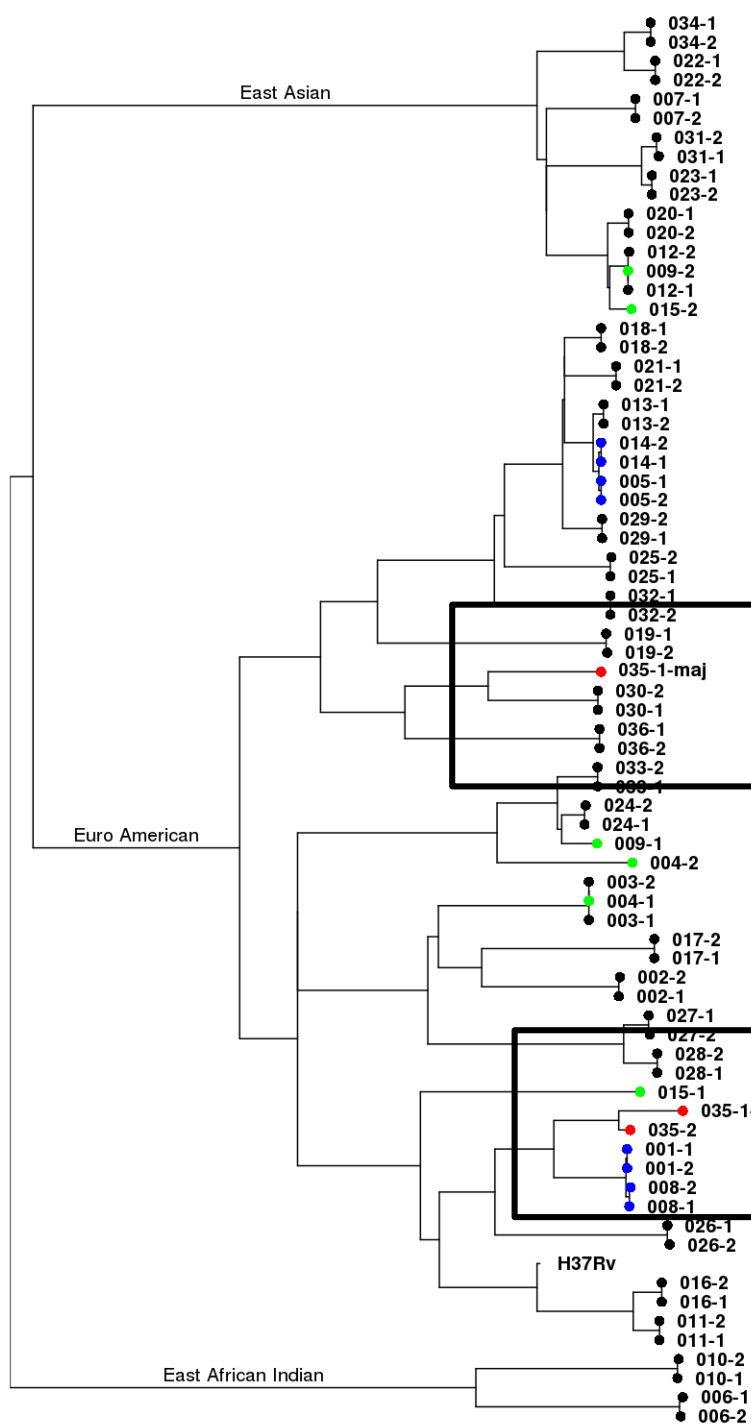


5123 sites

xxx-1 *baseline*
xxx-2 *post treatment*



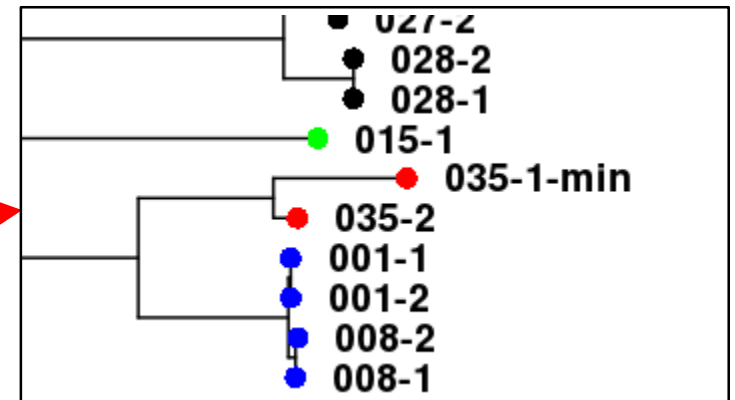
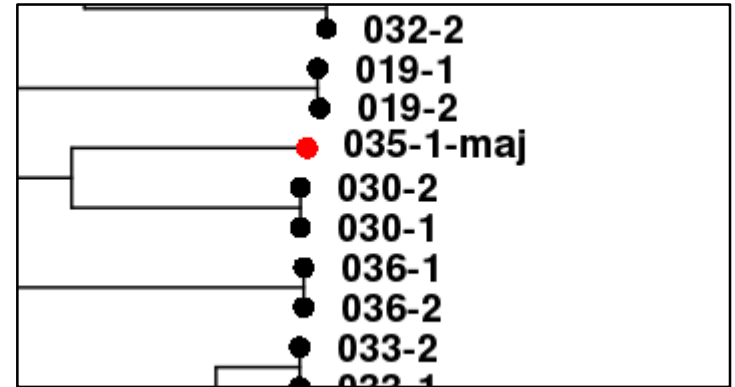
reinfection?



5123 sites

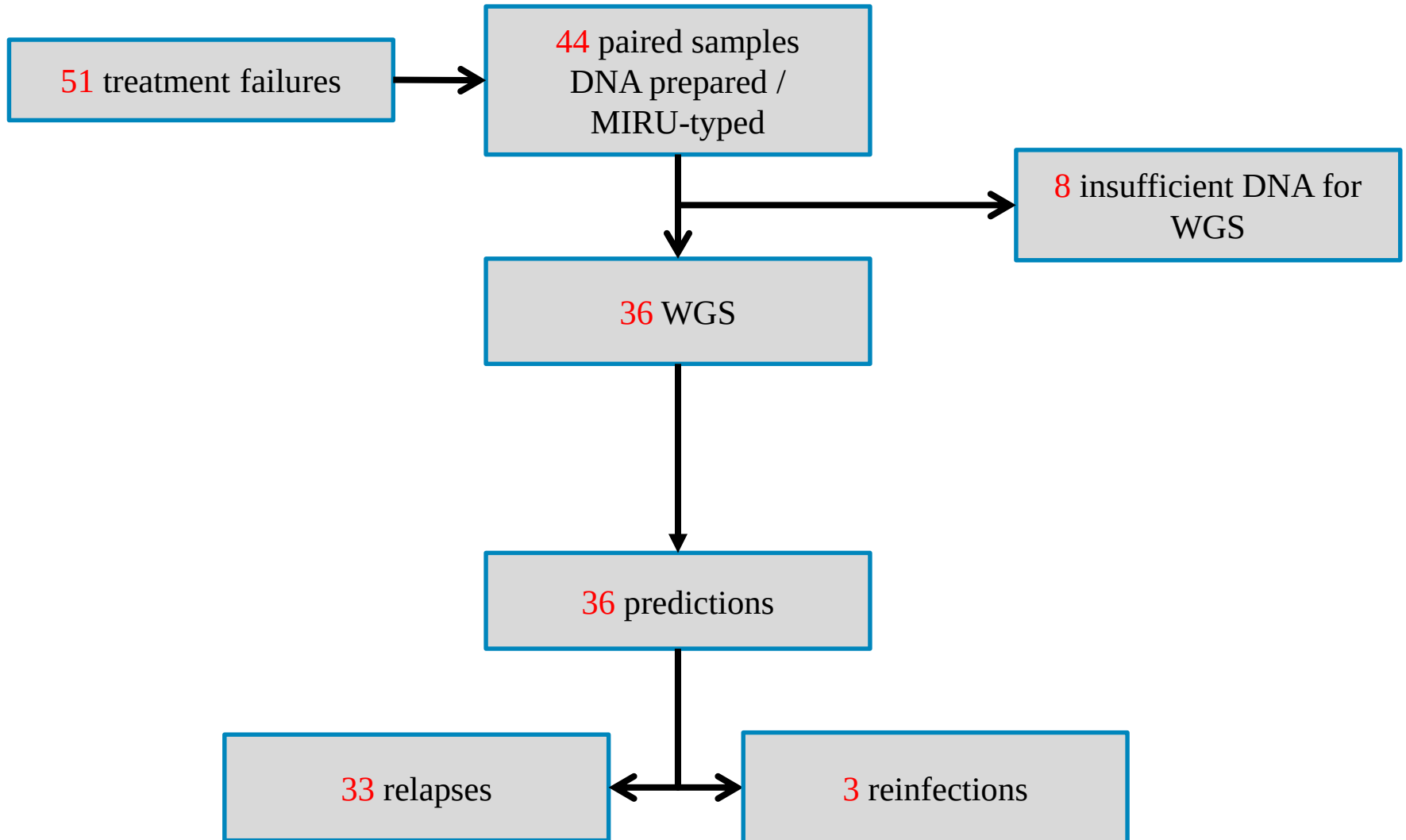
xxx-1 baseline

xxx-2 post treatment



mixed infection

Treatment failures



Summary

	MIRU-VNTR	WGS
Relapse	32	33
Reinfection	4	3

- **WGS technically more straightforward, comparable in cost (cost also decreasing)**
- **Greater resolution and likely enhanced accuracy**
- **Added value**
 - **Genotypic resistance markers**
 - **Transmission events**
 - **Greater comparability between studies**
 - **Growing resource of global TB genome sequence**

Acknowledgements



**David Coleman
Amina Jindani
Neil Stoker
Philip Butcher**



**Tim McHugh
Anna Bateson
Patrick Phillips**

RIFAQUIN Study Team

