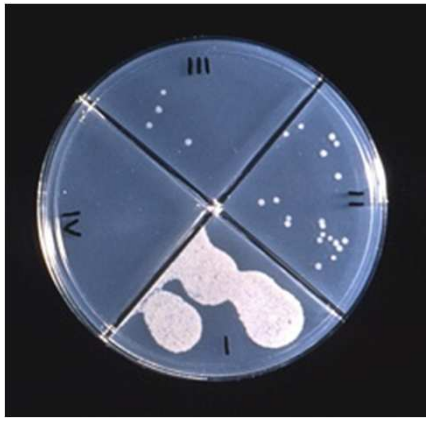
Sequencing of M.Tb directly from sputum

Professor Judy Breuer

MTb Drug susceptibility testing



Phenotypic

Still the gold standard

Very slow. Depending on type can take 7-10 days or 4 – 6 weeks

Molecular tests

- Gene Xpert

  Molecular detection of MTB and drug resistance markers within 2 hours

  Limited to rifampicin

  Can't distinguish between synonymous and non-synonymous mutations

- Line probe assays

  DNA strip test allows molecular detection of drug resistance markers directly from clinical samples within 5 hours

  Limited number of mutations it can detect

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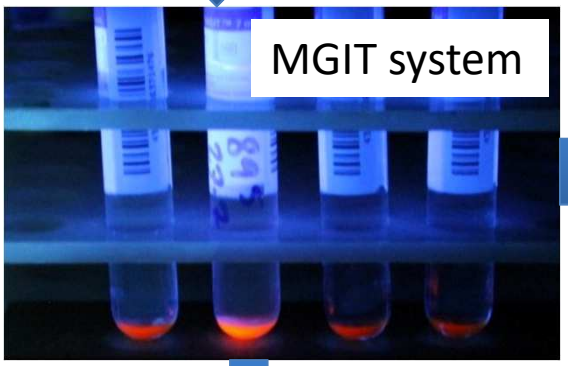
Sequencing early MGIT culture as an alternative

(1) Overcomes the need for culture, which can take weeks

(2) Overcomes possible biasing of genetic diversity from culture

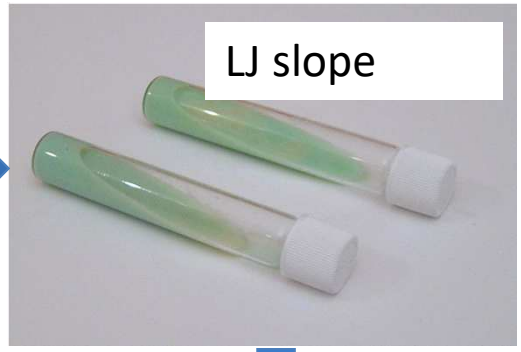
Koser et al NEJM 2013

Sputum sample



NGS sequencing

2 strains



NGS sequencing

1 strains

Especially important in context of superinfections

Quicker than conventional culture

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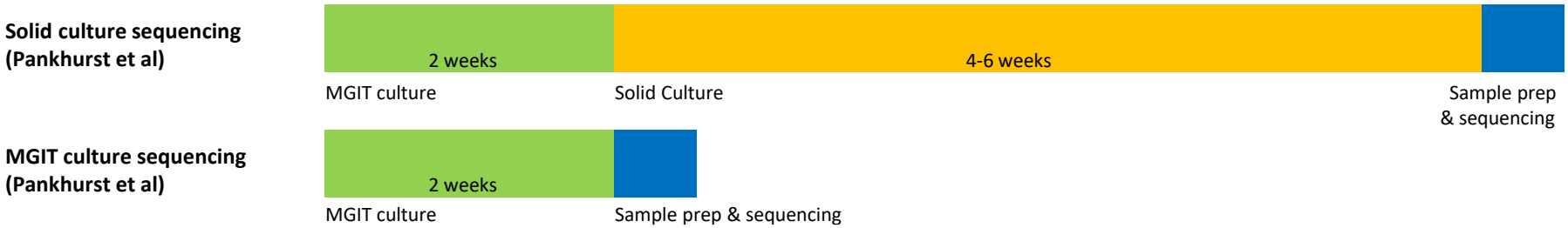
MGIT sequencing reduces time to AMR result



Pankhurst et al 2015 LID

WGS directly on sputum samples

Possible without any enrichment

But very low coverage: 0.002 to 0.7 fold

Not enough to accurately call resistance

Culture-independent detection and characterisation of *Mycobacterium tuberculosis* and *M. africanum* in sputum samples using shotgun metagenomics on a benchtop sequencer

Emma L. Doughty [1], Martin J. Sergeant [1], Ifedayo Adetifa [2], Martin Antonio [1,2], Mark J. Pallen [1]

Published September 23, 2014

PubMed 25279265

PeerJ Picks · 2015 Part of the PeerJ PeerJ Picks 2015 Collection

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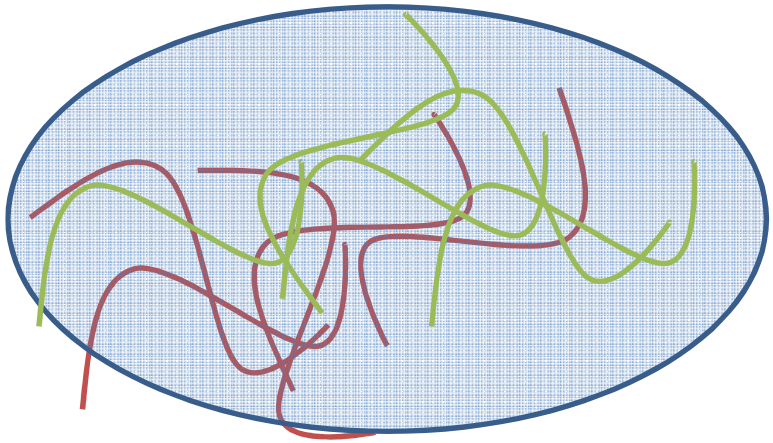
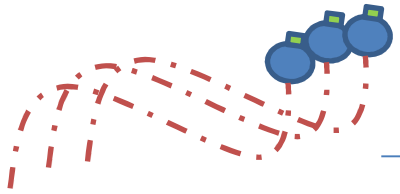
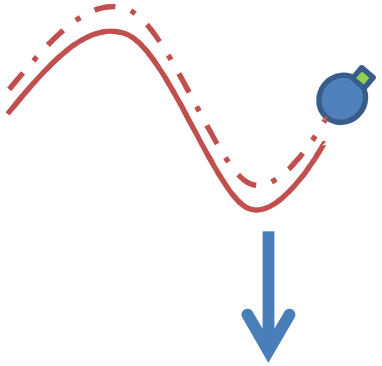
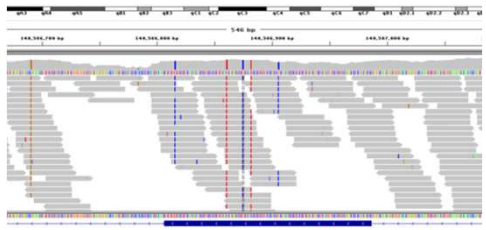
Sequencing of enriched pathogen nucleic acid







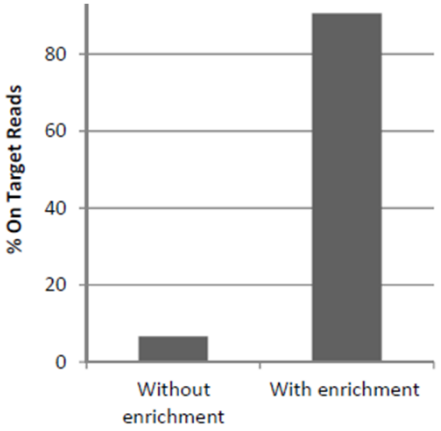


Pros
Can be used directly on sputum samples
Works for highly variable pathogens
Works for all sizes of pathogens
Good detection of mixtures
Automated high throughput

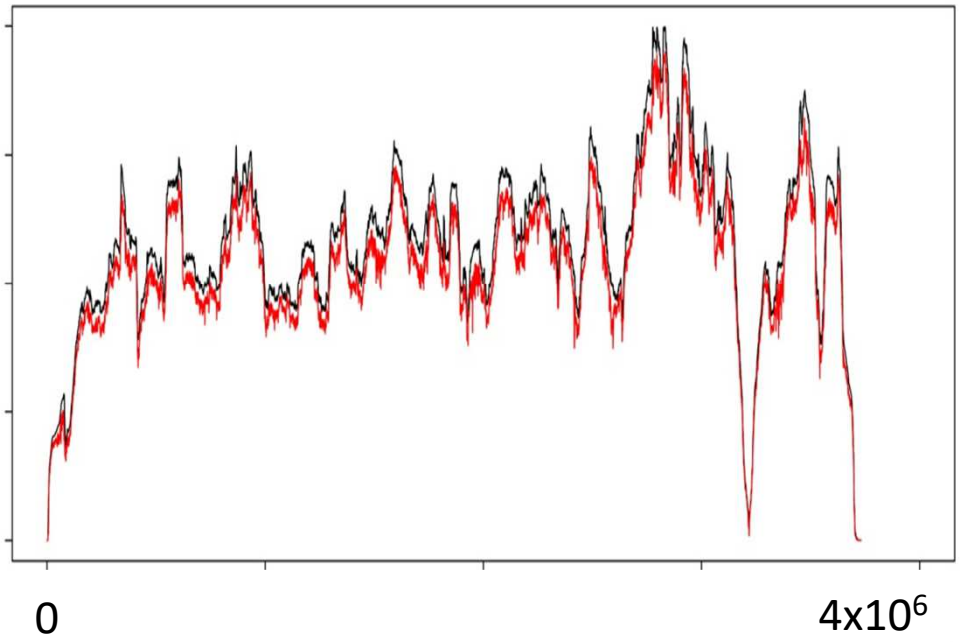
Cons
Expensive to set up
Expertise needed

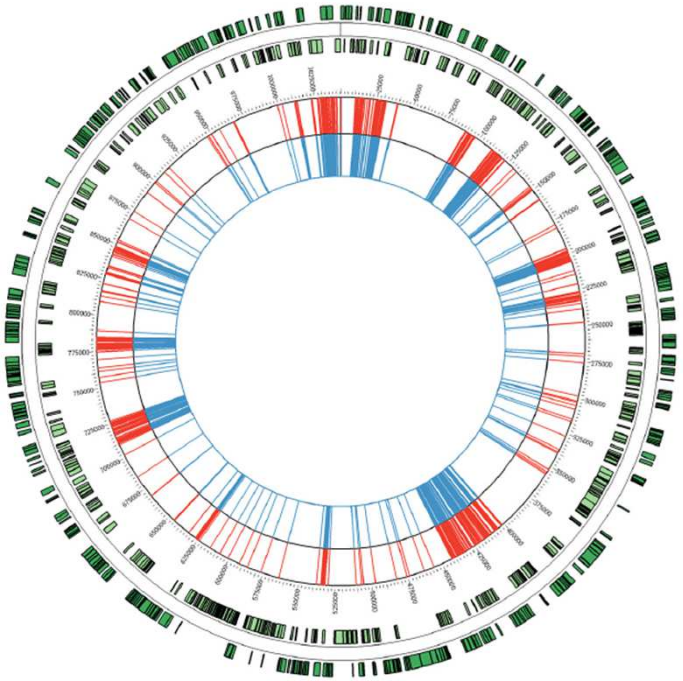
Targeted Enrichment Sequencing

- Semi automated method
- No prior PCR/culture
- Random access
- Sensitive – 100-1000 copies /ml



10 -10,000 fold enrichment



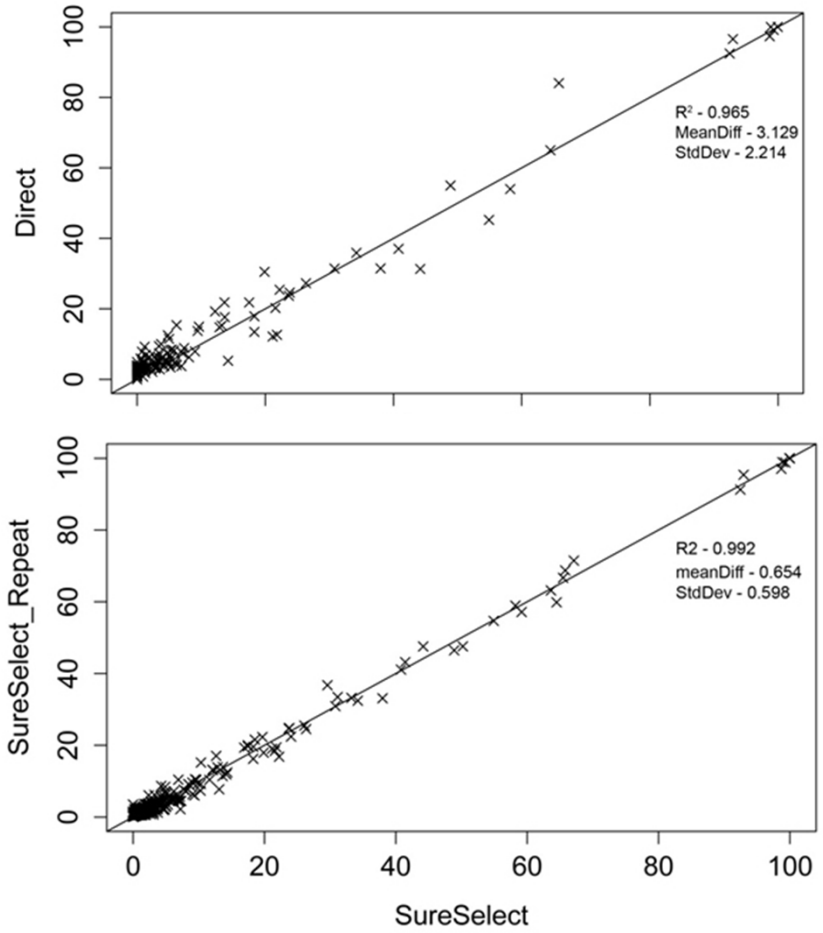


Identical genome

Brown 2015 JCM

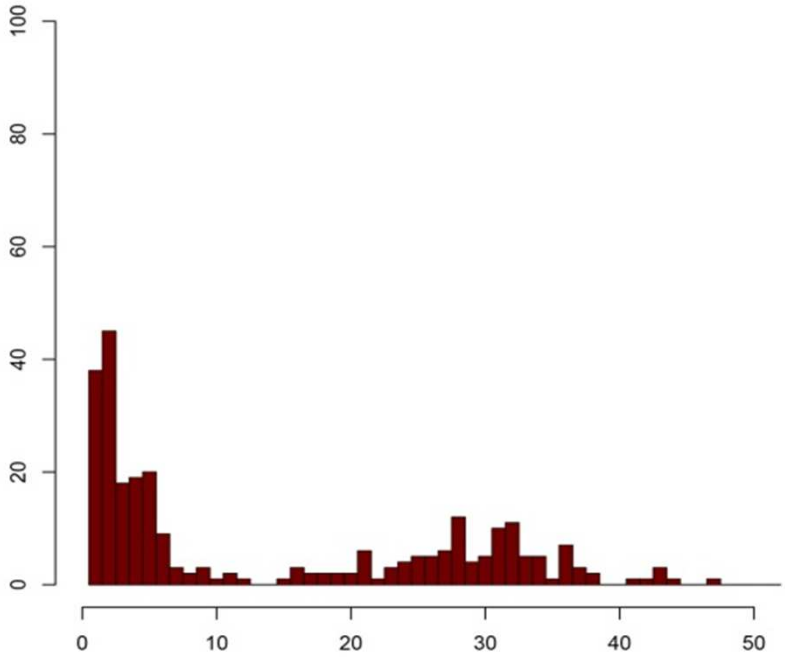
Christiansson 2014 BMC Infection

Targeted Enrichment Sequencing



Preserves variant frequencies

Depledge 2011 Plos One, Depledge 2014 MBE



Enables detection of mixed infections

Depledge , Ruis, Bryant, Doyle unpublished

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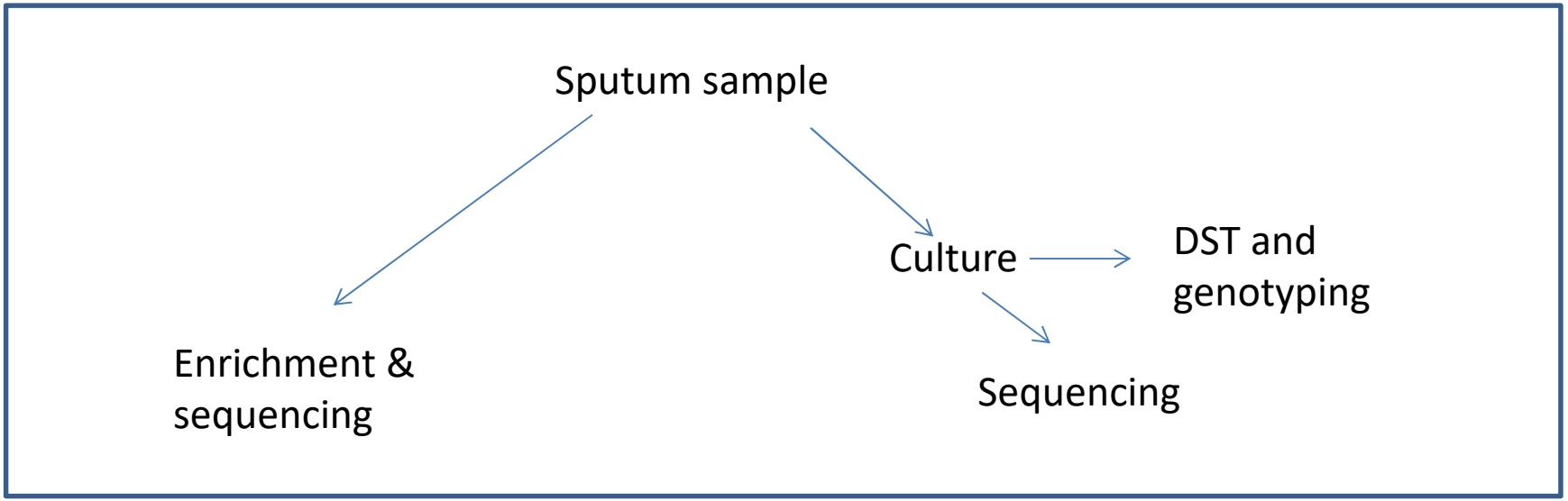
Pilot study to test Sure Select method on TB

Outcomes: Can we use targeted enrichment on TB?
Is it as good as culture?

Secondary outcome:
Are there any differences in the diversity obtained between sputum and culture?

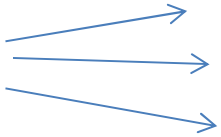


Brown et al, JCM 2015)

Routine samples collected from UK and Lithuania:

(1) 24 subjects with drug resistance data:

All sputum positive, culture positive

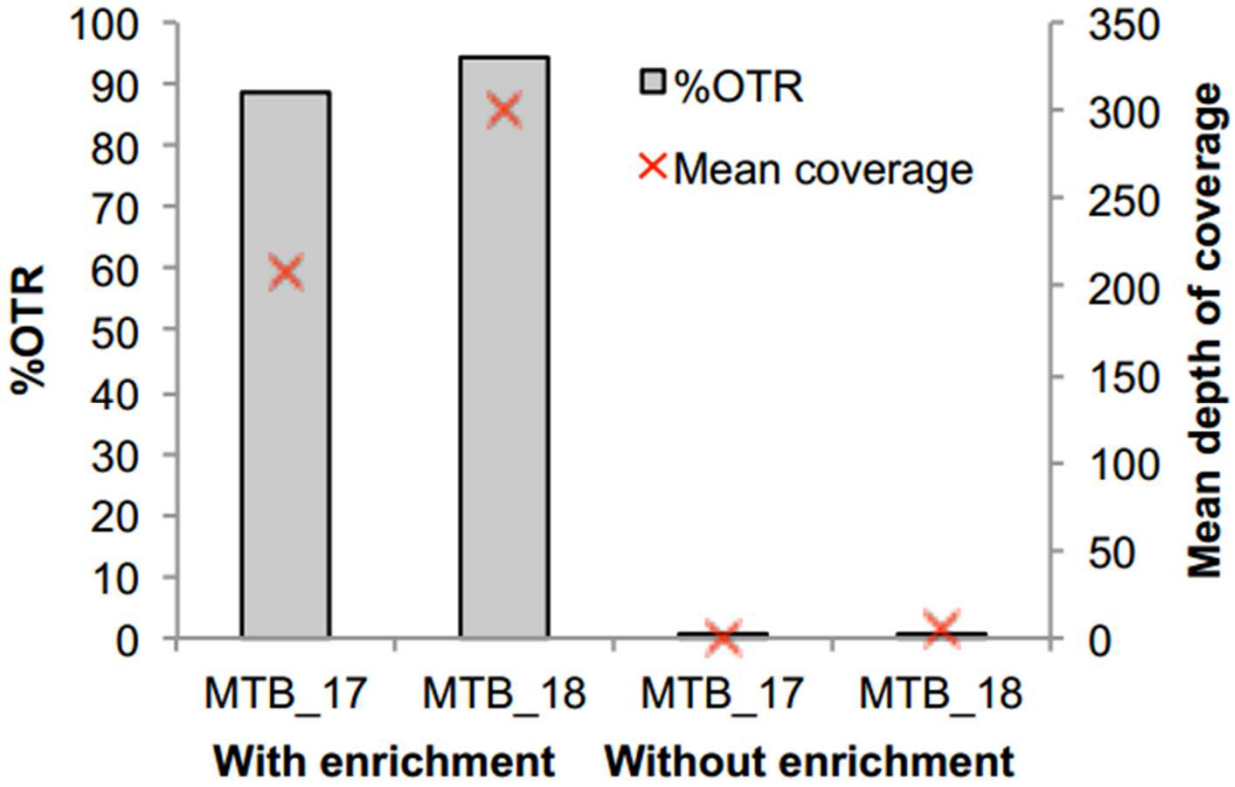


9 sensitive
8 MDR
3 XDR

(2) 10 subjects without drug resistance data:

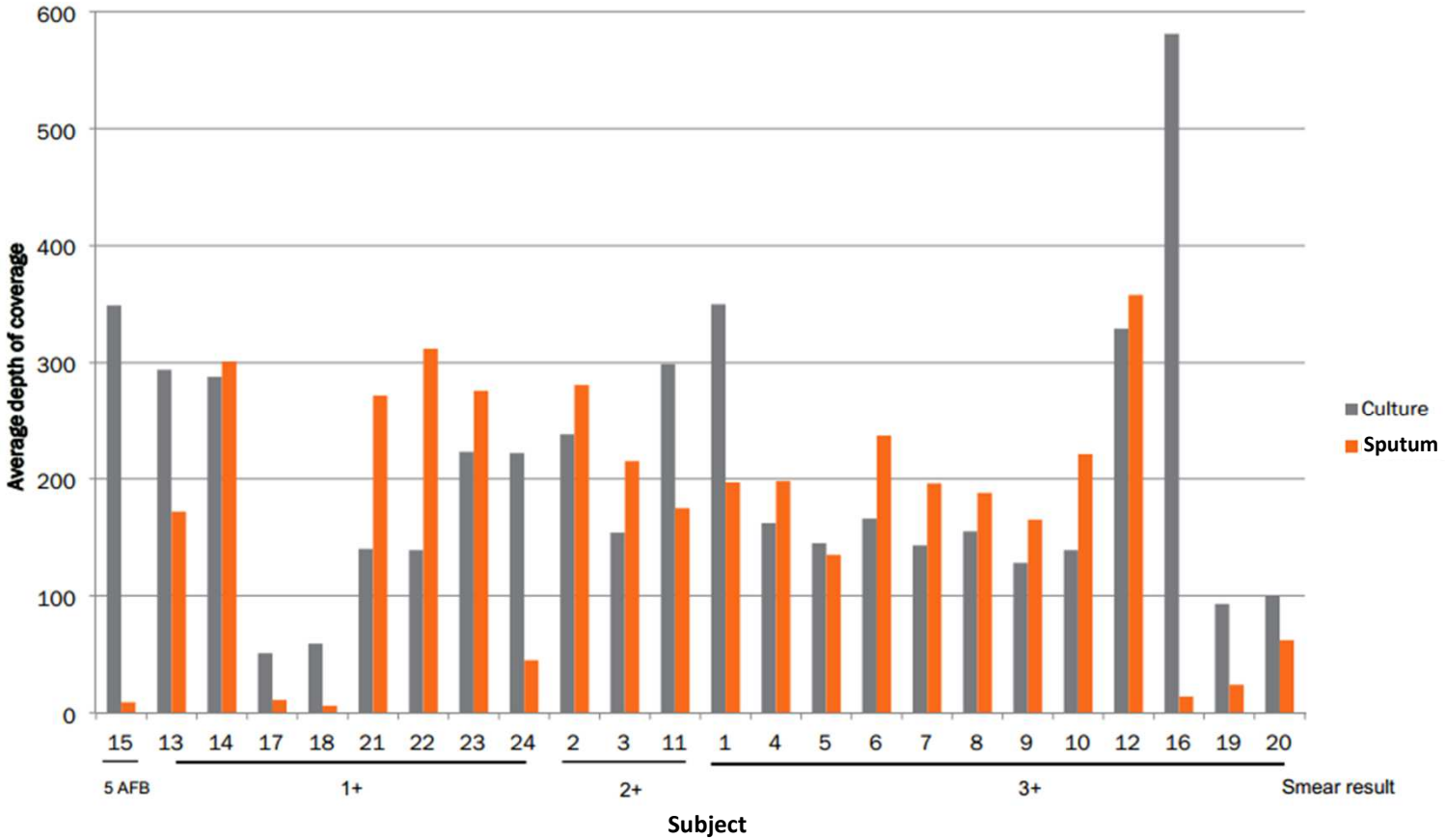
2 sputum positive, culture negative
8 sputum negative, culture negative

Does enrichment improve MTB recovery in absence of culture?



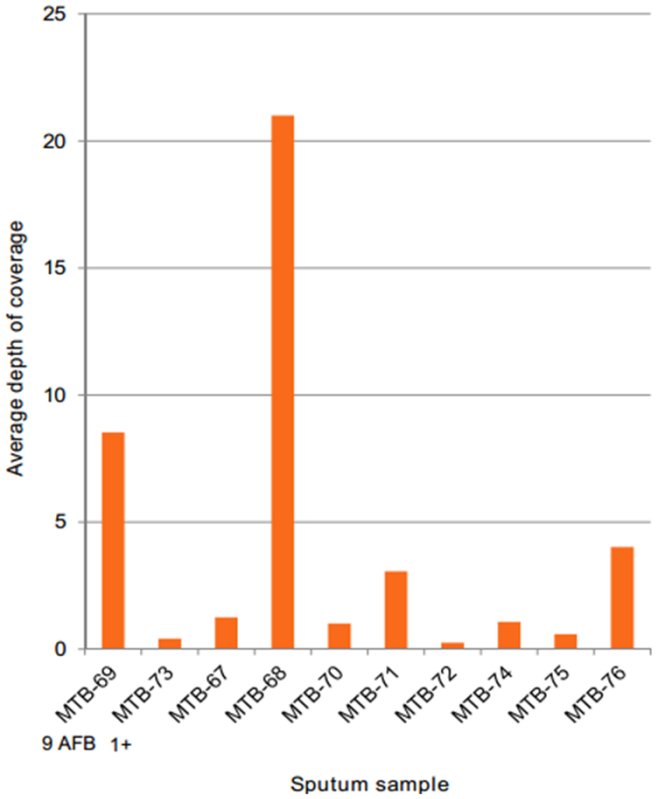
Brown et al J Clin Micro 2015

How does MTB sequence from enrichment compare with culture?



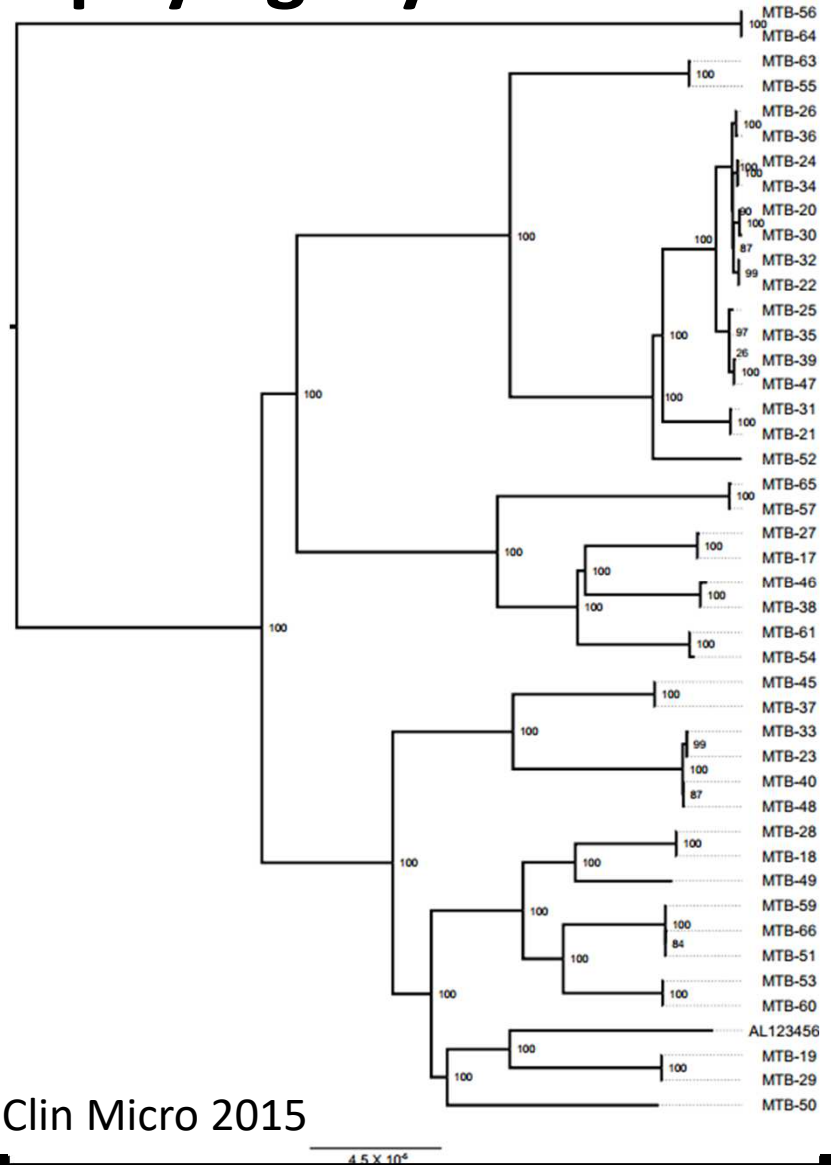
Brown et al J Clin Micro 2015

Can we recover MTB when culture doesn't work?



Brown et al J Clin Micro 2015

Is there enough information to construct a robust phylogeny?



Brown et al J Clin Micro 2015

Can we call resistance genotypes?

- Can only call resistance; (sensitivity can be inferred in the absence of a mutation)
- Used LSHTM drug resistance database
- 88 % of phenotypically resistance cases had a mutation
- 94 % of sensitive cases had no mutation
- With one exception all were homozygous

Brown et al J Clin Micro 2015

Discrepancies between phenotype and genotype

Patient	Sputum positivity	Sample	Type	Rif	Inh	Emb	Pza	Str*	Ofl*	Pas*	Amg*	Thi*
1	3+		Culture phenotype	S	S	S	S	NA	NA	NA	NA	NA
		MTB-27	Culture genotype									
		MTB-17	Sputum genotype									
2	2+		Culture phenotype	S	S	S	S	NA	NA	NA	NA	NA
		MTB-28	Culture genotype							R		
		MTB-18	Sputum genotype							R		
3	2+		Culture phenotype	S	S	S	S	NA	NA	NA	NA	NA
		MTB-29WE	Culture genotype									
		MTB-19	Sputum genotype									
4	3+		Culture phenotype	R	R	R	R	R	S	S	R (Kan)	S
		MTB-30WE	Culture genotype	R	R	Low R	R	R			R	
		MTB-20	Sputum genotype	R	R	Low R	R	R			R	
5	3+		Culture phenotype	S	R	S	R	R	R	S	R (Kan & Amk)	R
		MTB-31WE	Culture genotype	R	R	Low R		R	R		R	
		MTB-21	Sputum genotype	R	R	Low R		R	R		R (Kan)	
6	3+		Culture phenotype	R	R	S	R	R	R	S	R (Kan)	R
		MTB-32WE	Culture genotype	R	R	Low R	R	R	R		R	R
		MTB-22	Sputum genotype	R	R	Low R	R	R	R		R	R

phenotypically sensitive to rifampicin but has L452P mutation in the *rpoB* gene. This mutation has been associated with both high and low rifampicin resistance in the literature

Mutation in codon 306 of embB gene Confers borderline MIC

No previously described mutation identified

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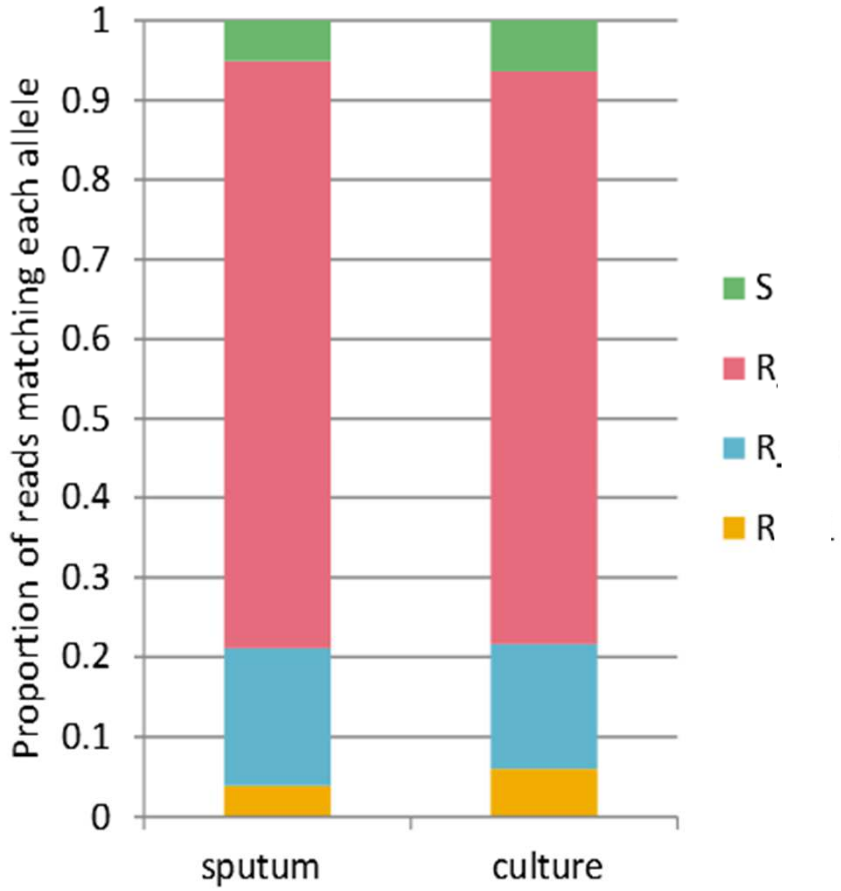
Brown et al J Clin Micro 2015

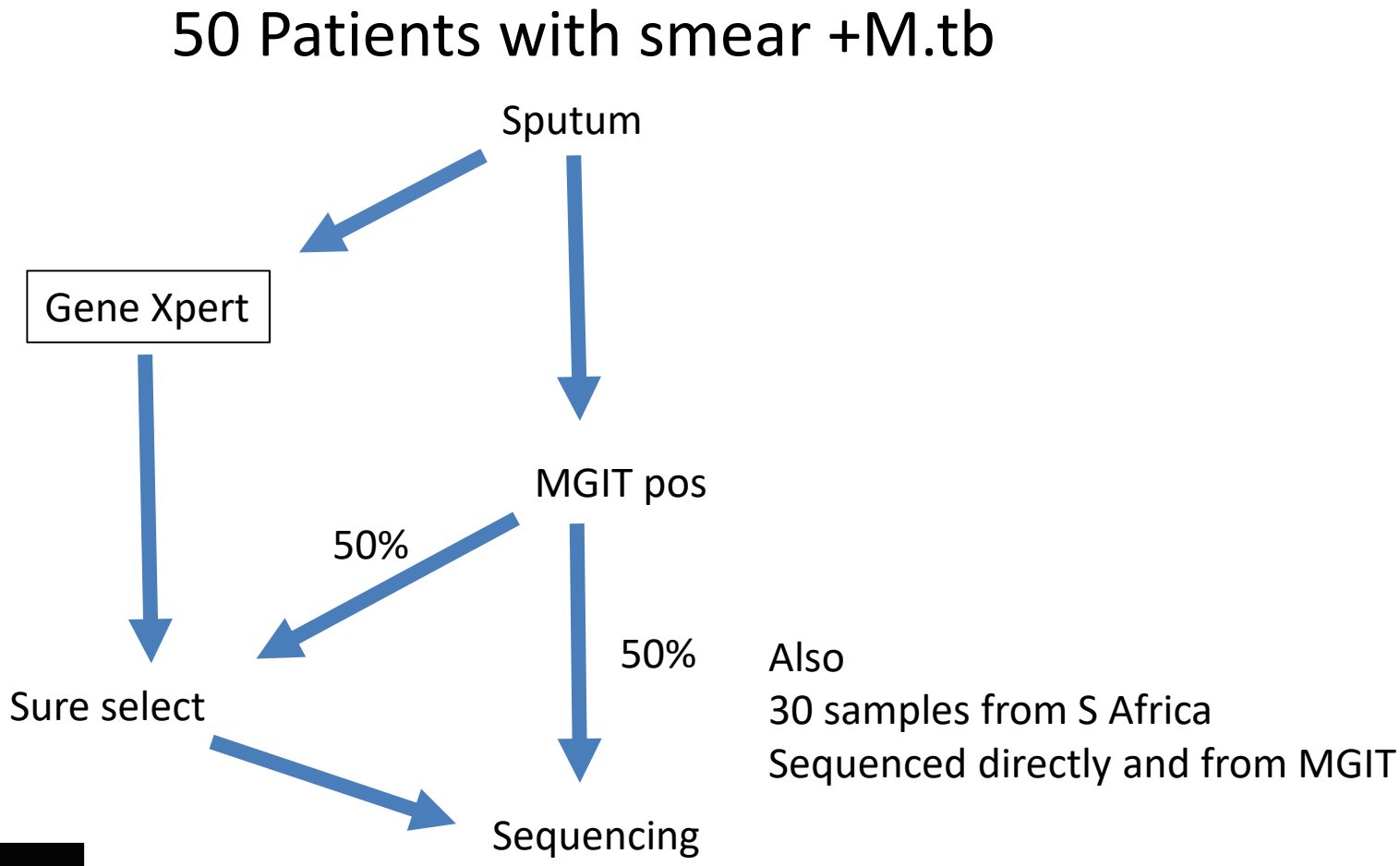
Does sequencing from sputum reveal more genetic diversity?



gyrA gene in subject 10

Brown et al J Clin Micro 2015

Prospective study to evaluate sequencing directly from sputum versus MGIT culture



Conclusions

- **Targeted enrichment successfully recovers genomes from sputum**
- **The data is at a high enough quality to accurately reconstruct phylogenies and call resistance mutations**
- **Consensus sequences are identical to MGIT and cultured samples**
- **Sensitivity is related to genome copy input (>90% for smear +)**
- **Can also recover genomes from smear negative sputum**
- **Turnaround times are faster than other methods for MTb AMR detection 1.5/2.5 days versus 14 days (MGIT) and 21-28 days (culture).**

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Acknowledgements

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