

**eurac**  
research

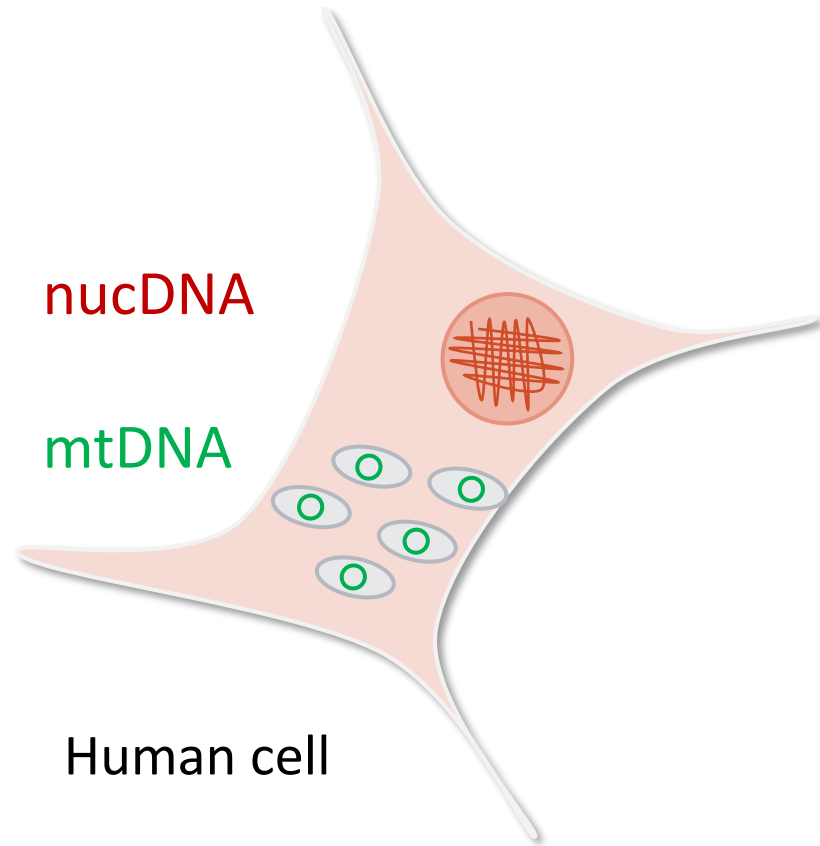


# Design and evaluation of a targeted enrichment assay capturing *Mycobacterium tuberculosis* complex DNA in ancient human remains

Heidi Y. Jäger-Ketikidés, Ildikó Pap, Ildikó Szikossy, György Pálfi, Frank Maixner, Albert Zink

# ANCIENT DNA

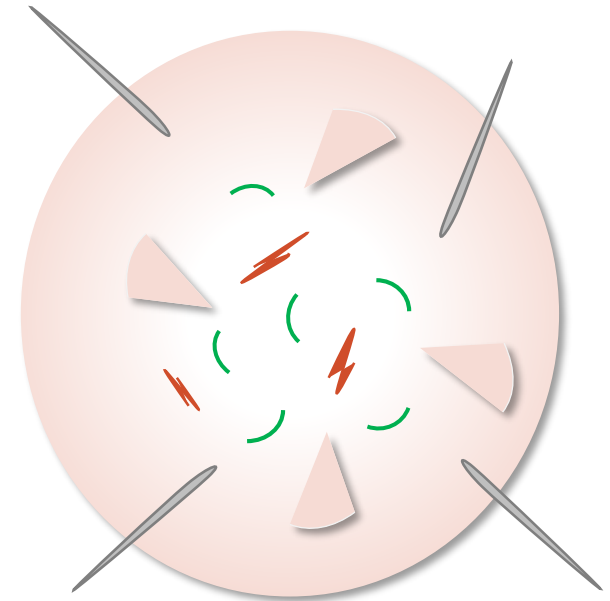
Highly fragmented



Cell lysis  
Post mortem



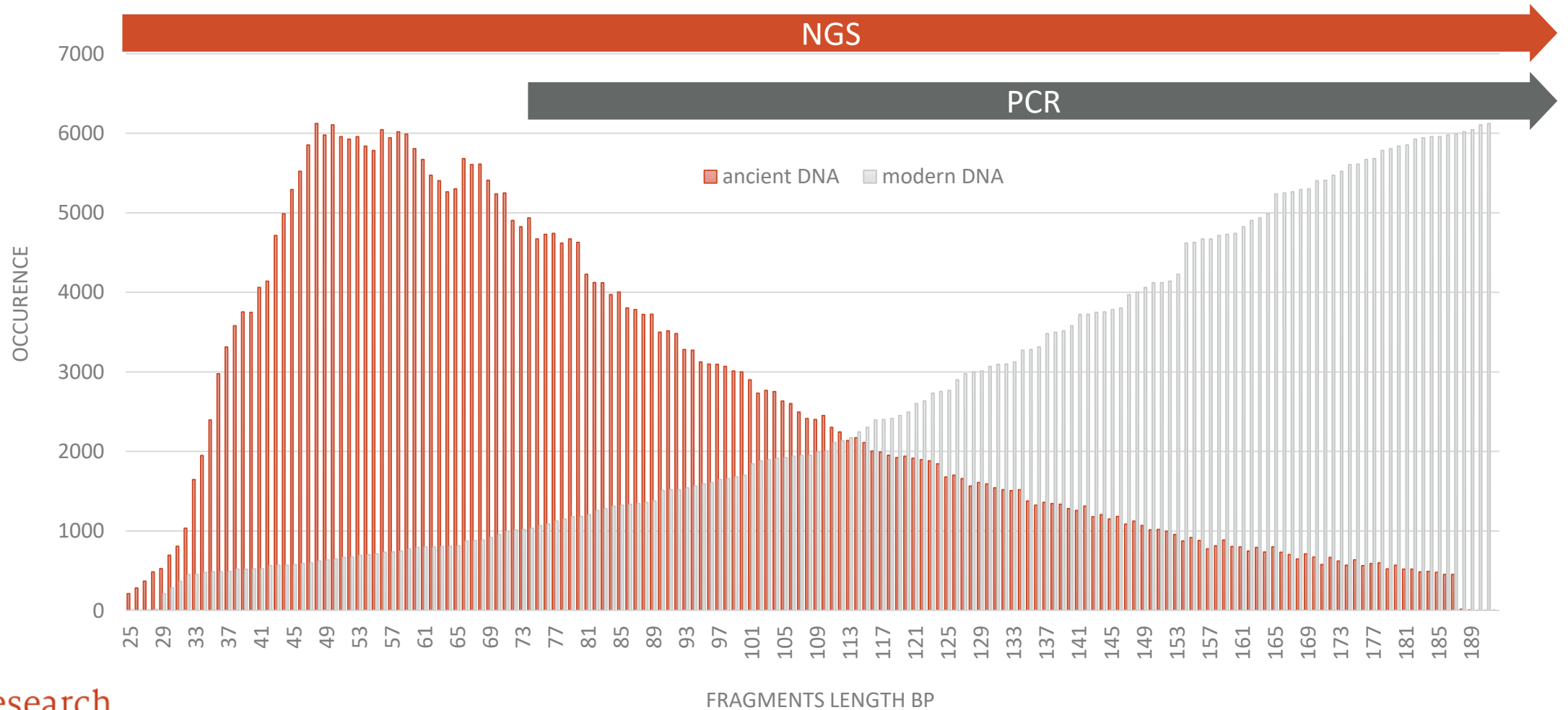
Microorganisms  
Temperature  
Humidity  
pH



Highly fragmented biomolecules  
Still detectable after 700.000  
years (Orlando 2013)

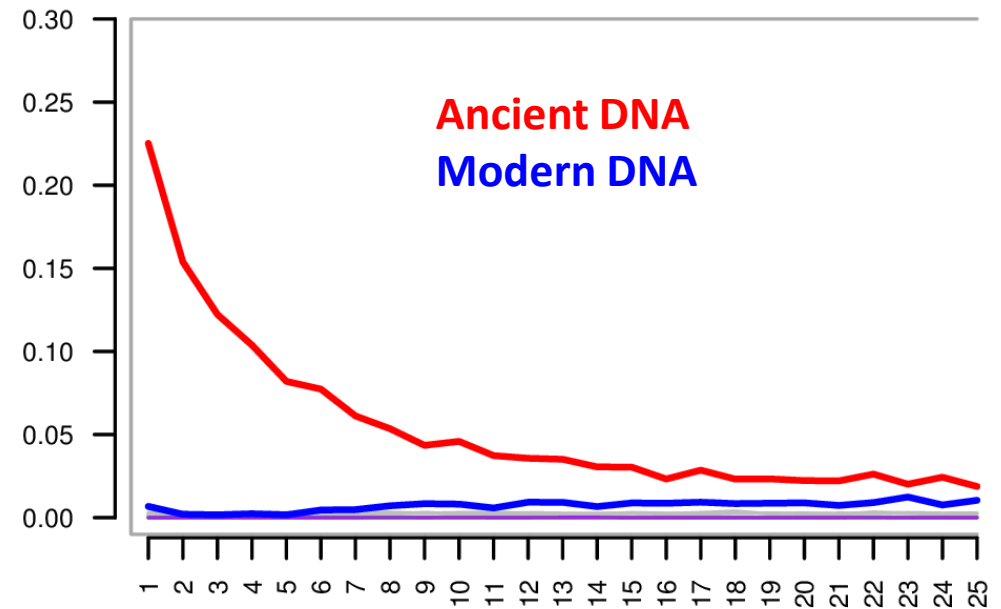
# ANCIENT DNA

Highly fragmented



# ANCIENT DNA

## Damage patterns





# THE VÁC MUMMY COLLECTION



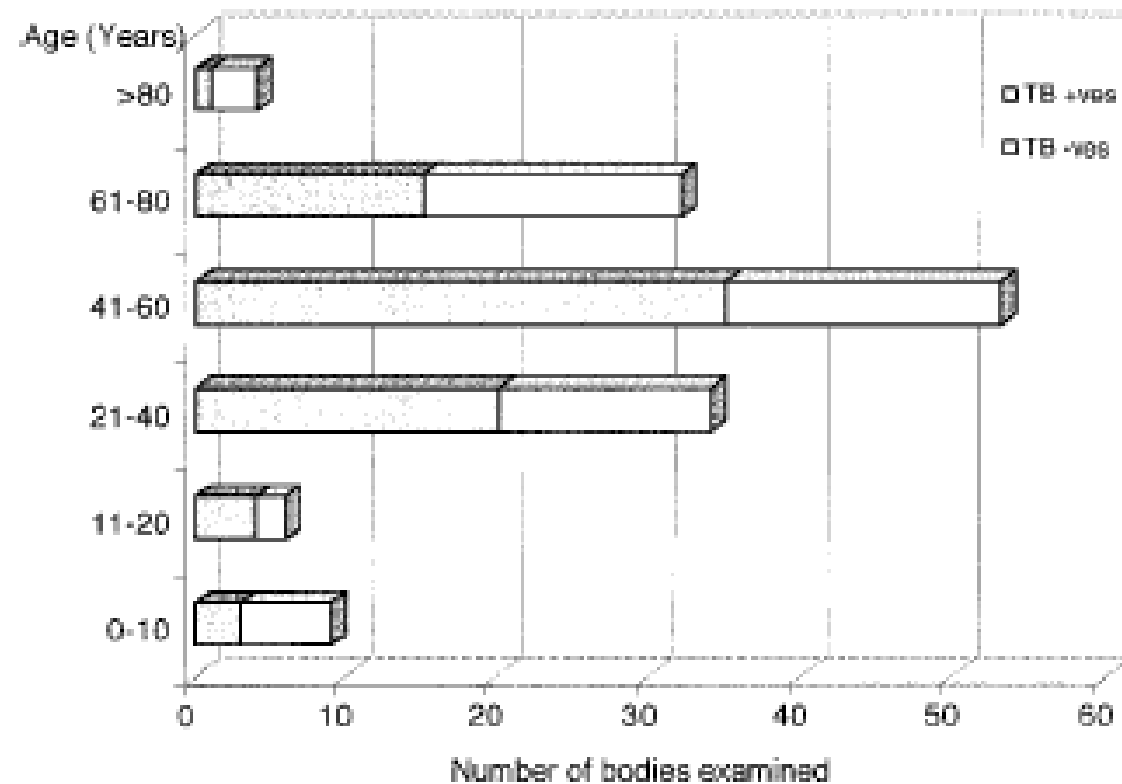
**Interdisciplinary Mummy Project I-II**  
(1998-2002, 2003-2008)  
Dept of Anthropology, HNHM Budapest  
UCL London, UK, Semmelweis University

# Widespread Occurrence of *Mycobacterium tuberculosis* DNA From 18th–19th Century Hungarians

Helen A. Fletcher,<sup>1</sup> Helen D. Donoghue,<sup>1\*</sup> John Holton,<sup>1</sup> Ildikó Pap,<sup>2</sup> and Mark Spigelman<sup>1</sup>

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**Fig. 2.** Distribution of individuals according to age at death and MTB positivity.



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## CORRESPONDENCE

### Metagenomic Analysis of Tuberculosis in a Mummy

N Engl J Med 2013; 369:289-290 | July 18, 2013 | DOI: 10.1056/NEJMc1302295

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**Chan J. Z., Sergeant M. J., Lee O. Y., Minnikin D. E., Besra G. S., Pap I., Spigelman M., Donoghue H. D. and Pallen M. J. (2013)**

## Eighteenth-century genomes show that mixed infections were common at time of peak tuberculosis in Europe

**Gemma L. Kay, Martin J. Sergeant, Zheming Zhou, Jacqueline Z.-M. Chan, Andrew Millard, Joshua Quick, Ildikó Szikossy, Ildikó Pap, Mark Spigelman, Nicholas J. Loman, Mark Achtman, Helen D. Donoghue & Mark J. Pallen**

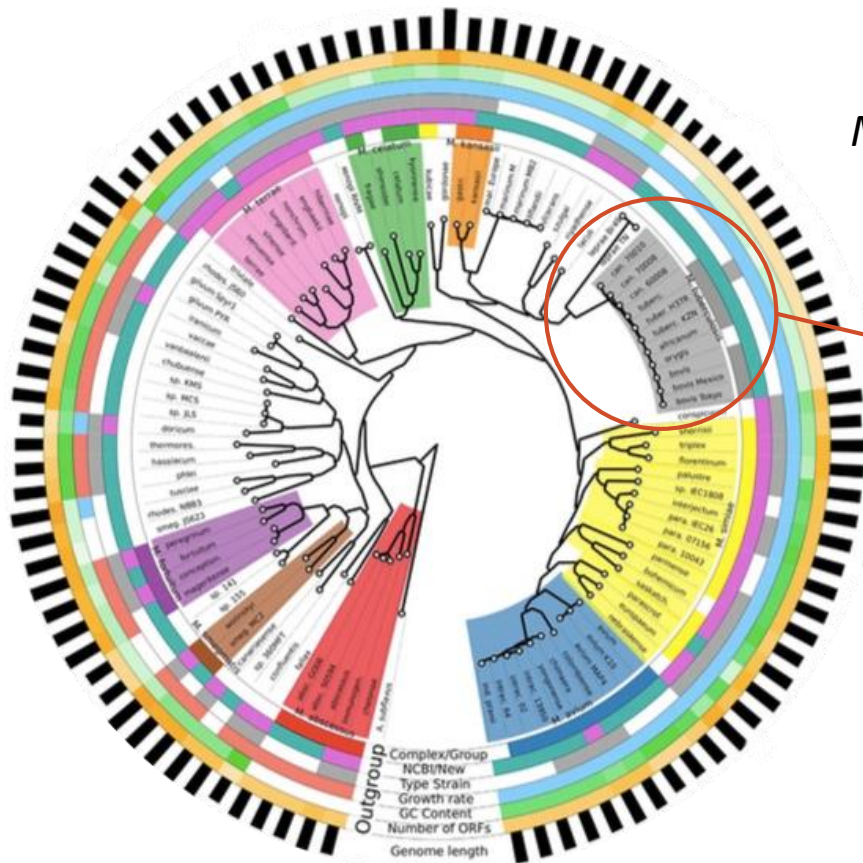
**[Affiliations](#) | [Contributions](#) | [Corresponding author](#)**

*Nature Communications* **6**, Article number: 6717 | doi:10.1038/ncomms7717

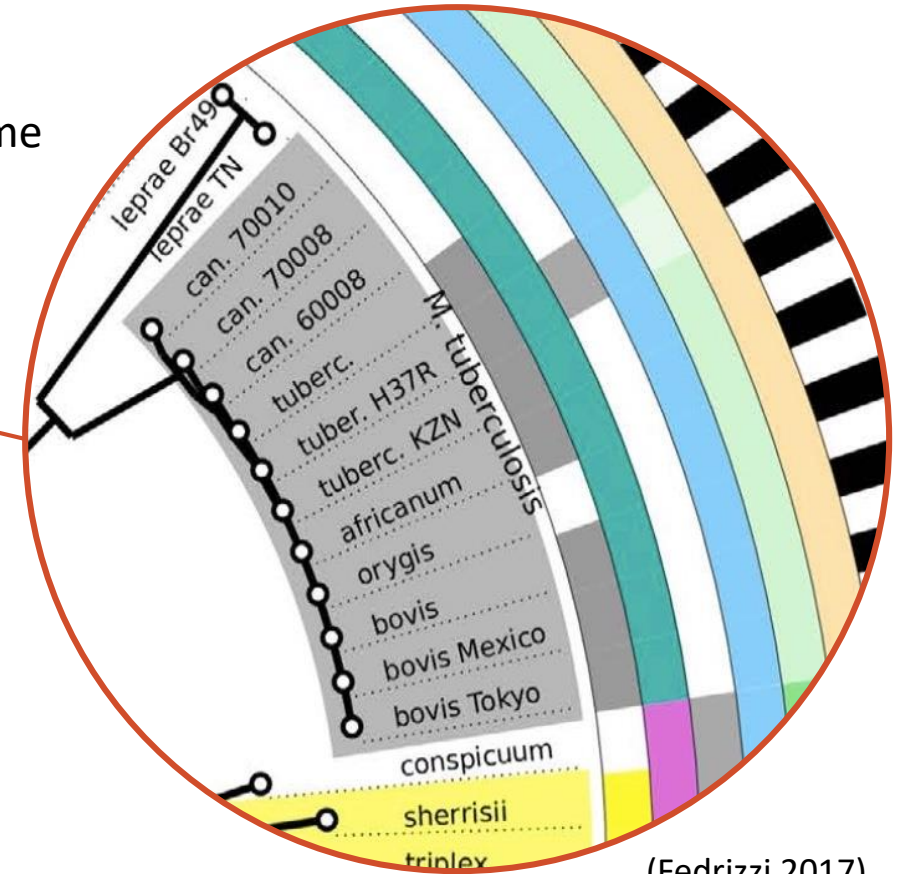
Received 24 October 2014 | Accepted 18 February 2015 | Published 07 April 2015



# M. TUBERCULOSIS COMPLEX (MTBC)



*M. tuberculosis* genome size  $\approx 4.4$  Mbp



(Fedrizzi 2017)

Outgroup

Non-tuberculous Mycobacteria

Ingroup

MTBC + *M. leprae*

# MTBC DNA CAPTURE DESIGN

= ,Ingroup' minus ,Outgroup'

- **Ingroup:** Currently available full genomes of the MTBC and *M. leprae* bacteria (Bos 2014)
- **Outgroup:** All other non-tuberculous *Mycobacteria* (NTMs) (Fedrizzi 2017)

**41.46 %**

39005 (58.54%) aligned 0 times  
26387 (39.60%) aligned exactly 1 times  
1237 (1.86%) aligned >1 times

**MTBC capture  
baits**

**47.32 %**

35103 (52.68%) aligned 0 times  
29271 (43.93%) aligned exactly 1 time  
2255 (3.38%) aligned >1 times

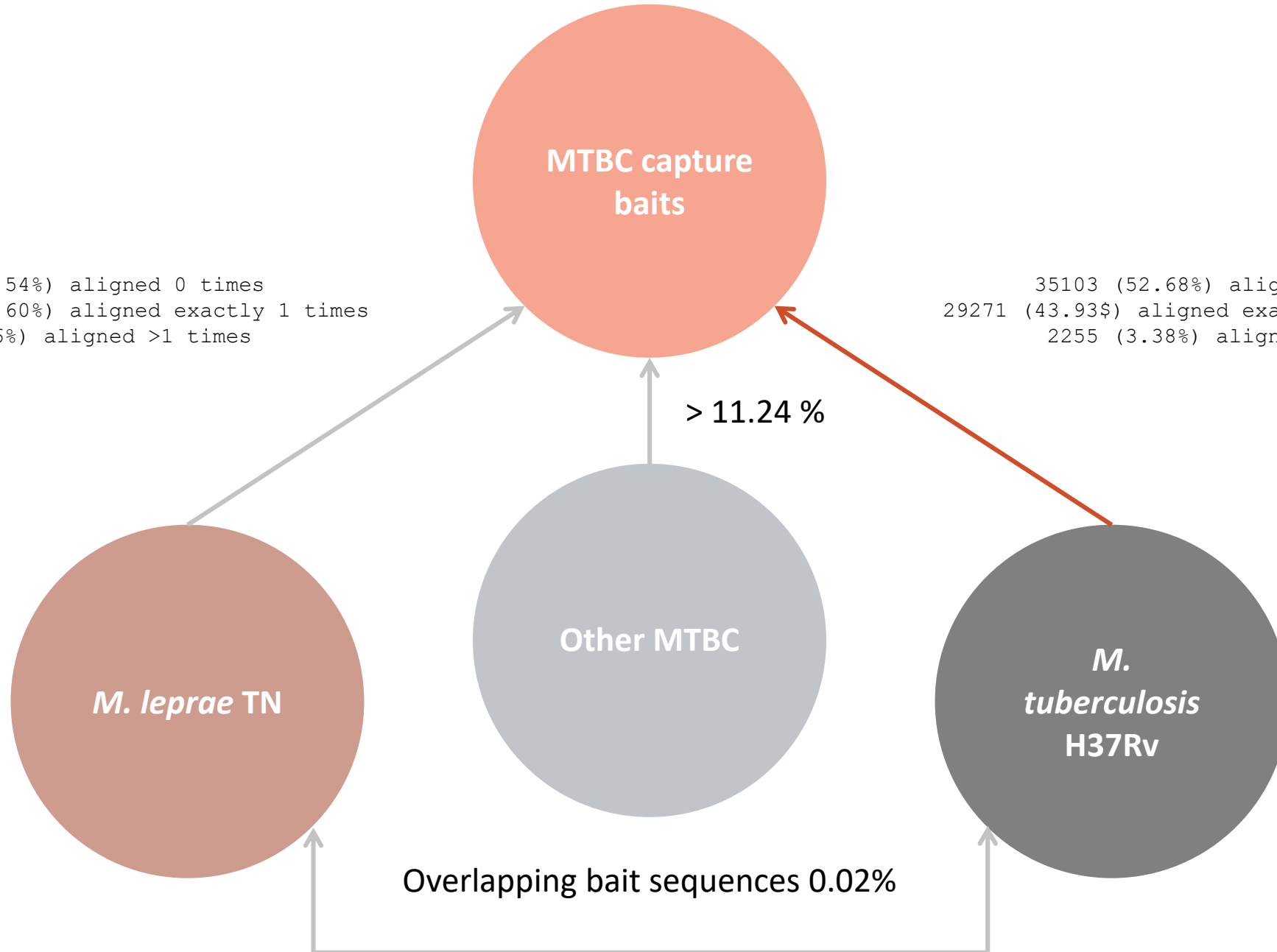
> 11.24 %

**Other MTBC**

***M. leprae* TN**

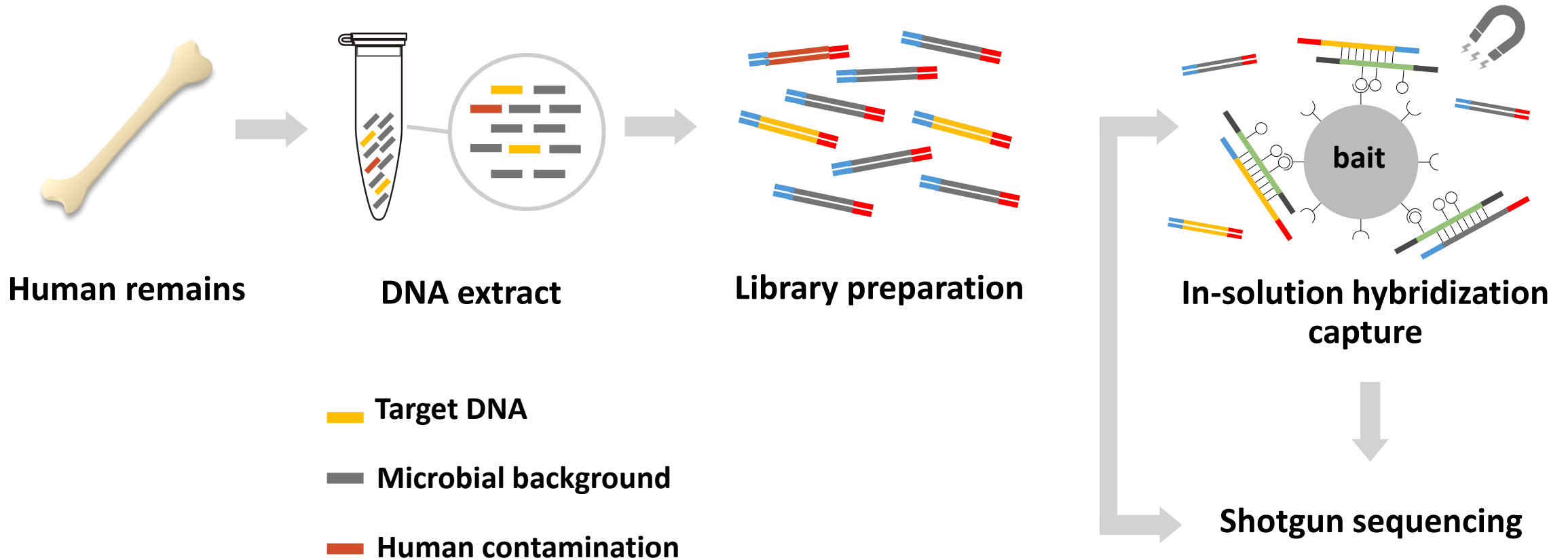
***M.  
tuberculosis*  
H37Rv**

Overlapping bait sequences 0.02%



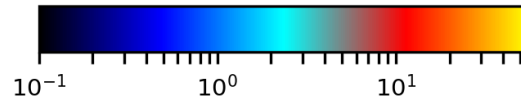
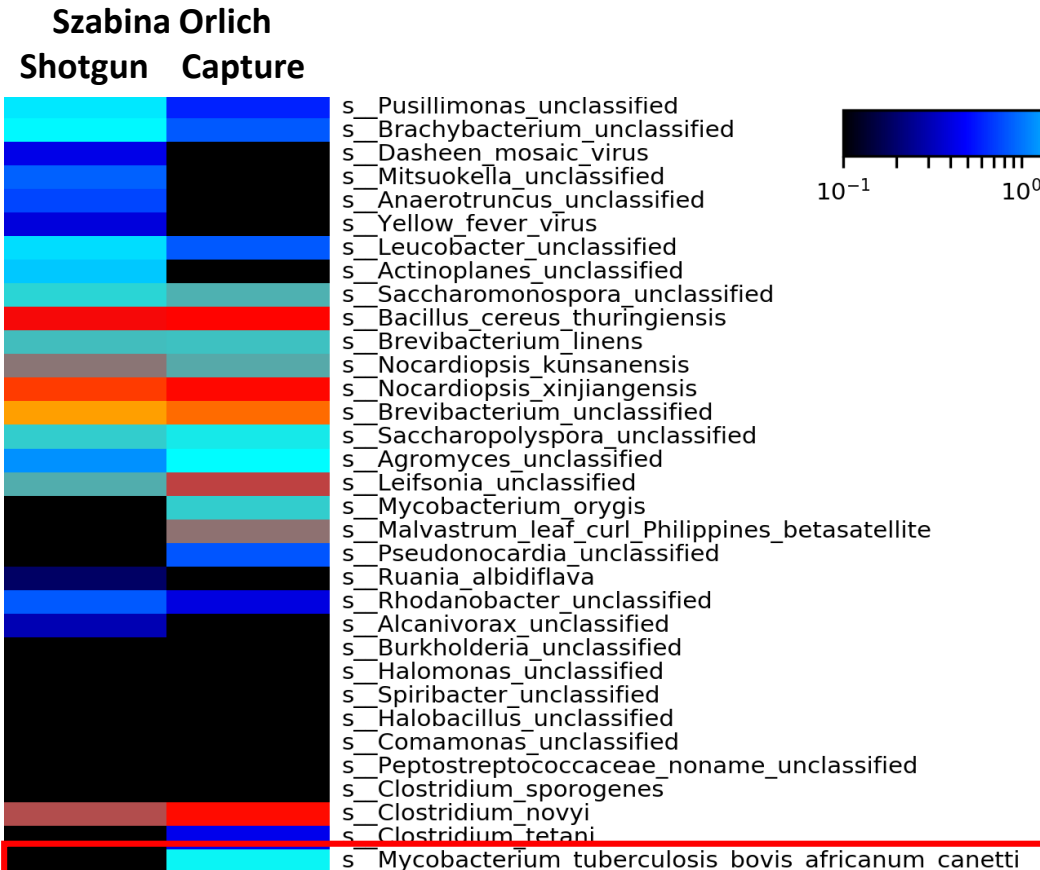
# HYBRIDIZATION CAPTURE: WORKFLOW

Pulling out the 1%



# HYBRIDIZATION CAPTURE: EVALUATION

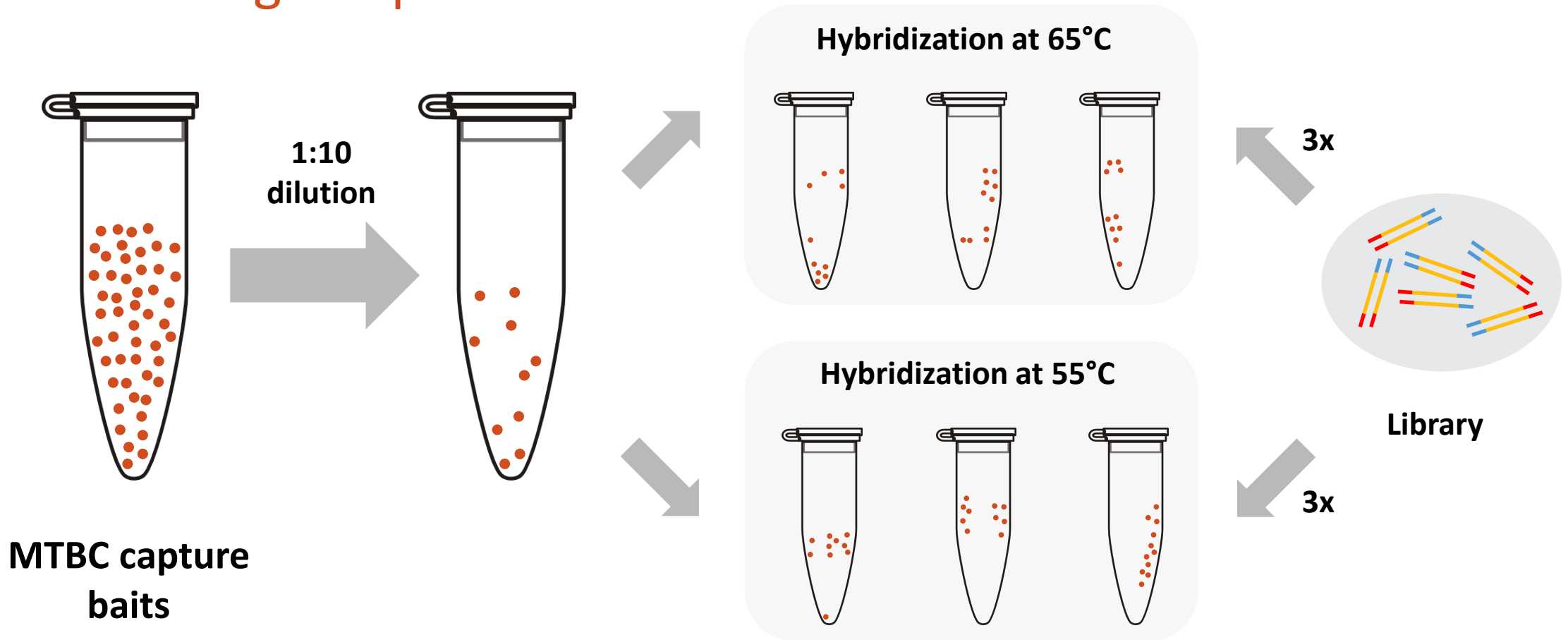
via MetaPhlAn, gene mapping, ...



	rpoB	katG	mpt40	gyrB	gyrA	IS6110	IS1081
Shotgun	86	2	0	2	2	0	0
Capture	66	5	0	5	5	21	8

# HYBRIDIZATION CAPTURE: OPTIMIZATION

Tweaking the protocol



# OUTLOOK

- Genome reconstruction
- Genotyping
- Evolutionary phylogenetic analyses
- Possible co-infections, epidemiological studies, strain diversity within individuals, ...
- Development of a cost-efficient tool

# eurac research

