# MIRU-VNTR genotyping of *M. bovis* and *M. caprae* from Swiss bovine samples using QIAxcel®

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## Introduction

Bovine tuberculosis (TB) is an infectious disease caused by *Mycobacterium bovis, M. caprae*, and rarely by *M. tuberculosis*. It affects cattle, but also other domestic and wild animals. It can also infect humans; notably farmers and slaughterhouse workers.

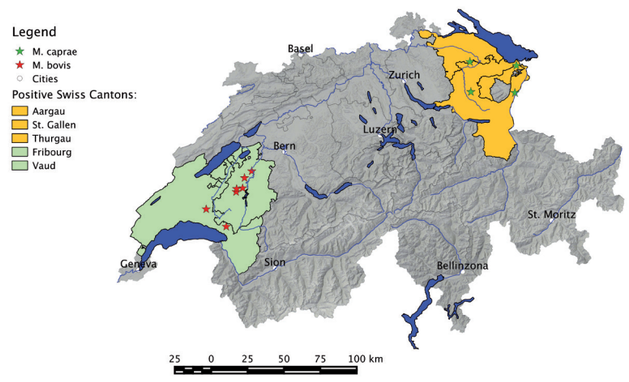
Genotyping based on mycobacterial interspersed repetitive unit – variable number of tandem repeat (MIRU-VNTR) has been established as the most reliable method for human TB strain genotyping.

**Project goals:**

- Test applicability of the MIRU-VNTR genotyping on bovine strains using automated capillary electrophoresis.
- Develop an appropriate VNTR loci panel for Swiss bovine samples, in addition to the conventional 24 MIRU-VNTR target panel.
- Epidemiological and etiological elucidation of the Swiss tuberculosis outbreak of 2013.
- Understand the transmission and spread of *M. bovis* and *M. caprae* in Switzerland.

## Bovine tuberculosis situation in Switzerland

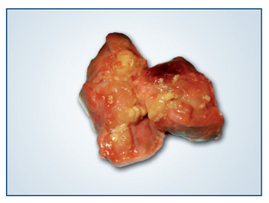
- Bovine tuberculosis was eradicated from Switzerland in 1960.
- Until 1980, there was a nationwide monitoring program using the periodic intradermal tuberculin test of the entire cattle population; thereafter, monitoring of bovine tuberculosis at slaughterhouses by standard post-mortem examination.
- Sporadic cases were registered in 1985 and 2000.
- Disease surveillance consisted of slaughterhouse monitoring only.
- In 2013, two outbreaks of bovine tuberculosis were detected: one caused by *M. bovis* and the other by *M. caprae*.



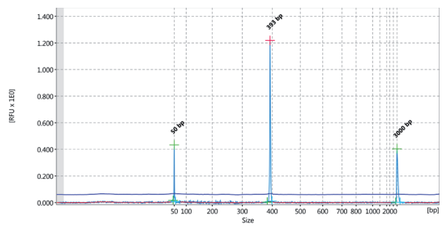

**Localization of farms with infected animals.** During the Swiss outbreak of bovine tuberculosis in 2013, suspicious samples from slaughterhouses were collected by the Institute of Veterinary Bacteriology (IVB), University of Zurich.

## Materials and methods

A small section of a suspicious lymph node was cultivated in a biosafety level 3 laboratory, using Stonebrink culture media. Genomic DNA was purified from lymph nodes using the QIAamp® *cador*® Pathogen Mini Kit and amplified by PCR using the 24 MIRU-VNTR target panel.[1,2] Automated native capillary electrophoresis was performed using the QIAxcel Advanced System in combination with the QIAxcel DNA High Resolution Kit, utilizing the OM1700 method. A QX Alignment Marker (50 bp/3 kb) and a QX DNA Size Marker (100 bp – 2.5 kb) were run with the samples.



Suspicious lymph nodes from slaughterhouse.



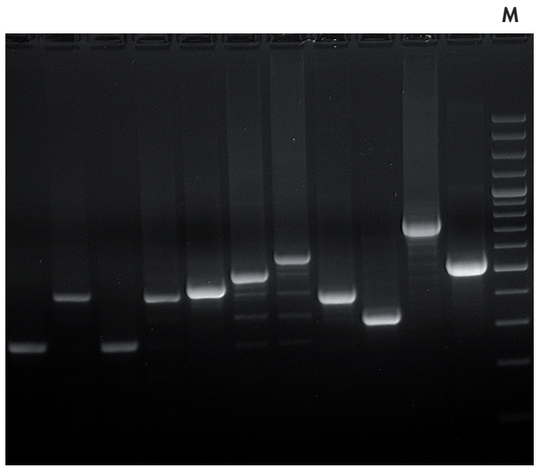

Electropherogram visualization using QIAxcel ScreenGel Software.

Analysis of PCR products using the QIAxcel Advanced System.

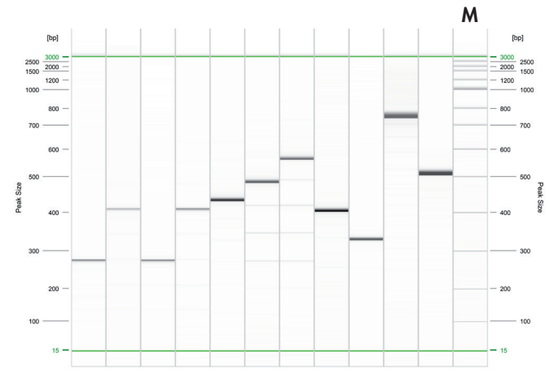
**Positive BBL™ Stonebrink culture (BBL Stonebrink TB Medium + PACT.** Becton, Dickinson and Company, USA). Institute of Veterinary Bacteriology (IVB), University of Zurich.

## Conventional agarose gel versus capillary electrophoresis

The QIAxcel Advanced System, which is based on automated native capillary electrophoresis, enables faster and more accurate DNA fragment size estimation, compared with conventional agarose gel electrophoresis




**Conventional agarose gel.** Five core loci with 2 different mycobacterial strains isolated in Switzerland were analyzed. The PCR products were visualized on a 2% agarose gel. **M**: 100 bp DNA size marker.




**Native capillary electrophoresis.** The same PCR products were analyzed using the QIAxcel Advanced System. **M**: QX DNA Size Marker 100 bp – 2.5 kb.

## Genotyping of *Mycobacterium bovis*

In order to do preliminary screening tests, we analyzed 16 different *M. bovis* and 7 *M. caprae* positive samples. As reference strains, *M. tuberculosis* strain H37Rv, BCG Pasteur, DSM43990 *M. bovis* and *M. bovis* isolated at the Institute for Medical Microbiology (IMM) in Zürich were used.

**Allele calling using the 24 standard MIRU-VNTR loci**

| | MIRU 02 | VNTR | ETR C | MIRU 04 | MIRU 40 | MIRU 10 | MIRU 16 | VNTR | MIRU 20 | QUB 2163b | ETR A | VNTR | VNTR | ETR B | MIRU 23 | MIRU 24 | MIRU 26 | MIRU 27 | VNTR | MIRU 31 | VNTR | QUB26 | VNTR | MIRU 39 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **154** | **424** | **577** | **580** | **802** | **960** | **1644** | **1955** | **2059** | **2163b** | **2165** | **2347** | **2401** | **2461** | **2531** | **2687** | **2996** | **3007** | **3171** | **3192** | **3690** | **4052** | **4156** | **4348** |
| H37Rv | 2 | 2 | 4 | 3' | 1 | 3 | 2 | 2 | 2 | 5 | 3 | 4 | 2 | 3 | 6 | 1 | 3 | 3 | 3 | 3 | 5 | 5 | 2 | 2 |
| *M. bovis* BCG from IMM | 2 | 0 | 5 | 2' | 2 | 2 | 3 | 1 | 2 | 3 | 5 | 2 | 2 | 5 | 4 | 2 | 5 | 3 | 3 | 3 | 2 | 5 | 0 | 2 |
| Swiss field strain *M. bovis* | 2 | 0 | 5 | 3 | 2 | 2 | 3 | 1 | 3 | 3 | 4 | 3 | 4 | 7 | 4 | 2 | 5 | 1 | 3 | 3 | 2 | 5 | 1 | 2 |
| Swiss field strain *M. caprae* | 2 | 4 | 5 | 2 | 2 | 6 | 4 | 2 | 2 | 5 | 5 | 3 | 4 | 3 | 4 | 2 | 4 | 3 | 2 | 3 | 1 | 3 | 3 | 2 |
| Reference DSM43990 | 2 | 0 | 5 | 1' | 3 | 2 | 3 | 1 | 2 | 3 | 5 | 2 | 2 | 5 | 4 | 2 | 5 | 3 | 3 | 3 | 2 | 5 | 0 | 2 |
| BCG Pasteur | 2 | 0 | 6 | 2' | 2 | 2 | 3 | 1 | 2 | 3 | 5 | 2 | 2 | 5 | 4 | 2 | 5 | 3 | 3 | 3 | 2 | 5 | 0 | 2 |

BCG: Bacillus Calmette–Guérin; ETR: Exact tandem repeats; IMM: Institute of Medical Microbiology (University of Zurich); MIRU: Mycobacterial interspersed repetitive units; QUB: Queen's University Belfast; VNTR: Variable number of tandem repeats.

As illustrated, the peak calling table from QIAxcel ScreenGel Software allows a rapid and automated export of the MIRU-VNTR alleles in an Excel® data file.

## Conclusion

- The QIAxcel Advanced System enables high resolution, high-throughput analysis for *M. bovis* and *M. caprae* genotyping.
- Identification of alleles at 24 loci was much more accurate compared to conventional agarose gel electrophoresis.
- Automatic allele identification utilized the peak calling function of the QIAxcel ScreenGel software.
- Straightforward and time-saving method.

**Outlook:**

- Our goal is to design a robust and more accurate VNTR loci panel, in combination with well-established and routinely used 24 MIRU-VNTR target panel, to better differentiate Swiss *M. bovis* and *M. caprae* field strains.
- The QIAxcel system provides a suitable tool for further development and testing of additional MIRU-VNTR loci.

References

1. Supply, P., et al. (2006) Proposal for Standardization of Optimized MIRU-VNTR Typing of Mycobacterium tuberculosis. J. Clin. Microbiol. **44**, 4498.
2. www.miru-vntrplus.org/MIRU/files/MIRU-VNTRtypingmanualv6.pdf [Accessed 15 May 2014].



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