

The differentiation of *Mycobacterium avium* subs. *avium* and *M. a. paratuberculosis* using RFLP analysis

Restriction fragment length polymorphisms (RFLPs) were the first type of molecular markers used in linkage studies. The RFLP method is based on differences in specific DNA sites occurring in related organisms, which are recognised by specific restriction enzymes. Differences in the sizes of restriction fragments between individuals can be detected by Southern blotting with a probe specific for insertion sequences that are present in the genome in multiple copies. A RFLP method for typing *M. a. paratuberculosis* (*MAP*) and *M. a. avium* (*MAA*) has been standardised at VRI (Pavlik et al., 1999; Dvorska et al., 2003).

OBJECT OF THE COURSE

The differentiation of *MAP* and *MAA* using chromosomal DNA restriction fragment length polymorphism (RFLP) analysis is the object of the course. The main goal will lie in providing to the participants theoretical knowledge and, in particular, practical skills in the RFLP analysis of mycobacteria.

The course is primarily intended for researchers involved in microbiology, molecular biology and in the medical diagnostics of mycobacteria, but all participants who are interested in learning the method are welcome. The participants should have a basic knowledge of microbiology and molecular biology and should be acquainted with recommended literature related to the course.

COURSE CONTENT

1. DNA isolation using the CTAB method - 2 days
2. The control of isolated DNA (gel electrophoresis) and digestion of DNA using restriction enzymes - 1 day
3. The control of digestion (gel electrophoresis); the separation of digested fragments on a gel - 1 day
4. Blotting of fragments from the gel to a membrane; fixation of fragments on a membrane using UV light; control of blotting -1 day
5. Preparation of a probe (PCR method) and buffers; hybridization of membrane with the probe; overnight incubation -1 day
6. Washing of the membrane and the development of the film - 1 day

During the course the methods will be practically demonstrated and explained by a supervisor accompanied by a participant. The minimum time of duration of the course is 7 working days. If the applicant wishes to carry out the method independently by him-(her)-self or to analyse their own samples the course could be prolonged for another 7 days (the total price of the course will then be adjusted according to the number of samples and days of visit).

All successful participants will receive a certificate of attendance.

GENERAL INFORMATION

Code: 06-RFLP-1
Course Leader: Mgr. Monika Moravkova
Duration: 7 working days
Registration fee: € 950 (materials for course, lunches)
Other stay expenses: about € 55 per day (accommodation + others)
Language: English
Location: Brno, Czech Republic

For more information please contact:

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