



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

PRACTICAL LESSONS IN MOLECULAR BIOLOGY: SEX DETERMINATION IN BIRDS

Eva Bártová, Lenka Dubská, Ivan Literák

Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology,
University of Veterinary and Pharmaceutical Sciences Brno

ABSTRACT:

During winter semester 2009-2010, new practicals focused on the method of molecular biology were introduced into the syllabus of Biology and Genetics. During practicals, students worked on a complex task of bird sex identification from biologic material. The principle of sex identification in birds is based on restriction analysis of PCR product of the gene CHD (Chromo-Helicase-DNA binding gene). At the beginning of the semester, the students obtained information about an innovation of the practicals and the possibility to examine blood sampled from their parrot or other birds. Otherwise, students were provided with tissues from pheasant male or female. The practicals run parallel in two rooms, both of them equipped with required material and machines. The pipettes, DNA isolation sets, reagents for PCR and restriction reaction and other material were shared by group of 3-4 students, however every student tested one sample. During three lessons, students prepared bird tissues or blood lysates, isolated DNA, prepared PCR and subsequently restriction reaction of PCR product, followed by gel electrophoresis and evaluation of the result in UV light. Students appreciated the innovated practicals, especially the possibility to acquire new experience with molecular biology methods that contributed to their understanding of the principles of described methods together with their application potential.

These practicals were financed by the European Social Fund and the State Budget of the Czech Republic by project "Innovation in Animal Protection and Welfare Education", number CZ.107/2.2.00/07.0165.

Key words: innovation of practical, DNA isolation, PCR, electrophoresis, restriction reaction, molecular biology

During winter semester 2009 – 2010, the methods of molecular biology were introduced into the practicals of Biology and Genetics in master study programme of both veterinary faculties (Faculty of Veterinary Medicine, Faculty of Veterinary Hygiene and Ecology). This innovation was financed by the European Social Fund and the State Budget of the Czech Republic with the project "Innovation in Animal Protection and Welfare Education".

Students used the methods of molecular biology to solve one complex task of bird sex identification from biologic material. The principle of bird sex identification is based on restriction analysis of PCR product of gene CHD (Chrom-Helicase-DNA binding gene).

The practicals run parallel in two rooms, both of them equipped with required material and machines. Students in each room formed groups of 3-4 students that shared set of automatic pipettes, DNA isolation sets, reagents for PCR and restriction reaction and other material. Students were provided with bird tissue from male or female pheasant to determine sex of examined bird individual. There was the possibility to determine the sex of parrot or other birds bred by students. In this case, they received tube with anti-coagulant EDTA for the bird blood sampling.

The sex determination of bird run in several steps. In the first lesson, students let the bird tissue lyse by enzymes. In the second lesson, they isolated DNA from lysate using column-based DNA isolation set, prepared mixture for PCR with specific primers and started the DNA amplification in the thermocycler. In the third lesson, they incubated the PCR product with restriction endonuclease *HaeIII*. While waiting for the result, students prepared agarose gel and electrophoresis device. Subsequently, they loaded processed PCR products, male and female



Fig. 1: Gel preparation for gel electrophoresis



Fig. 2: Sample loading into gel

control samples and DNA molecular weight standard into wells in gel. After electrophoresis, they placed the gel into UV light to obtain the result. Students were excited to see the result of their work represented by bands in the gel. They identified sex of bird by comparing their sample with male and female control samples. One band indicated male sex, or two bands indicated female. Students were disappointed by incorrect results, such as no PCR product at all or one band however representing uncleaved PCR product. Teachers explained critical steps in the process such as DNA isolation, proper preparation of PCR mixture and sample loading.

The innovation of the practical lessons was based on the use of fundamental methods of molecular biology to solve one complex task. Students used these methods by themselves and finally interpreted the result. All this helped them to understand the principles of methods and for what purpose they are used in praxis. Moreover, the practical experience with molecular methods contributes to students' understanding of widely used application procedures.

Address:

Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 61242 Brno

Contacts:

MVDr. Eva Bártová, Ph.D., e-mail: bartovae@vfu.cz
RNDr. Lenka Dubska, Ph.D., e-mail.: dubska@mou.cz
Prof. MVDr. Ivan Literák, CSc., e-mail: literaki@vfu.cz



Fig. 3: Visualization of restricted PCR product using UV light

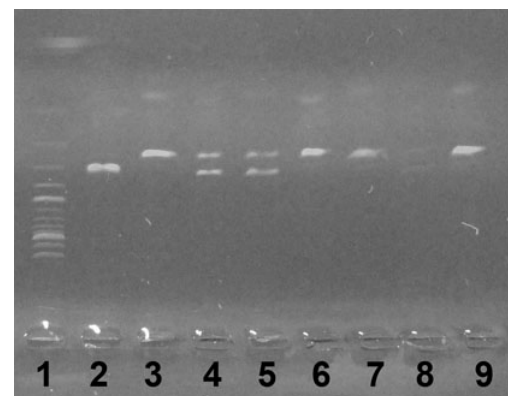


Fig. 4: Gel after gel electrophoresis (1-DNA molecular weight, standard, 2-non-restricted PCR product, 3,6,7,9-male, 4,5,8-female)