

Jiri Kalina: SABRE Placement Report

Copy number variation in Avidin, an egg antimicrobial gene

This SABRE placement in Roslin Institute was divided into two parts. The first was between May 14th and June 19th and the second between July 15th and September 19th. The reason for this was my participation on World Poultry Congress in Brisbane which was held at the beginning of July.

The objective of the visit was to study copy number variations (CNV) of the Avidin gene and Avidin related genes. Copy number variation is a form of structural variation in the genome and refers to differences in the number of copies of a particular region in the genome. Like other types of genetic variation, some gene copy number variants have been associated with susceptibility or resistance to disease. Avidin is homotetrameric protein produced in oviducts of birds and is deposited in egg white. Avidin makes up 0.05% of total protein fraction (approximately 1.8 mg per egg) and can bind to biotin (Vitamin H) with a high degree of affinity and specificity. The dissociation constant of avidin is $K_D \approx 10^{-15}$ M which is one of the strongest known non-covalent bonds. To the date there are seven described Avidin-related genes (Avr1-7) with known genomic sequence. Due to their high sequence similarity it is difficult to distinguish between each Avr and also their function and expression profile is still not well described.

RNA from selected tissues (magnum, isthmus, shell gland, cloaca, and small intestine) has been isolated using modified guanidinium thiocyanate-phenol-chloroform extraction and reversely transcribed into single strain cDNA. DNA samples were isolated from blood of the animals. Using the similarities between Avidin and Avrs, we have designed universal as well as Avidin specific oligonucleotide primers for Real-time PCR analysis and for simple PCR amplification from both genomic DNA and cDNA and optimized PCR reaction for maximal yield without non-specific amplification. Real-time PCR program has been optimized to amplify the product with required efficiency similar to the calibrator control gene of known copy number (in our case the Growth hormone receptor gene) so that we can use the difference in Ct values between the control and unknown samples to distinguish relative quantification of genes. Obtained amplicons from simple PCR of the length of 620bp containing Avidin and also Avrs genomic sequences were isolated from agarose gel and purified. Using comparative analysis of the sequences we have selected unique restriction site presented in Avrs but missing in Avidin. After restriction with specific endonuclease we have obtained restriction pattern that showed the ratio between Avidin gene and all Avrs (Fig.1). The ratio was measured using the Scion Image, a public domain, Java-based image processing program developed at the National Institutes of Health. Three different hen breeds were screened, broiler, layer and silky. We discovered significant differences between broilers and two other breeds indicating potential CNV for Avidin and/or Avrs. Obtained results may contribute to further research projects studying its biological significance, e.g. in antimicrobial activity.

Copy number variation in egg white protein genes; Measuring avidin related gene copy number

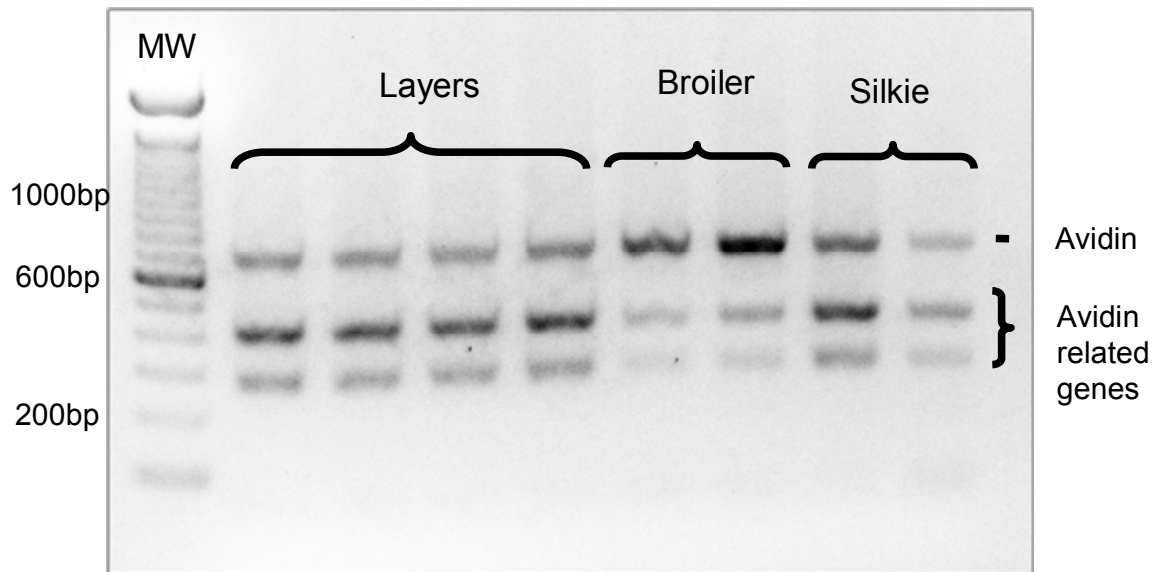


Fig.1 Restriction of amplified Avidin and AVR genes among 3 different chicken breeds (Lines: 1- ladder, 2-5 - layers, 6-7 - broilers, 8-9 - silkies)