GENETIC RESISTANCE
– WITH FOCUS ON MAJOR HISTOCOMPATIBILITY COMPLEX

Helle R. Juul-Madsen¹
Tina S. Dalgaard¹
Jan Salomonsen²
Dan E. Hel³

¹Danish Institute of Agricultural Sciences, DK-8830 Tjele, Denmark
²Royal Veterinary and Agricultural University, DK-1870 Frederiksberg, Denmark
³The Hebrew University of Jerusalem, Zip 76100, City Rehovot, Israel

TABLE OF CONTENTS

INTRODUCTION ................................................................. 2
Serological typing of MHC
  Introduction........................................................................ 3
  Equipment ................................................................. 3
  Reagents .................................................................. 3
  Preparation of Normal Chicken Plasma............................... 3
  Preparation of B-F and B-G allo-antisera........................... 3
  Preparation of “ready-to-use” allo-antiserum....................... 4
  Haemagglutination test.................................................... 5
  Serological MHC typing of offspring................................. 5
Restriction Fragment Length Polymorphism (RFLP) typing of MHC
  Introduction........................................................................ 5
  Equipment ................................................................. 6
  Reagents .................................................................. 6
  Purification of genomic DNA with Sodium Chloride............ 6
  Genomic DNA restriction digestion................................... 7
  Alkali blotting............................................................. 7
Micro-satellite fragment typing of MHC
  Introduction........................................................................ 8
  Equipment ................................................................. 8
  Reagents .................................................................. 8
  PCR amplification of micro-satellite................................. 8
  Fragment analyses ......................................................... 9
Suppliers ............................................................................ 9
Concluding remarks........................................................ 9
Acknowledgements.......................................................... 9
References........................................................................ 9
Introduction

Avian infectious diseases are a major problem in intensive poultry production systems. Avian infectious diseases are also a matter of concern to the human population as chickens may be carriers of human bacterial pathogens such as *Salmonella* or *Escherichia coli*. Furthermore, viral pathogens may evolve in chickens, cross species barriers, and infect humans. The outbreaks of avian influenza in Hong Kong in 1997 and 2003 are good examples (Kuhnlein et al., 2003).

The major tools to combat avian diseases have so far been antibiotics and vaccines. However, there are major concerns that both antibiotics and vaccines may contribute to the evolution of more resistant pathogens, which require the development of new and more efficient vaccines. This has been so for Infectious Bursal Disease Virus (IBDV) for several years. Increased response to vaccines in combination with better hygiene may therefore be a favourable production principle of choice.

The immune system is the natural means by which animals resist infection, and immunological parameters may reflect the functional capacity or immuno-competence of the immune system and, in turn, the ability of the animal to resist infection. So far only a few genes have been implicated in affecting disease resistance. Genes from the Major Histocompatibility Complex (MHC) involved in antigen presentation were among the earliest genes to be identified. These genes encode some of the most polymorphic molecules known in the animal kingdom. Diversity at the population level is thought to evolve via the interaction between host MHC molecules and pathogens leading to a balanced evolution of both species.

In chickens, the MHC genes are located in two chromosomal regions (the B system and the Rfp-Y system) that assort in a genetically independent way, although located on the same micro-chromosome (Briles et al., 1993, Miller et al., 1994; Fillon et al., 1996). The B system is considered the strongest histocompatibility antigen delivering locus and contains at least two B-F genes, which are homologous to mammalian MHC class I genes, and at least two B-LB genes which are homologous to mammalian MHC class IIB genes. In addition, the B system contains genes encoding a third group of molecules, namely the B-G genes. The B-G molecules have so far only been found in birds, and are just as polymorphic as the B-F and the B-L molecules. However, their function is still unknown (Guillemot et al., 1989; Kaufman et al., 1989; Miller et al., 1990). B-F antigens are expressed on almost all chicken cells including nucleated chicken erythrocytes whereas the B-L antigens primarily are expressed on B-cells, activated T-cells, and antigen presenting cells. The B-G molecules are primarily expressed on erythrocytes and thrombocytes although white blood cells also have B-G epitopes (Salomonsen et al., 1991). The Rfp-Y system is considered to be an MHC-like region rather that a second MHC since there is no report on rapid allograft rejection due to polymorphism in Rfp-Y (Kaufman and Wallny, 1996).

Genetic disease resistance is known to influence several diseases in poultry. In many cases, this resistance has been mapped to the MHC region. One of the most significant associations between MHC and disease resistance is Marek’s disease (Lamont, 1998), but resistance to other virus-induced diseases such as Rous sarcoma virus (Schierman and Collins, 1987) and avian leukosis (Yoo and Sheldon, 1992) have been linked to the MHC region as well. In addition to viral diseases, other diseases have been shown to be influenced by the MHC. These include fowl cholera (Lamont et al., 1987), coccidiosis (Lillehoj et al., 1989) and salmonella infections (Cotter et al., 1998).

In relation to IBDV, disease resistance between different chicken lines has been reported (Bumstead et al., 1993), however, no disease resistance has so far been associated with the B system. But recently Juul-Madsen et al. (2002) showed a difference in response to live attenuated IBDV vaccine that in part was related to the MHC.

Analysis of genetic variation in the chicken MHC can be approached by different methods. Originally MHC typing was performed with a haemagglutination technique based on allospecific polyclonal antisera. The serological agents, however, often cross-react between different MHC haplotypes and typing of outbred chicken populations can be difficult (Kroemer et al., 1990). DNA may easily be isolated from the nucleated chicken erythrocytes, and MHC genotyping by restriction fragment length analysis (RFLP) or by a PCR based test of a polymorphic micro-satellite located in the B complex (Zoorob et al., 1998) are useful supplements.
Serological typing of MHC

Introduction
Serological typing of MHC is performed by a haemagglutination test at room temperature in which the allo-antibody react (agglutinate) with the allo-antigen on the surface of erythrocytes. The haemagglutination is performed on open glass slides supplemented with 20% normal chicken plasma as a source of haemagglutination-enhancing factor (Simonsen 1975). Before a MHC haemaglutination test can be performed, specific B antisera must be produced by immunisation of chickens (recipients) with donor cells expressing different MHC antigens than the recipient. B-G allo-antiserum is produced by immunisation of chickens with Red Blood Cells (RBC) whereas B-F allo-antiserum is produced by immunisation of chickens with White Blood Cells (WBC) to avoid the simultaneous formation of anti-B-G specific antibodies.

Equipment

- Glass slides with 4x10 holes (Figure 1)
- A humid chamber
- A light table
- Table centrifuge e.g. Hereus Omnifuge 2.0
- Rotator
- Syringes (1, 2, and 20 mL)
- CPD (Citrate Phosphate Dextrose adenine) tubes (code VT-050SCPD17) (TE)
- Glass Pasteur pipettes
- 13 mL centrifuge tubes
- 4 mL polystyren test tubes
- 50 mL polypropylen tubes

Reagents

- 3.8 % Sodium citrate
- PBS buffer pH 7.4
- 10 % Na-azide
- 4 mL of Normal Chicken Plasma (NCP)
- Lymphoprep (Me)
- "Ready-to-use” allo-antisera (the antibody) reacting with different MHC haplotypes.

Preparation of Normal Chicken Plasma (NCP)

1. Centrifuge blood from a chicken stabilised in 1/5 vol. 3.8 % Sodium Citrate at 500xg for 8 min at 20°C.
2. Remove the upper phase and store the plasma in 4 mL aliquots at -20°C.

Preparation of B-F and B-G allo-antisera (antibodies)

1. Bleed a donor chicken for 8 mL of blood and stabilise the blood in 1/5 vol. 3.8% Natrium Citrate.
2. Purify the white blood cells (WBC) on a Lymphoprep gradient according to the manufacturer’s description.
3. Collect the WBC from the interphase and transfer the cells to a new 13 mL tube.
4. Pipette 100 μL of packed peripheral Red Blood Cells (RBC) from the bottom of the gradient and transfer the cells to a new 13 mL tube containing 5 mL PBS buffer.
5. Gently resuspend both kinds of cells in PBS buffer up to 10 mL and centrifuge the cells at 200xg for 10 min at 4°C.
6. Wash the cells gently 2 times in 5 mL cold PBS buffer and repeat the centrifugation.
7. For the RBC aspirate the supernatant down to 0.5 mL and add 4.5 mL cold PBS – mix gently.
8. For the WBC aspirate the supernatant down to 0.5 mL and add 0.5 mL cold PBS – mix gently.
9. Place the tubes on a rotator at room temperature until immunisation.
10. Immunise each recipient chicken in the wing vein with either 1 mL WBC or 1 mL RBC according to your immunisation plan.
11. For each kind of donor cells two recipients having different MHC haplotypes are chosen (two for WBC and two for RBC).
12. The immunisation is carried out once a week during 8 weeks, and bleeding for collection of allo-antibodies is initiated after the 3rd immunisation and then once a week.
13. Test-typing of the bled sera, diluted 1:2 in PBS buffer, must be analysed for specificity using the haemagglutination test (see below).
14. Add 0.2% Na-azide to the produced allo-antisera and store at -20°C in aliquots with batch numbers.
15. One animal may be bled for 20 mL for at least 4 times.

Preparation of “ready-to-use” allo-antisera

1. Defrost an allo-antisera diluted 1:2 in PBS buffer containing 0.2% Na-azide.
2. Prepare a panel of 2% blood suspension from animals with known MHC haplotypes (see below).
3. Make a test dilution series of the allo-antisera in PBS buffer (e.g. 1:2 to 1:128)
4. Test all the dilutions against the panel of known MHC haplotypes using the haemagglutination test described below. The test is performed to find the correct dilution for optimal reaction (agglutination in the highest dilution) and to test the allo-antisera for cross-reaction to other MHC antigens than the one the allo-antisera was raised against.
5. If a cross-reaction is found, the allo-antisera has to be absorbed to remove antibodies reacting with unwanted MHC antigens. Below an example of absorption is described.

Example: the allo-antisera anti-B-F19 cross-reacts with blood cells from animals carrying the B21 haplotype. These antibodies therefore have to be removed by absorption.

1. Bleed 10-20 mL blood from an animal carrying the B21 haplotype and stabilise the blood with 1/5 vol. 3.8% natrium-citrate.
2. Collect the blood cells by centrifugation at 500xg in 8 min at 20°C.
3. Wash the blood cells 2-3 times in 5-10 mL PBS buffer by vortexing followed by centrifugation at 500xg in 8 min at 20°C until the supernatant is clear.
4. After the last wash, the white layer of cells (the lymphocytes on top of the red blood cells) is removed with a Pasteur pipette and discarded. 20 mL blood gives about 5–6 mL packed RBC.
5. Make a test-absorption by mixing 1 mL anti-B-F19 allo-antisera and 1 ml packed RBC from the B21 animal.
6. Rotate the tube for 30 min on a rotator.
7. Centrifuge the solution at 500xg for 8 min at 20°C.
8. Transfer the supernatant to a new tube. Discard the RBC.
9. Test the anti-B-F allo-antisera for specificity by haemagglutination (see below) of various dilutions of the absorbed sera (1:2 to 1:128) against 2% blood suspensions from an animal carrying the B19 haplotype (positive control) and an animal carrying the B21 haplotype (negative control).
10. If there still seems to be a cross-reaction to B21 cells, it may be enough to dilute the absorbed allo-antisera further to solve the problem. Otherwise, the test-absorption must be repeated.
11. If the absorption is acceptable, larger portions of absorbed allo-antisera may be prepared. It is important that the antibody/antigen ratio is the same as in the test-absorption.
12. If an antibody is to be absorbed with various haplotypes, it may be an advantage to absorb it with one or two haplotypes first, and then test the allo-antisera against the panel of cross-reactive haplotypes. Sometimes the cross-reactive unspecific antibodies can be removed at the first absorption.
13. The absorbed allo-antisera is now “ready-to-use” in the haemagglutination test. The batches of “ready-to-use” allo-antisera are kept at -20°C.
Haemagglutination test

1. Bleed 1-2 mL blood from animals to be serotyped and stabilise the blood in 1/5 vol. 3.8 % Sodium Citrate.
2. Bleed 1-2 mL blood from animals with known MHC haplotypes (controls) and stabilise the blood in 1/5 vol. 3.8 % Sodium Citrate. The control blood may be kept for 2 weeks in the refrigerator if the blood is transferred to a CPD (Citrate Phosphate Dextrose adenine) tube.
3. Produce a 2 % suspension of the blood samples in PBS buffer in 4 mL test tubes (2-3 drops of blood + 2 ml PBS buffer - i.e. the blood colour turns into light orange). All the blood suspensions must have exactly the same colour. The suspensions may be kept for a maximum of 2 days in the refrigerator.
4. Drop onto the glass slide one drop of “ready-to-use” allo-antiserum (the antibody) and one drop of 2 % blood suspension (antigen).
5. Mix the two solutions gently by rocking the glass slide back and forth.
6. Incubate the glass slide in a humid chamber for 30 min.
7. Defrost a 4 mL NCP tube and dilute the plasma with PBS buffer up to 10 mL (20% dilution).
8. Filtrate the solution through a piece of gaze placed at the bottom of a 20 mL syringe into a 50 ml tube.
9. Adjust the vol. to 20 mL again with PBS buffer and repeat the filtration step using a new piece of gaze and the same 50 mL tube.
10. After incubation for 30 min take the glass slide out of the humid chamber and add one drop of 20% NCP. Mix the solution by rocking the glass slide back and forth.
11. Leave the glass slide for another 30 min in the humid chamber.
12. The haemagglutination is read on the light table and categorised as very positive (+++), positive (+), or negative (-). Please, be aware that a positive reaction may stick to the bottom so rock the glass slide back and forth when reading.
13. The slide may, if necessary, be placed in the humid chamber for another 10-15 min in order to have a more clear reaction, however; this may result in a fainter reaction.

Serological MHC typing of offspring

As the antibody titre in the allo-antiserum may change when frozen, “ready-to-use” allo-antisera are always test-titrated before use. Testing is carried out using a panel of 6 – 10 different haplotypes with known positive and negative reactions. The typing of animals with unknown haplotypes can then be performed on the glass slides. The schema below shows a simple result of a serological MHC typing.

<table>
<thead>
<tr>
<th>Antibody/Antigen</th>
<th>B15 animal</th>
<th>B19 animal</th>
<th>B21 animal</th>
<th>Unknown</th>
<th>Unknown</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-B-F15</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-B-F19</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Anti-B-F21</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Anti-B-G15</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-B-G19</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Anti-B-G21</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Typing result</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>B15</td>
<td>B19</td>
<td>B21</td>
</tr>
</tbody>
</table>

Restriction Fragment Length Polymorphism (RFLP) typing of MHC

Introduction
The use of RFLP analysis to molecular characterisation of genetic variation in the MHC is a valuable supplement to serological analysis. The RFLP analysis can examine sub-regions of genotypes for which the production of serological reagents has been elusive, analyse MHC chromosomal recombinants, and categorise newly identified genotypes. Identification of new MHC haplotypes by RFLP analysis makes it easier to make an immunisation plan for generation of new allo-antisera to unknown MHC antigens. Actually,
this was the method of choice when we distinguished between 5 to 6 new MHC haplotypes in a commercial breeding flock from the Lohmann Company (unpublished results).

The method is very time consuming and expensive compared to the serological typing but we use the method routinely in the lab as a supplement when we have problems with the serological typing. In our lab all our chicken lines containing different MHC haplotypes have been analysed by this method and a system has been worked out to distinguish between the different MHC haplotypes using one single restriction enzyme (Bgl II) and cDNA probes for B-F, B-L and B-G (Juul-Madsen et al., 1993 and 2000).

**Equipment**

- Micro-tube centrifuge
- High Speed centrifuge
- 37°C incubator
- Tubes (1.5 mL, 2.2 mL, 50 mL)
- Tea filters
- 10 ml pipettes
- 13 mL Sarsted centrifuge tubes
- Pasteur pipette
- Hybond N+ nylon membranes (RPN203B, AM)

**Reagents**

- TKM-1 buffer (10mM Tris-HCl** pH 7.6; 10mMKCL; 10mM MgCl₂; 2mM EDTA)
- TKM-2 buffer (10mM Tris-HCl** pH 7.6; 10mM KCL; 10mM MgCl₂; 2mM EDTA; 0.4M NaCl)
- NP-40
- SDS
- 6M NaCl (It is OK with some undissolved crystals in the bottle)
- 2-propanol
- TE buffer (Sambrook et al., 1989)
- 10 mg/mL BSA
- 0.2 M Spermidine
- 0.5 M EDTA (Sambrook et al., 1989)
- 7.5M NH₄-O-A
- 99% Ethanol
- DNA loading buffer (Sambrook et al., 1989)
- 0.25 M HCl
- 4M NaOH
- 10 mg/ml carrier DNA (Sambrook et al., 1989)
- ³²P labelled probe (Sambrook et al., 1989)

** Trizma Base 99.9% from Sigma # T-8524

**Purification of genomic DNA with Sodium Chloride**

This method is included because it is a very easy, safe and cheap method to get large amounts of genomic DNA but the method is not very known. The method is adapted from the paper of Lahiri and Nurnberg (1991).

1. Transfer a blood sample to a micro tube with a Pasteur pipette and centrifuge in a micro centrifuge at 300xg for 5 min.
2. Mix 200µl packed cells with 25mL TKM-1 buffer containing 2.5% NP-40 in 50mL tubes.
3. Centrifuge at 1000xg for 10 min at room temperature (RT).
4. Discard the supernatant and resuspend the pellet (the nuclei's) in 20 mL TKM-1 buffer and vortex thoroughly.
5. Centrifuge at 1000xg for 10 min at RT.
6. Discard the supernatant and immediately resuspend the pellet thoroughly in 300μL TKM-1 buffer.
7. Add 10 mL TKM-2 buffer containing 0.625% SDS and shake the tube vigorously – do not vortex at this step as it will break the DNA into smaller pieces.
8. Incubate for a minimum of 1 hour (possibly o/n) at 60°C in a water bath and shake a couple of times with your hands. The solution has to be viscously but clear for best results.
9. Transfer the DNA solution to a 13mL Sarstedt centrifuge tube containing 3.75mL 6M NaCL - mix well by shaking with your hands.
10. Centrifuge in a high-speed centrifuge at 10.844xg for 10 min at RT.
11. Filter the supernatant through a tea filter into a new 50 ml tube.
12. Add 1 vol. 2-propanol and mix carefully until the DNA precipitates.
13. Spool the DNA onto a Pasteur pipette, wash in 70% EtOH and dry the DNA by rotating the pipette on the inside of a clean tube.
14. Resuspend the DNA in 2 mL TE buffer in a 2.2 mL micro tube.
15. Incubate for 30 min at 65°C, then o/n at 4°C and shake until the DNA dissolves.
16. If the DNA has not dissolved, filter the DNA solution through a tea filter.
17. Measure OD 260 and 280 nm.
18. Store the DNA in 2.2mL micro tubes at -20°C.

Genomic DNA restriction digestion

1. Use 1.5 mL micro tubes.
2. 7.5 - 10μg DNA is digested in an end concentration of 0.075 - 0.1μg/μL.
3. Add in the following order:
   - dH2O
   - 0.1μg/μL BSA
   - 2 mM Spermidine
   - 1/10 end volume of 10 x restriction buffer
   - the DNA
4. Digest the DNA with a given restriction enzyme - 2U/μg DNA for 6-8 hours at an enzyme determined temperature.
5. Add an extra 2U/μg DNA and continue the digestion o/n.
6. Terminate the digestion by adding EDTA up to 10 mM, ½ volume 7.5M NH₄-O-Ac, and 3 volume 99% Ethanol. Mix and incubate the samples for a minimum of 1 hour at -20°C.
7. Centrifuge the samples at 26384xg for a minimum of 15 min at 4°C.
8. Discard the supernatant and dry the pellet. The pellet must not dry completely as it then is difficult for genomic DNA to dissolve again.
9. Resuspend the pellet thoroughly in 20μL TE buffer containing 1:5 amount DNA loading buffer.
10. Incubate the samples for 30 min at 37°C with the lid open to evaporate the alcohol.
11. The samples are ready to be loaded on an 0.6 – 1% agarose gel containing 0.5 μg/mL Etitiium Bromide – the percentage of agarose depends on how often the restriction enzyme cut the DNA.

Alkal Blotting - DNA hybridisation

1. Photograph the gel to check for successful digestion of the samples.
2. Place the gel in ½ L 0.25 M HCl on a shaking table for 20 min (gentle shaking). This treatment depurinizes the DNA and breaks the DNA into smaller pieces which makes the transfer to the Hybond N+ membrane easier.
3. Pour off the acid and rinse the gel several times in MilliQ H₂O to remove all the acid.
4. Make a Southern Blot according to the manufacturer’s description for alkali blotting using Hybond N+ and 0.4M NaOH.
5. Prehybridise in 10 - 20 mL prehybridisation buffer (6 X SSC; 1% SDS; 10 x Denhardt; 200 -400 μL Carrier DNA** (10mg/mL) at 67°C in shaker water bath for 2 – 4 hrs.
6. Hybridise in 10 – 20 mL hybridisation buffer (prehybridisation buffer + 32P-labelled probe**) at 67°C in shaker water bath over night.
7. Wash the filter in a 55°C hot water bath after the following program:
2 times in 1 x SSC, 0.1% SDS for 25 min
2 times in 0.1 x SSC, 0.1% SDS for 25 min.
2 times in 0.1 x SSC for 25 min.
8. The filter is then ready for autoradiography.

** Boil the probe and the carrier DNA for 5 min and place on ice for 5 min. Add $^{32}$P-labelled probe until there is $6-8 \times 10^6$ cpm/ml.

Micro-satellite fragment typing of MHC

Introduction
Micro-satellites are DNA sequences consisting of stretches of repeated short nucleotide motifs that may show polymorphism in the number of repeats. Micro-satellites are usually embedded in DNA with a unique sequence and can therefore easily be amplified by PCR. The LEI0258 micro-satellite (Zoorob et al., 1998; EMBL Accession no. Z83781) is a polymorphic 12-mer located within the B region of the chicken MHC on chromosome 16. The length of the LEI0258 was found to differ in 13 different MHC haplotypes from inbred lines with the exception of B2 that was identical with B15 (unpublished results). The use of fluorescent-labelled primers and automatic gel scanning makes this technique an attractive and rapid alternative to the other two MHC typing techniques.

Equipment
- PCR instrument
- 0.2 µL PCR tubes
- Automatic Gel Scanner (e.g. ALFexpress® DNA Sequencer AM)
- Analysis software (e.g. ALFwin Fragment Analyser 1.0 AM)

Reagents
- QIAamp® DNA Mini Kit (QI)
- PCR primers: 
  - [f]: 5’-(Cy5)-CACGCAGCAGAACTTGGTAAGG-3’
  - [r]: 5’-AGCTGTGCTCAGTCCTCAGTGC-3’
- Taq DNA Polymerase (27-0799-05, AM)
- PCR nucleotide mix (US77170, AM)
- PCR buffer (50 mM KCl, 1.5 mM MgCl$_2$, 10 mM Tris-HCl)
- Polyacrylamide (e.g. Reprogel High resolution, AM)
- Loading Buffer (5 mg/ml of dextran blue in 100 % deionised formamide)
- Fluorescent 50 bp fragment ladder (e.g. ALFexpress™Sizer, 27-4539-01, AM)

PCR amplification of micro-satellite

1. Purify genomic DNA from tissue or blood samples with the QIAamp® DNA Mini Kit according to the manufacturer’s instructions (15 - 40 µg DNA). Alternatively genomic DNA can be purified from red blood cells by the sodium chloride method mentioned above.
2. Set up a PCR reaction in a total volume of 25 µL PCR buffer including:
   - 1 µL of genomic DNA
   - 0.05 µM of each primer
   - 0.2 mM of each dNTP (PCR nucleotide mix)
   - 1 U of Taq DNA polymerase
3. Apart from samples, a panel of DNA from known MHC haplotypes can be included in the PCR step.
4. Perform PCR with a reaction profile of 94°C/5 min followed by 94°C/1 min, 56°C/1 min, 72°C/2 min for 25 cycles and finally 72°C/10 min.

Fragment analysis

5. Use 1.5 µL of the PCR reaction and mix it with 3-4 µL of Loading Buffer
6. Denature samples at 95°C for 2 min.
7. Keep samples on ice for 2 min.
8. Separate PCR products on polyacrylamide gel along with 50 bp fragment ladder and controls.
9. Evaluate results on automatic gel scanner.
10. Deduce MHC type of unknown samples by comparing fragment sizes with known MHC controls.

Suppliers
AM: Amersham Biosciences, Little Chalfont, UK
ME: Medinor ASA, Oslo, Norway
QI: Qiagen, GmbH, Hilden, Germany
TE: Terumo Europe NV, Paris, France

Concluding remarks

- The three typing methods do not replace but complement each other depending on the purpose of the typing.
- The fragment analysis may also be run on a 3% metaphor agarose gel or a 20 cm 6 – 9% polyacrylamid gel stained with ethidium bromide. A 40 cm 4% denaturing polyacrylamide gel visualised by silver staining may also be used.
- A table of inbreed and partly inbreed chicken lines with known MHC haplotype in the whole world will be published by Marcia Miller, USA (manuscript in preparation).

Acknowledgements
The author’s wish to thanks the technicians Lene Rosborg Dal and Hanne Svenstrup - the real experts in the lab for critical reviewing of the methods.

References


Figure 1: Glass plates