

## PCR-RFLP genotyping protocol for chicken *Mx* gene G/A polymorphism associated with the S631N mutation

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**ABSTRACT.** The *Mx* (myxovirus resistance) gene codes for a protein with antiviral activity. Non-synonymous G/A polymorphism at position 2032 of chicken *Mx* cDNA results in a change at amino acid 631 of the Mx protein. This mutation has been shown to affect the antiviral activity of the Mx molecule, although recent studies have not confirmed this effect in response to some influenza strains. Nevertheless, the G/A polymorphism could be important for the chicken's response to other viruses. A robust PCR-RFLP protocol for genotyping chicken *Mx* gene polymorphism associated with the S631N mutation was developed. The F primer anneals to the last intron of the *Mx* gene, and the R primer anneals to the last exon of the gene, with an expected PCR product of 299 bp. PCR products were digested with *Hpy*8I. This enzyme cuts the sequence 5'-GTN|NAC-3', 2 bp downstream of the *Mx* polymorphism for the G allele, whereas the fragment containing the A allele is not

cleaved. One hundred and twenty-seven chickens (commercial broilers, White Leghorn and New Hampshire) were genotyped using this protocol, and genotyping data were validated by sequencing. Full identity of results between the two genotyping methods was observed for all 127 samples, proving the reliability and robustness of this PCR-RFLP protocol.

**Key words:** Chicken; PCR-RFLP; Avian influenza virus; *Mx* gene; S631N mutation