

## Differentially expressed transcripts in shell glands from low and high egg production strains of chickens using cDNA microarrays

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### Abstract

We have constructed a tissue-specific in-house cDNA microarray to identify differentially expressed transcripts in shell glands from low (B) and high (L2) egg production strains of Taiwanese country chickens during their egg-laying period. The shell gland cDNA library was constructed from the high egg production strain. cDNA clones (7680) were randomly selected and their 5'-end sequences characterized. After excluding overlapping sequences, an in-house cDNA microarray, representing 2743 non-redundant transcripts, was generated for functional genomic studies. Using our microarray, we have successfully identified 85 differentially expressed transcripts from the two different strains of chicken shell glands. In this study, 34 of these transcripts were associated with signal transduction, protein biosynthesis,

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cell adhesion, cellular metabolism, skeletal development, cell organization and biogenesis. We selected a number of the differentially expressed transcripts for further validation using semi-quantitative RT-PCR. These included elongation factor 2 (EEF2), ovocalyxin-32 (OCX-32) and annexin A2 (ANXA2) which were expressed at high levels in the chicken shell glands of the B strain and, in contrast, the coactosin-like protein (COTL1), transcription factor SOX18 and MX protein were more highly expressed in the L2 strain. Our results suggest that these differentially expressed transcripts may be suitable to use as molecular markers for high rates of egg production, and now need to be investigated further to assess whether they can be applied for use in breeding selection programs in Taiwanese country chickens.

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**Keywords:** Chickens; Shell glands; Egg production; cDNA microarrays; RT-PCR

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## 1. Introduction

An important priority in the poultry industry is to develop a rapid and accurate method to select chicken strains, which have good rates of high quality egg production. Traditional methods currently used to improve egg production efficiency and quality are very time consuming, as they assess positive genetic progress by long-term monitoring of either egg number or laying rate (Fairfull and Gowe, 1990; Atzmon et al., 2002). Since 1982, two domestic Taiwanese country chicken strains, B and L2, have been established from a common base population. There is a significantly greater egg production rate in the L2 strain (0.8) compared to the B strain (0.4), and this difference has been maintained over 20 generations (Chao and Lee, 2001). Using these two strains of chicken, we investigated whether we could identify molecular markers associated with one or other chicken strain, which could then provide the basis of a rapid selection procedure based on cDNA microarray technology.

cDNA microarrays were developed in the mid-1990s and permit the simultaneous analysis of thousands of genes (Scheda et al., 1995). The majority of microarray experiments have been conducted with human and rodent material rather than livestock species (Davoli et al., 2002; van Hemert et al., 2003; Yao et al., 2004), and although there is extensive tissue-specific gene sequence data from chickens, the field of chicken functional genomics has been relatively little explored (Liu et al., 2001; Cogburn et al., 2003). In this paper, we describe the construction of tissue-specific cDNA libraries and the genomic analysis of expressed sequence tags (ESTs) detected in shell glands isolated from B and L2 breeds of Taiwanese country chickens. The chicken shell gland is regulated by estrogen and is the site of chicken eggshell formation and accumulation, whose quality, thickness and strength is determined by the action of ion transport binding proteins (Berg et al., 2004; Vetter and O'Grady, 2005). We reasoned that this tissue is a likely source of differentially expressed genes associated with either low or high rates of egg production. We coupled cDNA library construction with cDNA microarray technology to compare global gene expression in shell glands from either the low (B) or high (L2) strains of Taiwanese country chickens during their egg-laying period. Then, we characterized the differentially expressed genes between the B and L2 strains in more detail to identify candidate molecular markers correlated with either low or high rates of egg production. We propose that the molecular markers we identify could be used to achieve early and efficient breed selection during the egg-laying stage in Taiwanese country chickens.

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