

论文 鸡卵巢组织SSH cDNA文库构建及差异表达基因筛选与初步鉴定

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摘要:

为分离与鸡产蛋性能密切相关的重要基因或调控因子,利用抑制性消减杂交技术,以高产蛋鸡品种海兰褐商品代蛋鸡和产蛋量相对较低的地方鸡品种大骨鸡20周龄时的卵巢组织为试验材料,构建消减cDNA文库。文库的插入片段集中在500 bp左右,挑取720个克隆进行PCR筛选,获得657个阳性克隆,经过点杂交筛选后选择了536个阳性克隆,从中随机挑取100个阳性克隆进行测序。经过同源性比对并后得到52个差异表达基因,除3个未知基因外,其余涉及到细胞增殖与代谢调控、卵泡生长发育、钙磷和蛋白质营养代谢调节等多个方面。并对4个可能影响鸡产蛋性能的差异表达基因进行了RT-PCR半定量检测鉴定。

关键词: 鸡 cDNA文库 卵巢组织 差异表达基因 抑制性消减杂交

Construction of Suppression Subtractive cDNA Library and Screening and Preliminary Identification of Differentially Expressed Genes in the Ovary of the Different Egg Production Performance in Chicken

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Abstract:

In order to screen the chief genes and regulatory factors relevant to egg performance in chicken, suppression subtractive hybridization (SSH) was employed to construct the SSH cDNA library in the ovary from commercial Hyland brown layers of high egg production and Dagu layers of low egg production at 20 weeks of age in the present study. The inserted fragment sizes focused around 500 bp and 720 clones were verified by PCR, in which 657 clones were used to perform dot blotting hybridization. A total of 536 clones were obtained and 100 clones were randomly picked and sequenced. Fifty two differentially expressed genes were isolated by homologous BLAST search. Besides 3 unknown function genes, the others implicated in cell propagation and regulation, folliculogenesis, and nutritional metabolic pathway of calcium, phosphorus and protein, and so on. Four differentially expressed genes probably impacting egg production of chicken were identified by using semi-quantitative RT-PCR.

Keywords: chicken cDNA Library ovary tissue different expressed gene suppression subtractive hybridization

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