

# Annotating a partial transcriptome of the marine snail *Dicathais orbita*'s hypobranchial gland using suppressive subtractive hybridization and BLAST2GO

**Patrick W. Laffy**

patrick.laffy@flinders.edu.au

**Kirsten Benkendorff**

kirsten.benkendorff@flinders.edu.au

**Catherine A. Abbott.**

cathy.abbott@flinders.edu.au

School of Biological Sciences, Flinders University, GPO BOX 2100, Adelaide, South Australia 5001.

## **Abstract**

Analysis of the function of the hypobranchial gland of the marine snail *Dicathais orbita* was performed. Suppressive subtractive hybridization was performed comparing hypobranchial gland expressed genes to those expressed within the mantle tissue of the snail, maximizing identification of those uniquely expressed or up regulated genes within this gland. A total of 411 unique sequences were obtained from this cDNA library, and automated gene ontology annotation software BLAST2GO was utilized to assign function to the transcripts. Genes involved in electron transport, protein biosynthesis and modification, acetylcholine metabolism and cell structure formation were all identified, suggesting one of the key roles of the gland is in biosynthesis. An arylsulfatase gene, suggested to be involved in the formation of ancient dye compound Tyrian purple and its bioactive precursors, was also identified to be expressed within the gland, supporting the argument that Tyrian purple production is an internal process exclusive to the hypobranchial gland, and dye compounds are not derived from dietary sources.

**Keywords:** Suppressive subtractive hybridization, SSH, BLAST2GO, Muricid, Mollusc

## **1 Introduction**

The marine snail *Dicathais orbita* produces potent anti-cancer and antibacterial compounds as the chemical precursors of the dye Tyrian purple within its hypobranchial gland (HBG) [1, 3]. Further, choline esters have also been found in the HBG which may find clinical use due to their neuromuscular blocking action [4]. The identification of key enzymes expressed within the hypobranchial gland may allow us to develop biosynthetic methods using recombinant techniques for producing these pharmaceutically active compounds. It is known that there are three enzymes involved in the formation of these compounds, and although one has been previously identified in a

land snail, none of these sequences have been identified from *D. orbita* or any marine mollusc. A more detailed investigation into hypobranchial gene expression may also indicate the biological processes and the overall function of this organ in *D. orbita*.

## 2 Methods

Suppressive subtractive hybridization was performed to create a cDNA library from *D. orbita* HBG RNA, containing genes that are differentially expressed in the HBG that are not expressed in *D. orbita* mantle tissue. The resulting cDNA was cloned and sequenced. The automated sequence annotation software BLAST2GO [2] and our own pipeline for BLAST searches were used to identify and annotate the functions of these sequences.

## 3 Results and Discussions

411 unique sequences were obtained from the HBG of *D. orbita*. Of these sequences, 140 had their functions putatively identified, and the usage of an alternate codon translation system in some sequences identified the presence of ciliate protozoa within the hypobranchial gland. In total, 50 ciliate protozoan sequences were identified, including highly conserved ribosomal RNA sequences. A total of 90 sequences expressed by *D. orbita* had their functions identified, some sequences with roles in electron transport, protein biosynthesis and modification, acetylcholine metabolism and cell structure formation. These results indicate the HBG is a biosynthetic organ. A gene involved in acetylcholine metabolism was also identified, suggesting a cellular process for managing choline ester production exists within the HBG. An individual arylsulfatase sequence was identified within our cDNA library, which may be involved in the production of the bioactive Tyrian purple precursors. Further investigations into the enzymatic production of Tyrian purple will be required in order to elucidate the remaining genes.

## References

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