

## New MHC Genes In Immunized Sea Star *Asterias Rubens* To HRP

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### Abstract

MHC genes were recently discovered in 2 classes of Echinodermata : the Ophiurids and the Crinoïds. These genes belong to MHC Class I and MHC Class II. Blast against human was performed in sea star *Asterias rubens* genome. A MHC Class I gene (the well-known HLA-A gene) exists also in Asterids and particularly in sea star *Asterias rubens* immunized to HRP antigen (Horse-radish peroxidase)

### Introduction

MHC genes (Class I, Class II) were discovered, for the first time, in Invertebrates, and particularly in 2 classes of Echinodermata : the ophiurids and the crinoïds out of 5 classes

(1). It was important to research in the 3 other classes (and particularly in asterids) the presence or not of similar MHC genes. We recall this research(1) was concerning HLA E

gene, HLA B gene from MHC Class I and HLADRB1 gene, HLADQB1 gene from MHC Class II.

### Materials and Methods :

Sea stars *Asterias rubens* were immunized twice to HRP (Horse-radish peroxidase from Sigma Products) :

100µg/injection in sea star coelomic cavity. 5 days after the last injection, digestive coeca were removed and treated with Trizol (Invitrogen) to obtain RNA. Secondly, cDNA was normalized using double strand specific nuclease essentially as described by Zhulidov et al (2). cDNA was fragmented using DNA Fragmentase (New England Biolabs), according to the manufacturer's instructions. After ligation of adapters for Illumina's GSII sequencing system, the cDNA was sequenced on the Illumina GSII platform sequencing 100 bp from one side of the approximately 200 bp fragments. Sequences were assembled using Velvet Zerbino et al. (3)

Assembled nodes were used for further assembly including *Beta vulgaris* EST-Data from NCBI in MIRA.

### Results :

The two described MHC Class I genes (1) are present in sea star *Asterias rubens* genome. Furthermore, A HLA-A transcriptome was revealed in this last one ; the sequence of which, in 5'-3' was the following :

Contig 1 -Length : 152

```
5'CCACAGACAAGATCATACTGAATGAGCAAAAAGTGGGAAGCATTCCC
TTTGAAAAGTGGCACAAGACAGGGATGCCCTCTCTCACTACTCTATT
CAACATAGTGTGGAAGTTCTGGCCAGGGCAATCAGGCAGGAGAAGG
AAATAAAGGT 3'
```

The concern e-value was :3,50e-66 at a significant degree.

### Conclusion :

Results show clearly a HLA-A gene exists in *Asterias rubens* genome.

It appears as clearly as possible that 3 MHC Class I genes are present in Echinodermata : we recall next to HLA-A gene : the HLA-B, the HLA-E genes we described.



Further studies are necessary to clarify a) the existence or not of new MHC Class II genes in *Asterias rubens* b) the rôle of Immunization to HRP which can induce the appearance of new MHC genes.

#### References :

1. Leclerc M, Proteomics and Bioinformatics 2020 ; 2(1) :59-61.
2. Zhulidov PA, et al. Nucleic Acid Res 2004 ; 3 : 32-37.
3. Zerbino DR, et al. Gen Res 2007 ;18 : 821-829.