

Biosynthesis of the crinoid antedon bifida Ig kappa gene cloning in PUC-GW-KAN plasmid CDR regions in asterids

Abstract

The biosynthesis « de novo » of Antedon bifida IGKappa gene was performed in our laboratory. Then, it was cloned with success, into a pUC-GW-Kan plasmid. 3 Echinodermata (Invertebrates) out of 5 possess IG site, notably in Asterids, Ophurids, rinoïds. Furthermore CDR1, CDR2, CDR3? determining regions were described in the Asterid: *Asterias rubens*.

Keywords: oligonucleotides, cloning, transcriptome sequence, plasmids

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Introduction

Recently we have presented the transcriptome sequence of Antedon bifida IGKappa gene, as following (in 5'-3'). It presented 1536 bp.

Trinity

5,AGCGAATGAAAAAGAAGAACCGGCCAAAAAAGTACTTCTACCAAAGAAGCGAATGAAAAAG
AAGAACCGGCCAAAAAAGTACTTCTACCAAAGAAGAACTGAAATAGAAGAACTAACCGAAAC
AAGTATTCTACAAAATCAGTTTCTGCCAGTGATATATTCCTTGGTACAACCTTCACACTGGAGATG
GGATTCTGCGTAGGACCTGAACACAAACCGTTTACAGGAGATTTCGACGGTGACGGTAATGAAGA
TCTTCTGTTTCACAATTCAAAGACAGGCTCGAAAAAGATATACTATGCAAGTTGTGACGGCTCTTTT
AATGGTGATAGGTCGTGGAGAAGAGAGATGAATTTTGTCTACGTAAGTGGATATGATCTATACATTG
GTGATTTCACCGCGATGGTCGATCCGATATGCTGTGTATCGTCCTCAGTATGGTCAGATTGGGT
TGTGTTGGCGCAACCTGGGGGTGTATTCAGTCTAACCCGTGGTTCGTATAGTCCCAATTGGTGCAA
GGCCACCACTGATAAAGTATATATTGGAGACTTCAACGCAGACGGTCGGGATGATATTCTTTGCCAC
ACACAAAGTTCGGGTACATTGCAATATATTATGCATTATACACTGGTTATTTTCTACCTCTACAACA
TATCGCTTACACGAAGTATGAGTTGGTGCAGAGGTACATATCAAAGAGTGTATACTGGAGATTTC
ACGGAGACCGAAGGGTTGATATGCTCTGCCACGACTACTCATCTGGCTACATATATGTAGCAGTAGC
CACAGCGACTGGTGGATTACCTCTGCCACATGGAGCAGAAGTATGGGCTGGTGCAAGCATTCTGA
ACTCTAAGCTCAGCATTGGAGATTTCATAAAGATAACCGCGACGACATCATGTGCAGCGACACAA
ATGGTCTTACTGGATAGCATTCTCTGTACAACGGTTCGTTTTTCATCTAAAAGCTGGACCCGTAA
ACAAAAGTGGTGATCTGGCAATGATGTGTTAGTTTCGGATGTGAATGGAGATGGTGGGGATGA
TTTGATGTGCCATAATGAAGCCGACGGCATCAAGTACATATCGATCAACCATAAGGCCTAAAGCAA
GTTCTCTCAATATTACAGAAAAGTATTACACCAAAATGATTCATTTTGTACTGAACCTCAATTCAA
TTCAATTAAATTTACATAAACGTTAACGGAAGGATACAATCAACTAAAATAATGTTTCATTATT
TTTCGTCGATAACCTAAACAAAAATCAGATAAGAAATTATACAATAATATACTGTAAACGTATTATAC
AAAATAATTAAATGTATATTAAGCTACTGTACTTAGAAATGTACTTGTACGCTTATTAATATTAATAAG
CCTAATGCCCGGGTTGATAATAATAAATACATTTTGTCAAGTTCAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAACTCAAAAGTCCCAGGCCCCACCCCGACCTACTGA
ACCAGAAAG3.

We have tried, in the present paper, to synthetize « de novo » the corresponding gene by the help of pUC-GW Kan construct.

Materials and methods

The gene synthesis methods are the following ones. We see below:

- Synthesis of oligonucleotides with overlapping segments in sense and antisense direction
- Assembly of the oligonucleotides into a double stranded DNA, using a poly chain assembly method (PCA).

Synthetized sequence was given below

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5AGCGAATGAAAAAGAAGAACCGGCCAAAAAAGTACTTCTACCAAAGAAGCGAATGAAAAAGA
AGAACCGGCCAAAAAAGTACTTCTACCAAAGAAGAACTGAAATAGAAGAACTAACCGAAACA
AGTATTTCTACAAAATCAGTTTCTGCCAGTGATATATTCCTTGGTACAACCTTTCACACTGGAGATGG
GATTCTGCGTAGGACCTGAACACAAACCGTTTACAGGAGATTTCGACGGTGACGGTAATGAAGATC
TTCTGTTTCACAATTCAAAGACAGGCTCGAAAAAGATATACTATGCAAGTTGTGACGGCTCTTTTAA
TGGTGATAGGTCGTGGAGAAGAGAGATGAATTTTGTCTACGTAAGTGGATATGATCTATACATTGGT
GATTTCAACGGCGATGGTCGATCCGATATGCTGTGTCATCGTCCCTCAGTATGGTCAGATTGGGTTG
TGTGGCGCAACCTGGGGGTGTATTCAGTCTAACCCGTGGTTCGTATAGTCCCAATTGGTGCAAGG
CCACCACTGATAAAGTATATATTGGAGACTTCAACGCAGACGGTCGGGATGATATTCTTTGCCACAC
ACAAAGTTCGGGTACATTGCAATATATTATGCATTATACACTGGTTATTTTTCTACCTCTACAACATA
TCGCTTTACACGAAGTATGAGTTGGTGCAGAGGTACATATCAAAGAGTGTATACTGGAGATTTCAA
CGGAGACCGAAGGGTTGATATGCTCTGCCACGACTACTCATCTGGCTACATATATGTAGCAGTAGCC
ACAGCGACTGGTGGATTACCTCTGCCACATGGAGCAGAAGTATGGGCTGGTGCAAGCATTTCGAA
CTCTAAGCTCAGCATTGGAGATTTCATAAAGATAACCGCGACGACATCATGTGCAGCGACACAAA
TGGTCCTTACTGGATAGCATTCTCTGTACAACGGTTCGTTTTTCATCTAAAAGCTGGACCCGTAAG
CAAACTGGTGTACATCTGGCAATGATGTGTAGTTTCGGATGTGAATGGAGATGGTGGGGATGAT
TTGATGTGCCATAATGAAGCCGACGGCATCAAGTACATATCGATCAACCATAAGGCCTAAAGCAAG
TTCCTCTCAATATTACAGAAAATATTACCCACAAATGATTCATTTTGTACTGAACCTCAATTCAAAT
TCAATTAATAATTACATAAACGTTAACGGAAGGATACAATCAACTAAAATAATGTTTCATTCAATTTT
TTCGTCGATAACCTAAACAAAAATCAGATAAGAAATTATACAATAATATACTGTAAACGTATTATACA
AAATAATTAATGTATATTAAGCTACTGTACTTAGAAATGTACTTGTACGCTTATTAATATTAATAAGC
CTAATGCCCGGGTTGATAATAATAAAATACATTTTGTCAAGTTCAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACTCAAAAGTCCAGGCCCCACCCCGACCTACTGAA
CCAGAAAG 3
```

The various sequences issued from blasts were compared as shown (Table 2).

Table 2 Blastn original sequence/ synthetized sequence results

Size seq 1	Size seq 2	Max score	Total score	Query cover	E. value	Per. ident	Acc len
1536	1536	2837	2837	100%	0.0	100%	1536

Conclusion

It appears the 2 sequences are identical: it asserts the validity of the experiment by the use of cloning in pUC-GW-Kan plasmid.

On the other hand, such a fundamental work has allowed to determine

- a) the biosynthesis of the Crinoïd Antedon bifida ICKappa gene
- b) its conservation in pUC-GW-Kan plasmids for further experiments to perform.

At last, it can be said, 3 Ickappa genes from 3 different Echinodermata (The Asterids, The Ophuirids and The Crinoïds) possess IG sites.¹⁻³

Furthermore, the Asterid: *Asterias rubens* possesses CDR1, CDR2, CDR3? determining regions⁴ by the use of bioinformatics⁵ according the method of Ehrenmann and Lefranc. Strong homologies appear between sea star CDR1, CDR2 and *Pongo abelii* ones. How great are the Echinoderms!

Acknowledgments

None.

Conflicts of interest

The author declares that there is no conflicts of interest.

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