

Ophurid Ophiocomina Nigra HLA-E Gene Synthesis in PUC-GW-KAN Plasmid or HLA-E Echinodermata Gene Biosynthesis « De Novo » in E. Coli Sensu Lato Plasmid

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Abstract

HLA-E (Class 1) is a MHC gene which has been isolated in 2020, in our laboratory. We show now its biosyntheses « de novo » in a PUC-GW-KAN plasmid. Such experiment was performed with the Ophiocomina nigra IGHK gene one year ago.

Introduction:

We have isolated recently MHC genes in Echinodermata [1] in 3 classes: The Ophurids, the Crinoïds, the Asterids. At that time, we decided to synthesize one of these genes: The well-known HLA-E one in a PUC-GW-KAN plasmid (Yan Li gift).

Methods :

We operate according to the following method [2]. It was resumed in 4 parts:

1. Synthesis of oligonucleotides with overlapping segments in sense and antisense direction.
2. Assembly of the oligonucleotides into a double stranded DNA, using a poly chain assembly method (PCA).
3. For larger constructs, the sequence is split into smaller, intermediate fragments, to facilitate synthesis. Once the intermediated fragments have been obtained with correct sequence, they are assembled into the full-length sequence.
4. Cloning into the linearized vector by either

recombination or ligation-based cloning, mostly performed within the same step as full-length sequence assembly.

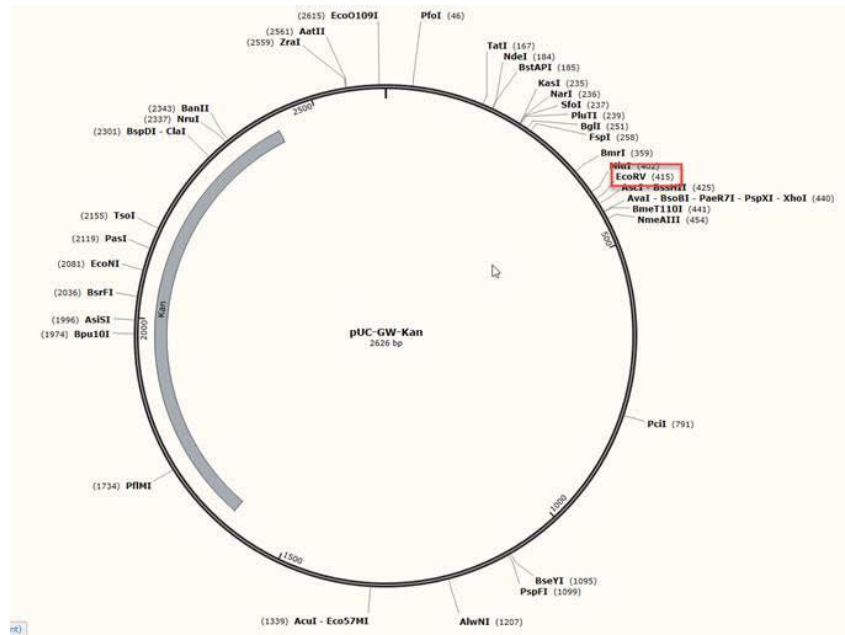
Regarding the restriction site, which was used for cloning, construct was cloned into vector pUC-GW by using the unique EcoRV restriction site. Please find below the primers used for sequencing.

M13F-77	GATGTGCTGCAAGGCGATTA
M13R-88	TTATGCTTCCGGCTCGTATG
U-SEQ4883	CCTCCAATCGGGTAACTC

Results:

- 1) Plasmid map:

The construct appears below



2) Recalling of Original sequence in 5'-3':

TGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCG
 GATCACGAGGTCAGGAGATCGAGACCATCCTGGCT
 AACACAGTGAAACCCCGTCTCTACTAAAAATACAA
 AAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGT
 CCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGC
 GTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAG
 ATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGC
 GAGACTCTGTCTCAAAAAAAAAAAAAAAAAAAAAA
 AA

3) Synthetized sequence in 5'-3':

TGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCG

GATCACGAGGTCAGGAGATCGAGACCATCCTGGCT
 AACACAGTGAAACCCCGTCTCTACTAAAAATACAA
 AAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGT
 CCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGC
 GTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAG
 ATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGC
 GAGACTCTGTCTCAAAAAAAAAAAAAAAAAAAAAA
 AA

4) Blastn original sequence/ synthetized sequence

The **table 1:** Resumes mainly the identities and the e-value between these 2 precedent sequences. Chromatograms were also performed:

Table 1: Comparisons between original sequence and synthetized one.

Size Seq1	Size Seq2	Max score	Total score	Query cover	E. Value	Per. Ident	Acc Len
281	281	520	520	100%	7e-152	100%	934

Conclusion:

We conclude our experiment is valid when compared to table 1. Furthermore, we assert, it is the first time such discovery:

- a) MHC Genes in Echinodermata (Invertebrates) were found
- b) biosynthesis of HLA-E echinodermata gene in a PUC-GW-KAN plasmid was performed.

References:

1. Leclerc M. (2020) Evidence of MHC Class I and Class II Genes in Echinodermata. 2(1): 59-61.
2. Leclerc M. (2021) Biosynthesis « De Novo » of the Ophurid Ophiocomina Nigra Igkappa Gene.1(1): 1-4.