

Short Commentary

Ophuirid Ophiocoma 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 an MHC gene which has been isolated in 2020, in our laboratory. We show now its biosynthesis «de novo» in a PUC-GW-KAN plasmid. Such experiment was performed with the Ophiocoma nigra IGKappa gene one year ago.

INTRODUCTION

We have isolated recently MHC genes in Echinodermata [1] in 3 classes: the Ophuirids, 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 table below for the primers used for sequencing (Table 1).

Table 1. Primers used for sequencing.

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

RESULTS

1) Plasmid map

The construct appears below (Figure 1):

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

TGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCG
GATCACGAGGTCAGGAGATCGAGACCATCCTGGCT
AACACAGTGAAACCCCGTCTCTACTAAAAATACAA
AAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGT
CCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGC
GTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAG
ATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGC
GAGACTCTGTCTCAAAAAAAAAAAAAAAAAAAAAAA
AA

3) Synthetized sequence in 5'-3':

TGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCG
GATCACGAGGTCAGGAGATCGAGACCATCCTGGCT
AACACAGTGAAACCCCGTCTCTACTAAAAATACAA
AAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGT
CCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGC
GTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAG
ATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGC
GAGACTCTGTCTCAAAAAAAAAAAAAAAAAAAAAAA
AA

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4) Blastn original sequence/ synthetized sequence

The Table 2 resumes mainly the identities and the e-value between these 2 precedent sequences. Chromatograms were also performed.

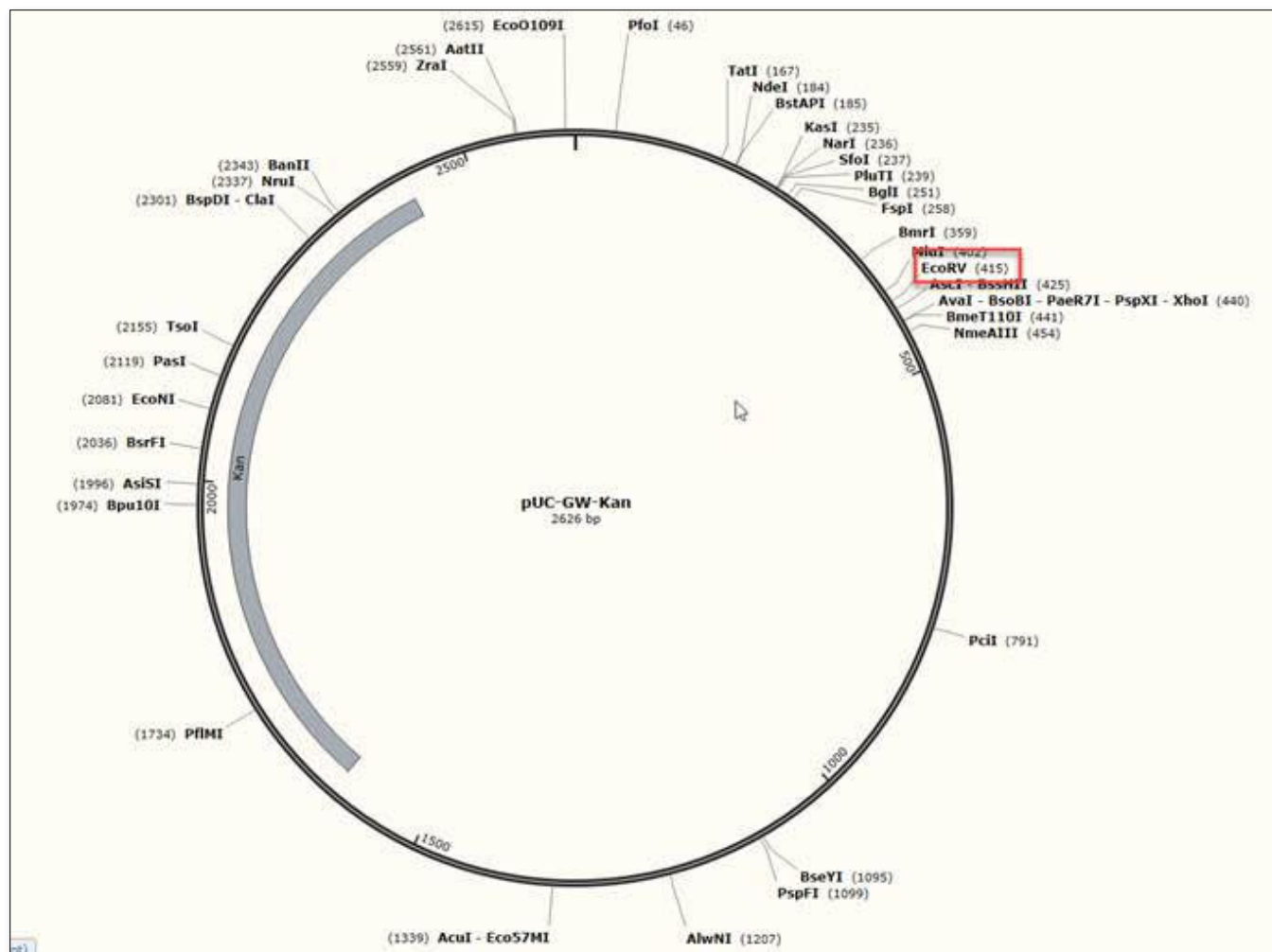


Figure 1. Plasmid map.

Table 2. 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 2. Furthermore, we assert, it is the first time such discovery:

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

REFERENCES

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