

DEVELOPMENT OF A NEW DIAGNOSTIC TOOL (MINI-ARRAY) FOR OSTREID HERPESVIRUS1 (OsHV-1) DETECTION IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS*

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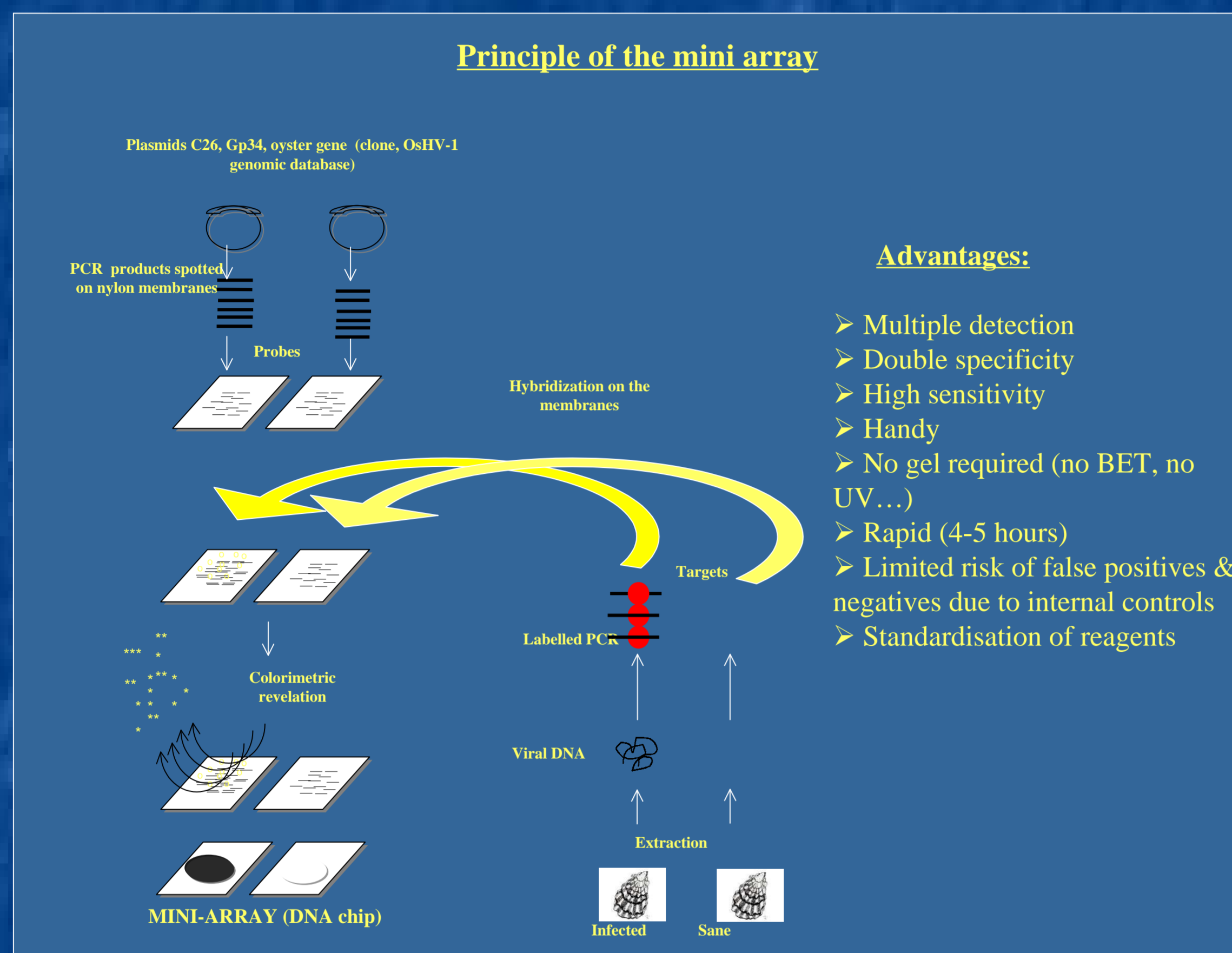
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OsHV-1 was the first herpesvirus to be identified in an invertebrate host and is associated to mortalities in the Pacific oyster (*Crassostrea gigas*) and other economically important bivalve species. Infections due to OsHV-1 are frequently reported and cause high mortality rates in larvae and spat of *C. gigas*. The present work focuses on the development of a new molecular diagnostic tools for OsHV-1 detection: the mini-array.



The mini array method combines target nuclei acid amplification by PCR and a specific hybridisation revealed by a colorimetric reaction on a nylon membrane (DNA chip).

Briefly, following DNA extraction from biological samples (oysters), a multiplex PCR involving specific primers allows amplification and labelling of two herpes virus sequences. Hybridisation on the membranes involves probes designed to specifically match to the PCR products. The detection of the obtained hybrids is performed using enzyme-linked antibodies, producing a blue precipitate (that can be observed with the naked eye) in presence of appropriate substrate.

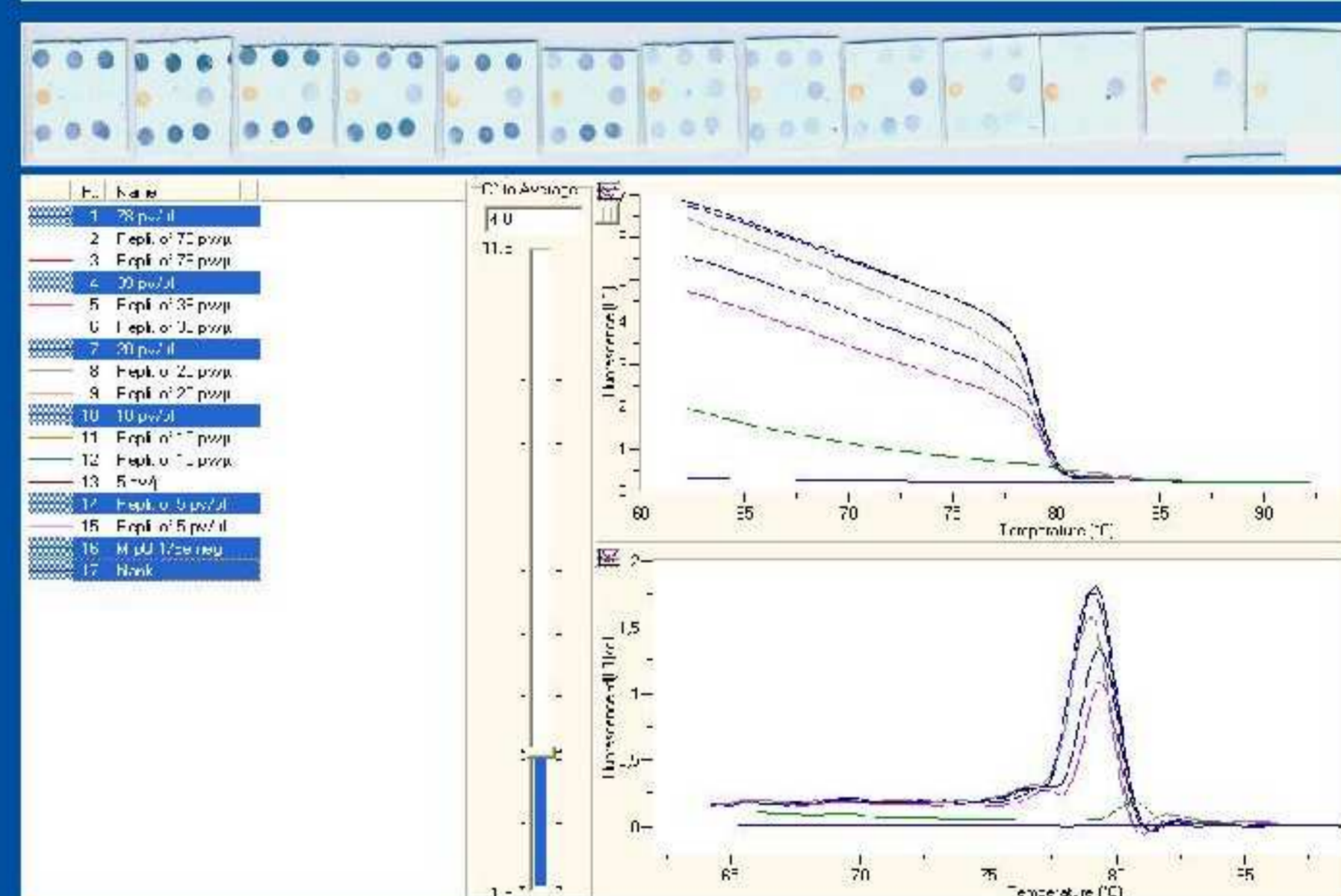
Following the development of the prototype, analytical sensitivity and specificity have been estimated and the efficiency of the mini-array has been assessed by means of a comparison with two other molecular methods developed by Ifremer LGP la Tremblade, namely PCR and real time PCR. The comparison, carried out on two samples populations displaying different prevalence for OsHV-1, revealed a high concordance between the three methods.

Finally, the repeatability of the mini-array has been assessed through a comparison test between the three diagnostic methods, using a pool of OsHV-1 positive samples and a pool of OsHV-1 negative samples. Quantitatively, concordance (between methods) and repeatability (intra assay) were 100%.

Assessment of analytical sensitivity and specificity : miniarray vs q PCR

Viral DNA (purified OsHV-1) copies per µl of sample

10000 5000 2500 1250 625 312 156 78 39 20 10 NEG Blanc



Miniarray

Light Cycler
(q-PCR)

The promotion of an integrated bio-security approach and the development of rapid, reliable and sensitive diagnosis tools that meet the time constraints experienced under hatching conditions, are of the utmost importance for efficient control of diseases in mollusc hatcheries.

The involvement of hatcheries in the control of OsHV-1 using the mini-array would offer producers the possibility to contribute proactively in the early detection of possible adverse conditions for oyster growth and survival before a major problem occurs, contributing to the minimisation of the effects of disease outbreak on their business and subsequently to the enhancement of their competitiveness. Adequate control of larvae and spat from hatcheries in case of intensive rearing in controlled facilities may avoid costly epizooties. Moreover, the availability of an efficient test for detection OsHV-1 may facilitate screening of brood stock, spat and larvae before commercial transactions and therefore constitute a guarantee of product quality for oyster producers.



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