(12) DEMANDE INTERNATIONALE PUBLIÉE EN VERTU DU TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

(19) Organisation Mondiale de la Propriété Intellectuelle
Bureau international

(43) Date de la publication internationale
26 juin 2014 (26.06.2014)

WIPO | PCT

(10) Numéro de publication internationale
WO 2014/096688 A3

(51) Classification internationale des brevets : *C12Q 1/68* (2006.01)

(21) Numéro de la demande internationale : PCT/FR2013/053144

(22) Date de dépôt international : 17 décembre 2013 (17.12.2013)

(25) Langue de dépôt : français

(26) Langue de publication : français

(30) Données relatives à la priorité :
1262140 17 décembre 2012 (17.12.2012) FR

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(81) États désignés *(sauf indication contraire, pour tout titre de protection nationale disponible)* : AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) États désignés *(sauf indication contraire, pour tout titre de protection régionale disponible)* : ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), eurasien (AM, AZ, BY, KG, KZ, RU, TJ, TM), européen (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Déclarations en vertu de la règle 4.17 :

— *relative à la qualité d'inventeur (règle 4.17.iv))*

Publiée :

— *avec rapport de recherche internationale (Art. 21(3))*

— *avec la partie de la description réservée au listage des séquences (règle 5.2.a))*

(88) Date de publication du rapport de recherche internationale : 13 novembre 2014

(54) Title : SIGNATURE PREDICTIVE OF THE BIOMINERALIZATION CAPACITY OF A GRAFT-DONOR PEARL OYSTER

(54) Titre : SIGNATURE PRÉDICTIVE DE LA CAPACITÉ DE BIOMINÉRALISATION D'UNE HUÎTRE PERLIÈRE DONNEUSE DE GREFFONS

(57) Abstract : The present invention relates to a signature predictive of the biomineralization capacity of a graft-donor oyster, said signature comprising the expression profile of at least one biomarker comprising or consisting of a nucleotide sequence selected from SEQ ID NO 1 to SEQ ID NO 42, variants thereof and fragments thereof. Another subject of the invention is a graft-donor pearl oyster with a high biomineralization capacity, characterized in that it has a predictive signature according to the invention. The invention also relates to a biomarker predictive of the biomineralization capacity of a graft-donor pearl oyster, said biomarker being selected from the list consisting of SEQ ID NO 1 to SEQ ID NO 42.

(57) Abrégé : La présente invention concerne une signature prédictive de la capacité de biominéralisation d'une huître donneuse de greffons, ladite signature comprenant le profil d'expression d'au moins un biomarqueur comprenant ou consistant en une séquence nucléotidique sélectionnée parmi SEQ ID NO : 1 à SEQ ID NO : 42, leurs variants et leurs fragments. Un autre objet de l'invention est une huître perlière donneuse de greffons de haute capacité de biominéralisation, caractérisée en ce qu'elle présente une signature prédictive selon l'invention. L'invention concerne également un biomarqueur prédictif de la capacité de biominéralisation d'une huître perlière donneuse de greffons, ledit biomarqueur étant sélectionné parmi la liste consistant en SEQ ID NO : 1 à SEQ ID NO : 42.

WO 2014/096688 A3

SIGNATURE PREDICTIVE OF THE BIOMINERALIZATION CAPACITY OF A GRAFT-DONOR PEARL OYSTER

FIELD OF THE INVENTION

The present invention relates to the field of pearl culture. This invention more specifically relates to a predictive signature of the capacity for biomineralization of a graft-donor pearl oyster. This invention also relates to a pearl oyster with a high capacity for biomineralization comprising the signature according to the invention.

PRIOR ART OF THE INVENTION

Pearl culture is a human activity involving the culture, in a natural environment, of *Pinctada sp.* pearl oysters for the production of cultured pearls. In a first step, the pearl oysters that will be used either for donor oysters or recipient oysters are collected and raised in order to reach the level of development necessary for graft success. Grafting is a surgical operation in which the graft, a portion of the mantle of the donor oyster (around 4 mm^2) is inserted into the pearl pouch of the recipient oyster, in combination with a nacre bead, the nucleus. Once inserted in the recipient pearl oyster, the mineralising epithelial border of the graft multiplies and lines the pearl pouch so as to form the pearl sac, which surrounds the nucleus. Said pearl sac then deposits layers of nacre around the nucleus, thus forming the pearl (Cochennec-Laureau et al, Aquatic Living Resource, 2010: 23, 131-140).

It is estimated that only 3 to 5%, on average, of the pearls produced are very high quality. Most pearls have surface defects, a non-homogeneous colour or an too thin nacre thickness. Because of the economic importance of pearl culture, in particular in French Polynesia, there is a need for tools or methods enabling the percentage of high-quality pearls produced to be increased.

To identify donor or recipient pearl oysters having specific properties enabling high-quality pearls to be produced, genetic, and in particular genomic and transcriptomic approaches, have been used.

The work of Wada and Komaru (Aquaculture, 1996, 142: 25-32) has thus made it possible to observe a "donor effect" corresponding to an influence of the donor pearl oyster on the characteristics of pearls, and in particular on their colour and lustre.

The American patent US 7 163 795 describes a method for identifying oysters capable of expressing the protein nacrein (a protein constituting the nacre and potentially involved in the biomineralization process), which may be used in pearl culture. This patent also describes a method for identifying the optimal conditions for the formation of the pearl, said method comprising the analysis of the expression of nacrein. However, as pearl culture is traditionally performed on the open sea, it appears to be difficult to adapt the culture conditions.

In two successive studies, Inoue et al. described the relationship between the quality of the pearl produced and the gene expression profile of six genes involved in biomineralization in the pearl sac (Inoue et al., Zoological Science, 2011, 28: 32-6; Inoue et al., Mar Biotechnol., 2011, 13: 48-55). The results presented by Inoue et al. constitute the first demonstration that the expression level of a gene involved in the process of biomineralization may be correlated with the quality of the pearl obtained.

In 2011, Kinoshita et al. published the results of a large-scale study on the identification of genes involved in biomineralization in *Pinctada fucata*, by a transcriptomic approach (Kinoshita et al., PLoS One, 2011; 6(6):e21238). However, this study does not establish a connection between gene expression and pearl quality.

In order to identify graft-donor pearl oysters with a high capacity for biomineralization, the inventors conducted a large-scale transcriptomic and proteomic study, and analysed the relationship between (1) the gene expression profile and (2) the quality and growth (nacre thickness) of the pearl during experimental grafting. This study enabled them to identify a particular gene signature that is predictive of pearl quality.

SUMMARY

This invention therefore relates to a predictive signature of the capacity for biomineralization of a graft-donor oyster, said signature comprising the expression profile of at least two biomarkers comprising or consisting of a nucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

According to an embodiment, said signature is predictive for the commercial quality of the pearls obtained and/or the rate of nacre deposition around the nucleus using said donor oyster.

This invention also relates to a predictive signature of the commercial quality of pearls obtained by using a donor oyster and/or the rate of nacre deposition around the nucleus using said donor oyster, said signature comprising the expression profile in the mantel of said donor oyster of at least one biomarker comprising or consisting of a nucleotide sequence selected from among SEQ ID NO: 1, 4, 5, 8, 11, 14, 18, 23, 26, 27, 28, 36, 39, 40 and 42, variants and fragments thereof.

According to an embodiment, the signature of the invention comprises the expression profile of at least one nucleotide sequence selected from SEQ ID NO: 3, 4, 5, 11 and 14, variants and fragments thereof, and said signature is predictive of the commercial quality of the pearls obtained using said donor oyster.

According to an embodiment, the signature of the invention comprises the expression profile of at least one nucleotide sequence selected from SEQ ID NO: 8, 18, 23, 26, 27, 36, 39 or 40, variants and fragments thereof, and said signature is predictive of the rate of nacre deposition around the nucleus using said donor oyster.

According to an embodiment, the signature of the invention comprises the expression profile of at least one nucleotide sequence selected from SEQ ID NO: 28 or 42, variants and fragments thereof, and said signature is also predictive of the number of surface defects of the pearls obtained using said donor oyster.

The invention also relates to a graft-donor oyster with a high capacity for biomineralization, characterised in that it has a predictive signature as defined above.

This invention also relates to a process for identification of a donor oyster according to the invention, in which:

(a) in a biological sample of said oyster, the expression level of at least two predictive biomarkers as described above is determined, and

(b) the expression level obtained in step (a) is compared with a control expression level.

This invention also relates to a process for selection of a donor oyster according to the invention, in which:

(a) in a biological sample of said oyster, the expression level of at least two predictive biomarkers as described above is determined, and

(b) the expression level obtained in step (a) is compared with a control expression level.

The invention also relates to a kit enabling the process according to the invention to be implemented, comprising means for determining the expression profile of at least one biomarker as described in the present invention. According to a first embodiment, said means make it possible to determine the expression profile at the transcriptomic level by RT-PCR, RT-qPCR and high-speed quantitative PCR. According to a second embodiment, said means make it possible to determine the expression profile at the protein level.

The invention also relates to a graft characterised in that it has a predictive signature as defined above, said graft being a portion of the epithelium of the mantle of a donor oyster according to the invention.

The invention also relates to a recipient oyster grafted with a graft as defined above, in which a nucleus is present.

The invention also relates to a process for producing at least one higher-quality pearl, comprising the culture of at least one recipient oyster according to the invention, said oyster comprising the predictive signature of the invention and/or having been identified according to the oyster identification and/or selection process according to the invention; as well as the pearl obtained by said process.

The invention also relates to a biomarker predictive of the capacity for biomineralization of a graft-donor oyster, said biomarker comprising or consisting of a nucleotide sequence selected from the list consisting of SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

The invention also relates to the use of at least one biomarker comprising or consisting of a nucleotide sequence selected from the list consisting of SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof, in order to determine the capacity for biomineralization of a graft-donor oyster. According to an embodiment, the biomarker is selected from the list consisting of SEQ ID NO: 1, 4, 5, 8, 11, 14, 18, 23, 26, 27, 28, 36, 39, 40 and 42, variants and fragments thereof.

The invention also relates to the use of at least one biomarker selected from the sequences SEQ ID NO: 3, 4, 5, 11 and 14, preferably the sequence SEQ ID NO: 4, in order to predict the capacity of a graft-donor oyster to produce high-quality commercial pearls.

The invention also relates to the use of at least one biomarker selected from the sequences SEQ ID NO: 28 and SEQ ID NO: 42, preferably the sequence SEQ ID NO: 28, in order to predict the capacity of a graft-donor oyster to produce pearls having a small number of surface defects.

The invention also relates to the use of at least one biomarker selected from the sequences SEQ ID NO: 8, 18, 23, 26, 27, 36, 39 and 40, in order to predict the capacity of a graft-donor oyster to produce pearls having a small number of surface defects.

DEFINITIONS

In this invention, the following terms have the following meanings:

The term "pearl culture" refers to a human activity intended for producing cultured pearls from pearl oysters, generally belonging to the genus *Pinctada sp.* According to an embodiment of the invention, the oyster belongs to the genus *Pinctada sp.*, preferably to the species *Pinctada fucata, Pinctada maxima* or *Pinctada margaritifera.*

The term "biomarker predictive of the high capacity for biomineralization of a donor pearl oyster", (hereinafter referred to as "predictive biomarker"), means a nucleotide sequence, preferably a gene, of which the expression profile in a donor oyster is predictive of the high capacity for biomineralization of said donor pearl oyster, and consequently is predictive of the quality of the pearls produced using said pearl oyster as a graft donor. According to an embodiment of the invention, the predictive biomarker of the invention comprises or consists of a nucleotide sequence selected from the 42 nucleotide sequences of Table 1 below, variants and fragments thereof:

Identifier	**Name**	**Accession Number (GeneBank)**
SEQ ID NO: 1	CLEC2-2	
SEQ ID NO: 2	C3	
SEQ ID NO: 3	CALC-1	HQ654770
SEQ ID NO: 4	CLEC3-1	
SEQ ID NO: 5	DERM-2	
SEQ ID NO: 6	C9	
SEQ ID NO: 7	1	
SEQ ID NO: 8	MUCO-2	
SEQ ID NO: 9	C88bis	
SEQ ID NO: 10	shem5-1	HE610376
SEQ ID NO: 11	shem7-2	
SEQ ID NO: 12	C85	
SEQ ID NO: 13	C14	
SEQ ID NO: 14	C18	
SEQ ID NO: 15	C22	
SEQ ID NO: 16	Protinh3-1	HE610400
SEQ ID NO: 17	C2	
SEQ ID NO: 18	C46	
SEQ ID NO: 19	Cbind-3	
SEQ ID NO: 20	C66ter	
SEQ ID NO: 21	C17	
SEQ ID NO: 22	PLW-2	
SEQ ID NO: 23	PIF-2	HE610401
SEQ ID NO: 24	46	
SEQ ID NO: 25	C6	
SEQ ID NO: 26	C26bis	
SEQ ID NO: 27	C54(C53bis)	
SEQ ID NO: 28	mp11-2	HE610374
SEQ ID NO: 29	PMMG1-2	
SEQ ID NO: 30	73	
SEQ ID NO: 31	C23	
SEQ ID NO: 32	C1	

SEQ ID NO: 33	MNK-1	
SEQ ID NO: 34	PLC-2	
SEQ ID NO: 35	mp9-2	
SEQ ID NO: 36	C75bis	
SEQ ID NO: 37	13	
SEQ ID NO: 38	C35bis	
SEQ ID NO: 39	2	
SEQ ID NO: 40	mp2-2	
SEQ ID NO: 41	C8	
SEQ ID NO: 42	Protinh2-1	HE610399

Table 1

According to an embodiment of the invention, the predictive biomarker of the invention comprises or consists of a nucleotide sequence selected from the following 15 nucleotide sequences, variants and fragments thereof: CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), MUCO-2 (SEQ ID NO: 8), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), mp11-2 SEQ ID NO: 28), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40), and Protinh2-1 (SEQ ID NO: 42).

According to an embodiment of the invention, the fragments are nucleic acid sequences of more than 25 nucleotides, preferably of more than 50, 100, 150, 200 or of more than 500 nucleotides. According to an embodiment of the invention, a fragment of a sequence SEQ ID NO: X corresponds to a nucleotide sequence of more than 25 contiguous nucleotides, preferably of more than 50, 100, 150, 200 or of more than 500 contiguous nucleotides of SEQ ID NO: X.

According to an embodiment, the phrase "variant of a sequence SEQ ID NO: X" means any nucleic acid sequence comprising more than 25 nucleotides, preferably more than 50, 100, 150, 200 or more than 500 contiguous nucleotides of a nucleotide acid sequence SEQ ID NO: X.

According to another embodiment of the invention, the phrase "variant of a sequence SEQ ID NO: X" means a sequence comprising the sequence SEQ ID NO: X between 1

and 500, preferably 1 and 200, more preferably 1 and 100 additional nucleic acids in 5' and/or in 3'.

According to another embodiment of the invention, the phrase "variant of a sequence SEQ ID NO: X" means a sequence having minor structural modifications and preserving the function of SEQ ID NO: X. Examples of such minor structural modifications include, without being limited to, deletions, substitutions or additions of bases, affecting 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleic acids.

According to another embodiment of the invention, the phrase "variant of a sequence SEQ ID NO: X" means a nucleotide sequence comprising more than 25, preferably more than 50, 100, 150, 200, 300, 400, 500, 1000, 1500, 2000 or 3000 nucleotides having an identity of more than 75%, 80%, 90%, 95% or more than 96%, 97%, 98% or 99% with the sequence SEQ ID NO: X.

In the sense of this invention, the term "identity", when used to refer to the relationship between the sequences of two or more nucleotide sequences, refers to the degree of similarity between said nucleotide sequences, as determined by the number of correspondences between the chains of two or more bases.

According to the invention, the "identity" corresponds to a percentage of identity between two (or more than 2) sequences. This percentage is defined as the number of positions for which the bases are identical when the sequences are optimally aligned, divided by the total number of bases of the smallest of the 2 sequences. The differences between the sequences can be randomly distributed and over their entire lengths.

Two sequences are said to be optimally aligned when the percentage of identity is maximal. In addition, as will be more clear to a person skilled in the art, it may be necessary to use additions of gap positions so as to obtain an optimal alignment between the two sequences. The percentage of identity between two nucleic acid sequences can therefore be determined by comparing said two optimally aligned sequences in which the nucleic acid sequence to be compared may comprise additions or deletions with respect to the reference sequence for an optimal alignment between said two sequences. The percentage of identity is then calculated by determining the number of identical

positions for which the nucleotide is identical between the two sequences, by dividing said number of identical positions by the total number of positions in the comparison window and by multiplying the result obtained by 100 so as to obtain the percentage of identity between said two sequences.

Preferably, the methods for determining identity are designed so as to have the greatest possible match between the sequences compared.

The percentage of identity can be determined by a particular mathematical model or by a computer program (generally designated by the term "algorithm"). Methods for calculating the identity between nucleotide sequences are well known to a person skilled in the art. Non-limiting examples of such methods include those described in the following documents: Computations Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data Part 1, Griffin, A. M., and Griffin H. G., eds. Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Grivskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., SIAM J. Applied Math., 48, 1073 (1988).

Methods for determining identity have been described in computer programs accessible to the public. Preferred examples of methods using computer programs include, without being limited to, the software package GCG, comprising GAP (Devereux et al., Nucl. Acid. Res. 2, 387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol. 215, 403-410 (1990)). The BLASTX program is available at the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., supra). The algorithm of Smith-Waterman, which is well known to a person skilled in the art, can also be used to determine the percentage of identity between two sequences.

The term "predictive signature" refers to a particular gene expression profile, the presence of which in a donor pearl oyster is predictive of the high capacity for

biomineralization of said donor oyster, and consequently is predictive of the quality of the pearls produced and/or the rate of nacre deposition around the nucleus using said donor oyster. According to an embodiment of the invention, the predictive signature of the invention comprises 1 to 42 predictive biomarkers as described above.

The phrase "donor oyster with a high capacity for biomineralization" refers to a graft-donor oyster enabling higher-quality pearls to be obtained, and/or higher-quality pearls to be obtained more quickly. Thus, according to an embodiment, a donor oyster with a high capacity for biomineralization enables a pearl to be obtained having a nacre thickness around the nucleus that is greater than or equal to 0.8 mm, preferably greater than or equal to 1.5 mm, in less than 24 months, preferably in less than 21 months, more preferably in less than 18 months. According to an embodiment of the invention, the donor oyster with a high capacity for biomineralization of the invention leads to 100% of the pearls obtained having a nacre thickness greater than 0.8 mm after 18 months of culture, and/or to 30.2% of the pearls obtained having a nacre thickness greater than 1.5 mm after 18 months of culture. By comparison, in the absence of selection of donor oysters, these percentages are respectively 80.9 and 13.4%.

The term "higher-quality pearls" refers to a pearl having a characteristic of nacre thickness around the nucleus (obtaining a nacre deposit around the nucleus that is greater within the same culture time), and/or a number of surface defects and/or a higher or lower commercial quality with respect to predetermined thresholds. In the context of this invention, the quality of a pearl may be evaluated by 3 variables: a "nacre thickness" variable, a "number of defects at the surface of the pearls" variable, and a "commercial quality" variable. According to the invention, a donor oyster with a high capacity for biomineralization is an oyster having at least one predictive biomarker according to the invention and/or at least one predictive signature of the invention.

According to an embodiment of the invention, the thickness of the nacre of a pearl corresponds to the thickness in millimetres of nacre around the nucleus of the pearl. This may be measured, for example, by subtracting the diameter of the nucleus from the smallest diameter of the pearl and dividing the difference in diameter by 2. According to an embodiment of the invention, the diameters of the pearls and the nuclei may be

measured by computation on the basis of scanned pearl and nucleus images. A non-limiting example of analysis software that may be used to measure the size of the pearls and the nucleus is ImagJ software. According to an embodiment of the invention, a higher-quality pearl has a nacre thickness of more than 0.8 mm, preferably of more than 1.2 mm, and even more preferably of more than 1.5 mm.

According to an embodiment of the invention, the estimation of the quality of pearls on the basis of the number of defects corresponds to the classification of each pearl in a classification system containing 4 classes, based on the number of defects observed at their surface. The defects considered for this classification are pits, blisters, and spots. According to an embodiment of the invention, the first class comprises pearls without defects; the second class comprises pearls having 1 to 5 defects; the third class comprises pearls having more than 5 defects and the fourth class comprises pearls that are entirely covered with defects. According to an embodiment of the invention, a higher-quality pearl belongs to the first class relative to the number of defects, i.e. does not have any defects.

According to an embodiment, the commercial quality of the pearl is assessed according to the surface state (presence or absence of imperfections) and the lustre (brilliance or brightness) of the pearl, in accordance with the definition provided in Deliberation no. 2005-42 of 4 February 2005 (Official Journal of French Polynesia). According to this deliberation, the assessment of the quality of the pearl corresponds to the classification of pearls into 5 quality categories, class A through class D and the "Reject" class. According to an embodiment of the invention, a higher-quality pearl belongs to class A.

In the sense of this invention, the term "around", placed in front of a number, means more or less 10% of the nominal value of said number.

DETAILED DESCRIPTION

This invention relates to a donor pearl oyster with a high capacity for biomineralization.

According to an embodiment of the invention, donor oysters with a high capacity for biomineralization make it possible to obtain, in less than 24 months of culture, preferably in les than 21 months of culture, more preferably in less than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, or even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm, preferably greater than or equal to around 1.2 mm, more preferably greater than or equal to around 1.5 mm.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain 100% pearls having a nacre thickness greater than or equal to around 0.8 mm, after a culture period of around 15 months, preferably of around 16 months, and more preferably of around 18 months.

According to another embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain more than around 30% pearls having a nacre thickness greater than or equal to around 1.5 mm, after a culture period of around 15 months, preferably of around 16 months, and more preferably of around 18 months.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain more than 5%, preferably more than 10%, more preferably more than 15, 20, 25, 30, 40, 50, 60, 70, 80, 90% pearls having no surface defect. According to an embodiment of the invention the donor oysters with a high capacity for biomineralization make it possible to obtain around 10 to 20%, preferably around 12.5 to 17.5%, more preferably around 14.3% pearls having no surface defects. By comparison, the inventors have demonstrated that the graft-donor oysters normally used make it possible to obtain only around 5.2% pearls having no surface defects.

According to an embodiment of the invention, the donor oysters having a high capacity for biomineralization make it possible to obtain more than 5%, preferably more than 10%, 20%, more preferably more than 30, 40, 50, 60, 70, 80, 90% pearls of category A

and/or B. According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain around 10 to 50%, preferably around 20 to 30%, more preferably around 24.3% pearls of category A. According to another embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain around 25 to 70%, preferably around 40 to 60%, more preferably around 51.4% pearls of category A or B. By comparison, the inventors have demonstrated that the graft-donor oysters normally used make it possible to obtain only around 4.6% pearls of category A and around 19.5% pearls of category A or B.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization (1) make it possible to obtain, in less than 24 months of culture, preferably in less than 21 months of culture, more preferably in less than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, or even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm, and (2) make it possible to obtain more than 5%, preferably more than 10%, more preferably more than 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% pearls having no surface defect.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization (1) make it possible to obtain, in less than 24 months of culture, preferably in less than 21 months of culture, more preferably in less than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, or even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm, and (2) make it possible to obtain more than 5%, preferably more than 10, 20, 30, 40, 50, 60, 70, 80 or 90% pearls of category A and/or B.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain (1) more than 5%, preferably more than 10%, more preferably more than 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% pearls having no surface defects, and (2) more than 5%, preferably more than 10, 20, 30, 40, 50, 60, 70, 80 or 90% pearls of category A and/or B.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization (1) make it possible to obtain, in less than 24 months of culture, preferably in less than 21 months of culture, more preferably in less than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, or even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm; and (2) make it possible to obtain more than 5%, preferably more than 10%, more preferably more than 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% pearls having no surface defect, and (3) make it possible to obtain more than 5%, preferably more than 10, 20, 30, 40, 50, 60, 70, 80 or 90% pearls of category A and/or B.

According to this invention, the donor oyster with a high capacity for biomineralization comprises a predictive biomarker of the quality of biomineralization, and/or a predictive signature of the quality of biomineralization.

This invention therefore also relates to a predictive biomarker of the capacity for biomineralization of an oyster.

This invention therefore also relates to a predictive signature of the capacity for biomineralization of a graft-donor oyster.

According to the invention, the predictive biomarker of the capacity for biomineralization of an oyster is a nucleotide sequence comprising or consisting of a nucleotide sequence selected from the list comprising sequences SEQ ID NO: 1 to SEQ ID NO: 42, presented in Table 1, variants and fragments thereof. According to an embodiment of the invention, the predictive biomarker of the invention comprises or consists of a nucleotide sequence selected from the following 15 nucleotide sequences, variants and fragments thereof: CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), MUCO-2 (SEQ ID NO: 8), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), mp11-2 SEQ ID NO: 28), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40), and Protinh2-1 (SEQ ID NO: 42).

According to the invention, the predictive signature of the invention comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from the list comprising sequences SEQ ID NO: 1 to SEQ ID NO: 42 presented in Table 1, variants and fragments thereof. According to an embodiment of the invention, the predictive signature of the invention comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from the following 15 nucleotide sequences, variants and fragments thereof: CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), MUCO-2 (SEQ ID NO: 8), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), mp11-2 SEQ ID NO: 28), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40), and Protinh2-1 (SEQ ID NO: 42).

According to an embodiment of the invention, the predictive signature of the invention comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 or 42 nucleotide sequences comprising or consisting of a nucleotide sequence selected from the list comprising sequences SEQ ID NO: 1 to SEQ ID NO: 42 presented in Table 1, variants and fragments thereof. According to an embodiment of the invention, the predictive signature of the invention comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 nucleotide sequences comprising or consisting of a nucleotide sequence selected from the following 15 nucleotide sequences, variants and fragments thereof: CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), MUCO-2 (SEQ ID NO: 8), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), mp11-2 SEQ ID NO: 28), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40), and Protinh2-1 (SEQ ID NO: 42).

According to an embodiment, the donor oyster with a high capacity for biomineralization according to the invention makes it possible to obtain, in less than 24 months of culture, preferably in less than 21 months of culture, more preferably in less

than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, or even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm, preferably greater than or equal to around 1.2 mm, more preferably greater than or equal to around 1.5 mm.

According to this embodiment, the predictive biomarker is predictive of the nacre thickness around the nucleus, and comprises or consists of a nucleotide sequence selected from the nucleotide sequences of Table 2, variants and fragments thereof.

Identifier	Name
SEQ ID NO: 1	CLEC2-2
SEQ ID NO: 2	C3
SEQ ID NO: 3	CALC-1
SEQ ID NO: 4	CLEC3-1
SEQ ID NO: 5	DERM-2
SEQ ID NO: 6	C9
SEQ ID NO: 7	1
SEQ ID NO: 8	MUCO-2
SEQ ID NO: 9	C88bis
SEQ ID NO: 10	shem5-1
SEQ ID NO: 11	shem7-2
SEQ ID NO: 12	C85
SEQ ID NO: 13	C14
SEQ ID NO: 14	C18
SEQ ID NO: 15	C22
SEQ ID NO: 16	Protinh3-1
SEQ ID NO: 17	C2
SEQ ID NO: 18	C46
SEQ ID NO: 19	Cbind-3
SEQ ID NO: 20	C66ter
SEQ ID NO: 21	C17
SEQ ID NO: 22	PLW-2
SEQ ID NO: 23	PIF-2
SEQ ID NO: 24	46
SEQ ID NO: 25	C6
SEQ ID NO: 26	C26bis
SEQ ID NO: 27	C54(C53bis)
SEQ ID NO: 28	mp11-2
SEQ ID NO: 29	PMMG1-2
SEQ ID NO: 30	73
SEQ ID NO: 31	C23
SEQ ID NO: 32	C1

SEQ ID NO: 33	MNK-1
SEQ ID NO: 34	PLC-2
SEQ ID NO: 35	mp9-2
SEQ ID NO: 36	C75bis
SEQ ID NO: 37	13
SEQ ID NO: 38	C35bis
SEQ ID NO: 39	2
SEQ ID NO: 40	mp2-2
SEQ ID NO: 41	C8

Table 2

According to an embodiment, the predictive biomarker is predictive of the nacre thickness around the nucleus, and comprises or consists of a nucleotide sequence selected from the following nucleotide sequences, variants and fragments thereof: MUCO-2 (SEQ ID NO: 8), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40).

According to this embodiment, the predictive signature is predictive of the nacre thickness around the nucleus and comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from Table 2, variants and fragments thereof.

According to an embodiment, the predictive signature comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 2, variants and fragments thereof.

According to an embodiment, the predictive signature comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8 nucleotide sequences selected from the following nucleotide sequences, variants and fragments thereof: MUCO-2 (SEQ ID NO: 8), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40).

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain more than 5%, preferably more than 10%, more preferably more than 15, 20, 25, 30, 40, 50, 60, 70, 80, 90% pearls having no surface defects.

According to this embodiment, the predictive biomarker is predictive of the number of defects at the surface of the pearl, and comprises or consists of a nucleotide sequence selected from the nucleotide sequences of Table 3, variants and fragments thereof.

Identifier	Gene name
SEQ ID NO: 1	CLEC2-2
SEQ ID NO: 2	C3
SEQ ID NO: 3	CALC-1
SEQ ID NO: 4	CLEC3-1
SEQ ID NO: 5	DERM-2
SEQ ID NO: 6	C9
SEQ ID NO: 7	1
SEQ ID NO: 8	MUCO-2
SEQ ID NO: 9	C88bis
SEQ ID NO: 21	C17
SEQ ID NO: 22	PLW-2
SEQ ID NO: 23	PIF-2
SEQ ID NO: 24	46
SEQ ID NO: 25	C6
SEQ ID NO: 26	C26bis
SEQ ID NO: 27	C54(C53bis)
SEQ ID NO: 28	mp11-2
SEQ ID NO: 42	Protinh2-1

Table 3

According to this embodiment, the predictive biomarker is predictive of the number of defects at the surface of the pearl, and comprises or consists of a nucleotide sequence selected from the nucleotide sequences mp11-2 (SEQ ID NO: 28) and Protinh2-1 (SEQ ID NO: 42).

According to this embodiment, the predictive signature is predictive of the number of defects and comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from Table 3, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the number of defects and comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 3, the variants or the fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the number of surface defects and comprises the expression profile of 1 or 2 nucleotide sequences selected form the nucleotide sequences mp11-2 (SEQ ID NO: 28) and Protinh2-1 (SEQ ID NO: 42), variants and fragments thereof.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain more than 5%, preferably more than 10%, 20%, more preferably more than 30, 40, 50, 60, 70, 80, 90% pearls of category A and/or B.

According to this embodiment, the predictive biomarker is predictive of the commercial quality of the pearl, and comprises or consists of a nucleotide sequence selected from the nucleotide sequences of Table 4, variants and fragments thereof.

Identifier	**Gene name**
SEQ ID NO: 1	CLEC2-2
SEQ ID NO: 2	C3
SEQ ID NO: 3	CALC-1
SEQ ID NO: 4	CLEC3-1
SEQ ID NO: 5	DERM-2
SEQ ID NO: 6	C9
SEQ ID NO: 7	1
SEQ ID NO: 8	MUCO-2
SEQ ID NO: 9	C88bis
SEQ ID NO: 10	shem5-1
SEQ ID NO: 11	shem7-2
SEQ ID NO: 12	C85
SEQ ID NO: 13	C14
SEQ ID NO: 14	C18
SEQ ID NO: 15	C22
SEQ ID NO: 16	Protinh3-1
SEQ ID NO: 17	C2
SEQ ID NO: 18	C46
SEQ ID NO: 19	Cbind-3

SEQ ID NO: 20	C66ter
SEQ ID NO: 28	mp11-2

Table 4

According to this embodiment, the predictive biomarker is predictive of the commercial quality of the pearl, and comprises or consists of a nucleotide sequence selected from the nucleotide sequences CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), variants and fragments thereof.

According to this embodiment, the predictive signature is predictive of the commercial quality of the pearl and comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from Table 4, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the commercial quality of the pearl and comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 4, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the commercial quality of the pearl and comprises the expression profile of 1 or a combination of 2, 3, 4, 5 nucleotide sequences selected from CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), variants and fragments thereof.

According to another embodiment of the invention, the donor oysters with a high capacity for biomineralization (1) make it possible to obtain, in less than 24 months of culture, preferably in less than 21 months, more preferably in less than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm, and (2) make it

possible to obtain more than 5%, preferably more than 10%, more preferably more than 15, 20, 25, 30, 40, 50, 60, 70, 80, 90% pearls having no surface defects.

According to this embodiment of the invention, the predictive biomarker is predictive of the nacre thickness around the nucleus and the number of defects at the surface of the pearl and comprises or consists of a nucleotide sequence selected from the nucleotide sequences of Table 5, variants and fragments thereof.

Identifier	Gene name
SEQ ID NO: 1	CLEC2-2
SEQ ID NO: 2	C3
SEQ ID NO: 3	CALC-1
SEQ ID NO: 4	CLEC3-1
SEQ ID NO: 5	DERM-2
SEQ ID NO: 6	C9
SEQ ID NO: 7	1
SEQ ID NO: 8	MUCO-2
SEQ ID NO: 9	C88bis
SEQ ID NO: 21	C17
SEQ ID NO: 22	PLW-2
SEQ ID NO: 23	PIF-2
SEQ ID NO: 24	46
SEQ ID NO: 25	C6
SEQ ID NO: 26	C26bis
SEQ ID NO: 27	C54(C53bis)

Table 5

According to this embodiment, the predictive signature is predictive of the nacre thickness around the nucleus and the number of defects at the surface of the pearl and comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from Table 5, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the nacre thickness around the nucleus and the number of defects at the surface of the pearl and comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 5, variants and fragments thereof.

According to another embodiment, the predictive signature is predictive of the nacre thickness around the nucleus and the number of defects at the surface of the pearl and comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 2, variants and fragments thereof; and (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 3, the variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the nacre thickness around the nucleus and the number of defects at the surface of the pearl and comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5, 6, 7, 8 nucleotide sequences comprising or consisting of a nucleotide sequence selected from MUCO-2 (SEQ ID NO: 8), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40), the variants and fragments thereof; and (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or 2 nucleotide sequences selected from the nucleotide sequences mp11-2 (SEQ ID NO: 28) and Protinh2-1 (SEQ ID NO: 42), variants and fragments thereof.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization (1) make it possible to obtain, in less than 24 months of culture, preferably in less than 21 months of culture, more preferably in less than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, or even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm, and (2) make it possible to obtain more than 5%, preferably more than 10, 20, 30, 40, 50, 60, 70, 80 or 90% pearls of category A and/or B,

According to this embodiment of the invention, the predictive biomarker is predictive of the thickness and the commercial quality of the pearl and comprises or consists of a nucleotide sequence selected from the nucleotide sequences of Table 6, variants and fragments thereof.

Identifier	Gene name
SEQ ID NO: 1	CLEC2-2
SEQ ID NO: 2	C3
SEQ ID NO: 3	CALC-1
SEQ ID NO: 4	CLEC3-1
SEQ ID NO: 5	DERM-2
SEQ ID NO: 6	C9
SEQ ID NO: 7	1
SEQ ID NO: 8	MUCO-2
SEQ ID NO: 9	C88bis
SEQ ID NO: 10	shem5-1
SEQ ID NO: 11	shem7-2
SEQ ID NO: 12	C85
SEQ ID NO: 13	C14
SEQ ID NO: 14	C18
SEQ ID NO: 15	C22
SEQ ID NO: 16	Protinh3-1
SEQ ID NO: 17	C2
SEQ ID NO: 18	C46
SEQ ID NO: 19	Cbind-3
SEQ ID NO: 20	C66ter

Table 6

According to this embodiment, the predictive signature is predictive of the thickness and the commercial quality of the pearl and comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from Table 6, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the thickness and the commercial quality of the pearl and comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 6, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the thickness and the commercial quality of the pearl and comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 2, variants and fragments thereof; and (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 4, variants and fragments thereof.

According to another embodiment, the predictive signature is predictive of the thickness and the commercial quality of the pearl and comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5, 6, 7, 8 nucleotide sequences comprising or consisting of a nucleotide sequence selected from MUCO-2 (SEQ ID NO: 8), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40), variants and fragments thereof; and (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5 nucleotide sequences selected from CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), variants and fragments thereof.

According to another embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible to obtain (1) more than 5%, preferably more than 10%, more preferably 15, 20, 25, 30, 40, 50, 60, 70, 80, 90% pearls having no surface defect, and (2) more than 5%, preferably more than 10, 20, 30, 40, 50, 60, 70, 80, 90% pearls of category A and/or B.

According to this embodiment of the invention, the predictive biomarker is predictive of the number of defects at the surface of the pearl and the commercial quality of the pearl

and comprises or consists of a nucleotide sequence selected from the nucleotide sequences of Table 7, variants and fragments thereof.

Identifier	Name
SEQ ID NO: 1	CLEC2-2
SEQ ID NO: 2	C3
SEQ ID NO: 3	CALC-1
SEQ ID NO: 4	CLEC3-1
SEQ ID NO: 5	DERM-2
SEQ ID NO: 6	C9
SEQ ID NO: 7	1
SEQ ID NO: 8	MUCO-2
SEQ ID NO: 9	C88bis
SEQ ID NO: 28	mp11-2

Table 7

According to this embodiment of the invention, the predictive signature is predictive of the number of defects and the commercial quality of the pearl and comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected form Table 7, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the thickness and the commercial quality of the pearl and comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 7, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the number of defects and the commercial quality of the pearl and comprises the expression profile comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence or 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 3, variants and fragments thereof; and (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence or 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 4, the variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the number of defects and the commercial quality of the pearl and comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or 2 nucleotide sequences selected from the nucleotide sequences mp11-2 (SEQ ID NO: 28) and Protinh2-1 (SEQ ID NO: 42), variants and fragments thereof; and (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5 nucleotide sequences selected from CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), variants and fragments thereof.

According to an embodiment of the invention, the donor oysters with a high capacity for biomineralization make it possible (1) to obtain, in un less than der 24 months of culture, preferably in less than 21 months of culture, more preferably in less than 18 months of culture, more than around 25%, preferably around 30%, more preferably more than around 40%, 50%, 60%, 70%, 80%, or even more preferably more than around 95% pearls having a nacre thickness greater than or equal to around 0.8 mm, and (2) make it possible to obtain more than 5%, preferably more than 10%, more preferably more than 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% pearls having no surface defect, and (3) make it possible to obtain more than 5%, preferably more than 10, 20, 30, 40, 50, 60, 70, 80, 90% pearls of category A and/or B.

According to this embodiment of the invention, the predictive biomarker is predictive of the thickness, the number of defects at the surface and the commercial quality of the pearl, and comprises or consists of a nucleotide sequence selected from the nucleotide sequences of Table 8, variants and fragments thereof.

Identifier	Gene name
SEQ ID NO: 1	CLEC2-2
SEQ ID NO: 2	C3
SEQ ID NO: 3	CALC-1
SEQ ID NO: 4	CLEC3-1
SEQ ID NO: 5	DERM-2
SEQ ID NO: 6	C9
SEQ ID NO: 7	1

SEQ ID NO: 8	MUCO-2
SEQ ID NO: 9	C88bis

Table 8

According to this embodiment of the invention, the predictive signature is predictive of the nacre thickness around the nucleus, the number of defects and the commercial quality of the pearl and comprises the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from Table 8, variants and fragments thereof.

According to another embodiment of the invention, the predictive signature is predictive of the nacre thickness around the nucleus, the number of defects and the commercial quality of the pearl and comprises the expression profile of 1 or a combination of 2, 3, 4, 5, 6, 7, 8 or 9 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 8, variants and fragments thereof.

According to this embodiment, the predictive signature is predictive of the number of defects, the thickness and the commercial quality of the pearl and comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence or 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 2, variants and fragments thereof; (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence or 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 3, variants and fragments thereof; and (3) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence or 1 or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotide sequences comprising or consisting of a nucleotide sequence selected from Table 4, variants and fragments thereof.

According to this embodiment, the predictive signature is predictive of the number of defects, the thickness and the commercial quality of the pearl and comprises the expression profile (1) of at least one nucleotide sequence comprising or consisting of a

nucleotide sequence of 1 or a combination of 2, 3, 4, 5, 6, 7, 8 nucleotide sequences comprising or consisting of a nucleotide sequence selected from MUCO-2 (SEQ ID NO: 8), C46 (SEQ ID NO: 18), PIF-2 (SEQ ID NO: 23), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), C75bis (SEQ ID NO: 36), 2 (SEQ ID NO: 39), mp2-2 (SEQ ID NO: 40), the variants and fragments thereof; (2) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or 2 nucleotide sequences selected from the nucleotide sequences mp11-2 (SEQ ID NO: 28) and Protinh2-1 (SEQ ID NO: 42), variants and fragments thereof; and (3) of at least one nucleotide sequence comprising or consisting of a nucleotide sequence of 1 or a combination of 2, 3, 4, 5 nucleotide sequences selected from CALC-1 (SEQ ID NO: 3), CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), shem7-2 (SEQ ID NO: 11), C18 (SEQ ID NO: 14), variants and fragments thereof.

According to an embodiment, the predictive signature of the invention is not constituted by the expression profiles SEQ ID NO: 28 and SEQ ID NO: 42.

According to an embodiment, the predictive signature of the invention is not constituted by the expression profiles SEQ ID NO: 42 or SEQ ID NO: 28.

According to an embodiment, the predictive signature of the invention comprises or is constituted by the expression profile of SEQ ID NO: 4. According to an embodiment, this predictive signature is predictive of the commercial quality of the pearls obtained using a donor oyster endowed with said signature.

According to an embodiment, the predictive signature of the invention comprises or is constituted by the expression profile of SEQ ID NO: 28. According to an embodiment, this predictive signature is predictive of the number of surface defects of the pearls obtained using a donor oyster endowed with said signature.

According to an embodiment, the predictive signature of the invention comprises or is constituted by the expression profiles of SEQ ID NO: 28 and SEQ ID NO: 4. According to an embodiment, this predictive signature is predictive of the number of surface defects of the pearls obtained and the commercial quality of the pearls obtained using a donor oyster endowed with said signature.

According to an embodiment of the invention, a donor oyster with a high capacity for biomineralization has an over-expression or an under-expression of at least one predictive biomarker with respect to a control sample.

According to an embodiment of the invention, the predictive signature of the invention corresponds to an over-expression or an under-expression of at least one predictive biomarker in the biomineralizing tissue of a graft-donor pearl oyster with respect to a control sample as defined below.

According to an embodiment of the invention, an over-expression or an under-expression of a nucleotide sequence with respect to a control sample corresponds to a difference in the expression level by a factor greater than or equal to 2, preferably greater than or equal to 5, more preferably greater than or equal to 10 and even more preferably a factor greater than or equal to 25 with respect to the control sample.

According to an embodiment, a donor pearl oyster with a high capacity for biomineralization has an over-expression or an under-expression of at least one predictive biomarker with respect to the control sample as defined below, according to the following Table 9.

Identifier	**Name**	**Variable affected**	**Over-expression/ Under-expression**
SEQ ID NO: 1	CLEC2-2	Thickness	+
		Number of defects	+
		Commercial quality	-
SEQ ID NO: 2	C3	Thickness	+
		Number of defects	+
		Commercial quality	-
SEQ ID NO: 3	CALC-1	Thickness	+
		Number of defects	+
		Commercial quality	-
SEQ ID NO: 4	CLEC3-1	Thickness	+
		Number of defects	+
		Commercial quality	-
SEQ ID NO: 5	DERM-2	Thickness	+
		Number of defects	-
		Commercial quality	-
SEQ ID NO: 6	C9	Thickness	+
		Number of defects	-
		Commercial quality	-

SEQ ID NO: 7	1	Thickness	-
		Number of defects	-
		Commercial quality	-
SEQ ID NO: 8	MUCO-2	Thickness	+
		Number of defects	+
		Commercial quality	+
SEQ ID NO: 9	C88bis	Thickness	+
		Number of defects	-
		Commercial quality	+
SEQ ID NO: 10	shem5-1	Thickness	+
		Commercial quality	+
SEQ ID NO: 11	shem7-2	Thickness	+
		Commercial quality	+
SEQ ID NO: 12	C85	Thickness	+
		Commercial quality	+
SEQ ID NO: 13	C14	Thickness	+
		Commercial quality	-
SEQ ID NO: 14	C18	Thickness	+
		Commercial quality	-
SEQ ID NO: 15	C22	Thickness	+
		Commercial quality	-
SEQ ID NO: 16	Protinh3-1	Thickness	+
		Commercial quality	-
SEQ ID NO: 17	C2	Thickness	+
		Commercial quality	-
SEQ ID NO: 18	C46	Thickness	-
		Commercial quality	+
SEQ ID NO: 19	Cbind-3	Thickness	-
		Commercial quality	-
SEQ ID NO: 20	C66ter	Thickness	+
		Commercial quality	+
SEQ ID NO: 21	C17	Thickness	+
		Number of defects	+
SEQ ID NO: 22	PLW-2	Thickness	+
		Number of defects	-
SEQ ID NO: 23	PIF-2	Thickness	+
		Number of defects	+
SEQ ID NO: 24	46	Thickness	+
		Number of defects	+
SEQ ID NO: 25	C6	Thickness	+
		Number of defects	-
SEQ ID NO: 26	C26bis	Thickness	+
		Number of defects	+
SEQ ID NO: 27	C54(C53bis)	Thickness	+
		Number of defects	+

SEQ ID NO: 28	mp11-2	Commercial quality	-
		Number of defects	+
SEQ ID NO: 29	PMMG1-2	Thickness	+
SEQ ID NO: 30	73	Thickness	+
SEQ ID NO: 31	C23	Thickness	+
SEQ ID NO: 32	C1	Thickness	+
SEQ ID NO: 33	MNK-1	Thickness	+
SEQ ID NO: 34	PLC-2	Thickness	+
SEQ ID NO: 35	mp9-2	Thickness	+
SEQ ID NO: 36	C75bis	Thickness	+
SEQ ID NO: 37	13	Thickness	+
SEQ ID NO: 38	C35bis	Thickness	-
SEQ ID NO: 39	2	Thickness	-
SEQ ID NO: 40	mp2-2	Thickness	-
SEQ ID NO: 41	C8	Thickness	+
SEQ ID NO: 42	Protinh2-1	Number of defects	-

+ : over-expression with respect to the control sample

- : under-expression with respect to the control sample

Table 9

According to an embodiment of the invention, the control sample corresponds to a group of graft-donor pearl oysters representative of the natural (wild) population of donor pearl oysters available to pearl culture professionals, and used in the context of grafts for commercial ends.

According to an embodiment of the invention, the relative expression level of the predictive biomarkers is determined by quantitative PCR according to the method of 2^-ΔΔCt (Livak and Schmittgen, 2001) according to the following formula: fold = 2^-ΔΔCt = ^-[DCt target - DCt calibrator], by comparison of the following two groups of donor pearl oysters:

- "Group A" (target), corresponding, according to this embodiment of the invention, to donor pearl oysters producing, on average, pearls of higher quality (high nacre thickness, few surface defects and/or better quality classification); and

- "Group B" (control or calibrator), corresponding, according to this embodiment, to a group of graft-donor pearl oysters representative of the natural (wild) population of donor pearl oysters available to pearl culture professionals, and used in the context of grafts for commercial ends.

According to an embodiment of the invention, the quantitative PCR method is based on the use of fluorescent probes. The Ct corresponds to the number of the PCR cycle at which the fluorescence emitted by said probes goes beyond a predetermined threshold.

According to a first embodiment, the target DCt corresponds to the average of the differences in Ct between the predictive biomarkers of interest and the reference genes 18S and SAGE1 for the graft or pearl pouch samples of the target group "A"; and the calibrator DCt corresponds to the average of the differences in Ct between the predictive biomarkers of interest and the reference genes 18S and SAGE1 for the graft or pearl pouch samples of the calibrator group "B".

According to a second embodiment, the target DCt corresponds to the median of the differences in Ct between the predictive biomarkers and the reference genes 18S and SAGE1 for the graft or pearl pouch samples of the target group "A"; and the calibrator DCt corresponds to the median of the differences in Ct between the predictive biomarkers and the reference genes 18S and SAGE1 for the graft or pearl pouch samples of the calibrator group "B".

This invention also relates to a graft from a donor pearl oyster with a high capacity for biomineralization according to the invention.

This invention therefore relates to a graft comprising a predictive biomarker according the invention and/or a predictive signature according to the invention.

This invention also relates to a grafted recipient oyster comprising a graft from a donor oyster identified by the identification process of the invention.

According to an embodiment, after the grafting of a recipient oyster with a graft from a donor oyster according to the invention, the predictive signature according to the invention can be found at the pearl sac of said recipient oyster.

According to an embodiment, said predictive signature in the recipient oyster is predictive of the number of defects at the surface of the pearl, and comprises the expression profile of at least one nucleotide sequence, preferably 1, 2, 3, 4, 5, 6, 7 or 8 nucleotide sequences selected from CLEC2-2 (SEQ ID NO: 1), C9 (SEQ ID NO: 6), MUCO-2 (SEQ ID NO: 8), C26bis (SEQ ID NO: 26), C54(C53bis) (SEQ ID NO: 27), CALC-1 (SEQ ID NO: 3); C18 (SEQ ID NO: 14) and Cbind-3 (SEQ ID NO: 19), variants and fragments thereof. According to an embodiment of the invention, said predictive signature corresponds to an over-expression of CLEC2-2 (SEQ ID NO: 1), C9 (SEQ ID NO: 6), MUCO-2 (SEQ ID NO: 8), C26bis (SEQ ID NO: 26) and/or C54(C53bis) (SEQ ID NO: 27), variants and fragments thereof; and/or to an under-expression of CALC-1 (SEQ ID NO: 3), C18 (SEQ ID NO: 14) and/or Cbind-3 (SEQ ID NO: 19), variants and fragments thereof.

According to an embodiment, said predictive signature in the recipient oyster is predictive of the commercial quality of the pearl, and comprises the expression profile of at least one nucleotide sequence, preferably 1, 2, 3, 4, 5, 6 or 7 nucleotide sequences selected from C3 (SEQ ID NO: 2), CALC-1 (SEQ ID NO: 3), CLEC31- (SEQ ID NO: 4), MUCO-2 (SEQ ID NO: 8), C2 (SEQ ID NO: 17), C46 (SEQ ID NO: 18) and Cbind-3 (SEQ ID NO: 19), variants and fragments thereof. According to an embodiment of the invention, said predictive signature corresponds to an under-expression of C3 (SEQ ID NO: 2), CALC-1 (SEQ ID NO: 3) CLEC3-1 (SEQ ID NO: 4) MUCO-2 (SEQ ID NO: 8), C2 (SEQ ID NO: 17), C46 (SEQ ID NO: 18) and/or Cbind-3 (SEQ ID NO: 19), variants and fragments thereof.

According to an embodiment, said predictive signature in the recipient oyster is predictive of the nacre thickness around the nucleus, and comprises the expression profile of at least one nucleotide sequence, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 nucleotide sequences selected from CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), MUCO-2 (SEQ ID NO: 8), C88bis (SEQ ID NO: 9), C18 (SEQ ID

NO: 14), Protinh3-1 (SEQ ID NO: 16), C17 (SEQ ID NO: 21), PIF-2 (SEQ ID NO: 23), C6 (SEQ ID NO: 25), C26bis (SEQ ID NO: 26), C54 (SEQ ID NO: 27), CALC-1 (SEQ ID NO: 3), C46 (SEQ ID NO: 18), PMMG1-2 (SEQ ID NO: 29) and C35bis (SEQ ID NO: 38), variants and fragments thereof. According to an embodiment of the invention, said predictive signature corresponds to an over-expression of CLEC3-1 (SEQ ID NO: 4), DERM-2 (SEQ ID NO: 5), MUCO-2 (SEQ ID NO: 8), C88bis (SEQ ID NO: 9), C18 (SEQ ID NO: 14), Protinh3-1 (SEQ ID NO: 16), C17 (SEQ ID NO: 21), PIF-2 (SEQ ID NO: 23), C6 (SEQ ID NO: 25), C26bis (SEQ ID NO: 26) and/or C54 (SEQ ID NO: 27), variants and fragments thereof; and/or to an under-expression of CALC-1 (SEQ ID NO: 3), C46 (SEQ ID NO: 18), PMMG1-2 (SEQ ID NO: 29) and C35bis (SEQ ID NO: 38), variants and fragments thereof.

This invention also relates to a process for identification of donor oysters with a high capacity for biomineralization.

According to this invention, the process for identification of donor oysters with a high capacity for biomineralization comprises:

(a) the determination of the expression profile of a predictive biomarker or a plurality of predictive biomarkers forming a predictive signature in a sample of a graft-donor oyster, said signature being predictive of the quality of biomineralization, and

(b) the comparison of the expression profile obtained in step (a) with a control expression profile obtained in a group of graft-donor pearl oysters representative of the natural population ("calibrator" group B).

According to an embodiment of the invention, the process of the invention comprises the determination of the presence of a signature predictive of the nacre thickness around the nucleus, the number of defects at the surface of the pearl and/or the commercial quality of the pearl, as described above.

According to an embodiment of the invention, the process of the invention enables the comparison of the biomineralization capacities of two groups of donor oysters ("target" group A and "calibrator" group B), and the expression level of the "calibrator" B group

of donor pearl oysters serves as a reference for the establishment of an expression profile in the "target" A group of donor pearl oysters with a high biomineralization property.

According to an embodiment of the invention, the analysis of the expression profile is performed on a graft of the donor oyster, preferably after the collection step.

According to an embodiment of the invention, the analysis of the expression profile is performed on the mantle of graft-donor oysters, before the grafting step.

According to an embodiment of the invention, the analysis of the expression profile is performed on the oyster pearl sac having received a graft, after the grafting step.

According to an embodiment of the invention, the determination of the expression profile is performed at the level of the RNA, the DNA and/or the protein.

According to an embodiment of the invention, the determination of the expression profile corresponds to the determination of the transcription level of the predictive biomarker(s) considered. The methods for determining the transcription level of a nucleotide sequence are well known to a person skilled in the art, and include, without being limited to, the following methods: RT-PCR, quantitative RT-PCR (RT-qPCR), high-speed RT-qPCR, DNA chip, RNA chip.

According to an embodiment of the invention, the determination of the expression profile is performed at the protein level, and corresponds to the level of translation of the predictive biomarker(s) considered. The methods for determining the level of translation of a nucleotide sequence (i.e. expression of the protein translated) are well known to a person skilled in the art, and include, without being limited to, the following methods: Western-Blot, immunochemistry, ELISA, ELISA Sandwich, PAGE...

This invention also relates to an oyster identified by the identification process of the invention. According to this embodiment, the oyster identified has an expression profile specific to at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

This invention also relates to a kit for determining the presence of a predictive biomarker according to the invention or a predictive signature according to the invention.

The kit according to the invention therefore enables the identification process of the invention to be implemented.

According to the invention, the kit comprises at least one means making it possible to determine the expression profile of at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from the list comprising sequences SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

According to an embodiment, the kit according to the invention comprises a control sample corresponding to biological material of biomineralizing tissue (mantle) of graft-donor pearl oysters from the natural population.

According to an embodiment of the invention, the kit according to the invention comprises means making it possible to determine the expression profile at the transcriptomic level by RT-PCR, RT-qPCR, or high-speed quantitative PCR.

According to this embodiment, the kit comprises means for extracting the total RNA, reverse transcription means, and/or means for amplifying at least one nucleotide sequence comprising or consisting of a nucleotide sequence selected from the list comprising sequences SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

According to an embodiment of the invention, the amplification means include a polymerase and a buffer, free nucleotides and at least one pair of primers, each primer of which is specifically and effectively hybridised with a sequence selected from SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

According to an embodiment of the invention, the pairs of primers are selected from the pairs in Table 10 below:

Identifier	Name	Sense primer	Antisense primer
SEQ ID NO: 1	CLEC2-2	AGCTACTGGCGCTC GATG (SEQ ID NO: 43)	TGCAGATGAAGCCA TGTGAG (SEQ ID NO: 44)
SEQ ID NO: 2	C3	ATTTCCACCACAGC CAGTAAAC (SEQ ID NO: 45)	CGTGGTACGGTGTGT CCTAAC (SEQ ID NO: 46)
SEQ ID NO: 3	CALC-1	CTCCATGCACAGAC ATGACC (SEQ ID NO: 47)	GCCAGTAATACGGA CCTTGG (SEQ ID NO: 48)
SEQ ID NO: 4	CLEC3-1	CATCGGTGACCATA GCCATAC (SEQ ID NO: 49)	CGTCTTTCAAGGAA GCTGTTG (SEQ ID NO: 50)
SEQ ID NO: 5	DERM-2	CACAGTGGTCACGA GGACAG (SEQ ID NO: 51)	TGATGCAACTTGGG GTCAG (SEQ ID NO: 52)
SEQ ID NO: 6	C9	CCGGACACTGGAAG TCATTAG (SEQ ID NO: 53)	TGCTCTGCACCCAGA ATATG (SEQ ID NO: 54)
SEQ ID NO: 7	1	CAATAAATAAAATA CGGCACAGTTC (SEQ ID NO: 55)	AGAAAATGCTGCTA TTAAATGGTG (SEQ ID NO: 56)
SEQ ID NO: 8	MUCO-2	AGTGGAACCGGAAC TGGTG (SEQ ID NO: 57)	CACATCTCCAACTCC AGCAAG (SEQ ID NO: 58)
SEQ ID NO: 9	C88bis	CCGGCTCCGTTACC ACTAC (SEQ ID NO: 59)	CCTATGGGCTGGTCG TACTC (SEQ ID NO: 60)
SEQ ID NO: 10	shem5-1	GTCCGAAACCAAAT CGTCTG (SEQ ID NO: 61)	CTGTGGTGATGGTG ACTTCG (SEQ ID NO: 62)
SEQ ID NO: 11	shem7-2	TAGCAAGCGCCAAA TATGC (SEQ ID NO: 63)	AAGTGATGCCGAAC CTAACC (SEQ ID NO: 64)
SEQ ID NO: 12	C85	AGTTCGTTTTGGTCC ACCTG (SEQ ID NO: 65)	TTCAGGGTGCCATCT TGTC (SEQ ID NO: 66)
SEQ ID NO: 13	C14	TGCATTTCGCATTGT TGTATG (SEQ ID NO: 67)	CATGTGCAGCCTCA AGGTATC (SEQ ID NO: 68)
SEQ ID NO: 14	C18	CCGCGTCAAGACCA TATACG (SEQ ID NO: 69)	GCAGCATTTGTAAA CCATTCG (SEQ ID NO: 70)
SEQ ID NO: 15	C22	CAACAAACCATCGG TCCTTAC (SEQ ID NO: 71)	TGCATAGGTCCCGGT AAAATAG (SEQ ID NO: 72)

SEQ ID NO: 16	Protinh3-1	TATGCGTGTGACTG TCCTGA (SEQ ID NO: 73)	TCAATGCAAACGAT CTGTCC (SEQ ID NO: 74)
SEQ ID NO: 17	C2	TGTTGGGTGGTCAT CGTAAG (SEQ ID NO: 75)	CCACATGCGGCAGA TACTC (SEQ ID NO: 76)
SEQ ID NO: 18	C46	CCTGTTTGCCTGATG TTGTG (SEQ ID NO: 77)	CGACAGCCATACTC ATCCAG (SEQ ID NO: 78)
SEQ ID NO: 19	Cbind-3	CATTGTGCGTGAGT TTGGAG (SEQ ID NO: 79)	TGCTGATTGGTTCAT TGAGG (SEQ ID NO: 80)
SEQ ID NO: 20	C66ter	GGCTGGTGGAGCCA ATATG (SEQ ID NO: 81)	TGACTTGAAATTCCC TTTTGTTG (SEQ ID NO: 82)
SEQ ID NO: 21	C17	CCCTTGGGAACAAA GGTACAG (SEQ ID NO: 83)	CATTGAATAAACGC CAACAAGTAG (SEQ ID NO: 84)
SEQ ID NO: 22	PLW-2	AATTCTTCAGCCCG GATATTG (SEQ ID NO: 85)	TCCTGACCTCCAGGG TGTAG (SEQ ID NO: 86)
SEQ ID NO: 23	PIF-2	TGATAACGGAGACG ACGATG (SEQ ID NO: 87)	AGGCACAATCACGA GAACTG (SEQ ID NO: 88)
SEQ ID NO: 24	46	TGAACAAACAAATT GCAAAGTC (SEQ ID NO: 89)	TCAAAGCAAAGAAA CAGTAAAGC (SEQ ID NO: 90)
SEQ ID NO: 25	C6	GCGATGACGACGTT TCTACC (SEQ ID NO: 91)	GGATTGGTTCGTTGT AAGCTATG (SEQ ID NO: 92)
SEQ ID NO: 26	C26bis	GGGAGTTTGTCAGG GATTACTCC (SEQ ID NO: 93)	GGTTCTCGCAAATTC CTTTCTG (SEQ ID NO: 94)
SEQ ID NO: 27	C54(C53bis)	TGCAACCCTGATCC TACTCC (SEQ ID NO: 95)	CGGAGATTGAACCA AAGCAG (SEQ ID NO: 96)
SEQ ID NO: 28	mp11-2	GTCCGAGGAATCAT GGTAGC (SEQ ID NO: 97)	GTGCAGAGGTCCCG ACAG (SEQ ID NO: 98)
SEQ ID NO: 29	PMMG1-2	CGGAAACTGGAAAG TTCAGG (SEQ ID NO: 99)	CATAGGACTGAAGG TCACATGG (SEQ ID NO: 100)
SEQ ID NO: 30	73	TTGAGCGTATCCAC ATCCAG (SEQ ID NO: 101)	GCAACACATTTGGA ATGCAC (SEQ ID NO: 102)

SEQ ID NO: 31	C23	CCCAACCCTCCTATT ATATGTCC (SEQ ID NO: 103)	TGACCGCGTCCTTAT GATTAC (SEQ ID NO: 104)
SEQ ID NO: 32	C1	ACCCAAAGGCTGGA GATACG (SEQ ID NO: 105)	TTCACCGTCAGGTCT TACGTAGTAG (SEQ ID NO: 106)
SEQ ID NO: 33	MNK-1	TAGGAAATCGTGAG GGCAAC (SEQ ID NO: 107)	TCGCTTAGCCAATCC TGAAC (SEQ ID NO: 108)
SEQ ID NO: 34	PLC-2	CAGCGCCGACGAAT TTAC (SEQ ID NO: 109)	AACCATAGCGTCCG TAGCAC (SEQ ID NO: 110)
SEQ ID NO: 35	mp9-2	TGGTTTGTTGGGTC AGATTG (SEQ ID NO: 111)	TTGCTACCCCTTGGC ATC (SEQ ID NO: 112)
SEQ ID NO: 36	C75bis	TTCATTTTGGTGGTT ATGGAATG (SEQ ID NO: 113)	CCGTTTCCACCTCCG TTAC (SEQ ID NO: 114)
SEQ ID NO: 37	13	TTCTCTTGACTTCAG TTCTTCATTG (SEQ ID NO: 115)	CAGTCAGGGACCAT TTTGTG (SEQ ID NO: 116)
SEQ ID NO: 38	C35bis	TGTGTCGGATTTGG CACTG (SEQ ID NO: 117)	GGGGTGTTTGTGTGT ATTTGTTC (SEQ ID NO: 118)
SEQ ID NO: 39	2	TCTGTGACCCACTCT TTCTGG (SEQ ID NO: 119)	ACAACAACGGCAAT GGAATC (SEQ ID NO: 120)
SEQ ID NO: 40	mp2-2	AGCTGATCCCGTCT TCACAG (SEQ ID NO: 121)	ATGCAAGGGGAAGA CAGATG (SEQ ID NO: 122)
SEQ ID NO: 41	C8	GGCTCCCAACATCT TTGTAC (SEQ ID NO: 123)	GTGGTTGCTGATTTG TCACTATC (SEQ ID NO: 124)
SEQ ID NO: 42	Protinh2-1	TGCAGGTTTTCGTAT GTCCA (SEQ ID NO: 125)	GCCCAGGAAAACAA AGTCAG (SEQ ID NO: 126)

Table 10

According to an embodiment of the invention, the kit according to the invention comprises means making it possible to determine the expression profile by determining the expression profile at the protein level (by proteomic analysis).

According to this embodiment, the kit comprises one or more antibodies specifically recognising one or more of the proteins encoded by the nucleotide sequences comprising or consisting of SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

According to an embodiment, the kit also comprises total protein extraction means.

This invention also relates to a device comprising a support comprising one or more probes specific to one or more nucleotide sequences identified under numbers SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof, for the implementation of the identification process according to the invention.

In the context of this invention, the term "probe" means any single-strand DNA or single-strand RNA fragment, the sequence of which is complementary to a sequence sought: it may thus be detected by hybridisation with the probe that is marked (by incorporation of nucleotides coupled to radioactive atoms/isotopes or fluorescent/fluorochrome groups), which acts as a molecular "hook".

According to preferred embodiments, the support of said device is chosen from a glass membrane, a metal membrane, a polymer membrane or a silica membrane.

Such devices are, for example, DNA or RNA chips comprising one or more nucleotide sequences identified under numbers SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof, or one or more sequences complementary to the nucleotide sequences identified under numbers SEQ ID NO: 1 to SEQ ID NO: 42, variants and fragments thereof.

The invention also relates to a process for grafting recipient pearl oysters comprising the insertion in the pearl pouch of the pearl oyster receiving a graft from a donor oyster with a high capacity for biomineralization according to the invention, corresponding to the epithelium of the mantle of the donor oyster, in combination with a nucleus. According to an embodiment of the invention, the donor oyster with a high capacity for biomineralization has been identified according to the process of the invention.

According to an embodiment of the invention, the grafting process comprises (i) opening the recipient pearl oyster, (ii) making an incision in the tissues of the recipient pearl oyster in order to have access to the pearl pouch and make it possible, in a third step (iii) to insert, in the pearl pouch of the recipient pearl oyster, a graft, corresponding to a portion of the epithelium of the mantle of the donor oyster according to the invention (around 4 mm^2), in combination with a nucleus.

This invention also relates to a process for producing a pearl of higher quality, comprising the grafting in a recipient oyster of a graft from a donor oyster with a high capacity for biomineralization according to the invention. It is understood that the recipient oyster comprises a nucleus. According to an embodiment of the invention, the pear production process comprises the grafting of a nucleus in the pearl pouch of a recipient oyster, and the grafting of a portion of the epithelium of the mantle of a donor oyster with a high capacity for biomineralization according to the invention.

According to an embodiment of the invention, said pearl production process comprises a first step comprising the collection and culturing of pearl oysters so as to obtain donor pearl oysters according to the invention and recipient pearl oysters; advantageously, the donor pearl oysters according to the invention are then cleaned so as to remove any possible parasites; then the recipient pearl oysters are placed in culture, preferably for a period of 10 to 24 months, preferably of 12 to 20 months, even more preferably of16 to 18 months. During the culture period, the mineralising outer epithelial border of the graft multiplies and lines the pearl pouch, in order to produce a pearl sack containing the nucleus, and which will deposit layers of nacre around the nucleus, thus resulting in the production of a pearl.

This invention also relates to the pearl obtained by the process for obtaining pearls as described above, using an oyster with a high capacity for biomineralization according to the invention as a graft-donor oyster.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Presentation of the experimental approach established.

Figure 2: Protocol used to validate candidate biomarkers of the growth and the quality of the pearl in *P. margaritifera.*

EXAMPLES

This invention will be easier to understand and interpret in view of the examples below.

The objective of this study was to develop a predictive signature of the quality of the pearl harvested, in order to select graft-donor oysters with a "high quality of mineralisation".

Experimental approach

The approach used consisted in developing a global approach to analysis of the transcriptome in order to identify nucleotide sequences expressed differentially in epithelial cells of tissue responsible for biomineralization of the shell (mantle) and the pearl (grafts and pearl sacs contained in the pearl pouches) (**Figure 1**). The approach consisted in developing two complementary transcriptomic approaches on the mineralising tissue (mantle, graft and pearl sac) and a global proteomic approach in parallel on the mineralised tissue (shells and pearls) of *P. margaritifera.* Four SAGE databanks were established for pearl mineralising tissue, grafts and pearl pouches, in order to identify nucleotide sequences differentially expressed according to different graft-donor pearl oysters potentially associated with contrasted pearl qualities. An EST mantle bank was also developed in parallel by pyrosequencing, on mantle, the biomineralizing tissue of the shell. Finally, the global proteomic approach was developed in parallel on the mineralised tissue of *P. margaritifera* in order to identify the proteins that comprise the shell and the pearl. The establishment of the global transcriptomic and proteomic approaches made it possible to arrive at the selection, by "candidate approach" or "blind", of biomarkers of the quality of the candidate pearl encoding proteins involved in the biomineralization processes in *P. margaritifera.* The validation of the biomarkers was performed by producing an experimental graft that made it possible, on the one hand, to evaluate the quality of the harvested pearls and to identify a "donor effect", and, on the other hand, to provide biological material (grafts

and pearl pouches) at the origin of the production of pearls of contrasted quality. The validation of candidate biomarkers was then performed by high-speed quantitative PCR and involved statistically correlating the expression level of the candidate biomarkers with a biological reality (pearl quality). The analysis of the expression level of the candidate predictive biomarkers in donor pearl oyster grafts and recipient pearl oyster pearl pouches, associated with the donor effect, resulted in the identification of biomarkers predictive of pearl quality.

Materials and methods

Oysters

P. margaritifera pearl oysters were used in the context of the global transcriptomic analyses, the global proteomic analysis and the experimental graft in order to validate biomarkers predictive of pearl quality.

Experimental graft (G1)

Forty *P. margaritifera* pearl oysters from French Polynesia, around 3 years old, were used as graft-donor oysters. Some thirty grafts were cut from the mantle of each valve of each donor pearl oyster. A minimum of 2 grafts from each donor was preserved. The remaining grafts from each valve of each donor pearl oyster were grafted in recipient pearl oysters. A total of 1978 recipient pearl oysters were grafted with 2.4 bio nuclei. Pearl pouch and pearl samples were collected at eight times: 1 day, 7 days, 21 days, 2 months, 3 months, 6 months, 12 months and 18 months.

RNA extraction, cDNA synthesis and quantitative PCR protocols

Extraction of total RNA

The RNA were isolated from a sample of mantle, graft or pearl pouch from the experimental graft and preserved in RNAlater® (Ambion). The tissue was rinsed with 1 mL of PBS, dilacerated with a scalpel in a Petri dish on ice, then placed in 1 mL of Trizol® (Invitrogen). The extraction was performed on an agitator overnight at 4°C.

The tubes were then incubated in a double boiler for 5 minutes at 30°C, then vortexed for 30 seconds. The ground material was centrifuged at 12000 g for 10 minutes at ambient temperature, and 1 mL of supernatant was collected and transferred to a new tube. The separation of the nucleic acids and proteins was performed by adding 200 µL of chloroform per tube, and by inversion mixing. After 15 minutes of incubation at ambient temperature, the tubes were centrifuged at 12000 g for 10 minutes at 4°C. The upper phase, around 500 µL and containing the RNA, was transferred to a new tube. The RNA were precipitated over night at -80°C by 250 µL of isopropanol and 250 µL of a "High salt" saline solution (0.8 M TriSodium Citrate; 1.2 M sodium chloride). The samples were centrifuged for 30 minutes at 15000 g. The supernatant was drained, the RNA base was washed twice in the vortex with 1 mL of 70% ethanol followed by a step of centrifugation of 15000 g at 4°C for 15 minutes. The bases were then dried under the hood for 10 minutes at ambient temperature, then placed in 50 µL of RNAse free water. The tubes were then incubated in a double boiler for 10 minutes at 55°C, before being homogenised by hand. The RNA were stored at 80°C. The quality and quantity of the RNA obtained were evaluated by electrophoresis on 1% agarose gel in TAE 0.5X at 100 mV, and by spectrophotometric assay with a Nanodrop ND1000 and an Agilent 2100 Bioanalyser (RNA 6000 Nano kit).

Reverse transcription

The cDNA synthesis of grafts and pearl pouches in order to determine the relative expression level of candidate biomarkers predictive of the pearl quality was performed with 800 ng of graft and pearl pouch RNA, using the SuperScriptTM II Reverse Transcriptase kit (InvitrogenTM- Cat. No: 18064-014) according to the supplier's instructions.

Quantitative PCR

The measurement of the expression level of candidate biomarkers predictive of pearl quality was performed by high-speed qPCR on the BioMark™ HD system (Fluidigm Corporation) using the 2XTaqManPreAmp Master Mix kit (Applied Biosystems - P/N 4384266) for the preamplification step and the 2XTaqMan Gene Expression Master

Mix kit (Applied Biosystems - P/N 4369016) for the amplification step, according to the supplier's instructions. Four Fluidigm 96.96 DynamicArray Real Time PCR chips (BMK-M-96.96) were used. The amplification program used is as follows: 2 minutes at 50°C, then 10 minutes of denaturation at 95°C, 35 amplification cycles (15 seconds of denaturation at 95°C, 1 minute of hybridisation and elongation at 60°C) with a fluorescence measurement at the end of each cycle, and a dissociation curve of 60°C to 95°C with a continuous fluorescence measurement every 1°C. The data was extracted and analysed using Fluidigm Real-time analysis software (Fluidigm Corporation) and Biomark Melting Curve analysis (Fluidigm Corporation). The relative expression level of the genes was determined by the method of 2^-ΔΔCt (Livak and Schmittgen, 2001) according to the following formula: fold = 2^-ΔΔCt = ^-[DCt target - DCt calibrator]. The DCt target corresponds to the average of the differences in Ct between the predictive biomarkers and the reference genes 18S and SAGE1 for the graft samples or pearl pouches of the target group "A". The DCt calibrator corresponds to the average of the differences in Ct between the predictive biomarkers and the reference genes 18S and SAGE1 for the graft samples or pearl pouches of the calibrator group "B", as described in this invention.

Evaluation of pearls quality

Prior to their evaluation, the pearls harvested in the context of the experimental graft were washed with coarse salt, rinsed in fresh water, wiped with a clean rag and lustred.

In order to perform the global evaluation of the pearl quality, the pearl quality parameters were broken down into different variables as follows: a "Nacre thickness at the surface of the pearl" variable, a "Number of defects at the surface of the pearls" variable, and a "Commercial quality" variable.

The "Nacre thickness at the surface of the pearl" variable corresponds to the measurement in millimetres of the minimum nacre thickness present at the surface of the nucleus. This was obtained by subtracting the diameter of the nucleus at the smallest diameter of the pearl and dividing the difference in diameter by 2. The diameters of the pearls and the nuclei were measured using scanned images of pearls and nuclei (300

pixels/cm, Epson Perfection 4990 Photo scanner) and processed with the image analysis software ImageJ (version 1.44).

The "Number of defects at the surface of the pearls" variable corresponds to the classification of each pearl into 4 quality classes on the basis of the number of defects observed at their surface (pearls without defects, pearls with 1 to 5 defects, pearls with more than 5 defects and pearls completely covered with defects). The defects considered are pits, blisters and spots. In a second phase, a grade ranging from 0 to 3 was assigned to each pearl on the basis of the defect class to which it belongs (0 for pearls covered with defects to 3 for pearls without defects).

The "Commercial quality" variable corresponds to the classification of pearls into 5 quality categories (A, B, C, D and Reject). This classification was performed in two phases in accordance with the criteria defined by Deliberation no. 2005-42 of 4 February 2005 (Official Journal of French Polynesia). In a second phase, a grade ranging from 0 to 4 was assigned to each pearl on the basis of the category to which it belongs (0 for pearls in the "reject" category to 4 for pearls in category "A").

In order to identify the donor effect, the graft-donor pearl oysters were classified in increasing order of quality on the basis of each variable. This classification was performed by assigning a grade to each donor pearl oyster on the basis of the evaluation of the quality of the pearls harvested at 18 months in the context of the experimental graft. For the "Nacre thickness at the surface of the pearl" variable, this grade corresponds to the average of the thicknesses of the pearls harvested per donor. For the "Number of defects at the surface of the pearls" and "Commercial quality" variables, the grade of each donor pearl oyster corresponds to the average of the grades of the pearls harvested per donor.

<u>Development of graft and pearl pouch sampling plans for validation of the pearl growth and quality biomarkers</u>

In order to form groups for sampling grafts and pearl pouches from donor pearl oysters used in the production of pearls of contrasted quality, two groups of graft-donor pearl oysters were created for each of the "Thickness", "Quality" and "Defect" variable, on

the basis of the donor effect and the quality of the pearls at 18 months harvested in the context of the experimental graft. "Group A" (target), corresponding to the donor pearl oysters producing, on average, higher-quality pearls (high nacre thickness, few surface defects and/or better quality classification). "Group B" (control or calibrator) corresponding to a group of graft-donor pearl oysters representative of the natural (wild) population of donor pearl oysters available to pearl culture professionals, and used in the context of grafts for commercial ends.

The development of "Groups A" (target) was carried out by performing an analysis of the raw data on pearl quality and the classification of the graft-donor pearl oysters, a Dunn's multiple comparison by pairs procedure and an ascendant hierarchical classification. The development of these groups of donor pearl oysters then made it possible to define, for each variable, two plans for sampling grafts and pearl pouches at the origin of the contrasted pearl production: a plan for sampling grafts from donor pearl oysters constituting groups A and B, and a plan for sampling pearl pouches from the grafting of donor pearl oyster grafts constituting groups A and B.

Statistical analyses

All of the statistical analyses performed in the context of the validation of biomarkers predictive of pearl quality were performed using the Xlstat software (version 2009.4.02) for the Shapiro-Wilk, Kruskall-Wallis tests, the Dunn's multiple comparison procedure and the ascendant hierarchical classification, and the R software (version 2.10.0) for the Wilcoxon test. The Shapiro-Wilk normality test was used with a threshold α of 5%. The Kruskall-Wallis test was performed with a threshold α of 5%. The Dunn's multiple comparison procedure was performed by applying a Bonferroni correction to the signification level (corrected threshold α = 0.01%). The ascendant hierarchical classification was performed using the distance of $\chi 2$ as a dissimilarity index and the Ward method as an aggregation method. No class number was assigned and the truncation level was defined automatically. The Wilcoxon test was used with a threshold α of 5% and unilaterally.

Results

The global approach to analysis of the transcriptome made it possible to identify a large number of nucleotide sequences expressed in tissue responsible for biomineralization of the shell and the pearl. The development of the EST mantle bank allowed us to identify 82 nucleotide sequences encoding proteins potentially involved in the biomineralization of the shell in *P. margaritifera*. The development of SAGE graft and pearl pouch banks made it possible to identify more than 5000 gene sequences annotated and expressed in tissue responsible for the biomineralization processes of the pearl. Finally, the global proteomic approach established for the constituents of the shell and pearls made it possible to identify 130 protein sequences constituting mineralised structures in *P. margaritifera*. Finally, the use of these proteomic and transcriptomic data banks made it possible to select a group of 268 candidate biomarkers predictive of pearl quality, and 205 of which encode proteins unknown at present in *P. margaritifera*. Of the 268 biomarkers identified, 224 effective and specific primer pairs were identified and 188 candidate predictive biomarkers were selected and tested.

The validation of the candidate predictive biomarkers as biomarkers predictive of pearl quality involved correlating the expression level of said predictive biomarkers with the commercial quality, the thickness and the number of defects in the pearls obtained in the context of the graft (**Figure 2**).

The analysis of the expression levels of the candidate biomarkers was thus performed in biomineralising tissue of the pearl and made it possible to identify nucleotide sequences differentially expressed on the basis of the quality of the harvested pearls. A nucleotide sequence is considered to be differentially expressed if the relative expression of said nucleotide sequence in group A is greater than or less than a factor, or "fold", greater than or equal to 2 with respect to the expression of the same nucleotide sequence in group B, as described in the present invention.

The combined analysis of the analyses of the expression level of the nucleotide sequences in correlation with pearl quality resulted in the validation of a group of 42

biomarkers predictive of the growth and quality of the pearl in *P. margaritifera* (Table 11).

Variable	Direction of the expression level of the biomarkers (group A vs. group B)	Number of biomarkers differentially expressed in the grafts	Total number of non-redundant biomarkers between the variables
Thickness	Over-expression	37	42
	Under-expression	6	
Defects	Over-expression	11	
	Under-expression	7	
Commercial quality	Over-expression	7	
	Under-expression	14	

Table 11: *Number of biomarkers of the quality of the pearl in P. margaritifera, having a fold greater than or equal to 2.*

The complete list of 42 biomarkers predictive of the quality of the pearl obtained is presented in Tables 1 and 12.

		Difference in expression between the oysters of group A and the oysters of group B for the criteria					
		Thickness		**Commercial quality**		**Defects**	
Identifier	**Name**	Graft	Pouches	Graft	Pouches	Graft	Pouches
SEQ ID NO: 1	**CLEC2-2**	4.986	0.581	0.040	0.847	2.626	19.279
SEQ ID NO: 2	**C3**	9.535	1.000	0.254	0.229	2.707	1.097
SEQ ID NO: 3	**CALC-1**	3.990	0.335	0.003	0.004	2.218	0.024
SEQ ID NO: 4	**CLEC3-1**	3.399	6.109	0.067	0.202	15.906	1.507
SEQ ID NO: 5	**DERM-2**	3.245	3.649	0.188	1.171	0.188	1.781
SEQ ID NO: 6	**C9**	9.793	1.482	0.094	0.960	0.094	2.672
SEQ ID NO: 7	**1**	0.210	1.608	0.136	0.569	0.206	0.569
SEQ ID NO: 8	**MUCO-2**	19.752	2.164	2.128	0.245	8.262	3.504
SEQ ID NO: 9	**C88bis**	11.366	2.350	4.244	1.323	0.193	1.563
SEQ ID NO: 10	**shem5-1**	45.682	1.478	10.583	0.784	0.528	1.516
SEQ ID NO: 11	**shem7-2**	7.064	1.825	7.670	0.905	0.918	0.972
SEQ ID NO: 12	**C85**	6.565	1.959	2.523	1.224	0.633	1.640
SEQ ID NO: 13	**C14**	29.368	1.612	0.211	0.978	1.723	0.581
SEQ ID NO: 14	**C18**	17.062	2.323	0.173	0.733	0.925	0.242
SEQ ID NO: 15	**C22**	12.098	1.714	0.427	0.560	1.722	2.742

SEQ ID NO: 16	**Protinh3-1**	8.790	2.573	0.439	1.340	0.572	2.265
SEQ ID NO: 17	**C2**	6.480	1.091	0.257	0.433	0.643	0.998
SEQ ID NO: 18	**C46**	0.199	0.040	2.902	0.462	0.517	1.217
SEQ ID NO: 19	**Cbind-3**	0.470	1.508	0.135	0.131	1.136	0.118
SEQ ID NO: 20	**C66ter**	5.562	1.777	2.696	0.762	0.925	1.541
SEQ ID NO: 21	**C17**	22.072	2.210	0.990	1.040	3.654	0.388
SEQ ID NO: 22	**PLW-2**	19.542	1.336	0.823	0.942	0.317	1.789
SEQ ID NO: 23	**PIF-2**	7.020	5.150	0.642	1.303	3.094	1.310
SEQ ID NO: 24	**46**	6.497	1.936	0.623	0.719	3.023	1.465
SEQ ID NO: 25	**C6**	6.153	2.619	0.563	1.198	0.260	0.565
SEQ ID NO: 26	**C26bis**	5.437	6.844	1.156	1.257	2.553	2.880
SEQ ID NO: 27	**C54(C53bis)**	9.155	4.211	0.965	0.448	6.646	2.288
SEQ ID NO: 28	**mp11-2**	1.178	3.283	0.441	1.618	5.525	2.593
SEQ ID NO: 29	**PMMG1-2**	12.967	0.268	0.502	0.129	1.296	3.741
SEQ ID NO: 30	**73**	8.999	1.052	0.869	1.004	0.936	0.509
SEQ ID NO: 31	**C23**	7.991	1.515	0.721	0.816	0.788	0.760
SEQ ID NO: 32	**C1**	6.654	1.289	0.985	0.650	0.736	0.867
SEQ ID NO: 33	**MNK-1**	6.218	1.294	1.674	0.745	1.384	1.030
SEQ ID NO: 34	**PLC-2**	6.153	1.316	1.338	0.747	0.856	0.107
SEQ ID NO: 35	**mp9-2**	6.140	1.569	1.169	0.978	1.169	1.248
SEQ ID NO: 36	**C75bis**	5.703	1.512	0.604	1.155	0.604	0.882
SEQ ID NO: 37	**13**	5.210	1.724	1.129	0.990	1.129	1.501
SEQ ID NO: 38	**C35bis**	0.198	0.364	0.986	0.589	0.566	1.269
SEQ ID NO: 39	**2**	0.153	1.061	1.012	0.716	0.593	1.586
SEQ ID NO: 40	**mp2-2**	0.056	1.054	1.839	1.007	1.220	1.007
SEQ ID NO: 41	**C8**	5.595	1.280	1.163	0.543	0.808	0.950
SEQ ID NO: 42	**Protinh2-1**	1.175	1.335	0.619	0.972	0.025	1.022

Table 12: *List of biomarker genes predictive of the quality of the pearl in P. margaritifera (the results correspond to the difference in expression between the oysters of group A and the oysters of group B).*

This work therefore made it possible to identify a signature predictive of the capacity for biomineralization of a donor pearl oyster.

15 of the 42 biomarkers SEQ ID NO: 1 to 42 were then evaluated on the basis of a data set resulting from two additional grafting projects independent of that described above.

Experimental grafts

In graft G2, 67 graft-donor oysters were used. A graft G3, 64 graft-donor oysters from a batch other than that of the G2 oysters were used. Fifty grafts were cut from the mantle of each valve of each donor pearl oyster. Two grafts from each donor were preserved (1 graft per valve) in order to determine, according to the method described in this invention, the expression levels of 15 of the 42 biomarkers SEQ ID NO: 1 to 42. The remaining grafts from each valve of each donor pearl oyster were grafted in recipient pearl oysters, 3208 oysters for G2 and 3051 oysters for G3, using nuclei of identical diameter. The pearls of G2 were harvested at 15 months post-graft and those of G3 at 16 months post-graft.

Classification of graft donors for grafts G2 and G3

A grade was assigned to each donor pearl oyster on the basis of the evaluation of the quality of the harvested pearls and according to the calculation mode described in this invention. A total of 2074 and 1879 pearls from grafts G2 and G3, respectively, were analysed on each of the 3 variables "Nacre thickness at the surface of the pearl", "Number of defects at the surface of the pearls" and "Commercial quality". For each of grafts G2 and G3, 4 donor pearl oysters producing, on average, higher-quality pearls (high nacre thickness, few surface defects and/or better quality classification) and constituting Group A (target), were selected (**Table 13**). Group B (control or calibrator) corresponds to a group of 23 graft-donor pearl oysters representative of the pearl oyster population used in grafts G2 and G3.

Table 13: Comparison of the means of the variables "Nacre thickness at the surface of the pearl", "Number of defects at the surface of the pearls" and "Commercial quality" according to the groups of donor oysters used in grafts G2 and G3. The gain in quality of the pearls produced by the donors of group A with respect to those of group B, expressed as a percentage, is indicated in bold and between parentheses.

	Mean value of the quality variables according to the donor oyster groups studied, for each of grafts G2 and G3					
Grafts	**G2**			**G3**		
Means of the variables / Donor group	**All donors**	**B**	**A**	**All donors**	**B**	**A**
Thickness	1.707	1.688	2.196 **(+30%)**	1.597	1.585	2.022 **(+28%)**
Number of defects	1.529	1.447	0.771 **(+47%)**	1.688	1.673	0.970 **(+42%)**
Commercial quality	1.557	1.594	2.429 **(+52%)**	1.356	1.276	2.118 **(+66%)**

Results

With regard to the analysis of the pearls of graft G2, the gain in quality of the pearls produced by the donors of group A with respect to those of group B on each of the variables "Nacre thickness at the surface of the pearl", "Number of defects at the surface of the pearls" and "Commercial quality" is 30%, 47% and 52% respectively (**Table 13**). For graft G3, the gain in quality of the pearls produced by the donors of group A is 28%, 42% and 66%, respectively. The mean values of each variable obtained in group B and in the group consisting of all of the donors used in the graft are similar, regardless of whether graft G2 or G3 is considered. These results illustrate the representativity of the donors of group B selected from the population of donors used in the two independent grafts G2 and G3.

The relative expression levels of 15 biomarkers predictive of the quality of the pearl in *P. margaritifera* were determined by real-time PCR according to the methodology described in the present invention. Thus, the expression levels in the donor groups A and B were calculated for each of grafts G2 and G3 and each of the 3 variables of interest: Thickness / Commercial quality / Defects.

The expression levels measured were compared with those obtained in the first graft (G1) and confirm the results previously obtained. The meaningful levels of over-expression or under-expression (fold greater than or equal to 2, designated "+" and "-" respectively) for each of the 15 biomarkers with a high capacity for biomineralization, obtained in grafts G1, G2 and G3, are indicated in **Table 14**.

Table 14: Comparison of the levels of over-expression or under-expression of the biomarkers of high capacity for biomineralization of the donor pearl oysters of group A with respect to the control sample according to the graft considered.

Identifier	**Name**	**Variable impacted**	**Over-expression / Under-expression**
SEQ ID NO: 3	CALC-1	Commercial quality	-
SEQ ID NO: 4	CLEC3-1	Commercial quality	-
SEQ ID NO: 5	DERM-2	Commercial quality	-
SEQ ID NO: 8	MUCO-2	Thickness	+
SEQ ID NO: 11	shem7-2	Commercial quality	+
SEQ ID NO: 14	C18	Commercial quality	-
SEQ ID NO: 18	C46	Thickness	-
SEQ ID NO: 23	PIF-2	Thickness	+
SEQ ID NO: 26	C26bis	Thickness	+
SEQ ID NO: 27	C54(C53bis)	Thickness	+
SEQ ID NO: 28	mp11-2	Number of defects	+
SEQ ID NO: 36	C75bis	Thickness	+
SEQ ID NO: 39	2	Thickness	-
SEQ ID NO: 40	mp2-2	Thickness	-
SEQ ID NO: 42	Protinh2-1	Number of defects	-

The combined analysis of the analyses of the expression level of 15 of the 42 biomarkers SEQ ID NO: 1 to 42 in correlation with the quality of the pearls obtained in three independent experimental grafts therefore resulted in the definition of a refined list of 15 biomarkers predictive of the growth and quality of the pearl in *P. margaritifera* (**Table 14**).

CLAIMS

1. Signature predictive of the commercial quality of pearls obtained using a donor oyster and/or the rate of nacre deposition around the nucleus using said donor oyster, said signature comprising the expression profile in the mantle of said donor oyster of at least one biomarker comprising or consisting of a nucleotide sequence selected from SEQ ID NO: 1, 4, 5, 8, 11, 14, 18, 23, 26, 27, 28, 36, 39, 40 and 42, variants and fragments thereof.

2. Predictive signature according to claim **1**, said signature comprising the expression profile in the mantle of said donor oyster of at least one nucleotide sequence selected from SEQ ID NO: 3, 4, 5, 11 and 14, variants and fragments thereof, and said signature being predictive of the commercial quality of the pearls obtained using said donor oyster.

3. Predictive signature according to claim **1** or claim **2**, said signature comprising the expression profile in the mantle of said donor oyster of at least one nucleotide sequence selected from SEQ ID NO: 8, 18, 23, 26, 27, 36, 39 or 40, variants and fragments thereof, and said signature being predictive of the rate of nacre deposition around the nucleus using said donor oyster.

4. Predictive signature according to any one of claims **1** to **3**, said signature comprising the expression profile in the mantle of said donor oyster of at least one nucleotide sequence selected from SEQ ID NO: 28 or 42, variants and fragments thereof, and said signature also being predictive of the number of surface defects of the pearls obtained using said donor oyster.

5. Process of identification and/or selection of a graft-donor oyster with a high capacity for biomineralization, in which:

(a) in a biological sample of said oyster, the expression level of at least two predictive biomarkers as described in any one of claims **1** to **4** is determined, and

(b) the expression level obtained in step (a) is compared with a control expression level.

6. Kit enabling the process of claim **5** to be implemented, comprising means for determining the expression profile of at least one biomarker as described in any one of claims **1** to **4**, in which said means make it possible to determine the expression profile at the transcriptomic level by RT-PCR, RT-qPCR or high-speed quantitative PCR.

7. Kit enabling the process of claim **5** to be implemented, comprising means for determining the expression profile of at least one biomarker as described in any one of claims **1** to **4**, in which said means make it possible to determine the expression profile at the protein level.

8. Process for production of at least one higher-quality pearl, comprising the culture of at least one recipient oyster comprising the predictive signature according to any one of claims **1** to **4**, or identified according to the identification process of claim **5**.

9. Use of at least one biomarker comprising or consisting of a nucleotide sequence selected from the list consisting of SEQ ID NO: 1, 4, 5, 8, 11, 14, 18, 23, 26, 27, 28, 36, 39, 40 and 42, variants and fragments thereof, in order to determine the capacity for biomineralization of a graft-donor oyster.

10. Use of at least one biomarker comprising or consisting of a nucleotide sequence selected from the sequences SEQ ID NO: 3, 4, 5, 11 and 14, preferably the sequence SEQ ID NO: 4, in order to predict the capacity of a graft-donor oyster to produce pearls of high commercial quality.

11. Use of at least one biomarker comprising or consisting of a nucleotide sequence selected from the sequences SEQ ID NO: 28 and SEQ ID NO: 42, preferably the

sequence SEQ ID NO: 28, in order to predict the capacity of a graft-donor oyster to produce pearls having a small number of surface defects.

12. Use of at least one biomarker comprising or consisting of a nucleotide sequence selected from the sequences SEQ ID NO: 8, 18, 23, 26, 27, 36, 39 and 40, in order to predict the capacity of a graft-donor oyster to produce pearls having a small number of surface defects.

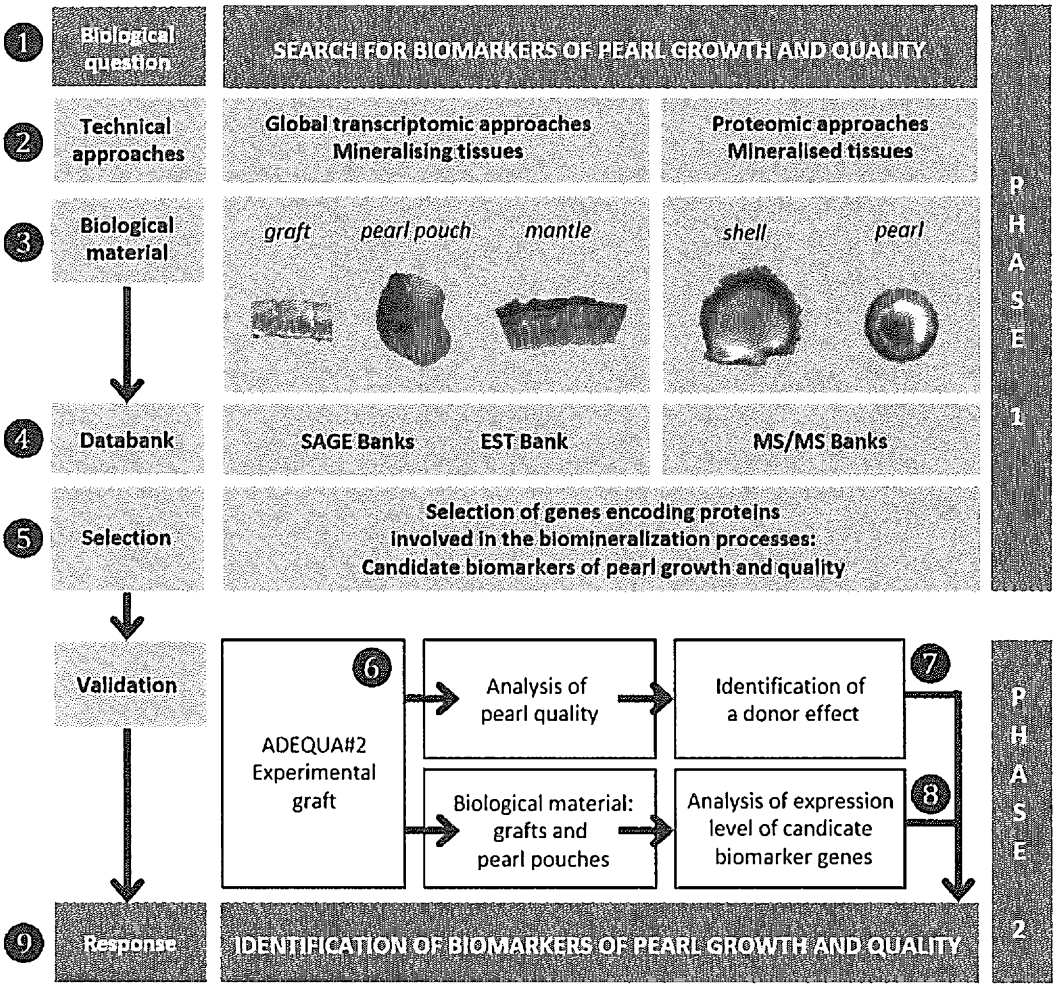
1
Biological question
SEARCH FOR BIOMARKERS OF PEARL GROWTH AND QUALITY
2
Technical approaches
Global transcriptomic approaches
Mineralising tissues
Proteomic approaches
Mineralised tissues
3
Biological material
graft
pearl pouch
mantle
shell
pearl
4
Databank
SAGE Banks
EST Bank
MS/MS Banks
5
Selection
Selection of genes encoding proteins
involved in the biomineralization processes:
Candidate biomarkers of pearl growth and quality
PHASE 1
Validation
6
ADEQUA#2
Experimental
graft
Analysis of
pearl quality
Identification of
a donor effect
7
Biological material:
grafts and
pearl pouches
Analysis of expression
level of candicate
biomarker genes
8
9
Response
IDENTIFICATION OF BIOMARKERS OF PEARL GROWTH AND QUALITY
PHASE 2

FIG.1

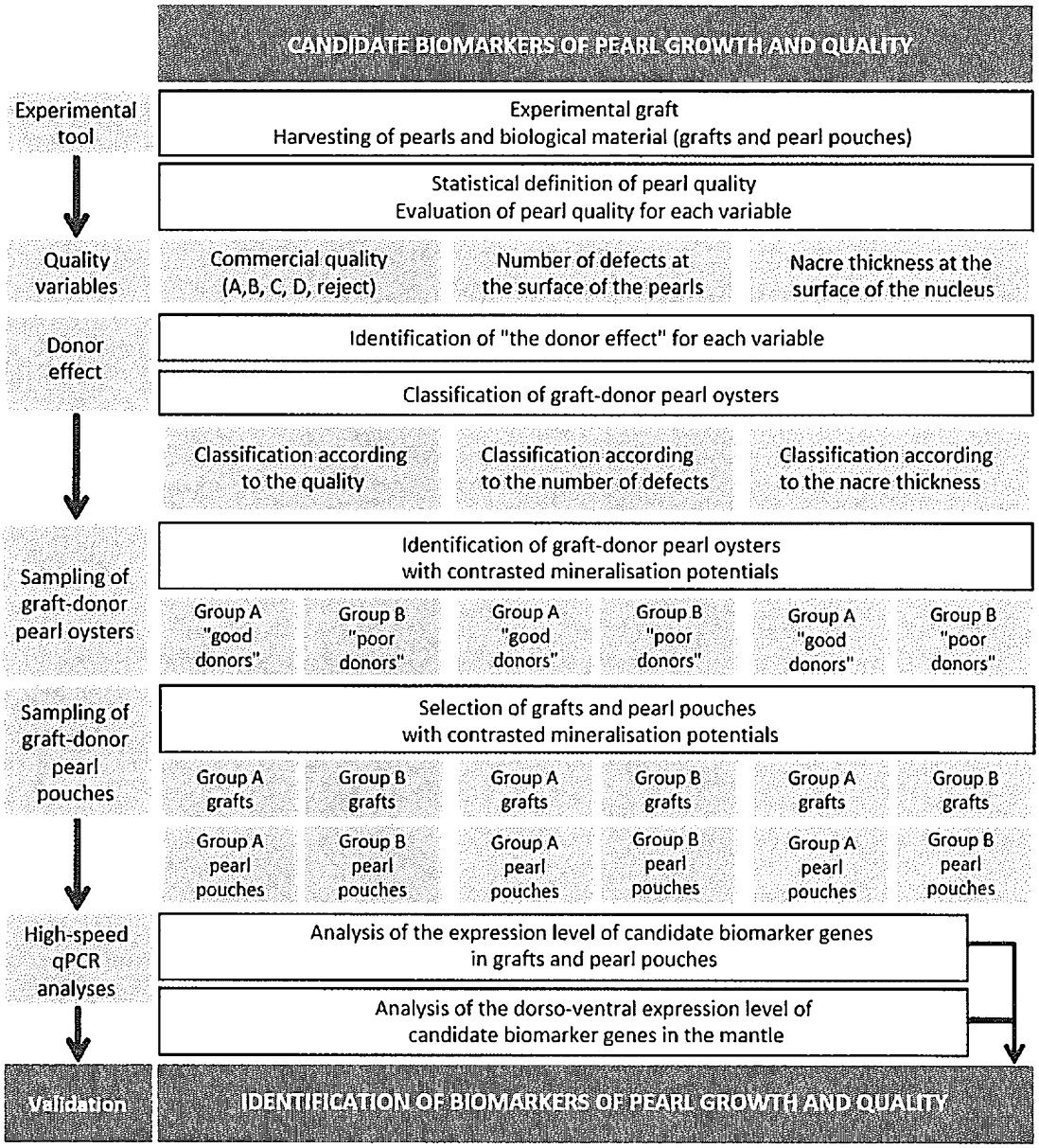
CANDIDATE BIOMARKERS OF PEARL GROWTH AND QUALITY
Experimental tool
Experimental graft
Harvesting of pearls and biological material (grafts and pearl pouches)
Statistical definition of pearl quality
Evaluation of pearl quality for each variable
Quality variables
Commercial quality (A,B, C, D, reject)
Number of defects at the surface of the pearls
Nacre thickness at the surface of the nucleus
Donor effect
Identification of "the donor effect" for each variable
Classification of graft-donor pearl oysters
Classification according to the quality
Classification according to the number of defects
Classification according to the nacre thickness
Sampling of graft-donor pearl oysters
Identification of graft-donor pearl oysters with contrasted mineralisation potentials
Group A "good donors"
Group B "poor donors"
Group A "good donors"
Group B "poor donors"
Group A "good donors"
Group B "poor donors"
Sampling of graft-donor pearl pouches
Selection of grafts and pearl pouches with contrasted mineralisation potentials
Group A grafts
Group B grafts
Group A grafts
Group B grafts
Group A grafts
Group B grafts
Group A pearl pouches
Group B pearl pouches
Group A pearl pouches
Group B pearl pouches
Group A pearl pouches
Group B pearl pouches
High-speed qPCR analyses
Analysis of the expression level of candidate biomarker genes in grafts and pearl pouches
Analysis of the dorso-ventral expression level of candidate biomarker genes in the mantle
Validation
IDENTIFICATION OF BIOMARKERS OF PEARL GROWTH AND QUALITY

FIG. 2

SEQUENCE LISTING

<110> IFREMER (Institut Français de la Recherche pour l'Exploitation de la Mer)
DIRECTION DES RESSOURCES MARINES
SKULD TECH
GUEGUEN, Yannick

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<213> Pinctada margaritifera

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ctgcccccac aaccgtaact acagcatcgt tgattgtcct cacaatcctg gtcagaaagg 660

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<213> Pinctada margaritifera

<220>
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<212> DNA
<213> Pinctada margaritifera

<220>
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<222> 1..489

<223> /mol_type="unassigned DNA"
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taatcaacat gaagaccgta ggatggagtt ccgctgctgc agtcttccag gaatgtgtgt 360

gttgaattgt agaacaacag gctgggtaaa tgattatgac caggaagttc tacatactgt 420

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gttggaggtt tgtatggctt gtatgaagct gaggtgaatg caaataaagc gacgatgaca 480

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<211> 276
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<213> Pinctada margaritifera

<220>

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<223> /mol_type="unassigned DNA"
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tttgacattt attcgtatag ttttctttta ttaacaccat ttaatagcag cattttcttc 180

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<211> 1017
<212> DNA
<213> Pinctada margaritifera

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<210> 11
<211> 1421
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<213> Pinctada margaritifera

<220>
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ccagaaccta gacctttagg tgggttaggt tcggcatcac ttgctggatc aggaccagga 300

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<213> Pinctada margaritifera

<220>
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gaaattaggt tttgtccaaa ctccatctga tccacagaat attgtaccgt ttccatccgg 240

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<212> DNA
<213> Pinctada margaritifera

<220>
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agtgcgtttt caattcgtac gattatatta aaataaaaga acaaatgaaa tacattggga 180

caaaagaatg acattagaaa tactgattct gttggtactg cccatattat gtgaattcca 240

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aggaattcat caaaagtaaa ctccattctc gatttgtttg aaatataaaa gtagatatca 660

gcctgattta aataatgaat attgttgtaa aactattgca caaatactgt aggatttcac 720

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attctttaaa acaatcttgc ttcctttgtc ttccacctta atttgtacaa aaagttaaag 3420

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<213> Pinctada margaritifera

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aacaaacaaa ttgcaaagtc tgattaagtc tttttaaatt ttgtcaaata atttgctcaa 240

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acagcattac tgttattgga agttaagata tgaagatttt actgttatgc tttactgttt 360

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gtaccgacgc caactgatgt tcccgtatta tctgggcctc cattcatttg caaatctatt 240

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aatcccggtg gtccttctat ctctactgcc tgctttggtt caatctccgg aacttttatc 420

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aatttttatc tctggtcttt gtaatatttc tatttccggt gttttgactt ccatagcttg 540

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tgacgattat agcagcgatc ctttttgtca ccatcgtatc atgtaatgct agaaaaagrt 240

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tacagttact tcaaacgttt atacaatagt ttacttttt taaatacaaa aagaaggaaa 720

aaatgtatct ttaattaagt ttcgaacttc atgttgatag ttatccatac ataggttgct 780

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gttcccccac cctcaagact gtgacaaatt cctgaactgt tttgatgata ttccatacat 360

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tcatttttgc tgtaatgctt gcagcttcgg aggctcaaga taattggttt gttgggtcag 180

attgtgtctg tacactggaa tataaccctg tatgcggcgt ggatggtgtt acctacggaa 240

atgattgtca acgacgatgc caaggggtag caaaagcatc agatggcgaa tgtagtggaa 300

acgattgtat ttgtccccgg aactatgatc ctgtttgtgg tgtaaatggt aggacgtatg 360

gcaatgactg tatacgacaa tgcagtggag tgcaactagc atatcctggc gaatgcagaa 420

gaggtccttg tgtttgtccc tttatccttc gcccagtgtg tggaagagat ggcaggacat 480

atccaaattt gtgcgtaatg cgttgcaggg gagtgaggtt ggcatatttc ggtgaatgca 540

gaggatgtcc aaatcccaga gtcttttgtt tcgcaccccc ttgcagtgtt tcctcatgcc 600

cacgctatcc acgagcaagc tgtagagcag gatgcaattg caatgccaga ttcttttatg 660

gaggaagaga agtcactaac gattgcagag tctagacaag aaaaaaaggg tcataataga 720

ccgaggcagt ttctggatga attatttatg aacggattta aaaaaaataa agatagattt 780

caataatgaa gtcattttaa gaaggagaat aacatatctt ttattttcgt agagaaaaaa 840

aatacacact tcacaactaa caagatagga cgcactaa 878

<210> 36

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<212> DNA
<213> Pinctada margaritifera

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tggtgagcaa aatggaggcg aaaagaaaaa ggataacaaa gatatcatta aaatgattgg 180

agtacctatt ttgaaactac ttttgaaaga agaatttcaa agcgaaggac aaaatggtgg 240

aaacggcgga aatgaacaag gtggtggaaa cgaacaaggt ggtggaaacg gacaaggtgg 300

tggaaatgaa caaggtggtg gaaacggaca aggtggcgga aacggtcaag gtggtggaaa 360

tggtaagaac ggcggaaatg gacaaggcgg tggatatgga tatggttttc attttggtgg 420

ttatggaatg gacggcggaa atggacaaaa tggtggtaat ggtggtggca atggaggcgg 480

taacggaggt ggaaacggag gacaaggaaa tggtcaaaat ggaggcggag gcaagggcgg 540

caacggagga tacggctttg atgtaatgaa aatgatccaa ccacctcatt accttaaaaa 600

gacttattga tgatgaaaat taatcacgcc aaaccataca caatcttgta aatattctct 660

aatttatttg taaataaatt ttatttgcat taacatacaa aaaaa 705

<210> 37
<211> 368
<212> DNA
<213> Pinctada margaritifera

<220>
<221> source
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<223> /mol_type="unassigned DNA"
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<400> 37
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gacttcagtt cttcattgaa ctgcaataaa tagtacataa atcacaatta cacaaacatt 120

caaatgtgta agacaaaaac aagttaattt atgaaagtac aaaatatttt acaaacatct 180

acatgtaatt tacaacacaa ccttttctac ataaatacac aaaatggtcc ctgactgaaa 240

ttgccttggt ctctgtgaca cttctgaata tacacatatt gtagtttctc ttaccaaaaa 300

acaaactgct taaacttttg cttttataag tctttatcct ttataatcat atcatcatta 360

ttatgtaa 368

<210> 38
<211> 262
<212> DNA
<213> Pinctada margaritifera

<220>
<221> source
<222> 1..262
<223> /mol_type="unassigned DNA"
/organism="Pinctada margaritifera"

<400> 38
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gaacgcaaca cttgtgtcgg atttggcact ggttgttata tcgattgctc tcaatgtagt 120

cttctggaca gtcatatttg aagtatacgc aatagcagtc aacctcgacc tcttcctgca 180

agaagaagaa caaatacaca caaacacccc ggccgtaatg gccactctgc gttgatacca 240

ctgcttattc tagaggccga gg 262

<210> 39
<211> 257
<212> DNA
<213> Pinctada margaritifera

<220>
<221> source
<222> 1..257
<223> /mol_type="unassigned DNA"
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<400> 39
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actagttttc ggcgctgtca tcaatctgag acgtatttac aagtactgcc agtttcttgc 120

tagtgacgac gtttcatcgt tgtgacgtac gtacatggca ttttcaaacc tgattccatt 180

gccgttgttg tatttccctc ccagagcatt tttcttccat aagatgatga caacaattat 240

gataaaaaca agacaag 257

<210> 40
<211> 1719
<212> DNA
<213> Pinctada margaritifera

<220>
<221> source
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<223> /mol_type="unassigned DNA"
/organism="Pinctada margaritifera"

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ctcgcgaaaa ttatgtcctt gtgaataaaa gttgwttacg atggttcata tcctgcacaa 120

catacaatgt cagtaaattg acatttttaa catgttaaaa aagcacttct aaatgtagat 180

acattacaga aatatacttc acgacggttc ttgtgatcag aggaaagact tgacgtgtaa 240

aggaaaactg gcaagatcat gatacaatgg agtaaatgtg tatgtacttg gtatttgatc 300

agagtgattc tttgttaaag ctttatcaac acaaattatg tcagcaccta tgaaatgacc 360

ctttctgtat agaattctgt tgaaagagct gaaatatcta taatgctgaa cagtttcatt 420

ctctacaagg acacttaaca ctgcttaaga agccagtaca atatgtataa caatctaaat 480

gtgtattgcc attttaaaaa tttgctagaa cagatctaca tgataggata aaataaattc 540

agtctacaat atgacgcgta tttccaatat tcatccctga ctggtttgct cctttgttcg 600

ttcccatttg taaaccaatt ataccctgtc ctgctttcaa ttgttcatct gtgaattccc 660

ttttgttcgc cttggcttct ttgggaccaa atgcaccttg ctttcttgct aatgctgtta 720

aacacaaaac aacttggttc aaattctgag cttcatacaa atctacagtt tggaaacact 780

cgtggcctgc aactccattt gctctacacc actcaaggaa ctgtccaatc atttccatct 840

gtttgaaggc caatttttgt ggagctgatc ccgtcttcac agcaccatta gtcaacttag 900

cgagatagaa tccattttta agttgttcat ggaaatttgc ggtatcccca gaagtattaa 960

agttttctcc tattttctgg cttatccatt taaggcatac ctcggcttgc tctggttcat 1020

attttgcatc tgtcttcccc ttgcattctg cggcaagtcc ggatttccct gctctgtaag 1080

acattttgta aactttaaca gctcctgaaa tcgcaaattg tttaattgaa tgaagttcga 1140

cgaaaccccc ggccgtaatg gccactctgc gttataccac tgcttaagca gtggtatcaa 1200

cgcagagtgg ccattacggc caggcaagat gcagatcttt gtgaaaactt tgactggcaa 1260

aacaataacc cttgaggttg agccaagtga tactattgaa aatgtaaagg ccaaaataca 1320

agacaaagaa ggaatccccc ctgaccagca gaggttgatt tttgctggta agcagttgga 1380

agatggccgc actttgtccg actacaatat ccagaaggaa tctaccttac acttggtgtt 1440

acgtctgaga ggaggtgcca agaaaaggaa gaagaagaac tacactaccc caaaaaagaa 1500

caagcataag aagaagaagg tcaaactggc agtgctgaaa tactacaagg ttgacgaaaa 1560

tgggaagatc actcgcctgc gacgagagtg tcctaatgag gaatgtgggg caggagtctt 1620

catggctgct cgctttgaca gacagtactg tggaaaatgc tgcctaacat atgtatttaa 1680

caagccagaa gacaaataag aaggaataaa tctccctct 1719

<210> 41
<211> 559
<212> DNA
<213> Pinctada margaritifera

<220>
<221> source
<222> 1..559
<223> /mol_type="unassigned DNA"
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taatggcact gaaaacttat tgaagatgcc ttatggatgt ggggagcaga acatgcttaa 120

cttcgctccc aacatctttg tacagaaata tttagctgct acaaacaaac ttacaccaca 180

actagagtcc agaatcaaag actatacaat aaaaggatac caaagagaga tgacgtacca 240

gcattctaac tcaggyggtt ttagtgcctt tggagatagt gacaaatcag caaccacgtg 300

gttgacatca tttgtgatca aatcgtttgc ccaagcttca agttttatca ccattgatga 360

tgattcagtt ttgagagctg ttcaatttgt cattgatcaa cagaatccgg acggatcgtt 420

ccgagaaccc ggacgtgtac ttcataaaga aatgcaggga gattcagcca agggggaggg 480

acccctcaca gcctttgttc ttattgcact gaaagaggct acagttttta atcgagtccg 540

agaagtggct caggatgca 559

<210> 42
<211> 652
<212> DNA
<213> Pinctada margaritifera

<220>
<221> source
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<223> /mol_type="unassigned DNA"
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atatcgtggg caaatgcatg ttcctgtttt ccgcctgatg aaacaagaca gcaaaaatgc 120

cgtagggctg actttgtttt cctgggccgt ggctatgtta caggaataca acaaataggt 180

tccttttttct atttaagata ctgttttctc attgatcgtg tgtttaagga cagggcttca 240

tcgttgaata ttccttgtgc tttaacaaat gttgagtctt cctattgtgg tgtcagattc 300

gaacgtgggc gaagatatat tgtatcaggt tatcttacaa ggagcggaaa ccaaattgga 360

gcttgtgagt ggaatgaaag atggagtaac gtgccttttc ttacaagatt acaacttttc 420

aatgatccgc agtggtgttt gccttgaact ggttgcctca atatttgtaa aaccaggcta 480

cctttagagc aactacgcat aaaaaaawtt acatgwtatg aggacaacat tttcgtttaa 540

aacgtcgttt cacgaagtat gtcaatttgc aaaacttgat aatcttttaa tacttttca 600

atacaaaaag gaaaaacaag ctaacgaaaa actaaacaaa aatagagtag ta 652

<210> 43
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<220>
<221> source
<222> 1..18
<223> /mol_type="unassigned DNA"
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/organism="artificial sequences"

<400> 43
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<210> 44
<211> 20
<212> DNA
<213> artificial sequences

<220>
<221> source
<222> 1..20

<223> /mol_type="unassigned DNA"
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/organism="artificial sequences"

<400> 44
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<210> 45
<211> 22
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<220>
<221> source
<222> 1..22
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<400> 45
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<210> 46
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<220>
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/organism="artificial sequences"

<400> 46
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<210> 47
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<220>
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<210> 48
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<220>
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/organism="artificial sequences"

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<210> 49
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<220>
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/organism="artificial sequences"

<400> 49
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<210> 50
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<220>
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<210> 51
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<400> 51
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<210> 52
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<400> 52
tgatgcaact tggggtcag 19

<210> 53
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<220>
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<210> 54
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<400> 54
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<210> 55
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<220>
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<222> 1..25
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<210> 56
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<220>
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<210> 57
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/organism="artificial sequences"

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<210> 58
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<210> 59
<211> 19
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<220>
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/organism="artificial sequences"

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<210> 61
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/organism="artificial sequences"

<400> 63
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<210> 64
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/organism="artificial sequences"

<400> 64
aagtgatgcc gaacctaacc 20

<210> 65
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<210> 67
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<210> 68
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<210> 69
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<220>
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<210> 70
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<400> 70
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<210> 71
<211> 21
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<220>
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<223> /mol_type="unassigned DNA"
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<400> 71
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<210> 72
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<220>
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<400> 72
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<210> 73
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<400> 73
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<210> 74
<211> 20
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<220>
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/organism="artificial sequences"

<400> 74
tcaatgcaaa cgatctgtcc 20

<210> 75
<211> 20
<212> DNA
<213> artificial sequences

<220>
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<222> 1..20
<223> /mol_type="unassigned DNA"
/note="C2 - Amorce sens"
/organism="artificial sequences"

<400> 75
tgttgggtgg tcatcgtaag 20

<210> 76
<211> 19
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<220>
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<400> 76
ccacatgcgg cagatactc 19

<210> 77
<211> 20
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<220>
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<222> 1..20
<223> /mol_type="unassigned DNA"
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<400> 77
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<210> 78
<211> 20
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<220>
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<222> 1..20

<223> /mol_type="unassigned DNA"
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/organism="artificial sequences"

<400> 78
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<210> 79
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<400> 79
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<210> 80
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/organism="artificial sequences"

<400> 80
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<210> 81
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/organism="artificial sequences"

<400> 81
ggctggtgga gccaatatg 19

<210> 82
<211> 23
<212> DNA
<213> artificial sequences

<220>
<221> source
<222> 1..23
<223> /mol_type="unassigned DNA"
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/organism="artificial sequences"

<400> 82
tgacttgaaa ttccctttg ttg 23

<210> 83
<211> 21
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<220>
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<222> 1..21
<223> /mol_type="unassigned DNA"
/note="C17 - Amorce sens"
/organism="artificial sequences"

<400> 83
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<210> 84
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<400> 84
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<210> 85
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/organism="artificial sequences"

<400> 85
aattcttcag cccggatatt g 21

<210> 86
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