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OPTIMIZATION OF CONDITIONSPOLYMERASE CHAIN REACTION FOR DETECTION ALLELES GDF-9GENE OF COWS

Abstract

Growth differentiation factor 9 (GDF9) belongs to the transforming growth factor β superfamily and plays a critical role in ovarian follicular development and ovulation rate. This article discusses the possibility of using the polymerase chain reaction for the study of gene polymorphism GDF 9 cows black-and-white breed. Conducted optimized to PCR conditions, ie the optimal annealing temperature of the primers and the concentration of magnesium chloride in the reaction mixture.

Key words: PCR, RFLP, polymorphism, Growth differentiation factor 9, folliculogenesis.

Introduction

Growth differentiation factor 9 (GDF9) belongs to the transforming growth factor β superfamily and plays a critical role in ovarian follicular development and ovulation rate [1,2]. Previous studies have shown that GDF9 is involved in cumulus expansion, hyaluronic acid synthesis signaling, maintenance of an optimal oocyte microenvironment, and synergistic action along with bone morphogenetic protein 15 through the regulation of several key granulosa cell enzymes that are essential for normal ovulation, fertilization, and female reproduction [2, 3,4]. Given the central role of GDF9 in ovulation and reproduction, GDF9 is a good candidate gene for mutations associated with reproductive performance. This gene has been widely studied in humans, sheep, and goats [5]. However, studies of GDF9 and bovine reproduction are relatively rare.

Studies have established the influence of genetic polymorphism of BMP and GDF 9 15 sheep on the number ovulated follicles and ovulation rate. For example, gene alleles, Bone morphogenetic protein - BMP 15 sheep, influence the process of folliculogenesis and for this locus heterozygous sheep ovulate two or three oocytec. The authors of this study recommend the use of BMP 15 gene polymorphism in sheep as a DNA marker for improving fertility in sheep breeding animals [6]. In 2013 there was the first report of gene polymorphism GDF 9 (Growth differentiation factor 9) in cattle and the relationship of alleles of this gene with suitable access for transplantation embryos donor cows, with the total number of embryos. 9 GDF gene in cattle has length 3824 nucleotide pairs, exon portion of the gene was conserved and hasn't mutations, in the intron portion two point mutations were detected. It is known that the gene products GDF 9 process control follicle growth and its development in cows. Polymorphism of this gene is well studied in medicine, in women with a mutation in the coding region of the gene GDF 9 found signs of premature ovarian failure [7,8].

Chinese scientists found that high yields of high-quality embryos for transplantation was in donor cows with the CT genotype A485, also was positive correlation with the total number of embryos and genotype donor cows A625 AA. Thus, the authors suggest the use of polymorphism GDF 9 cows as a DNA marker for predicting the reproductive function in cows. In connection with the above, has been tasked to study gene polymorphism GDF 9 cows of black-and-white breed. in the conditions of breeding farm LLP "Bayserke-Agro" and put technology diagnostic PCR mutation locus GDF 9.

Materials and methods Blood for the experiments was taken from cows of black-motley breed from the jugular vein of vacuum tube with EDTA. The blood samples were delivered to

the laboratory an were stored in a freezer at -20 ° C. Isolation of DNA from blood was performed according to the instruction set "DNA sorbitol " (made in Russia) . Carrying out PCR to determine the point mutation of the gene in intron 9 GDF comprises selecting primers. The first step is to identify sequence of forward and reverse primers for amplification of the desired DNA fragment. Firstly, from the site NCBI was taken out of database complete gene sequence of GDF 9 (format Pasta), animal species - Bostaurus. In the next step, using the known sequences of the primers identified site-specific point mutations in intron 9 of GDF gene. Work on genotyping cows gene locus GDF 9 held in 2013 in educational research and diagnostic laboratory Kazakhstan-Japan Innovation Center Kazakh National Agrarian University. Instruments used: Centrifuge Eppendorf company, Vortex, thermostat, horizontal electrophoresis apparatus, Thermocyclers" Tertsik " and " Eppendorf " gel documenting system. For amplification of the desired gene fragment GDF 9 A485T were used primers, that developed by the authors Tang K.Q et al(2013), which have the following sequences: forwardprimer -F 5'-AGGGAAGAAGAAAGATCTTTTGC -3 'and antisense primer R: 5'-TCTACCCAGGCTTTAGTCCC - 3'. Using this pair of primers amplify a region of the gene allows GDF 9 208 base pairs in length. In this case, the animals used for genotyping of the restriction enzyme NsiI, which has a restriction site ATGCA / T:

For the detection of a point mutation in the second intron of the gene under study A625T, we used primers : direct F: 5'-ATGCCCTCATGGGTTGATGTAGGCTA -3 ' and reverse R: 5'-CTCCCATCTCTCTCATACACACAAG - 3' . Complementary sequence of the above primers were tested by us, by a computer program, the two pairs of primers were complementary to the gene under study GDF portion 9. Restriction of PCR product in the second experiment was conducted with a restriction enzyme DraI, which has a restriction site TTT/ AAA.

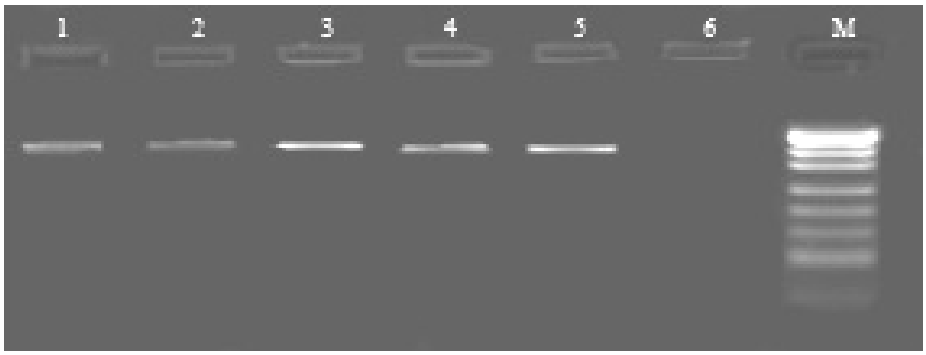

Figure 1.Polymerase chain reaction product, Lanes 1, 2, 3, 4, 5 - amplificate, 376 bp, 6 - negative control, M – molecular size marker, pUC19 DNA/MspI.

Success with the polymerase chain reaction depends on two factors: the concentration of $MgCl_2$ in the reaction mixture and the primer annealing temperature. Optimum MgCl2 concentration determined experimentally, primer annealing temperature was calculated by using the computer program «calculators for calculating the melting temperature of the primers».The optimum temperature for annealing the forward primer F 5'-AGGGAAGAAGAAAGATCTTTTGC -3 ' was 58,08 ° C, and reverse primer R: 5'-TCTACCCAGGCTTTAGTCCC - 3' temperature of 56,80 ° C.

Another important factor is that the concentration of $MgCl_2$, in the reaction mixture. It is now established that by increasing the $MgCl_2$ concentration increased synthesis of DNA molecules, but non-specific amplification is observed. Successful amplification passed at a concentration of 1.5 mM $MgCl_2$ and primers, an optimum annealing temperature was 58.0 C. The reaction volume was 50 ul having a composition: 5 ul of 10 X PCR buffer, 1.5 mM $MgCl_2$,

2,5 l of 25 mM direct and reverse primer, 5 l of 0.2 mM concentration of each dNTP, 0,5 l of an enzyme with the activity of Taq Polymerase 5u/µl, 5 microliters of DNA and 26.5 l of distilled water.

Using polymerase chain reaction together with RFLP allows for 5-6 hours to conduct animal genotyping loci A485T and A625T gene differentiation growth factor 9 (GDF 9).

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СИЫРЛАРДА GDF-9 ГЕНІНІҢ АЛЛЕЛЬДЕРІН АНЫҚТАУ ҮШІН ПОЛИМЕРАЗДЫҚ ТІЗБЕК РЕАКЦИЯСЫН ЖҮРГІЗУ ШАРТТАРЫН ОҢТАЙЛАНДЫРУ

Мақалада өсу факторының дифференциясы 9 трансформалық β супертуыстығына жататынын және осы фактордың аналық жануарлардың жұмыртқалықтарында фоллкулогенез үрдісіне және овуляция өту жылдамдығына байланысты екені зерттелген. Осы мақсатта авторлар қара-ала тұқымдас сиырларда GDF 9 генінің полиморфизмін анықтауға полимераздық тізбек реакциясын пайдалану мүмкіндігін көрсеткен. ПТР жүргізу шарттары оңтайландырылған, праймерлер жабысу температурасы мен реакциялық қоспадағы магния хлоридінің концентрациясы анықталған.

Кілт сөздер: ПТР**,** РФҰП, полиморфизм**,** өсу факторының дифференциясы 9, фолликулогенез.

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ОПТИМИЗАЦИЯ УСЛОВИЙ ПРОВЕДЕНИЯ ПОЛИМЕРАЗНОЙ ЦЕПНОЙ РЕАКЦИИ ДЛЯ ДЕТЕКЦИИ АЛЛЕЛЕЙ ГЕНА GDF-9 У КОРОВ

Фактор дифференциации роста 9 (GDF9) принадлежит трансформирующему фактору роста β суперсемейства и играет важнейшую роль в регуляции роста фолликулов и скорости овуляции. В статье рассматривается возможность применения полимеразной цепной реакции для изучения полиморфизма гена GDF 9 у коров черно-пестрой породы. Проведена оптимизиация условий ПЦР, т.е. установлена оптимальная температура отжига праймеров и концентрация магния хлорида в реакционной смеси.

Ключевые слова: ПЦР, ПДРФ, полиморфизм, фактор дифференциации роста 9, фолликулогенез.

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МЕТОДЫ ВЫДЕЛЕНИЯ ДНК PASTEURELLA MULTOCIDA ИЗ ОБРАЗЦОВ БИОЛОГИЧЕСКОГО МАТЕРИАЛА ДЛЯ ИСПОЛЬЗОВАНИЯ В ПЦР: СРАВНЕНИЕ И ОЦЕНКА

Аннотация

В статье на основании литературных источников и собственных исследований приведены результаты исследований по выбору оптимального метода выделения ДНК Pasteurellamultocida из биологического материала.

В результате выбора оптимальных методов выделения ДНК Pasteurellamultocida из биологического материала, определили, что все использованные в работе методы выделения ДНК вполне приемлемы для экстракции геномной ДНК Pasteurellamultocida, но наибольшее количество ДНК выделено с помощью ФХЭ с гуанидином. Отношение оптической плотности (E_{260}/E_{280}) полученных препаратов ДНК Pasteurellamultocida имело средние значение 1,7 > 0,04. Несмотря на многоступенчатость и продолжительность анализа в сравнении с новыми высокочувствительными и простыми в исполнении методами выделения ДНК, этот метод является оптимальным для выделения аналитических количеств ДНК в случаях, когда нет большого потока исследований.

Ключевые слова: Pasteurellamultocida, выделение, ДНК, спектрофотометрия, электрофорез.

Введение

К настоящему времени в арсенале исследователей имеется довольно большой набор методов экстракции и очистки ДНК, причем эти методы продолжают совершенствоваться и модифицироваться применительно к новым объектам исследования. В связи с разнообразием живых объектов универсальных методов выделения ДНК не существует. Использование того или иного метода выделения ДНК диктуется, во-первых, спецификой