A new set of multiplexes PCR suitable for genomic studies in Saudi sheep breeds

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Abstract. A total of 36 microsatellite markers (Bl42, BM1861, BM2504, BM864, BMC1222, BMS1620, BMS1948, BMSS522, BMS975, BRN, CSRDR2111, CSRDR263, CSSM008, CSSM015, CSSM25, CSSM41, CSSM43, CSSM66, FAS, HMH1R, ILSTS011, LSCV29, MAF33, MCM1, MCM136, MCM42, MCMA26, OARCP23, OARCP49, OARCP134, OARKP6, OCAM, RBP3, TGLA53, TGLA322, URB48) were used to investigate the possibility of being amplified set of multiplexes PCR. The markers are located in 20 different autosomic ovine chromosomes. The results showed that the 36 markers used in this study could be amplified together in nine different multiplexes. Each three multiplexes labeled by different fluorescent color (FAM, VIC and NED) gathered to be loaded in the same lane. The three multi-loads contained 11, 13 and 12 markers, respectively. 91.67% of the 36 markers have more than 4 alleles. These results are good indicator of the high variability of the selected markers in our population.

Key words: Microsatellite, Multiplex PCR, Sheep markers
Introduction

Kingdom of Saudi Arabia has a rich and diverse population of farm animal breeds which under traditional practices are posed at risk. The total number of Saudi’s sheep reached 5230859 head according to the Ministry of Agriculture, Year Book No 24 (MOA, 2012). As reported by Food and Agriculture Organization sheep is one of the most widely distributed and adaptable domestic species in this region. In the last twenty years the importance of studying the entire genome and diversity which led us to conservation and sustainable use of genetic resources for food and agriculture is a widely supported international objective contributing to efforts to eliminate global poverty and achieve world food security (FAO, 2007). Maddox and Cockett (2007) published a linkage map of sheep and showed that the map spans approximately 3580 cM, and is comprised of 1374 markers representing 1333 loci.

In genomic studies, the power of using multiplex PCR, then loading 3 multiplexes (multi-loads) which include primer of markers that previously marked by fluorescent label, gives us the facility to looking for more loci in the same lane. Multiplex PCR is a variant of PCR which enables simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers. Since its first description by Chamberlain et al., (1988) this method has been applied in many areas of DNA testing, including analyses of deletions, mutations, and polymorphisms, or quantitative assays and reverse transcription PCR. Typically, it is used for genotyping applications where simultaneous analyses of multiple markers are required, detection of pathogens or genetically modified organisms (GMOs), or for microsatellite analyses. Multiplex assays can be tedious and time-consuming to establish, requiring lengthy optimization procedures (Protocol-online, 2011). The present study presents three multi-loads suitable for genomic studies like genetic diversity by using molecular markers in Saudi local sheep population.

Material and Methods

In the present study 100 blood samples were taken from two Saudi sheep breeds (Naemi and Najdi; 50 from each breed) DNA from each sample was isolated according to salting out procedure (Miller et al., 1988). After isolation, DNA was taken to determine its quantity and quality using NanoDrop, followed by making the optimum dilutions depending on DNA concentration in each sample. The original samples were kept frozen (-20°C) until used. A total of 36 microsatellite markers were chosen (Table 1).
Table (1). List of microsatellite markers used in the study.

<table>
<thead>
<tr>
<th>Microsatellite Marker</th>
<th>Chromosome location</th>
<th>Primer sequence 5′ to 3′</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
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<td>BM1861</td>
<td>3</td>
<td>F-TGGTGAGCGCTCCTATGACCA</td>
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</tr>
<tr>
<td>BM2504</td>
<td>8</td>
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<td>Bishop et al., 1994</td>
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<td>F-TGGTGTCGCTTTCATGACCA</td>
<td>de Gortari et al., 1998</td>
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<tr>
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<td>de Gortari et al., 1998</td>
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<tr>
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</tr>
<tr>
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<td>BM97</td>
<td>9</td>
<td>F-ACACATCCTGCTCCTGA</td>
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<td>14</td>
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<td>8</td>
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<td>Moore et al., 1994</td>
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<td>26</td>
<td>F-ATGTCGCTTGCCTTGTCTCTA</td>
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<td>22</td>
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<td>OCAM</td>
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<td>RBP3</td>
<td>25</td>
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</tr>
<tr>
<td>TGLA51</td>
<td>12</td>
<td>F-CAACCTCCTCCTCTCTCTCTCT</td>
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</tr>
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<td>URB48</td>
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<td>Ma et al., 1996</td>
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</table>
Results and Discussion

All individual markers were tested in a small DNA sample from each breed to confirm that the marker will be amplified in these breeds. From the whole markers selected, different sets of microsatellites were chosen for amplifying a single PCR with selection criteria of sharing the same fluorochrome (multiplex reactions). Several multiplexes (between three and five) were mixed together in order to identify the alleles. The reactions of amplification were performed in a thermocycler. The PCR amplifications were developed in a volume of 10 µl using 5 µl of DNA and 5 µl of PCR master mix.

An automatic sequencer was used to determine each allele for each marker. The detection of the fluorochromes was carried out through a laser of Argon. Each of the fluorochromes has a distinct wavelength, enabling the incorporation of three colors in the same lane in the automatic sequencer. By this way, each lane has the possibility of utilizing four distinct colors for each reaction of amplification (FAM, NID and VIC). The fourth color, the ROX is assigned to the size standard. In general, three multiplexes PCR for each multi-load were mixed and a denaturalization chemical agent (formamide) was added. In each load, 16 animals were placed in an automatic sequencer. Results of the selected multiplex and multi-loads are shown in table (2 to 4).

Table (2). Melting temperature, fluorescent label and number of alleles observed for each marker in multiplexes 1, 2 and 3

<table>
<thead>
<tr>
<th>Multi-load</th>
<th>Fluorescent</th>
<th>Marker</th>
<th>Melting Temperature</th>
<th>Allele Number</th>
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<tbody>
<tr>
<td>A</td>
<td>FAM</td>
<td>BM1861</td>
<td>55°C</td>
<td>10</td>
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<td></td>
<td></td>
<td>MCM136</td>
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<td></td>
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<td>6</td>
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<tr>
<td></td>
<td></td>
<td>BMS522</td>
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<td></td>
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<td></td>
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<td>9</td>
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<tr>
<td></td>
<td>NED</td>
<td>BM975</td>
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<td></td>
<td></td>
<td>TGLA53</td>
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<td></td>
<td></td>
<td>HMH1R</td>
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<td>9</td>
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As shown in table (2), eleven microsatellite markers were joined together in three different multiplex labeled by the three fluorescence reagent (FAM, VIC and NED). Each contains 4, 4 and 3 markers, respectively. The melting temperature for every multiplex was similar in most cases (55 °C). Thereafter, the three different multiplexes migrated in the same lane of the automatic sequencer.
As shown in table (3), thirteen microsatellites markers were joined together in three different multiplex labeled by the three fluorescent (FAM, VIC and NED) each contains 5, 4 and 4 markers, respectively. The melting temperatures for every multiplex were 55, 60 and 55 ºC, respectively. Then the three different multiplexes were migrating in the same lane of the automatic sequencer.

As shown in table (4), eleven microsatellites markers were joined together in three different multiplexes labeled by the three fluorescent (FAM, VIC and NED). Each contains 4, 4 and 3 markers, respectively. The melting temperature for every multiplex was similar (55 ºC). Thereafter, the three different multiplexes were migrating in the same lane of the automatic sequencer.

**Table (3). Melting temperature fluorescent label and number of alleles observed for each marker in multiplexes 4, 5 and 6.**

<table>
<thead>
<tr>
<th>Multi-load</th>
<th>Fluorescent</th>
<th>Marker</th>
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<th>Allele Number</th>
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<tbody>
<tr>
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<td></td>
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<td>B</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>BMC1222</td>
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<td>5</td>
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</table>

**Table (4). Melting temperature fluorescent label and number of alleles observed for each marker in multiplexes 7, 8 and 9.**

<table>
<thead>
<tr>
<th>Multi-load</th>
<th>Fluorescent</th>
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<th>Melting Temperature</th>
<th>Allele Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>FAM</td>
<td>CSSM25</td>
<td>55ºC</td>
<td>7</td>
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<tr>
<td></td>
<td></td>
<td>BM864</td>
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<td></td>
<td>OARCP134</td>
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<td></td>
<td></td>
<td>BRN</td>
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<td></td>
<td>VIC</td>
<td>MAF33</td>
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<td>9</td>
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As reported by Al-Qassab et al. (2010) PCR multiplexes were formulated to allow simultaneous detection of more than one repetitive sequence at the same time as reported by the researchers that this approach leads to a rapid and robust method that allows the simultaneous assessment of diversity in each studied species.

Generally, the markers have a high variability index: 35 of them exhibited from 4 to 12 alleles, 1 marker presented 2 alleles and 10 markers presented 9 or more alleles in studied samples. In conclusion, 91.67% of the 36 markers have more than 4 alleles. These results are good indicator of the high variability of the selected markers in our population. The current findings are in agreement with that found by Maddox (2013), Moreover, Maddox et al (2000 a and b) reported a high variability for the selected markers.

Conclusion

In conclusion the current study developed 9 Multiplex-PCR reactions to analyse the genotypes of different microsatellite markers. The researcher designed 3 multiplex combinations “multi-loads”, and analysed them using an automatic sequencer that utilises 4 colours-one lane technology. As a result, it was able to clearly identify the allelic variants of 36 microsatellites, distributed uniformly on the ovine chromosomes and are suitable for genome analysis in addition to the study of diversity in Saudi sheep.

References


جموَّعة جَديدَة من تفاعل البِلمرة المَتسلسل المتعدد مَوافِقة للدراسات الجَينية في بعض سلالات الأَغنام في المملكة العربية السعودية

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(قدم للمنشور 13/10/2012م، قبل المنشور 25/11/2012م)

ملخص البحث: استخدمت في هذه الدراسة ست و ثلاثون عَلامة وراثية من نوع الميكلروستالايت بِغرض دراسة إمكانية دمجها في تكوين تفاعلات البلمرة المتسلسل المتعددة وتأثر هذه الانتقائية على تطبيقات الدراسات الجينية في قطعان الأغنام المحلية بالملكة العربية السعودية. أوضحت هذه الدراسة إمكانية الجمع (FAM, VIC, NED) بين عدة علامات وراثية بواسطة سبع مَعَدَّات مَعَالِمة بالفلوروسنت من أنواع ونُفِّخت ثلاثة مَعَدَّات مَعَالِمة بالفلوروسنت مختلفه تم استخدام 3 من مَعَدَّات التحَمِيل تحت كل منها على 11 و12 و13 عَلامة وراثية. تعتبر هذه الدراسة عامة لاستخدام مَعَدَّات تُحَمِّل صاحبة لسَلاَلات المحلية المستخدمة في دراسة المواقع الجينية ودراسات التنوع الوراثي. 77% من إجمالي 36 عَلامة وراثية مستخدمة في هذه الدراسة أعطت أكثر من 4 الالوان. هذه النتائج تعتبر مؤشرًا جيدًا لاختلافات العالية للعلامات المختصرة في عشرة الأَغنام تحت الدراسة وتشير مُحِالا جَديدًا لدراسة التنوع الوراثي للسَلاَلات الحيوائية في المملكة.