Primers for multiplex PCR identification for source of origin and pathogenic E.Coli contamination in meat samples: An electronic PCR experience

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Data Note

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Abstract
Meat fraud is an ongoing issue. So, identification of meat adulterant as well as bacterial contamination is utmost need. Advance technology like Multiplex and digital droplet PCR are the two currently used methods for diagnosing contamination of meat. However, simultaneous testing of adulterant and bacterial contamination in livestock are not done profoundly. Here, we have design PCR primers which can be used to identify adulterant and bacterial contamination concurrently.

Introduction
Meat adulterant is an ongoing phenomenon. To counteract such menace identification of meat contaminants by various techniques are also an ongoing research. There are several publications dealing with source of origin of meat identification by multiplex PCR approach. However, contamination of meat livestock with additives along with pathogenic bacteria is never attempted in a single goal in a multiplex PCR based approach. Like presence of meat additives, contamination by food poison causing bacteria is also an alarming issue. Therefore, identification of meat additives and contamination of cooked and/or uncooked meat by pathogenic/ non-pathogenic bacteria simultaneously is a definite advancement. Here we present PCR primer designing for simultaneous screening of meat adulterant along with meat contamination with pathogenic or non-pathogenic E Coli by multiplex PCR approach.

Materials and methods
Amplification of cytochrome b gene was used as a marker for present of cow, goat, pig and sheep meat in the livestock. Cytochrome b gene sequences of these livestock were retrieved from Nucleotide database. To identify the presence of E. Coli in the livestock, 16s rDNA gene was amplified. The primer was also generated using nucleotide sequences at Primer-BLAST website. The primer for amplification of eaeA gene was taken from Holland et al., 2000. Out of 10 probable primers for each nucleotide sequences, first two primers were
done for BLAST separately and primers which showed no similarity with other nucleotide sequences except self-species were chosen. Here, the amplification length of the product was also considered to avoid the overlapping in the gel band. Electronic PCR products with these chosen primers were performed by online servers (in case of E.Coli).

Results

The chosen primer sequences along with other parameters – accession no, gene name and amplified product length were given in Table 1. The amplified product length and sequences were checked by electronic PCR mentioned in the materials and methods section. The results were given in Figure 1. The product length, amplified sequences and uniqueness for cow, pig and sheep species identification were successful. The goat species was not checked by this server because it did not provide options. However, with the primers for goat species identification no other sequences of any species was found out. Further, E. Coli 16s rDNA was also identified by E. Coli gene identification server as mentioned in the materials and methods section and result was presented in Figure 1. Not all E.coli produces intimin toxin. Pathogenic E. Coli which produces this toxin and non-pathogenic strain which does not produces such toxin, that was also identified by the chosen primers and Electronic PCR.

Discussion

From the results we have seen that each primer designed has a different base pair length. If basepair difference is kept around 50 then in two tubes multiplex PCR amplification can be done from isolated DNA of meat sample to understand the source of origin and E Coli contamination. In one tube isolated DNA can be subjected to multiplex PCR amplification with designed primers for sheep, pig and goat. In another tube similar testing can be done for E Coli, E coli toxin and cow. In each of these cases the base pair difference of the predicted amplicons is around 50 and so distinguishable following electrophoresis on agarose gel (Zhao et al., 2021). The BLAST search of the designed primers predicts that there is no cross match with species /sequences other than the intended sequences (species). Therefore, we feel that the designed primers will serve the purpose. Further, screening for pathogenic E Coli and E Coli toxin in the sample will be doubly confirmed simultaneously. We believe that this approach will go a long way for assuring quality control of meat.
**Table 1.** Primers selected for electronic PCR. F denotes the forward primer and R denotes the Reverse primer.

<table>
<thead>
<tr>
<th>Species</th>
<th>gene</th>
<th>accession no.</th>
<th>Product length</th>
<th>Primer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>cytb</td>
<td>YP_209217</td>
<td>94</td>
<td>AAACAGGCTCCAACAACCCA (F) AGGGCCCCTAAGATGTCCCTT (R)</td>
</tr>
<tr>
<td>Goat</td>
<td>cytb</td>
<td>YP_004111308</td>
<td>104</td>
<td>CTCACCCGATTCTTTCGCTTTT (F) GTGGGGTTGTCTCGATCTCTTT (R)</td>
</tr>
<tr>
<td>Pig</td>
<td>cytb</td>
<td>NP_008646</td>
<td>168</td>
<td>TTGGCCCTAGTAGCTCCCAT (F) GAACGGGTGGTTCCTACGGGT (R)</td>
</tr>
<tr>
<td>Sheep</td>
<td>cytb</td>
<td>NP_008418</td>
<td>306</td>
<td>CGCCTGACTTACTCGGAGAC (F) TGTAGGCTGTCCTACCTTG (R)</td>
</tr>
<tr>
<td>E. Coli</td>
<td>16s rDNA</td>
<td>NR_024570</td>
<td>150</td>
<td>TATTGCACATGGGCAGCAAG (F) GGAGTTAGCCGCGTTCTTCTT (R)</td>
</tr>
<tr>
<td>E. Coli</td>
<td>eaeA</td>
<td>NC_002695</td>
<td>450</td>
<td>AAGCGACTGAGGTCACT (F) ACGCTGCTCAGATGT (R)</td>
</tr>
</tbody>
</table>
Figure 1. Electronic PCR results along with species names are shown.
References


Figure 1

Electronic PCR results along with species names are shown