(05-1-66)

Optimization of PCR protocols for detecting lignin biosynthesis genes (4CL and F5H) from *Zea mays* L. genomic DNA

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Objectives

Complete information of certain gene including exons and introns should be derived from the genomic DNA. However, unless full genome sequences exist, such information should be obtained from the enomic DNA. However, in general PCR protocols, amplification of genomic DNA (over 2kb) often failed. In this experiment, various PCR protocols were applied to amplify the maize genomic DNAs so as to amplify specific lignin biosynthesis genes at genomic levels.

Materials and Methods

Efficient PCR protocols should be developed for identifying lignin biosynthesis genes (4CL and F5H) directly from genomic DNAs. The optimization of PCR condition for 4CL and F5H genes included touch-down PCR with and/or without DMSO and long-PCR as well.

Results and Discussion

Detecting over 2kb size from PCR amplification using genomic DNA sometimes is hard to optimize. In our preliminary experiment, general PCR condition using primers failed to amplify or showed smaller size than expected.

To obtain full size of genomic DNAs, as first step;

Touch-down PCR were employed (from 72°C to 58 °C touch-down program) for 10 cycles and then 20 normal cycles were run. In this experiment, a little longer DNA was amplified but not exact size.

Secondly, addition of DMSO in PCR reaction, the same PCR condition was employed. However, in this condition, smaller size than expected (2kb to 4kb) amplified.

Finally, general long PCR condition with DMSO amplified expected size of genes from genomic DNA. Therefore, we recommend long PCR with addition of DMSO for amplification of specific genes using genomic DNA. We successfully amplified 4 different maize speices (Suwon, Kwanganok, Iksan/Tamra, Pioneer) and two different genes (4CL and F5H) with the same size each. Partial DNA sequencing with those amplified products showed over 97% homology.

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