Abtract
Cloning and analysis of promoter sequences are often used to study the mechanism of gene regulation. Researchers are mostly used sub-cloning method to increase the cloning efficiency, however this method is expensive and time consuming. An ideal alternative to overcome these problems is the use of ‘‘promoterless’’T-vectors containing reporter gene. Although the T/A cloning method is generally used to clone the PCR products, but a promoterless T/A vector contaning reporter gene that can be used for *Agrobacterium*-mediated transformation is not commercially available. Therefor in this research, 500bp fragment containing restriction site of the *Eam1105* I was cloned in to binary vector pCAMBIA1305.2, in order to construct a promoterless T/A binary vector for functional analysis in plant systems. Hence a 500bp fragment was amplified using primers containing restriction site of the *Eam1105* I by PCR. In addition to increase cloning efficiency of the 500bp fragment in pCAMBIA1305.2 and confirmation of recombination, restriction sites of *Hind* III and *Nco* I were added at 5' end of the primers. Then efficiency of the T/A vector was evaluated by cloning of 100bp, 200bp, 500bp and 981bp fragments. The results showed that these fragments were located within the recombinant binary vector. In order to determine the direction of the cloned fragment in the vector, enzymatic digestion of the T/A vector contaning 200bp fragment by *BamH* I enzyme was performed on agarose gel electrophoresis. Results of enzymatic digestion revealed the correct direction of the cloned 200bp fragment. Finally, after confirmation of the recombination of clones by PCR, to provide a rapid and cost-effective method to confirm the transformation, colorimetric assay based on gold nanoparticle was performed. In this way, the gold nanoparticles with a diameter of about 20nm was synthesized and in the presence of primers, cloning of the 500bp fragment in T/A recombinant vector was confirmed and the maximum Change of the gold nanoparticles wavelength was observed in the range of 614-624 nm that was indicating the presence of the 500bp fragment in the recombinant vector.

Keywords: pCAMBIA1305.2, T/A vector, Direction of cloning, Gold nanoparticles probe

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Designing of T/A binary vector and confirming of recombinant vector by gold nanoparticles probe

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