Isolasi RNA dan pengklonaan gen tripsin kation dari pankreas sapi ke escherichia coli DH5-945 = RNA isolation and gene cloning of bovine pancreatic trypsin cation into escherichia coli DH5-945

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Abstrak

Tripsin kation memiliki peran penting dalam teknik kultur sel mamalia adherent. Enzim tersebut berperan sebagai enzim hidrolitik yang berfungsi untuk mendispersi sel yang melekat pada cawan petri atau botol tempat kulturnya sehingga mempermudah proses kultur sel mamalia baik pada proses pemanenan sel maupun subkultur. Penelitian bertujuan untuk mengisolasi dan mengklon gen tripsin kation dari pankreas sapi ke dalam E. coli DH5 dengan vektor pGEM-T Easy. Fragmen gen tripsin kation target berukuran 780 pb diamplifikasi dari cDNA yang berasal dari mRNA sel pankreas sapi dengan primer spesifik gen tripsin kation. Gen tripsin kation target diligasi pada plasmid pGEM-T Easy. Vektor rekombinan ditransformasi dengan metode kejutan panas pada sel E. coli DH5 dan diseleksi menggunakan medium ampisilin. Vektor rekombinan di digesti menggunakan enzim SfoI dan XmaI dan menghasilkan pita DNA berukuran 780 pb pada elektroforesis gel agarosa. Hasil penelitian menunjukkan gen tripsin kation telah berhasil diklon ke dalam plasmid pGEM-T Easy.

.....Cationic trypsin has an important role in adherent mammalian cell culture. The enzyme is a hydrolytic enzyme that disperses the cells attached to petri dishes and culture bottle making the harvesting and subculturing procedures easier. This research aims to isolate the RNA and clone the bovine pancreatic cationic trypsin gene into E. coli DH5. The 780 bp cationic trypsin gene was amplified from cDNA derived from mRNA isolated from bovine pancreas using cationic trypsin gene-specific primers. Cationic trypsin gene was ligated to pGEM-T Easy plasmid. Recombinant vector was transformed by heat shock method into E. coli DH5 cells, which were then selected using amphicillin containing medium. The recombinant vector was digested using enzymes SfoI and XmaI to confirm the presence of the target gene. Agarose gel electrophoresis showed a 780 bp DNA band, confirming that the cationic trypsin gene was successfully cloned into the plasmid pGEM-T Easy.