DYS SEE

DNAse I Treatment.

iGEM Thessaly

Protocols

DNase I Treatment Protocol

DNase I is an endonuclease, which cleaves DNA non-specifically and creates fragments. Its main purpose in molecular biology is to eliminate DNA contamination in RNA extraction protocols. However, we used the enzyme to simulate the fragmentation occurring naturally in the human body.

Procedure

10 ul reaction

In a 0.2ml PCR tube add:

1. 1ul 10X DNase I reaction buffer
2. 1 unit of DNase I (0.2 ul from a 5U/ul stock)
3. 800 ng of DNA in less than 8.5 ul (more can be used if DNA is highly concentrated)
4. ddH2O to 10ul
   - Incubate at 37C for desired amount of time (3-10 min depending on template)
   - To stop the reaction, add 1ul 50mM EDTA and incubate at 75C for 10min. EDTA stops the reaction by chelating Magnesium cations, which are important co-factors of DNase I. Heating denatures the enzyme's secondary structure.
   - Quick-spin to collect liquid at the tube's bottom
   - Analyze a portion (~2ul) of reaction in a gel to check if the reaction was performed correctly

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