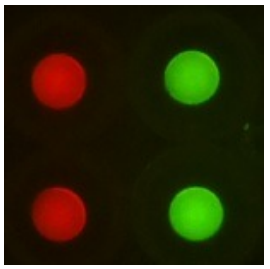
Nov 27, 2017 Version 2

Preparation of cell-free RNAPT7 reactions V.2



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http://federicilab.org

Protocol status: Working
We use this protocol in our group and it is working.

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Collection Integer ID: 8986

Abstract

This collection contains the protocols and recipes for the preparation of cell-free RNAPT7 transcription/translation coupled reactions.
The crude extracts are obtained from IPTG-treated E.coli BL21 DE3 STAR cells. IPTG triggers RNAPT7 expression and accumulation in cells before collection. Reactions take place when the cell extract is mixed with a maltodextrin-based energy solution along with amino acids solution and other salts (Magnesium and Potassium). This system allows transcription/translation coupled reactions from plasmid DNA that contains T7 promoter transcriptional units. In our hands, it has been possible to express different fluorescent reporters, including deGFP which is highly efficient. Products of the reaction can be measured during the whole reaction time in a plate reader with appropriate optic filters, open hardware cameras, and microscopes. When set up correctly, deGFP fluorescence intensity is detected as fast as ~30 minutes after the reaction starts and it shows a rapid increase during the first ~2.5-3.0 hours.

The system presented here contains the features highlighted bellow, and is an adaptation of protocols and findings from the following listed papers:

1.- This system uses maltodextrin and Polyphosphate-based energy solution: These reagents do not need refrigeration while transporting.

Kim, H.-C., Kim, T.-W., Kim, D.-M., . Prolonged production of proteins in a cellfree protein synthesis system using polymeric carbohydrates (2011)

Caschera, F.; Noireaux, V., A cost-effective polyphosphate-based metabolism fuels an all E. coli cell-free expression system. Metabolic engineering (2015)

2.- The S12 crude extract is prepared from E.coli cells (expressing Pol T7) using bead beater. We use an S12 extract which is simple and d not require ultra-centrifugation nor dialysis

Sun, Z. Z.; Hayes, C. A.; Shin, J.; Caschera, F.; Murray, R. M.; Noireaux, V., Protocols for implementing an Escherichia coli based TX-TL cell-free expression system for synthetic biology. Journal of visualized experiments (2013)

Kim, TW., Kim, HC., Oh, IS. et al. A highly efficient and economical cell-free protein synthesis system using the S12 extract of Escherichia coli. Biotechnol Bioproc E (2008)

3.- The amino acid stock solution is highly concentrated (each amino acid is at 12 nM in a stable mix):

Caschera, V. Noireaux Preparation of amino acid mixtures for cell-free expression systems. Benchmarks (2015)

Files

SEARCH

Protocol

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Step 3: Performing cell-free RNAPT7 reactions

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Step 1: Preparing S12 cell extracts using bead-beater

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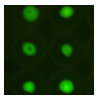

NAME

Step 2: Preparing amino acid, polyphosphates, and maltodextrin-based energy solutions for cell-free reactions

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