Culturing Escherichia coli cells

Created on: 20-03-2019 - Last modified on: 03-11-2022

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Organisation

Name of the organisation Vrije Universiteit Brussel (VUB)

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SCOPE OF THE METHOD

The Method relates to	Other: Recombinant DNA technology
The Method is situated in	Basic Research, Regulatory use - Routine production
Type of method	In vitro - Ex vivo

DESCRIPTION

Method keywords

bacterial cells

cell culture

protein expression

E. coli

prokaryote

Scientific area keywords

microbiology

biotechnology

Recombinant DNA technology

Method description

E. coli is one of the organisms of choice for the production of recombinant proteins. DH5 alpha cells are commonly used for maintenance, propagation and mutation, whilst BL21(DE3) and C43(DE3) are mainly used for expression of the transgene. The advantage of C43(DE3) is that is used to produce proteins that are expressed poorly in BL21 (DE3) or that are very toxic to the host organism. All strains can be cultured in Lysogeny Broth (LB) medium or LB agar plates with an appropiate antibiotic for positive selection of the clones. For induction of protein expression, isopropyl-b-thiogalactoside (IPTG) in a concentration of 0.2 mM - 1 mM can be used. In case you have a problems with leaky expression, 1 % w/v glucose can be added to the LB medium for excellent growth of the bacteria. Transformation of the cells can be achieved by heat shock or electroporation.

Lab equipment

Biosafety cabinet;

Bunsen burner;

Petri dishes.

Method status

Still in development

History of use

Internally validated

PROS, CONS & FUTURE POTENTIAL

Advantages

Fast growth kinetics (doubling time 20 mins);

High cell density cultures are easily achieved;

Readily available and inexpensive components for media;

Easy transformation.

Challenges

No post-translational modifications (i.e. prokaryote).

Future & Other applications

Every researcher that will need a prufied protein can obtain it in a recombinant form.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

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