SEP 24, 2015

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dwi7cd

External link:
http://www.agilent.com/cs/library/usermanuals/Public/200314.pdf

Protocol Citation: Harold Bien: Transform Stratagene's XL-10 Gold Ultracompetent cells (simplified). **protocols.io** https://dx.doi.org/10.17504/protocols.io.dwi7cd

Protocol status: Working

Transform Stratagene's XL-10 Gold Ultracompetent cells (simplified)

Forked from Transform Stratagene's XL-10 Gold Ultracompetent cells

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ABSTRACT

Protocol for transforming XL-10 Gold Ultracompetent cells from Stratagene (now Agilent). Protocol adopted from manufacturer's instructions and simplified to remove use of special media and beta-mercaptoethanol

Created: Sep 24, 2015

Last Modified: Feb 28, 2018

PROTOCOL integer ID: 1706

GUIDELINES

Use of 14-ml BD Falcon polypropylene round-bottom tubes: It is important that 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) are used for the transformation protocol, since other tubes may be degraded by β²-mercaptoethanol. In addition, the duration of the heat pulse has been optimized using these tubes.

Aliquoting Cells: Keep the cells on ice at all times during aliquoting. It is essential that the polypropylene tubes are placed on ice before the cells are thawed and that the cells are aliquoted directly into pre-chilled tubes. It is also important to use 100 µl of cells per transformation. Decreasing the volume will reduce efficiency.

Use of Î²-Mercaptoethanol (β²-ME): β²-ME has been shown to increase transformation efficiency. The β²-ME mixture provided is diluted and ready to use. Stratagene cannot guarantee results with Î²-ME from other sources.

Use of NZY+ Broth: Transformation of the supplied ultracompetent cells has been optimized using NZY+ as the medium for outgrowth following the heat pulse. Substitution with another outgrowth medium may result in a loss of efficiency.

Quantity and Volume of DNA: The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl supercoiled pUC18 DNA per 100 µl of cells. When transforming a ligation mixture, add 2 µl of the ligation mixturepер 100 µl of cells. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/µg) may be lower. The volume of the DNA solution added to the reaction may be increased to up to 10% of the reaction volume, but the transformation efficiency may be reduced.

Heat Pulse Duration and Temperature: Optimal transformation efficiency is observed when cells are heat-pulsed at 42°C for 30 seconds. Efficiency decreases sharply when cells are heat-pulsed for <30 seconds or for >40 seconds. Do not exceed 42°C.

Plating the Transformation Mixture: If plating <100 µl of cells, pipet the cells into a 200 µl pool of medium and then spread the mixture with a sterile spreader. If plating ≥100 µl, the cells can be spread on the plates directly. Tilt and tap the spreader to remove the last drop of cells. If desired, cells may be concentrated prior to plating by centrifugation at1000 rpm for 10 minutes followed by resuspension in 200 µl of NZY+ medium or alternative medium.

MATERIALS

STEP MATERIALS

Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

XL-10 Gold Ultracompetent cells **Agilent Technologies Catalog #200314**

XL-10 Gold Ultracompetent cells **Agilent Technologies Catalog #200314**

Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

XL-10 Gold Ultracompetent cells **Agilent Technologies Catalog #200314**

XL-10 Gold Ultracompetent cells **Agilent Technologies Catalog #200314**

PROTOCOL MATERIALS

Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

In Materials, Materials, Step 2

XL-10 Gold Ultracompetent cells **Agilent Technologies Catalog #200314**

In Materials, Materials, Materials, Materials and 2 steps

1 Pre-chill 14mL sterile culture tubes on ice

2 Pre-heat 0.9mL of LB broth to 42°C

1 mL

Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

3 Thaw XL-10 Gold Ultracompetent cells on ice then mix gently after completely thawed

XL-10 Gold Ultracompetent cells **Agilent Technologies Catalog #200314**

4 Aliquot cells into pre-chilled sterile culture tubes

100 µL

XL-10 Gold Ultracompetent cells **Agilent Technologies Catalog #200314**

5 Swirl tube gently then incubate on ice for 10 minutes swirling gently every 2 minutes

00:10:00

6 Add 0.1-50ng of DNA or 2uL of ligation product

2 µL

7 Swirl gently then incubate on ice for 30 minutes

00:30:00

8 Heat pulse tube at 42°C for exactly 30 seconds. The duration of the heat pulse is critical.

00:00:30

9 Incubate cells on ice for 2 minutes

00:02:00

10 Add 0.9mL of pre-heated LB to each tube

1 mL

11 Incubate at 37°C for 1 hour with shaking at 225-250 rpm

01:00:00

12 Plate no more than 200uL of transformation mixture per LB-agar plate with antibiotic selection

13 Incubate plates at 37°C overnight.

15:00:00