

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.

Citation: Harold Bien Transform Stratagene's XL-10 Gold Ultracompetent cells. **protocols.io**

dx.doi.org/10.17504/protocols.io.dtz6p5

Published: 17 Sep 2015

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 β -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 μ l of cells per transformation. Decreasing the volume will reduce efficiency.

Use of β -Mercaptoethanol (β -ME): β -ME has been shown to increase transformation efficiency. The β -ME mixture provided is diluted and ready to use. Stratagene cannot guarantee results with β -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 μ l of 0.01 ng/ μ l supercoiled pUC18 DNA per 100 μ l of cells. When transforming a ligation mixture, add 2 μ l of the ligation mixture per 100 μ l of cells. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/ μ g) 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 $^{\circ}$ C for 30 seconds. Efficiency decreases sharply when cells are heat-pulsed for <30 seconds or for >40 seconds. Do not exceed 42 $^{\circ}$ C.

Plating the Transformation Mixture: If plating <100 μ l of cells, pipet the cells into a 200 μ l pool of medium and then spread the mixture with a sterile spreader. If plating \geq 100 μ l, 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 at 1000 rpm for 10 minutes followed by resuspension in 200 μ l of NZY+ medium.

Protocol

Step 1.

Pre-chill 14mL sterile culture tubes on ice

Step 2.

Pre-heat 0.9mL of NZY+ broth to 42°C

 AMOUNT

1 ml Additional info:

 REAGENTS

✓ NZY broth by Contributed by users

Step 3.

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

 REAGENTS

XL-10 Gold Ultracompetent cells [200314](#) by [Agilent Technologies](#)

Step 4.

Aliquot cells into pre-chilled sterile culture tubes

 AMOUNT

100 µl Additional info:

 REAGENTS

XL-10 Gold Ultracompetent cells [200314](#) by [Agilent Technologies](#)

Step 5.

Add beta-mercaptoethanol

 AMOUNT

4 µl Additional info:

 REAGENTS

XL-10 Gold Beta-mercaptoethanol mix by [Agilent Technologies](#)

Step 6.

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

 DURATION

00:10:00

Step 7.

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

 AMOUNT

2 µl Additional info:

Step 8.

Swirl gently then incubate on ice for 30 minutes

 DURATION

00:30:00

Step 9.

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

 DURATION

00:00:30

Step 10.

Incubate cells on ice for 2 minutes

 DURATION

00:02:00

Step 11.

Add 0.9mL of pre-heated NZY+ broth to each tube

 AMOUNT

1 ml Additional info:

Step 12.

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

 DURATION

01:00:00

Step 13.

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

Step 14.

Incubate plates at 37°C overnight.

 DURATION

15:00:00