



Aug 20, 2020

© Bacterial Transformation - Mix & Go Competent Cells - CHEM 584

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In Development dx.doi.org/10.17504/protocols.io.bj2tkqen



THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Adapted from

https://files.zymoresearch.com/protocols/_t3001_t3002_mix_go_e._coli_transformation_kit_buffer_set.pdf

DOI

dx.doi.org/10.17504/protocols.io.bj2tkqen

PROTOCOL CITATION

Ken Christensen 2020. Bacterial Transformation - Mix & Go Competent Cells - CHEM 584 . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bj2tkqen

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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CREATED

Aug 20, 2020

LAST MODIFIED

Aug 20, 2020

PROTOCOL INTEGER ID

40755

SAFETY WARNINGS

You should follow standard BSL-1 procedures when using this protocol.

BEFORE STARTING

Get an aliquot of the Mix & Go competent cells from the -80 Freezer (ask a TA to get them for you)

- 1 Prewarm culture plates in a § 37 °C incubator for © 00:30:00.
- Add 11 μl to 15 μl plasmid DNA to a 150 μl aliquation of thawed competent cells (Mix & Go) on ice, mix gently for a few seconds (try to keep the added volume of DNA less than 5% of the total). Place tube on ice for ② 00:05:00

Citation: Ken Christensen (08/20/2020). Bacterial Transformation - Mix & Go Competent Cells - CHEM 584 . https://dx.doi.org/10.17504/protocols.io.bj2tkgen

minutes.



When selecting with Kanamycin, Tetracycline, etc., an outgrowth performed in SOC medium is required for efficient transformation. After the transformation mixture has incubated on ice for at least 5 min, add 4 volumes of SOC (e.g., 400 μ l of SOC to 100 μ l of the transformation mixture) and incubate for 1 hour at 37°C with gentle shaking at 200-300 rpm.

- 3 Spread **30 μl** to **100 μl** of the mixture onto a pre-warmed (37°C) LB plate containing Ampicillin (or other selection antibiotic).
 - Using chilled LB plates will decrease the transformation efficiency.
- 4 Incubate overnight at § 37 °C in the bacterial incubator.
 - You can also incubate on the bench or in a drawer at room temperature for a longer time if this is convenient for scheduling. For example, leaving a plate on the bench for 2-3 days at room temperature usually generates nice sized colonies and you avoid having to come into the lab the next day (e.g., over the weekend).