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Natural Transformation

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Protocol status: Working

We use this protocol and it's working

Created: October 20, 2018

Last Modified: October 20, 2018

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Abstract

Following this protocol, *V. Natriegens* cells can be transformed with linear fragments and plasmids based on natural transformation as described in Multiplex Genome Editing by Natural Transformation (MuGENT) for Synthetic Biology in *Vibrio natriegens* Dalia *et. al*, 2017. *ACS Synthetic Biology*.

PRE CULTURE

- 1 Of a strain containing tfoX regulated by an inducible promoter (in this case an IPTG inducible promoter).

🌡️ 30 °C Shaking 🧪 3 mL LBv2 [M] 100 Molarity (M) uM IPTG

[M] 100 Mass / % volume ug/ml Carbenicillin

🕒 12:00:00 Hours to 🕒 16:00:00 Hours

Transformation reaction

- 2 🧪 3.5 µL Pre culture 🧪 350 µL 28g/l XIO [M] 100 Molarity (M) um IPTG

Are mixed and 🧪 50 ng Transformation DNA are added. A control without addition of transformation DNA is prepared, too.

🌡️ 30 °C Static 🕒 04:00:00 Hours to 🕒 06:00:00 Hours

Recovery

- 3 Add 🧪 1 mL LBv2

🕒 02:00:00 Hours 🌡️ 30 °C Shaking



Selection

- 4 Plate complete reaction onto LBv2 agar plates containing antibiotic corresponding to transformation DNA.

Incubation  12:00:00 Hours  37 °C .