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Transform pCas9

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DISCLAIMER

ABSTRACT

this protocol is aimed to provide instructions of transforming pCas9, which is a key step of knock-out gene by Crispr/Cas9

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PROTOCOL CITATION

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transformation, Crispr/Cas9, pCas9

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MATERIALS TEXT

competent MG1655, pCas9 plasmids, LB agar mediums (contain chloramphenicol), LB broth

1 Mix **50 µL** of competent MG1655 with **10 µL** of pCas9

2 Keep the reaction mix on ice for **00:30:00 minutes** 30m

3 Then, transfer the mix to the water bath set at **42 °C** for **00:00:45** 45s

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4 Next, keep the mix on the ice for **00:02:00** 2m

5 Add **940 µL** LB broth and incubate the cells at **37 °C** with vigorous shaking (200 rpm) for **01:00:00** 1h

6 Plate the cells on LB-agar plates containing chloramphenicol

7 Incubate at **37 °C** **Overnight**

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