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Transform pCas9

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1 Works for me



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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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KEYWORDS

transformation, Crispr/Cas9, pCas9

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MATERIALS TEXT

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

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- 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

- 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) ^{1h}
for ⌚ **01:00:00**
- 6 Plate the cells on LB-agar plates containing chloramphenicol
- 7 Incubate at 🌡 **37 °C** ⌚ **Overnight**