

08. (August) 2019

Project: iGEM_Munich2019 Shared Project

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Colony PCR (E16: AL with His-loop in CD63), according to Colony PCR protocol.

- colonies #1, 2, 9 were inoculated for MiniPrep

DNA Digest with DNase/Dpn1 test

A-F: epis in which 1-3 components should be added according to workflow below.

| 07082019_DNase_Dpn1_DNA_digest_test | | | | | | |
|-------------------------------------|--------------------------|----------------------------|---------------------------|-------------------------------|-------------------------------|-------------------------------|
| | A | B | C | D | E | F |
| 1 | 100ul DMEM | 100ul DMEM | 100ul DMEM | 100ul DMEM | 100ul DMEM | 100ul DMEM |
| 2 | 5ul DNA, 50ul OptiMem | 5ul DNA, 50ul OptiMem | 5ul DNA, 50ul OptiMem | 56,8ul transfection mix | 56,8ul transfection mix | 56,8ul transfection mix |
| 3 | 50ul ddH2O | 50ul DNase reaction mix | 50ul Dpn1 reaction mix | 50ul ddH2O | 50ul DNase reaction mix | 50ul Dpn1 reaction mix |

| DNase_reaction_mix | | |
|--------------------|----------------|--------|
| | A | B |
| 1 | DNase 1 Buffer | 5ul |
| 2 | DNase 1 | 0.5ul |
| 3 | DNA | 5ul |
| 4 | milliQ H2O | 39.5ul |

| Dpn1_reaction_mix | | |
|-------------------|-----------------|------|
| | A | B |
| 1 | CutSmart Buffer | 5ul |
| 2 | Dpn 1 | 1ul |
| 3 | DNA | 5ul |
| 4 | milliQ H2O | 39ul |

Workflow

1. Prepare Transfection Mix & OptiMem mix under the hood, incubate 15min
2. Prepare DNase and Dpn1 reaction mixes (without enzymes) on the bench.
3. Add 100ul complete DMEM to each incubated eppi under the hood.
4. Add enzymes to previously prepared DNase & Dpn1 reaction mixes and join eppi contents.
5. DNase: incubate 10min on 37°C, to stop reaction add 1ul 0.5% EDTA and then incubate 10min on 75°C. Dpn1: 5-15min on 37°C.
6. Agarose gel electrophoresis (1.5%, 150V, 20min), according to protocol.