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Feacalis_colony_PCR

Elizabeth Fozo¹¹In-house protocol

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Works for me

This protocol is published without a DOI.



Eadewunm

ABSTRACT

E. faecalis colony PCR using taq polymerase

PROTOCOL CITATION

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<https://protocols.io/view/feacalis-colony-pcr-bp4pmqvn>

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ABSTRACT

E. faecalis colony PCR using taq polymerase

E. faecalis colony PCR using taq polymerase

- 1 Transfer 1 colony, depending on size to 50-100µL water. Normal size colonies can go in ~65µL water.
- 2 To a PCR tube add:
 - 15 µL colony water*
 - 1.25 µL primer 1 (20 µM stock)
 - 1.25 µL primer 2 (20 µM stock)
 - 2.5 µL 10X thermopolbuffer

- 0.5 μ L 10 mM dNTPs
 - 0.5 μ L Taq polymerase
 - 4 μ L water
- Total = 25 μ L

*Master mix can be made with all ingredients except the colony water for multiple reactions using the same primers.

3 Use the following cycle in the thermocycler:

94°C 10 min 1 cycle

94°C 15 sec

54°C 30sec(5°C below T_m)

72°C 1 min/kb 25-30 cycles

72°C 10 min 1 cycle

15°C HOLD