

MATERIALS

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Proteinase K

MATERIALS TEXT

Proteinase K from Tritirachium album

lyophilized powder, ≥30 units/mg protein

Made up to 20mg/ml in Tris-HCl

Prepare 1mL 1X PCR buffer with 0.4mg/ml Proteinase K:

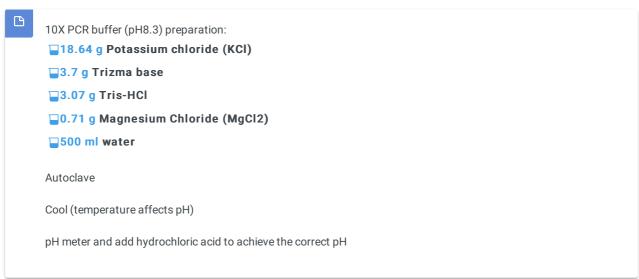
3855 μl water

95 μl 10X PCR buffer (pH8.3)

50 μl 20mg/ml Proteinase K solution

1000 μl Total volume

nb. volumes can be scaled as required



2 Prepare worm lysis tubes with PCR buffer and allow 115 μl 1X PCR buffer + PK per worm.

Pipette buffer into lid of PCR tube (up to 45ul can fit into tube lid)

- ${\tt 3} \qquad {\tt Pick\ a\ single\ worm\ at\ a\ time\ into\ the\ liquid\ in\ the\ 200uL\ PCR\ tube.\ Make\ sure\ you\ can\ see\ the\ worm\ swimming\ in\ the\ buffer}$
- 4 Close the lids onto the tubes of the 200uL tube and spin in a microfuge so that the liquid and worms are at the bottom of the tube
- Burst the cells open by freezing the tubes in the -80°C freezer for 10 minutes
- 6 Transfer tubes to thermocyler and incubate on worm lysis program:

60°C - 1hr 10 mins

95°C - 15 mins

7 Store gDNA in -20°C freezer until required. Use

2ul per 25uL PCR reaction.

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