

Protocol for DNA purification from gram (+) bacteria with PickPen®
(Lactobacillus sp.)

1. Prepare enzyme buffer:
 20 mM Tris-Cl, pH 8
 2 mM EDTA
 1.2 % Triton X-100
 20 mg/ml lysozyme or 200 µg/ml lysostaphin
2. Pellet 1.5 ml bacterial culture by centrifuging 8000 x *g* for 5 min. Discard the supernatant. Resuspend the pellet in 50 µl 1 X PBS. Add 50 µl enzyme buffer and mix well. Incubate for 1h at +37°C.
3. After incubation add Proteinase K and Lysis Buffer according to the table 1. Mix the tube properly by inverting the tube and pipetting up and down several times.
4. Pulse-vortex the tube for 15 seconds and incubate for 30 minutes at +56°C. During the lysis step pipette the rest of the reagents into tubes according to the table 1.
5. Follow the protocol starting from combining the lysed sample, Binding Buffer and Magnetic Particles as described in QuickPick™ SML gDNA kit insert.
6. Elute the DNA for 2 - 10 minutes or until magnetic particles are uniformly dispersed.
7. The volume of Elution buffer can be decreased or increased depending on the desired DNA concentration for the downstream application.

Table 1. Reagent volumes for genomic DNA purifications

Reagent	Reagent volume per preparation
Sample amount	1.5 ml bacterial culture
Lysis Buffer	50 µl
Proteinase K	10 µl
Binding Buffer	250 µl
Magnetic Particles	8 µl
Wash Buffer 1	2 x 500 µl
Wash Buffer 2	500 µl
Elution Buffer	10 - 100 µl

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