Isolation DNA from Milk using E.Z.N.A® Tissue DNA Kit

Protocol A: Isolate DNA from leukocyte in Milk: (up to 50ml milk can be used)

1. If the Milk has been stored at 2-8 °C, a fatty layer of cream should form. Remove the fatty layer of cream from the top of milk by using water suction pump.
2. Heat the milk to 37C for 15 minutes. Mix the milk few times during incubation.
3. Transfer the milk to the centrifuge tube and centrifuge at 6000 x g for 10 minutes. A solid fatty clot will be present in the centrifuged samples. Remove this fatty clot with the milk supernatant.
4. Add 10 ml PBS to the sample tube. Mix throughly and centrifuge at 6000 x g for 10minutes to wash the leukocytes pellet. Discard the supernatant.
5. Resuspend the pellet with 200µl TL Buffer and 20µl Proteinase K. Mix thoughly by vortexing. Incubate at 60C for 10-20 minutes
6. Add 220ul BL Buffer and mix thoughly by vortexing. Incubate at 60C for 10 minutes.
7. Continue with standard protocol for washing and Elution.

Protocol B: Isolate Bacteria DNA from Milk

8. Incubate the 500ul -1000µl milk to 37C for 15 minutes. Mix the milk few times during incubation.
9. Centrifuge at 6,000 x for 10 minutes. A solid fatty clot will be present in the centrifuged samples. Remove this fatty clot with the milk supernatant.
10. Add 1 ml PBS to the sample tube. Mix throughly and centrifuge at 6,000 x g for 5 minutes to wash the cell pellet. Discard the supernatant.
11. Add 200µl TE Buffer and 20µl of lysozyme (50mg/ml). Incubate at 37C for 10 minutes.
12. Centrifuge at 6,000 x g for 5 minutes and discard the supernmatant.
13. Resuspend the pellet with 200µl TL Buffer and Resuspend the pellet with 200µl TL Buffer and 20µl Proteinase K. Mix thoughly by vortexing. Incubate at 60C for 10-20 minutes.
14. Add 220ul BL Buffer and mix thoughly by vortexing. Incubate at 60°C for 10 minutes.
15. Continue with standard protocol for washing and Elution