

DNA Isolation

Sample preparation:

1. Use at least 1×10^4 cells to a maximum of 5×10^5 cells.
2. Wash the cells once with 1x PBS
3. Add 300 μL of Wizard[®] SV Lysis Buffer to the washed cells in a tissue culture plate. Mix lysate by pipetting

Purification of Genomic DNA from cells

4. Transfer each sample lysate to a Wizard[®] SV Minicolumn Assembly
5. Spin the Assembly at 13,000 x *g* for 15 minutes
6. Remove the minicolumn from the Assembly and discard the liquid in the Collection Tube. Replace the minicolumn into the Collection Tube.
7. Add 650 μL Wizard[®] SV Wash Solution (with 95% ethanol added) to each assembly. Centrifuge at 13,000 x *g* for 1 minute. Discard the liquid from the Collection Tube. Repeat this step for a total of 4 washes.
8. Discard the liquid from the Collection Tube and reassemble the minicolumn assembly. Centrifuge for 2 minutes at 13,000 x *g* to dry the binding matrix
9. Transfer the Wizard[®] SV Minicolumn to a new 1.5 mL tube that contains 2 μL RNase solution
10. Add 250 μL Nuclease-Free water to the minicolumn and incubate for 2 minutes at room temperature
11. Centrifuge the minicolumn tube assembly at 13,000 x *g* for 5 minutes. Total elution volume will be 250 μL .
12. Let sample stay at room temperature for 10 minutes (for the RNase solution)
13. Remove the minicolumn and measure the yield using a nanoDrop
14. store the purified DNA at -20 or -70°C

Hydrolyzing and concentration of sample

15. Add 250 μL of milliQ water to sample and 3 μL of internal standard (50 nM stock)
16. Put sample in heating block for 1 hour at 90°C
17. Cool down the sample and freeze it overnight at -20 °C
18. Put sample in freeze dryer until all water is gone
19. Reconstitute the sample in methanol by extracting the sample 3 times with 300 μL of methanol
20. Filter the sample through a 0.45 μm nylon filter
21. Let the methanol evaporate (in fume hood or by using the MiVac)
22. Reconstitute the sample in 20 μL 50% Methanol / 50% milliQ water in an autosampler vial
23. Inject sample onto the HPLC system