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Procedures to remove Endotoxin from DNA preps

During the early stage of DNA preparation: This is based on the alkaline lysis of E.coli DH5a cells. The endotoxins can be removed immediately after alkaline lysis, neutralization, and clarification step (for examples: after adding P1, P2, or N3 treatment, spin to remove the cell debris and before loading to the columns):

1. Add 0.1 x volume of 10% Triton X-114 to the cold and crude DNA solution.
2. Incubate on ice and mix occasionally by inversion the tubes.
3. Incubate at 37*C for 20-30 minutes or till the phase separate.
4. Spin for 5 minutes at low speed (3000xg) at room temperature.
5. Transfer the upper aqueous phase to an endotoxin free tube or load it directly to DNA mini (EZC101), Tin (EZC106) or Maxi (EZC111) spin columns.
6. Proceed with the rest of the DNA purification steps. Use endotoxin-free buffers and tubes.

During the final stage of DNA preparation: This is for the DNA purified by any method.

1. Add 500ul of the DNA solution into sterile micro-centrifuge tube.
2. Add 50ul cold 10x Triton X-114 (10%) to the tube and incubate the tube. Mix thoroughly and incubate on ice for 5 minutes. The solution should be clear.
3. Incubate the tube at 37*C for 20-30 minutes or till the phase separate.
4. Spin at 3000xg for 5 minutes in a microcentrifuge.
5. Carefully transfer the upper phase (containing DNA) to a clean microcentrifuge tube.
6. Repeat step 2-5 twice.
7. Add 50ul of the 3M sodium acetate (or 0.1x volume) and 0.6x volume of 2-propanol (or add 2.5 volume of 100% ethanol and incubate at -80*C for 20 minutes before spin).
8. Mix by inversion at room temperature and centrifuge at 15,000xg for 30 minutes at 4*C.
9. Carefully remove the supernatant.
10. Wash the pellet with 70% ethanol. Remove the supernatant.
11. Air-dry the pellet and resuspend the DNA in 100ul of endotoxin free water or TE buffer.
12. Determine DNA concentration and endoxoxin levels.