

<b>Title</b>	<b>Plasmid Extraction from Bacteria (KIT)</b>		
<b>Protocol Number</b>	MOLBIO19001	<b>Written by</b>	Duha A.
<b>Adapted from</b>	<a href="#">Gene Jet</a>	<b>Version</b>	1.0
<b>Date created</b>	13 <sup>th</sup> Nov 19	<b>Version update</b>	NA

### **Materials**

*Gene Jet Kit (Thermoscientific)*

Sterile eppendorfs

### Protocol:

1. Centrifuge the bacteria for 15 mints at 3000 rpm (take a rack with you upstairs)
2. Remove the supernatant be decanting
3. Resuspend the pallet in 250uL Resuspension solution (stored at 4°C)
4. Add 250 uL Lysis solution. Mix by inverting 6 times.
5. Quickly add 350 uL Neutralization solution. Mix by inverting 6 times.
6. Quickly centrifuge the tubes at 13000 RPM for 5 mints
7. Transfer the supernatant to clean geneJET columns. Don't disturb the precipitate.
8. Centrifuge for 1 mint at 13000 RPM.
9. Discard the flow through.
10. Add 500 uL wash buffer (make sure alcohol has been added).
11. Centrifuge for 1 mint at 13000 RPM.
12. Discard the flow through.
13. Repeat steps 10-12
14. Centrifuge for 1 mint at 13000 RPM to get rid of any residual wash buffer.
15. Transfer the geneJET columns to new clean eppendorfs
16. Add 35-50 uL elution buffer to the center of the filter without touching it. (It's better to elute the plasmid at 2 twice)
17. Wait for two mints
18. Centrifuge for two mints.
19. Quantify the plasmid using nandrop
20. Store Plasmid at -20°C