**Extraction of eDNA From Soil using Enrichment Cultures:**

1. **Cover flasks with foil and autoclave to sterilize.**
2. **Collect a sample (3-5 grams) of soil from an area of interest (try to avoid roots and other plant parts)**
3. **In a one-liter flask combine 10ml of M9 Minimum Media (10x), 90ml of water, 450µl glucose (40%), (prepare by sterile filtering) and 3 grams of your soil sample. Mix under sterile conditions and cover flasks with foil.**
4. **Incubate for 48 hours in 30° C orbital shaker.**

**Transfer of Culture to New Media:**

1. **Remove flask. Prepare new media as above in a fresh (sterile)1 liter flask (10ml of M9, 90ml of water, 450µl glucose).**
2. **Add 2ml of original culture to the newly prepared media. Return to 30° C orbital shaker for 48 hours.**
3. **Remove flask. Repeat media exchange as specified above and return to 30° C orbital shaker for 48 hours.**
4. **Pour remaining 98ml of flask into two 50ml falcon tubes and Spin tubes at 4,000 rpm for 20min at 4° C.**
5. **Pour off supernatant, label tubes. Store in -20° C freezer (long-term storage).**