

## Transfer of methyl cellulose/DNA to wounds

### 1. Prep 1% methyl cellulose:

- 0.3 g/30 mL sterile dH<sub>2</sub>O → 50 mL Falcon tube
- rock @ RT ~3 hrs, mixing with sterile pipet in biosafety cabinet after ~1.5 hrs (note: chilling can help)
- will appear as bubbly colloidal suspension with no visible chunks
- place upright @ -20 degrees C ~20 min to reduce bubbles and let settle
- Store at 4 degrees for up to 1 year.

### 2. Prep DNA/Methyl cellulose mixture:

- Dilute plasmid DNA to 1 ug/ul in ddH<sub>2</sub>O
- For each 0.8 cm diameter wound, prepare 50 ul of a 50/50 mix of DNA and 1% methyl cellulose to a final concentration of 0.5% methyl cellulose + 0.5 ug/ul plasmid DNA. Make enough for all wounds to be treated plus a couple extra. I.e., for 8 wounds, make enough for 10: 250 ul 1% methyl cellulose + 250 1 ug/ul plasmid DNA = 500 ul. Vortex briefly to mix, then let sit for ~ hour at RT.

3. Label bacterial plates (NOT cell culture plates!) and, in biosafety cabinet, spot 50 ul of DNA/methyl cellulose mixture for each wound (plus 1 extra). For ease in spotting, cut end off of 200 ul tip. Avoid introducing bubbles.

4. Place plates with spots in back of cabinet with lid off to dry. Place note on cabinet "NO U.V.!" (Check at ~1.5-2 hrs). Spots should be completely dry but not OVER-dry.

5. Peel pellets up with forceps and let sit in plate (now keep lid on, or DNA may fly away) until use (use same day). Place each on a fresh wound with forceps = 25 ug plasmid DNA/wound. Leave wound undressed.