TRANSFECTION INTO DICTYOSTELIUM:

PROTOCOL:

1. Count cells. Put 5 x 10⁶ to 1 x 10⁷ cells on ice for 20 minutes.

2. Spin cells in:

	FALCON	EPPENDORF
Rate of Spin	1000 g	1000 g
Temperature	4°C	4°C
Time	4 minutes	4 minutes

3. Resuspend in one mL of H-50 Buffer. Spin in an eppendorf tube.

4. Resuspend in 800 μ L of H-50 Buffer. Transfer to a cold cuvette (cool down the holder).

5. Add 10 µg of DNA. Mix DNA, buffer, and cells. Let sit on ice for 20 minutes.

6. 2X Electroporation.

[Settings: Resistance in OHMS= 200 and Capac. MFD= 25.]

7. Set on ice for 5 minutes.

8. Plate in 10 cm plates using 50-75 µL of cuvette contents and 10 mL of HLmedia

+ P/S.

Day After:

1. Change media and add marker.

2. Feed once between 5 and 7 days for about 1-3 weeks until colony formation is present.