

DNA SEQUENCING PROTOCOL

DATE: _____ INITIALS: _____ AMOUNT OF DNA: _____

Directions: Write Number and Primer in blank. Use the plate numbers to number the tubes.

| | | | |
|----------|----------|----------|----------|
| A1 _____ | A2 _____ | A3 _____ | A4 _____ |
| B1 _____ | B2 _____ | B3 _____ | B4 _____ |
| C1 _____ | C2 _____ | C3 _____ | C4 _____ |
| D1 _____ | D2 _____ | D3 _____ | D4 _____ |
| E1 _____ | E2 _____ | E3 _____ | E4 _____ |
| F1 _____ | F2 _____ | F3 _____ | F4 _____ |
| G1 _____ | G2 _____ | G3 _____ | G4 _____ |
| H1 _____ | H2 _____ | H3 _____ | H4 _____ |

| | | | |
|----------|----------|----------|----------|
| A5 _____ | A6 _____ | A7 _____ | A8 _____ |
| B5 _____ | B6 _____ | B7 _____ | B8 _____ |
| C5 _____ | C6 _____ | C7 _____ | C8 _____ |
| D5 _____ | D6 _____ | D7 _____ | D8 _____ |
| E5 _____ | E6 _____ | E7 _____ | E8 _____ |
| F5 _____ | F6 _____ | F7 _____ | F8 _____ |
| G5 _____ | G6 _____ | G7 _____ | G8 _____ |
| H5 _____ | H6 _____ | H7 _____ | H8 _____ |

| | | | |
|----------|-----------|-----------|-----------|
| A9 _____ | A10 _____ | A11 _____ | A12 _____ |
| B9 _____ | B10 _____ | B11 _____ | B12 _____ |
| C9 _____ | C10 _____ | C11 _____ | C12 _____ |
| D9 _____ | D10 _____ | D11 _____ | D12 _____ |
| E9 _____ | E10 _____ | E11 _____ | E12 _____ |
| F9 _____ | F10 _____ | F11 _____ | F12 _____ |
| G9 _____ | G10 _____ | G11 _____ | G12 _____ |
| H9 _____ | H10 _____ | H11 _____ | H12 _____ |

PROCEDURES:

1. Label tubes.
2. Dilute sequencing primers as necessary (10 uL PCR primer + 90 uL ddH₂O).
3. Prepare the Master Mixes, as below.

| | |
|--|---|
| _____ uL * _____ = _____ uL ddH ₂ O | 1 uL template = 4 uL ddH ₂ O |
| 2 uL * _____ = _____ uL 5X buffer | 2 uL template = 3 uL ddH ₂ O |
| 2 uL * _____ = _____ uL reaction mix | 3 uL template = 2 uL ddH ₂ O |
| 1 uL * _____ = _____ uL primer | 4 uL template = 1 uL ddH ₂ O |
| _____ uL MM/tube | 5 uL template = 0 uL ddH ₂ O |
| + | |
| _____ uL template = 10 uL | |

4. Briefly vortex MM's.
5. Pipet MM's into appropriate tubes.
6. Pipet template into appropriate tubes.
7. Place tubes in thermocycler for sequencing (Program 04 Sequencing)

CLEANUP

1. Briefly spin reaction tubes. Transfer into plate.
2. Add 2.5 µL of 125mM EDTA to each tube. Be sure that EDTA reaches bottom of wells.
3. Add 30 µL of 100% ETOH to each well. Close tubes.
4. Mix briefly by vortexing.
5. Incubate at room temperature for 15 minutes.
6. Centrifuge tubes on **Program 2** for 30 minutes.
7. **IMMEDIATELY** add 30 µL of 70% ETOH to each well.
8. Centrifuge tubes on **Program 1** for 15 minutes.
9. Open tubes. Invert on paper towel
10. Place paper towel and inverted tubes in centrifuge. Centrifuge on **Program #** for 1 minute
11. Store tubes in dark place in freezer. Resuspend in 15 µL formamide.