

VI. DNA Sequencing

A. CEQ Dye Terminator Cycle Sequencing

1. Example Sample sheet (make like to a compatible excel file)

Gene	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

2. Reaction mix

Total Sample:

File name:

Chemicals	Stock	Per reaction 10µl	Total volume				
DNA template		4µl					
RNase/DNase free water		1µl					
Primer	2µM	1µl					
DTCS quick start master mix		4µl					
Total		10µl					

3. PCR program (55bec30): 3.1 hours in total. Start: _____ finish: _____.

a. 95°C hold

- b. Step 1: 96°C 20 sec
- c. Step 2: 96°C 20 sec, 55°C 20 sec 60°C 4 min
- d. Step 3: 60°C for 4 min
- e. Step 4: Hold temperature: 4°C

4. *Ethanol Precipitation of Sequencing reactions*

- a. Prepare fresh Stop Solution/Glycogen mixture in 1.5ml microtube (The following components are for 2 plates 192 samples. Dispense 67µl in 8-strip tube if necessary):
 - 110µl of 20mg/ml of glycogen
 - 220µl 100mM Na₂-EDTA (Ph 8.0)
 - 220µl 3M Sodium Acetate (pH 5.2)
- b. Separate the PCR reaction tubes in 2 racks: 1 rack with forward primer reaction, the other rack with reverse primer reaction.
- c. Dispense 2.5µl mix to each PCR tube and mix thoroughly (leave tube open).
- d. Spin at 300rpm for 30 seconds on the rack.
- e. Add 30µl cold 95% (v/v) ethanol/dH₂O from -20°C freezer, put lid on and mix thoroughly by inverting 3 times.
- f. Immediately centrifuge at 5600rpm for 20 minutes.
- g. Discard lids. Invert onto 2 paper towers (fold once).
- h. Spin racks upside down at 300rpm for 30 seconds.
 - i. Add 150µl cold 70% (v/v) ethanol/dH₂O from -20°C freezer.
 - i. Immediately centrifuge at 5600rpm for 7 minutes (do not invert).
 - j. Invert onto 2 paper towers.
- l. Spin racks upside down at 300rpm for 30 seconds.
- m. Add 150µl cold 70% (v/v) ethanol/dH₂O from -20°C freezer.
- n. Immediately centrifuge at 5600rpm for 7 minutes (do not invert).
- o. Invert onto 2 paper towers.
- p. Spin plates upside down at 300rpm for 30 seconds.
- q. Turn pump power on, Gel pump on, put sample in, turn concentration on, set temperature to low and turn vacuum on (on Close). Dry in speedvac for 1 hour.
- r. Turn air button to O slowly then to open, turn concentration off, gel pump off, turn power off, open the speedvac.
- s. Resuspend the sample in 40µl Sample Loading Solution (606083).
- t. Spin plates at 300rpm for 30 seconds.
- u. Mix samples up and down gently using pipette tips and transfer the resuspended samples to the CEQ sample plate (P/N609801, nontransparent).
- v. Spin plates at 500rpm for 2 minutes.
- w. Dispense 285µl CEQ Separation Buffer (608012, leave at 4°C) to another plate (transparent).
- x. Overlay each of the resuspended samples with one drop of light mineral oil (Sigma Cat#M5904).
- y. Load the sample plate into the CEQ and start the desired method.