

TEMPERATURE GRADIENT CAPILLARY ELECTROPHORESIS (TGCE) RELATED PROTOCOLS

(Last Revised: April, 2007)

These protocols were developed by the Schnable Laboratory at Iowa State University. Please contact Dr. Patrick Schnable (schnable@iastate.edu) regarding questions or corrections.

PCR Program used to amplify from genomic DNA

(Used on an MJ Research Tetrad)

- (1) 95°C 10:00 min (initial denaturation)
- (2) 94°C 3:00 min
- (3) 94°C 30 sec (denaturation)
- (4) 60°C 45 sec (primer annealing)
- (5) 72°C 1 minute 30 sec (primer extension)
- (6) Repeat steps 3-5 30 times
- (7) 72°C 10:00 min (final extension)
- (8) 12°C hold
- (9) End

PCR reaction recipe

	For 1 rxn
DNA (pre-aliquot)	2.5 μ l
10x PCR Buffer	1.5 μ l
2mM dNTPs	1.5 μ l
50mM MgCl ₂	0.6 μ l
5uM Forward Primer	1.5 μ l
5uM Reverse Primer	1.5 μ l
AmpliTaq	0.09 μ l
PfuTaq	0.02 μ l
dH ₂ O	5.79 μ l
Total	15 μ l

Taq Info:

AmpliTaq Gold™ 1000U, 5U/ μ l, Applied Biosystems
Cloned Pfu DNA polymerase 100U, 2.5U/ μ l, CAT#600153-81, Stratagene

PCR program used to denature/re-anneal mixed PCR products

After denaturation, the temperature is very gradually decreased to the final annealing temperature.

(PCR Program used on an MJ Research Tetrad)

- Step#1: 95°C, 2 minutes 40 seconds
- Step#2: 95°C, 20 seconds, INC -1/cycle (decreases by 1 degree/20 seconds)
- Step#3: Go To Step 2. Repeat 15 times
- Step#4: 80°C, 1 minute, INC -1/cycle (decreases by 1 degree/minute)
- Step#5: Go To Step 4, Repeat 25 times
- Step#6: 55°C, 18 minutes
- Step#7: 55°C, 1 minute, INC -1/cycle (decreases by 1 degree/minute)
- Step#8: Go To Step 7, Repeat 10 times
- Step#9: 45°C, 30 seconds, INC -1/cycle (decreases by 1 degree/30 seconds)
- Step#10: Go To Step 9, Repeat 10 times
- Step#11: 12°C hold
- Step#12: End

Reveal running program

Bottle#1&2: Capillary Wash (WASH-500-002)
Bottle#3: Reveal Running Buffer without dye (BRUR-500-002)
Bottle#4: Reveal Mutation Discovery Running Buffer (BRUR-500-001)
Gel: Matrix, Reveal Mutation Discovery (#MREV-240-001)

1. Bottle#1, Line Purge, Purge rate: 20.0 ml/min for 15 min
2. Bottle#1, Flow rate: 22.5 ml/min for 5 min
3. Bottle#2, Flow rate: 25.0 ml/min for 5 min
4. Bottle#3, Line Purge, Purge rate: 10.0 ml/min for 3 min
5. Bottle#3, Flow rate: 6.0 ml/min for 6 min
6. Gel, Line Purge, Purge rate: 12 ml/min for 12 min
7. Gel, Gel Inject, Vol. delivered/cap: 42 µl, Delivery Time: 10 min
8. Bottle#4, Gel PreRUN, Flow rate: 5.0 ml/min, 10.0 kV, 5 min
9. Bottle#4, Sample Inject, Flow rate: 5.0 ml/min, 6.0 kV, 50 sec
10. Bottle#4, Electrophoresis + Current Monitor, Flow rate: 5.0 ml/min, 9.0 kV, 15 min
11. Bottle#4, DATA Acquisition, Flow rate: 5.0 ml/min, 9.0 kV, 50 min