

PCR Programs

Program 5 (single mutation for quickchange, 5-6kbp, Pfu polymerase, for PCDNA3 or PCDNA3')

1. stage 1, step 1, 95oC, 30 sec, cycle 1
2. stage 2, for 16 cycles.
 - step 1, 95oC, 30 sec
 - step 2, 55oC, 1 min
 - step 3, 68oC, 18 min

Program 4 (single mutation for quickchange, 5-6kbp, Pfu polymerase, for PRSETb)

1. stage 1, step 1, 95oC, 30 sec, cycle 1
2. stage 2, for 18 cycles.
 - step 1, 95oC, 30 sec
 - step 2, 55oC, 1 min
 - step 3, 68oC, 12 min

Program 32 (PCR SH2 domain, 5-6kbp)

1. stage 1, for 2 cycles, hold temperature.
 - step 1, 95oC, 2 min,
 - step 2, 40oC, 2 min
 - step 3, 72oC, 2 min 30 sec
2. stage 2, for 28 cycles
 - step 1, 95oC, 1 min
 - step 2, 52oC, 1 min
 - step 3, 72oC, 2 min 30 sec
3. stage 3, step 1, 72oC, 10 min

Program 10 (PCR for difficult annealing primers)

1. stage 1, step 1, 95oC, 5 min
2. stage 2, cycles 2.
 - step 1, 95oC, 1 min
 - step 2, 40oC, 1 min

- step 3, 72oC, 2 min 30 sec
3. stage 3, cycles 5
- step 1, 95oC, 1 min
 - step 2, 43oC, 1 min
 - step 3, 72oC, 2 min 30 sec
4. stage 4, cycles 5
- step 1, 95oC, 1 min
 - step 2, 45oC, 1 min
 - step 3, 72oC, 2 min 30 sec
5. stage 5, cycles 20
- step 1, 95oC, 1 min
 - step 2, 52oC, 1 min
 - step 3, 72oC, 2 min 30 sec
6. stage 6, step 1, 72oC, 10 min, cycle 1