

SGED_SOP_1.1.1: PCR Amplification of cDNA inserts

Effective Date: 08/01/03

I. PCR amplification

cDNA clone inserts can be amplified by PCR directly from clones in culture.

We use universal M13F and M13R amplification primers:

M13 FWD: 5' GTT TTC CCA GTC ACG ACG TTG 3'

M13 REV: 5' TGA GCG GAT AAC AAT TTC ACA CAG 3'

Clone inserts are amplified using the following protocol:

1. Selected clones are inoculated into 96 well deep-well blocks (Qiagen; Cat # 19573) containing 1.2 ml of LB/Ampicillin (50 µg/ml), sealed with QIAGEN air pore strips (Cat# 19571) and incubated for 16 hours at 37°C and 200 rpm in a shaking incubator. A 100 µl aliquot of each is archived for future use in microtiter plates containing 10% glycerol at -80°C.
2. Following overnight growth, 5µl of culture suspension are transferred into a 96 well PCR reaction plates (Perkin-Elmer Applied Biosystems Cat # N801-0560) containing 95µl of MilliQ water.
3. Microtiter plates containing diluted culture are heated to 95°C for 10 minutes in a PCR machine to lyse the cells and release the plasmid clones.
4. Prior to PCR, cellular debris is removed by centrifugation at 1200×g for 3 minutes in a centrifuge equipped with microtiter plate carriers.
5. Clone inserts are amplified in 100µl PCR reactions in 96 well reaction plates (Denville Scientific, Cat# C18096). A reaction master mix is prepared for each reaction plate:

MilliQ water	6.4ml
10× PCR buffer	1 ml
MgCl ₂ (50mM)	1 ml
M13 Forward primer (10µM)	200µl
M13 Reverse primer (10µM)	200µl
dNTP mix (2mM per dNTP)*	1 ml (Life Technologies Cat# 10297-018)
(* 20µl of each dNTP as 100 mM stock in 920µl of MilliQ water)	
Taq Recombinant (5U/µl)	40µl (Life Technologies; Cat# 10342-046)
Total:	9.84 ml

6. For each clone, add 96µl of master mix to 4µl of culture supernatant in 96 well PCR plate.
7. Reactions are amplified in an thermocycler (MJ Research; PTC-225 Tetrad) using the following cycling protocol:

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|-------------------------------|----------------------|
| 1) 95°C × 4 min | Initial Denaturation |
| 2) 95 C 30 sec | Denaturation |
| 3) 52 C 30 sec | Annealing |
| 4) 72 C 2 min | Extension |
| 5) Repeat steps 2-4, 34 times | |
| 6) 72 C 5 min | |
| 7) 4°C forever | |

II. Reaction clean-up

For efficient binding of the amplified clone inserts to the slides, it is essential to remove unincorporated nucleotides and primers from the reaction products. While there are a variety of techniques that can be used, we have found filtration using 96 well multiscreen filter plates (Millipore; Cat # MANU 03050) to give excellent DNA product recovery without any significant contamination at relatively low cost.

PCR products are cleaned using the following filtration protocol:

1. Transfer PCR product (100 μ l) to the Millipore filter plate.
2. Place the filter plate on a vacuum manifold filtration system (Qiagen, Cat # 19504 or Millipore Cat # MAVM0960R) and filter at a pressure of 15in (380 mm) Hg for 10 minutes or until the plate is dry.
3. Remove plate from the manifold filtration system. Add 100 μ l of MilliQ water to each well and place on a shaker. Shake vigorously for 15 minutes to resuspend the DNA.
4. Manually pipet the purified product to a new 96 well plate.

Plates containing the purified PCR products are then sealed using an aluminum foil seal (CIC, Cat# FS100) and stored at 4°C for future arraying.