

Preparing and labelling probe from a complex oligo library

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Introduction

This protocol employs emulsion PCR (ePCR) to enrich sub libraries from a complex oligo library. I have prepared this protocol based on “*Amplification of complex gene libraries by emulsion PCR*” R. William, *et.al*, *Nature Methods*¹ and “*Preparing Single-Stranded Labelled Probes from MYtags Immortal Library*”², Arbor Biosciences. Labelling protocol is based terminal transferase protocol from Roche.

Materials

- Oligo nucleotide library
- Enrichment primer sets
- Nuclease-free water
- Span 80 (Sigma S6760)
- Tween 80 (Sigma P5188)
- Triton X-100 (Sigma T8787)
- Mineral Oil (Sigma M5904)
- dNTP, 10mM (NEB N047S)
- Bovine Serum Albumin (BSA) (Sigma B6917)
- *Pfu* Turbo DNA Polymerase
- diethyl ether

Equipments

- BRAND magnetic stirring bar, PTFE, cylindrical with pivot ring (Sigma Z329061). pre-cleaned (See “Cleaning stir bars”).
 - Magnetic stirrer with speed control (Burrell Scientific)
 - PCR cleanup kit
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Before you begin

Prepare library

1. Prepare a stock solution of your library.
2. Resuspend at 1 ng/ μ l in 10 mM Tris-HCl pH 7.5 or nuclease-free water.
 - Keep the stock at -80° C.
3. Prepare working stock aliquots (0.08 ng/ μ l) by adding 2 μ l of immortal library to 23 μ l of nuclease-free water (total volume:25 μ l).

¹<https://www.ncbi.nlm.nih.gov/pubmed/16791213>

²Version 1.4

Prepare primers

Prepare forward and reverse primers working stocks(10 μ M) by diluting the 100 μ M stock in nuclease-free water.

Prepare water saturated diethyl ether.

Mix 100 ml of diethyl ether with 100 ml of nuclease-free water in a glass bottle by shaking for 30s. Allow the phases to settle before use (the top phase is diethyl ether). The water saturated solvent can be stored at RT for 3 months.

Procedure

Prepare ePCR mix.

1. Prepare the oil-surfactant mixture by thoroughly mixing the following components in a 50ml conical tube.

Component	Amount	Final concentration
Span 80	2.25ml	4.5% (vol/vol)
Tween 80	200 μ l	0.4% (vol/vol)
Triton X-100	25 μ l	0.05% (vol/vol)
Mineral oil	to 50ml	

2. Transfer 400 μ l of the oil-surfactant mixture to a CryoTube vial, and add a 3x8 mm stir bar(pre-cleaned)
3. Begin stirring the mixture at 1,000 r.p.m. on the magnetic stirrer.
4. Prepare the aqueous phase for the emulsion by mixing the following:

Component	Amount
10X Cloned <i>Pfu</i> buffer	26 μ l
BSA (50 mg/ml stock)	52 μ l
Forward primer (10 μ M stock)	7.8 μ l
Reverse primer (10 μ M stock)	7.8 μ l
dNTPs (10mM stock)	5.2 μ l
<i>Pfu</i> Turbo DNA Polymerase	5.2 μ l
Template DNA	$\leq 10^9$ molecules (1.66 fmol)
Nuclease-free water	to 260 μ l

5. Add 200 μ l of the aqueous phase to the oil-surfactant mixture in dropwise manner over a period of 1.5 min.
 - After the addition is complete, continue stirring for another 5 minutes.
 - Keep the aqueous phase leftovers as the without emulsion control.
- ▲ CRITICAL STEP**
6. Pipet the emulsion into the wells of a PCR plate as 10 aliquots of 50 μ l.
 - Pipet 50 μ l of the aqueous phase left over to a well as nonemulsified control.
 - Overlay the emulsified and nonemulsified reactions with mineral oil.

PCR

7. Subject the PCR plate to the following program.

Cycle number	Denaturation	Annealing	Polymerization
1	2 min at 95°C		
2-26	30 sec at 95°C	30 sec at A ¹	B ¹ min at 72°C
27			10 min at 72°C

¹A = C-5°C where C is the T_m of the primer with lowest T_m. B is equal to length of amplification in kbp.

8. Place the nonemulsified reaction aside.

■ **PAUSE POINT** You can keep both the w and w/o emulsion reaction at 4°C overnight!

9. Pool the ePCR reactions in a 1.7 ml eppendorf tube.

- Centrifuge at 13,000g for 5 minutes.
- Discard the upper (oil) phase.

10. To remove the remaining oil from the emulsion, perform the following extraction twice:

- Add 1 ml of water-saturated diethyl ether, vortex the tube and let it settle.
- Discard the upper (solvent) phase.

▲ **CRITICAL STEP**

11. Remove the residual solvent from the broken emulsion by centrifuging under vacuum for 5 min at 25°C.

12. Clean the emulsified and nonemulsified PCR products using a PCR clean-up kit.

13. Analyze Aliquots of the emulsified and nonemulsified PCR products by electrophoresis.

- 2% agarose gel in 1X TBE.