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 Lirary"*[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)
- Minral 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

Before you begin

Prepare library

1. Prepare a stock solution of your library.
2. Resuspend at 1 ng/μl 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/μl) by adding 2 μl of immortal library to 23μl of nuclease-free water (total volume:25μl).

[1] https://www.ncbi.nlm.nih.gov/pubmed/16791213
[2] Version 1.4

Prepare primers

Prepare forward and reverse primers working stocks(10μM) by diluting the 100μM 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 througly mixing the following components in a 50ml conical tube.

Component	Final concentration	For 5ml	For 25ml	For 50ml
Span 80	4.5% (vol/vol)	225µl	1.125ml	2.25ml
Tween 80	0.4% (vol/vol)	20µl	100µl	200µl
Triton X-100	0.05% (vol/vol)	2.5µl	12.5µl	25µl
Mineral oil		to 5ml	to 25ml	to 50ml

2. Transfer 400μl of the oil-surfactant mixure to a CryoTube vial, and add a 3x8 mm stir bar(pre-cleaned)
3. Begin stiring 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 *Pfu* buffer	26μl
BSA (20 mg/ml stock)	16.5μl
Forward primer (10µM stock)	7.8μl
Reverse primer (10µM stock)	7.8μl
dNTPs (10mM stock)	5.2μl
Pfu Turbo DNA Polymerase	5.2μl
Template DNA	$\leq 10^9 molecules$ (1.66 fmol)
Nuclease-free water	to 260 μl

5. Add 200μl of the aqueous phase to the oil-surfactant mixure 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 contol.

   ▲ CRITICAL STEP
6. Pipet the emulsion into the wells of a PCR plate as 10 aliquots of 50 μl.
   - Pipet 50 μl of the aqueos 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

[1] *A = C-5°C where C is the Tm of the primer with lowest Tm. 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 13,000*g* for 5 minutes.
   - Discard the upper (oil) phase.

10. To remove the remaining oil from the emulsion, perform the folowing 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 vaccum for 5 min at 25°C.

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

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

    - 2% agarose gel in 1X TBE.