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)
- Bovine Serum Albumin (BSA) (Sigma B6917)
- 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 (0.07 ng/μl) by diluting 2 μl of immortal library in 28 μl nuclease-free water.

[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	Amount	Final concentration
Span 80	2.25ml	4.5% (vol/vol)
Tween 80	200µl	0.4% (vol/vol)
Triton X-100	25µl	0.05% (vol/vol)
Mineral oil	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 PCR buffer	26μl
BSA (100 mg/ml stock)	26μl
Forward primer (10µM stock)	7.8μl
Reverse primer (10µM stock)	7.8μl
dNTPs (5mM stock)	10.4μl
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.
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!