

# 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*<sup>1</sup> and “*Preparing Single-Stranded Labelled Probes from MYtags Immortal Library*”<sup>2</sup>, 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
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## Before you begin

### Prepare library

1. Prepare a stock solution of your library.
2. Resuspend at 1 ng/ $\mu$ l in 10 mM Tris-HCl pH 7.5 or nuclease-free water.
  - Keep the stock at -80° C.
3. Prepare working stock aliquots (0.07 ng/ $\mu$ l) by diluting 2  $\mu$ l of immortal library in 28  $\mu$ l nuclease-free water.

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

<sup>2</sup>Version 1.4

## Prepare primers

Prepare forward and reverse primers working stocks(10 $\mu$ M) by diluting the 100 $\mu$ 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 $\mu$ l	0.4% (vol/vol)
Triton X-100	25 $\mu$ l	0.05% (vol/vol)
Mineral oil	to 50ml	

2. Transfer 400 $\mu$ 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 PCR buffer	26 $\mu$ l
BSA (100 mg/ml stock)	26 $\mu$ l
Forward primer (10 $\mu$ M stock)	7.8 $\mu$ l
Reverse primer (10 $\mu$ M stock)	7.8 $\mu$ l
dNTPs (5mM stock)	10.4 $\mu$ l
DNA polymerase	5.2 $\mu$ l
Template DNA	$\leq 10^9$ molecules (1.66 fmol)
Nuclease-free water	to 260 $\mu$ l

5. Add 200 $\mu$ 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  $\mu$ l.
    - Pipet 50  $\mu$ 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 <sup>1</sup>	B <sup>1</sup> min at 72°C
27			10 min at 72°C

<sup>1</sup>  $A = C - 5^{\circ}\text{C}$  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.