Method: 20mL Emulsion PCR (ePCR) Setup for Oligopaints (Part 1 of 3)

By Nicholas Apostolopoulos

Principle: We use a modified emulsion PCR protocol (Williams *et al.*, Nature Methods 3(7):545-50, 2006) to amplify our oligonucleotide libraries. In this strategy, PCR master mix is beaten into a water-in-oil emulsion, with each oil droplet receiving on average 1-2 template molecules. Thus, a complex library can be amplified in a great many small, parallel reactions rather than one large pooled reaction. This approach is designed to limit the effects of template competition, template bridging, and intermolecular recombination that can distort and skew the complexity of the library during exponential amplification.

The following protocol outlines how to setup 8 x 20mL ePCR reactions done in bags optimized for use in KBiosciences HC-16 Hydrocycler. Note that these reactions could also be cycled in a Peltier thermal cycler (e.g. MJ Research/BioRad machines) in 96-well plates.

Time Required:

Setting up the emulsion mixture can be done within 1-2 hours. The PCR itself takes ~4 hours, and can be stored overnight at 4 ºC if you wish to break the emulsion the next day.

Reagents:

- ABIL EM 90, a surfactant (Degussa)
- Bovine serum albumin (BSA) (for molecular biology, powder; EMD)
- Deoxynucleoside triphosphate (dNTP) mix (ABI)
- Mineral oil (for molecular biology, light oil; Sigma-Aldrich; # M5904)
- KAPA Taq (Kapa Biosystems)
- Triton X-100, a surfactant (general-purpose grade; Fisher Scientific)
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Equipment:

- Hydrocycler (KBiosciences/LGC Genomics; 16 plate Thermal Cycler)
- Thermal Bag Sealer
- Plastic bags (KBiosciences; KBS-0004-007)
- Glass Bottle (500ml, round bottoms; Pyrex)
- Magnetic stirrer with speed controller
- Stir bar (3.7 x 0.8 cm, pivot rings, polytetrafluoroethylene; VWR International)

Preparation:

Before setting up your aqueous PCR mix, you should first place your 500mL glass bottle in 4ºC cold room, and let it chill. It is very important that the emulsion is set up in the cold room, and kept cold until placed on the hydrocycler.

Procedure:

1. Prepare the oil-surfactant mixture by adding the following components to your 500mL chilled glass bottle in the cold room

Component	Final concentration	Vol. [134.4mL]
ABIL EM90	4% (v/v)	5.4 mL
Triton X-100	0.05% (v/v)	67.2 µl
Mineral Oil	96% (v/v)	129 mL

2. Add the 3.7 x 0.8 cm stir bar to the glass bottle, and begin stirring oil-surfactant mixture at 1,500 r.p.m on a magnetic stir plate in cold room. *Allow mixture to stir for at least 30min before adding aqueous phase and creating emulsion.*

3. Meanwhile, prepare aqueous phase for the emulsion by mixing the following components in a 50mL conical tube:

	Reagents		
	1X 2.8 mL aqueous ePCR		**16X ePCR Mix**
280	µL 10X KAPA Buffer	4480	µL 10X KAPA Buffer
56	µL 10mM dNTPs	896	µL 10mM dNTPs
14	µL Primer Forward (100µM)	224	µL Primer Forward (100µM)
14	µL Primer Reverse (100µM)	224	µL Primer Reverse (200µM)
56	µL Template (10ng/µL)	**448**	**µL Template (10ng/µL)**
140	µL BSA (10mg/mL)	2240	µL BSA (10mg/mL)
14	µL KAPA Taq	224	µL KAPA Taq
2233	µL dH2O	36064	µL dH2O
2800	**µL ePCR aqueous mix**	**44800**	**µL ePCR aqueous mix**

Thaw all components on ice, and keep aqueous mixture on ice at all times.

Note: It is best to keep fluorescently labeled primer in the dark at all times in order to maintain the integrity of the fluorophore. For the rest of the protocol, try to minimize exposure to light.

4. Invert conical tube 6-7 times to ensure aqueous phase is properly mixed. Afterwards, aliquot 44.8mL dropwise to oil-surfactant mixture in cold room while oil phase is still stirring. After addition is complete, continue to stir for an additional 15 minutes to create emulsion.

Note: To ensure that emulsion is properly made, you can take small aliquots of w/o emulsion and place on a microscope slide and look at aqueous phase droplets using

bright field microscopy. Droplets should range from 8 – 10 µm in size. If you find that droplets are either too small or too large, you can adjust stirring time and speed to create droplets of desired size.

5. Once emulsion is created, aliquot 20mL per seal-able ePCR bag. Place bags on ice and cover.

6. Once bags are filled, seal top of bags using adhesive strip, and further seal using a heat-sealer. Place bags in racks, and load carefully onto hydrocycler arm

Note: Make sure bag is completely sealed in order to prevent emulsion from spilling out into hydrocycler during PCR!

7. Use the following conditions for PCR amplification:

Step	Temperature (ºC)	Time (min)	Cycles
1	95	7	1
2	95	1	
3	60	2	**35**
4	72	1.5	
5	72	12	1
6	4	∞	

The annealing temperature (step 3) should be set to 2-5 ºC below the T_m of the primer being used

Note: For cycling in a Peltier machine, use a cycle of: 95 ºC for 5'; 35 cycles of 95 ºC for 30s, 60 ºC for 60s, 72 ºC for 30s; 72 ºC for 10'; 4 ºC until the reactions are removed from the machine.

8. Let reaction run till completion, or reaction can be left in hydrocycler O/N at 4ºC

9. Refer to 20mL emulsion breaking protocol for how to break emulsion

References:

This protocol is adapted from Williams et al. **Amplification of complex gene libraries by emulsion PCR.** *Nature Methods* 3(7):545-550 (2006) and from MYcroarray's "Emulsion PCR for Oligonucleotide Library Amplification" protocol.