Supplementary Material

Optimized methodology for recovery of products following emulsion PCR: applications for amplification of aptamer libraries and other complex templates.

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Supplementary Protocol.

Detailed protocol for ePCR and recovery of products using butanol extraction.

The following protocol provides step-by-step instructions to assemble emulsion PCR reactions and recover products using the final 2-butanol extraction procedure described in the main manuscript.

1. Materials and Equipment

1.1 Materials

- 10X Standard Taq Buffer (New England BioLabs, Ipswich, MA; Cat. No. B9014S),

  **Note that the use of a detergent-free buffer mix is essential for generating stable emulsions*.
- dNTP mix, 10 mM stock concentration in H_2O (New England BioLabs, Ipswich, MA; Cat. No. N0447S)
- Taq DNA Polymerase, 5000 units/uL stock concentration (New England BioLabs, Ipswich, MA; Cat. No. M0273S)
- Acetylated Bovine Serum Albumin, 1 mg/mL stock concentration in H_2O (Promega, Madison, WI; Cat. No. R3961)
- Primer (forward), 100 uM stock concentration, preferably in H_2O
- Primer (reverse), 100 uM stock concentration, preferably in H_2O
- DNA template, preferably in H_2O
- Ultrapure H_2O
- Tegosoft DEC (Evonik, Birmingham, AL; Cat. No. 270173-L151)
- Light Mineral Oil (Sigma Aldrich, St. Louis, MO; Cat. No. M5904)
- Abil WE 09 (Evonik, Birmingham, AL; Cat. No. 100267-L151)
- 2-butanol (Acros Organics, Waltham, MA; Cat. No. 107700010)
- Isopropanol (Fisher Scientific, Hampton, NH)
- QIAquick Gel Extraction Kit (Qiagen, Germantown, MD; Cat. No. 28704)
- 0.5 mL thin-walled PCR tubes
- 5-15 mL conical tubes
- 1.5 mL microcentrifuge tubes

1.2 Equipment

- Thermal cycler capable of accommodating 0.5 mL PCR tubes
- Benchtop vortex mixer equipped with a tube holder capable of accommodating 0.5 mL PCR tubes
- 4ºC walk-in cooler
- Microcentrifuge

2. Procedure

2.1 Emulsion PCR

- In a single conical tube, prepare an oil surfactant mix containing 147 uL Tegosoft DEC, 40 uL mineral oil, and 13 uL of Abil WE 09 per ePCR reaction, mix gently and set aside on ice.
- In 0.5 mL PCR tubes on ice, assemble the aqueous phases of ePCR reactions, each containing 10 uL of taq buffer (1X final concentration), 2 uL of DNTP mix (200 uM final concentration), 0.5 uL of Taq DNA

polymerase (2.5 units), 1 uL of acetylated BSA (0.01 mg/mL final concentration), 2 uL of forward primer (2 uM final concentration), 2 uL of reverse primer (2 uM final concentration), 10^9 copies of template, and H_2O to a final volume of 100 uL.

**Note that the amounts of BSA and template provided here are those which we have determined to work well in our laboratory for our specific workflow; optimal amounts may vary by application, and we suggest they be determined empirically by titration experiments for best results.*

- Add 200 uL of prechilled oil surfactant mix to each 100 uL aqueous phase, and vortex at full speed on a tube-holder equipped mixer for 5 minutes in a 4ºC walk-in cooler to generate emulsions. Emulsions should be creamy and appear milky white.

  **Note that if a cold room is not available, emulsions can be mixed by placing a vortex mixer in a refrigerator equipped with internal power outlets.*

- Thermal cycle emulsions using a program most appropriate for the specific template/primer combination. For example, we use an initial denaturation step of 95ºC for 5 minutes, followed by 30 cycles of 60s at 95ºC, 60s at 55ºC, 60s at 72ºC, along with a final extension step of 72ºC to amplify the random aptamer library described in the main manuscript.

  **Note that longer than usual step times may be necessary to unsure even heating of reactions due to the large reaction volume.*

  **Note that if a thermal cycler with a 0.5 mL block is not available, reactions can be aliqouted into three 0.2 mL PCR tubes, thermocycled using a 0.2 mL block, and repooled after amplification.*

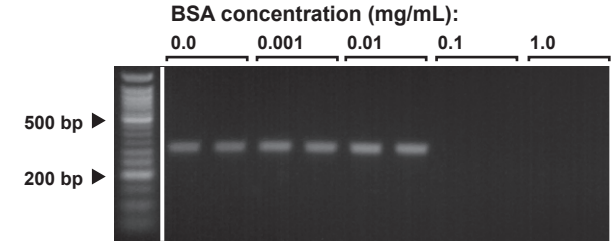
2.2 Recovery of products

- Following amplification, move emulsions to 1.5 mL microcentrifuge tubes.
- Add 1000 uL of 2-butanol, and vortex vigerously for 1 minute to break emulsions.
- Add 200 uL of H_2O and mix by inversion for 5 minutes.

  **Note that the 2-butanol and the water need to have maximal surface contact in order to facilitate the transfer of DNA; appropriately mixed samples will appear slightly opaque.*

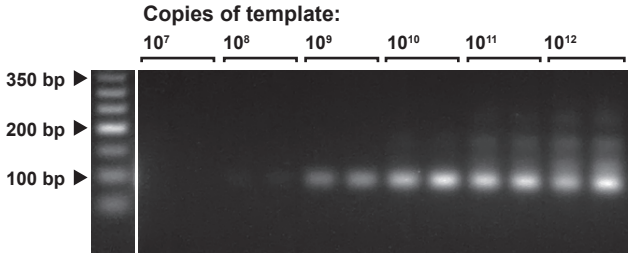
- Centrifuge for 2 minutes at 20,000xg at RT to separate phases.
- Carefully remove and discard a majority of the upper organic phase, leaving a small buffer layer of organic phase (roughly 50 uL), any interphase present, and the DNA-containing aqueous lower phase.
- Add 300 uL of Qiagen Buffer QG and 100 uL of isopropanol, and mix by vortexing for 60s.
- Add samples to QIAquick spin columns, and spin for 30s at 5,000xg at RT to bind DNA.
- Discard flow through, add 650 uL of Qiagen Buffer PE, and 30s at 5,000xg at RT.
- Discard flow through, and spin for 3 minutes at 20,000xg at RT to dry columns.
- Move spin columns to clean 1.5 mL microcentrifuge tubes, and add 50-100 uL of pre-warmed 70ºC H_2O.
- Incubate at RT for 3 minutes, and spin for 60s at 20,000xg to elute.
- Purified DNA can be visualized via gel electrophoresis or used in downstream preparations.

Supplementary Figure 1.



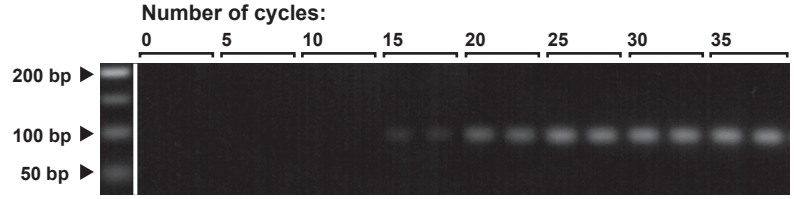
Determination of optimal BSA concentration. The amount of acetylated bovine serum album used in ePCR reactions was determined by preliminary titration experiments; a 315 bp fragment of a 22kb plasmid was amplified via ePCR in the presence of increasing concentrations of BSA ranging from 0-1 mg/mL. Products were isolated via isobutanol extraction and visualized via agarose gel electrophoresis to determine yields. Increasing BSA concentration up to 0.01 mg/mL increased yield of product, however, greater amounts inhibited the reaction completely. 0.01 mg/mL was used in all subsequent experiments.

Supplementary Figure 2.



Determination of optimal template concentration. The amount of template used in ePCR reactions was determined by preliminary titration experiments; 84 base random aptamer library was amplified via ePCR starting with increasing initial copies of template ranging from 10^7-10^{12} copies per reaction. Products were isolated via isobutanol extraction and visualized via agarose gel electrophoresis to determine yields and levels of background product formation. Increasing initial template concentration up to 10^9 copies per reaction increased yield of product without generation of background products, however, greater amounts produced visible background. An initial template concentration of 10^9 copies per reaction was used for all subsequent experiments.

Supplementary Figure 3.



Determination of optimal cycle number. The number of thermal cycles used for ePCR reactions was determined by preliminary experiments; 84 base random aptamer library was amplified for increasing numbers of cycles ranging from 0-35. Products were isolated via isobutanol extraction and visualized via agarose gel electrophoresis to determine yields and levels of background product formation. Product formation plateaued at 25 cycles. No background product was detected at any number of cycles. 25 cycles were used for all subsequent experiments.