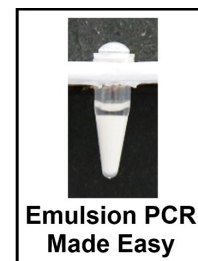


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Quick Emulsion PCR Extraction Protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

Emulsion PCR (ePCR) is an important technique that permits amplification of DNA molecules in physically separated picoliter volume water-in-oil droplets, and thus avoids formation of unproductive chimera and other artifacts between similar DNA sequences. However, the recovery of ePCR products involves repeated extraction with hazardous organic solvents followed by purification using silica-based columns, making the overall process cumbersome. In this report, we have described a 'Quick ePCR extraction protocol' for the purification of ePCR products, which directly employs silica-based DNA purification columns and the products purified using this method have been found to be compatible with gene cloning and NGS applications. The method described here makes ePCR easy, safe and within the reach of every laboratory.

Guidelines

1. The user is expected to be well versed with routine molecular biology and laboratory safety techniques.
2. The protocol provided here for extraction of emulsion PCR product using QIAquick PCR purification kit. However, our experiments have shown that the protocol also works with other PCR purification kits, namely, PureLink Quick PCR purification kit (Thermo Fisher Scientific), Monarch PCR and DNA Cleanup kit (NEB), DNA Clean & Concentrator – 5 (Zymo Research), and GenElute PCR Clean-Up Kit (Sigma-Aldrich).

Materials

MATERIALS

 QIAquick PCR purification kit **Qiagen**



Safety warnings

! Same as those suggested for using QIAQuick PCR purification kit.

Before start

- As per our knowledge, the protocol is valid for emulsion prepared using 1 part of aqueous PCR and 2 parts of oil-surfactant mixture (containing 4.5 % Span 80 (v/v), 0.4 % Tween 80 (v/v) and 0.05 % Triton X-100 (v/v) in mineral oil). [As described in Williams R, Peisajovich SG, Miller OJ, Magdassi S, Tawfik DS, Griffiths AD. Amplification of complex gene libraries by emulsion PCR. *Nat Methods* 3(7), 545-550 (2006)]
- Before starting this protocol, along with common lab equipment (vortex, centrifuge), you will need emulsified PCR reaction (post thermocycling) and a silica-column based QIAQuickPCR product purification kit.



- 1 Transfer entire emulsion PCR mixture (100 μ l + the overlaid mineral oil) to a fresh 1.5 ml microfuge tube and add 500 μ l of QIA PB purification buffer (5 x volume) directly the tube. For larger ePCR mixture, appropriate volume of PB buffer can be added.
- 2 Vigourously vortex the tube for 1 min.
- 3 Centrifuge the suspension at 13,000g for 1 min at room temperature (25°C).
- 4 Load the entire suspension on the QIAquick column. Centrifuge at 13,000g for 1 min at room temperature (25°C). Discard the flow-through. This step may be repeated if the suspension is more than 700 μ l.
- 5 Add 700 μ l of QIA PE buffer to the column. Centrifuge at 13,000g for 1 min at room temperature (25°C). Discard the flow-through.
- 6 Centrifuge at 13,000g for 2 min at room temperature (25°C) to remove excess ethanol. Transfer the column to a fresh microfuge tube.
- 7 Add 40 μ l of QIA EB buffer to the column and incubate at room temperature (25°C) for 5 mins. Centrifuge at 13,000g for 1 min at room temperature (25°C).
- 8 The eluted DNA is ready to use for downstream work and can be quantified using agarose gel electrophoresis or Nanodrop or any other method available with the user.