Emulsion PCR

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Emulsion PCR (EmPCR) is a commonly employed method for template amplification in multiple NGS-based sequencing platforms. The basic principle of emPCR is dilution and compartmentalization of template molecules in water droplets in a water-in-oil emulsion. Ideally, the dilution is to a degree where each droplet contains a single template molecule and functions as a micro-PCR reactor.

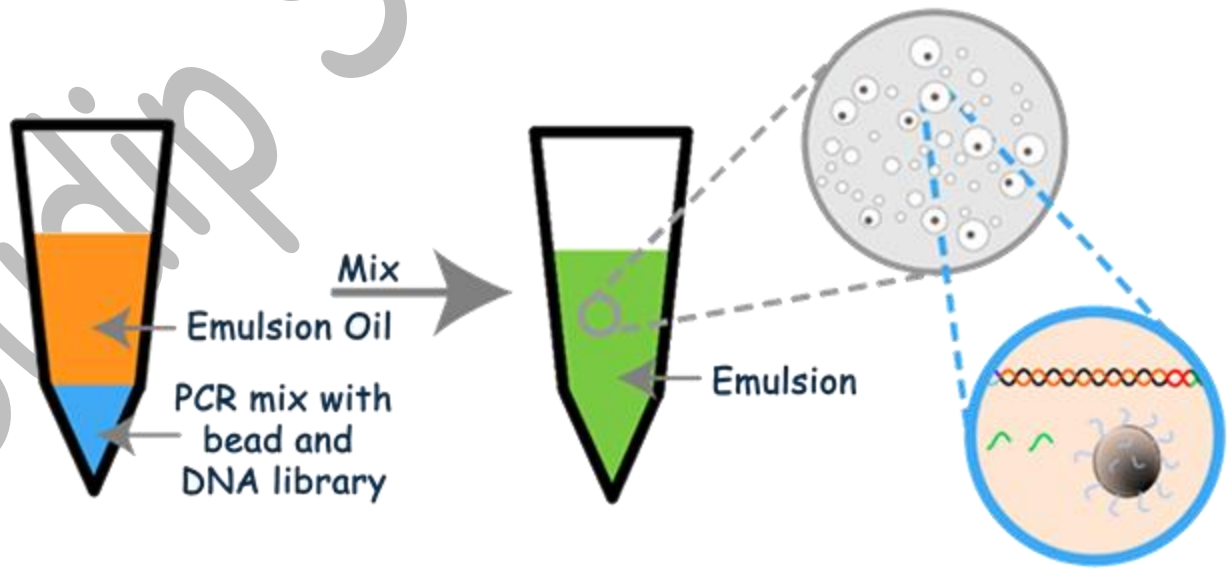
Procedure:

1) Fragmentation of DNA: The library is first fragmented either by sonication (high sound energy) or nebulization (forces DNA through a small hole) to fragments ranging from 300 to 800 bp.

2) Adapters are ligated: Adapters are then ligated onto the DNA fragments. These allow the strands to bind to the emulsion beads.

3) Denature to single strands: The double stranded DNA's with adapters are then denatured by heating the DNA up to 95 °C.

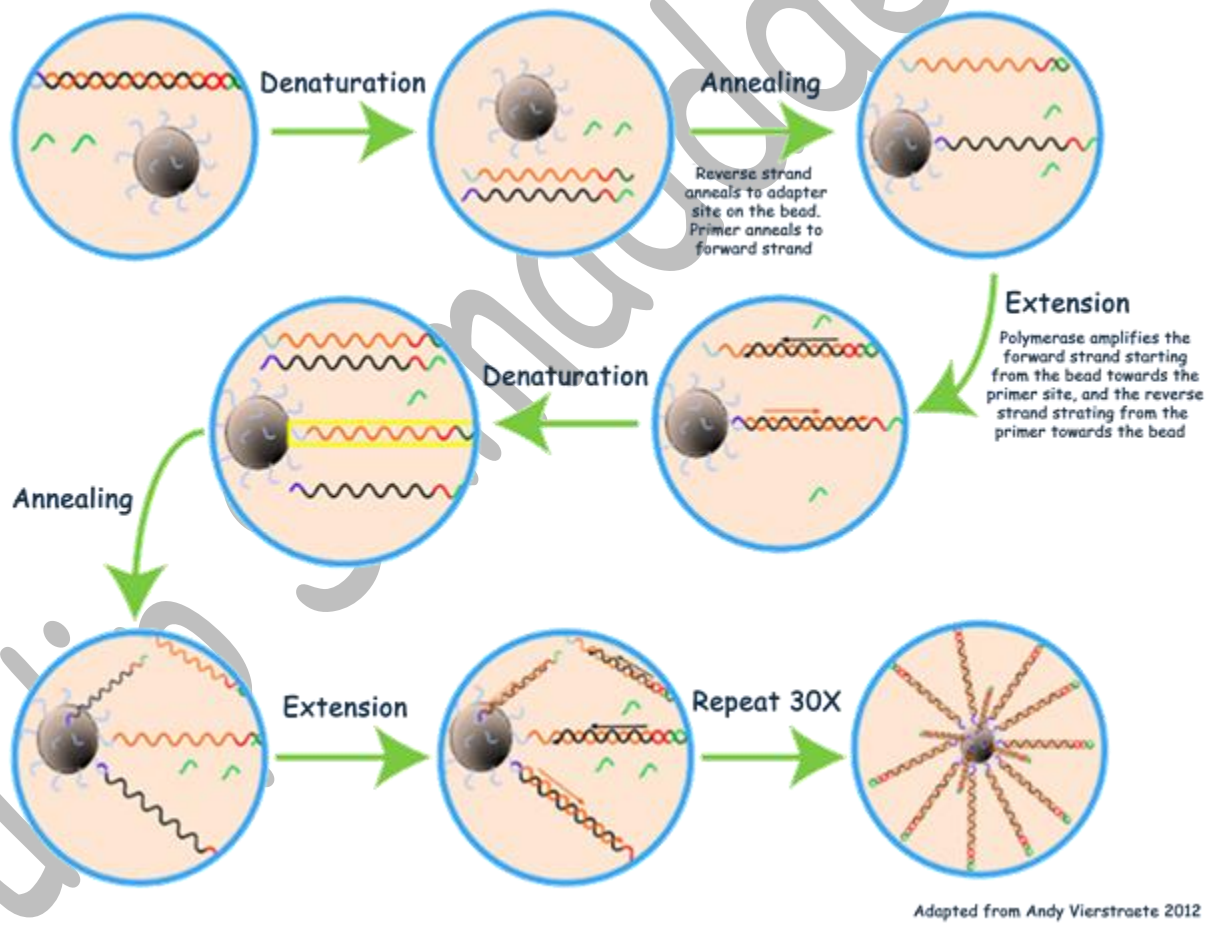
4) Formation of clonal bead populations: Each bead coated with **streptavidin**, which is resistant to organic solvents, denaturants, detergents, proteolytic enzymes and extremes of temperature and pH. Over a billion beads are used with a primer that



matches the adapters attached earlier. The ssDNA is then attached to these beads. Each bead is emulsified in a water-in-oil droplet with PCR reagents (DNA polymerase, primers, buffers, dNTPs).

5) ePCR amplifies DNA strands on beads: Within these droplets, PCR is conducted. This involves the steps Denaturation, Annealing, Elongation. Firstly, the strand is elongated with DNA polymerase and dNTPs. Then the double-strand is denatured, allowing for the strand to ligate to another site on the surface of the bead. Eventually, 1 million copies of the target is amplified on the surface of each bead. The water-in-oil droplet is approximately 1-um.

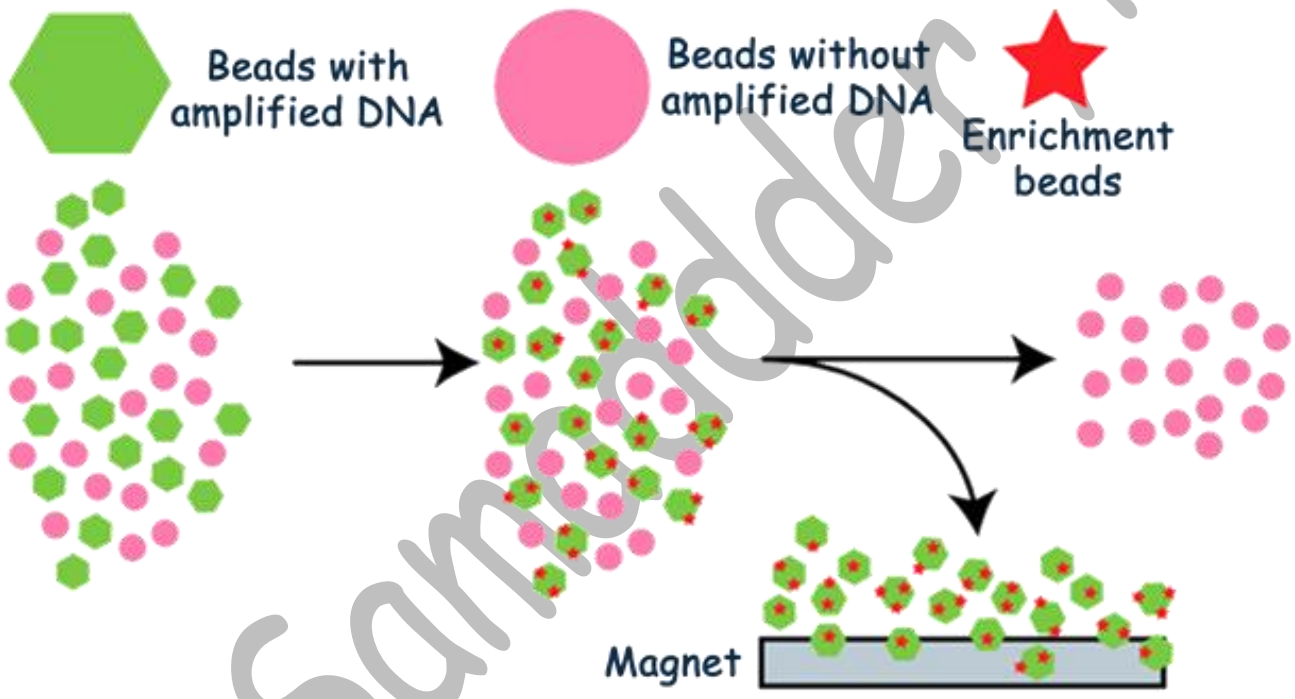
Follow the figure to see how each bead is able to replicate DNA on its surface.



6) Emulsion Breaking: After the DNA strands are amplified, the emulsion from the preceding step is broken using isopropanol and detergent buffer. The solution is then

vortexed, centrigued, and magnetically separated. The resulting solution is a suspension of empty, clonal and non-clonal beads, which will be filtered in the next step.

7) Bead enrichment: We may take out the enriched beads by attaching streptavidin coated magnetic enrichment bead. With a magnet, we can then pull out the beads with amplified DNA.



8) Bead Capping: Attach a capping oligonucleotide to the 3' end of both unextended forward ePCR primers and the RDV segment of template DNA.

9) Result: The beads with amplified sequences are then placed on a slide and are sequenced. Due to their high density of the same DNA molecule, the signal is amplified, allowing computers to read the sequencing data.