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# FAQs for Polymerases and Amplification

1. **Which thermophilic DNA polymerase should I use?**
   The choice of a DNA polymerase for use in PCR-based applications is highly dependent on the sequence you intend to amplify as well, as the desired result. For routine amplifications or high AT or high GC amplifications where yield and ease of use are priorities, NEB's OneTaq DNA Polymerases and Q5 High-Fidelity DNA Polymerases are the recommended choices. For routine amplifications where cost per reaction and yield are the priorities, NEB's Taq DNA Polymerase (NEB #M0267, NEB #M0273) is the industry standard.
   OneTaq Hot Start DNA Polymerase (NEB #M0481) offers high specificity and yield, while Q5 (NEB #M0491) and Q5 Hot Start High-Fidelity DNA Polymerases (NEB #M0493) lead the industry for extreme fidelity, yield and robustness. For our product offerings that address specific applications such as long or difficult PCR, PCR from blood, and multiplex PCR, in addition to convenient kits and master mixes, refer to our PCR Polymerase Selection Table.

2. **What should I take into consideration when designing a set of PCR primers?**
   Avoid complementarity between the primers to prevent primer-dimer formation.
   2. Avoid inverted repeats (self-complementarity).
   3. GC-content of the primer should be around 50%.
   4. Avoid Gs and Cs at the 3'-end of the primers.
   There are many computer programs which will help you to design a primer pair, such as Primer3, which can be found at http://frodo.wi.mit.edu/??

3. **How can I facilitate the amplification of templates with hairpin-loop structures or high GC-content?**
   One*Taq* DNA Polymerase has been developed with a GC Buffer and High GC Enhancer that aid in amplifications of difficult templates. Q5 High-Fidelity DNA Polymerase is supplied with buffers and High GC Enhancer, and has been optimized for robust amplification, regardless of GC content. Alternatively, you can try final concentrations of 5-10% DMSO, up to 10% glycerol, 1-2% formamide or combinations of these. Note: The use of co-solvents will lower the optimal annealing temperatures of your primers (e.g. 5.5-6°C in 10% DMSO). You could also try 7-deaza-dGTP in conjunction with normal dGTP in order to destabilize difficult structures. Note: 7-deaza-dGTP attenuates the signal of ethidium bromide staining.

4. **How important is the quality of my DNA template in long PCR?**
   Template preparation becomes particularly important when performing longer amplifications (>15 kb) and when using high-fidelity DNA Polymerases. Therefore, it is recommended to verify the length of the DNA by agarose gel electrophoresis.

5. **What type of reaction tubes are recommended?**
   We recommend thin-walled tubes especially for long PCR.

6. **What is two-step PCR?**
   If the $T_m$ of both of your primers is high enough (over 65°C), the annealing and the extension steps can be combined into a single step.

7. **What if my primer extension reaction yields no product or a smear?**
   Follow recommended conditions, included in our product literature
   * Optimize annealing and extension temperatures
   * Optimize $Mg^{2+}$ level
   * Optimize amount of polymerase
   * Purify DNA template by phenol/chloroform extraction and alcohol precipitation
   * Avoid high salt carry-over into primer extension reaction
   * Make sure dNTP solution has not undergone hydrolysis - try fresh dNTPs

8. **What causes an occasional smear in a "negative control" with no template present?**
   DNA polymerases with low Km values for DNA, especially those with proofreading exonuclease functions, can cause primer artifacts to form if the DNA polymerase cannot bind to its preferred substrate (a 3´ end of an annealed primer). This primer artifact possesses single-stranded and double-stranded regions, and can appear either as DNA barely migrating out of the gel well or as a smear originating at the gel well. OneTaq (NEB #M0480), OneTaq Hot Start (NEB #M0481), Q5 Hot Start (NEB #M0493), Phusion Hot Start Flex (NEB #M0535) and Taq DNA Polymerases (NEB #M0267, NEB #M0273) can minimize this artifact.

9. **Can PCR products be phosphorylated in the PCR mixture?**
   No, because the ammonium sulfate found in most PCR buffers inhibits T4 Polynucleotide Kinase (NEB #M0201). Instead, gel purify your DNA or use a G25 spin column for purification. In a 50 µl reaction of 1X Kinase Reaction Buffer use up to 200 pmol of 5´-hydroxyl DNA termini with 1 mM ATP. Heat to 70°C for 5 minutes, then chill on ice. Add 20 units T4 Polynucleotide Kinase (NEB #M0201) and incubate at 37°C for 30 minutes. Alternatively, treat your primers with T4 Polynucleotide Kinase (NEB #M0201) prior to PCR amplification. An alternative strategy, since primer synthesis is relatively inexpensive, is to use phosphorylated primers in the PCR reaction.

10. **How can I improve blunt-end ligation efficiency of PCR products?**
    Vectors should be treated with alkaline phosphatase and the insert should have a 5´ phosphate. Other possibilities to improve ligation include: lowering the ATP level in the ligation buffer, increasing the insert:vector ratio or trying our Quick Ligation Kit (NEB #M2200).

11. **What is the enzyme of choice for chewing back 3' overhangs and filling in 5' overhangs (3' recessed ends)?**
    DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210) and T4 DNA Polymerase (NEB #M0203) are the best choices for this application. DNA Polymerase I, Large (Klenow) Fragment can be used at 25°C or room temperature, but T4 DNA Polymerase must be used at 12°C due to its robust exonuclease. Both work well in a wide variety of buffers. Vent DNA Polymerase (NEB #M0254) and Deep Vent DNA Polymerase (NEB #M0258) can also be used but ThermoPol buffer must be used, the

reaction temperature is high, and the enzyme cannot be heat inactivated. Mung Bean Nuclease (NEB #M0250) will chew back 3' overhangs but the strong exonuclease activity combined with the lack of polymerase activity yield a lower percentage of blunt ends.

**12. What is touchdown PCR?**

It is a method for increasing specificity of PCR reactions. Touchdown PCR uses a cycling program where the annealing temperature is gradually reduced (e.g. 1-2°C in every second cycle). The initial annealing temperature should be several degrees above the estimated $T_m$ space of the primers. Annealing temperature is then gradually decreased until it reaches the calculated annealing temperature of the primers or some degrees below. Amplification is then continued using this annealing temperature.

**13. Which NEB DNA polymerases can incorporate fluorescently-labeled nucleotides during PCR?**

Taq DNA Polymerase (NEB #M0267, NEB #M0273) and Vent (exo-) DNA Polymerases (NEB #M0257) are the best choices for incorporating fluorescently-labeled nucleotides (Anderson, J.P., Angerer, B. and Loeb, L.A. (2005) Biotechniques 38, 257-264.)

**14. How can I amplify DNA from single cells?**

The Single Cell Amplification Kit (NEB #E2620) amplifies DNA from single cells with high sensitivity, specificity and reproducibility.