**Thermophilic enzymes**

**Taq Polymerase**

* First isolated from the bacterial thermophile ***Thermus aquaticus***, the Taq polymerase has become the staple enzyme in molecular biology. Commercial producers have since cloned and expressed the enzyme in *E. coli.*
* Recombinant Taq is a 94-kDa enzyme with an optimal 5’-3’ polymerase activity between 75-80 °C in the presence of a magnesium cofactor.
* At 72 °C it can replicate a DNA strand of 1000 base pairs in less than 10 seconds.
* Taq lacks 3’-5’ exonuclease activity and is prone to errors at a rate around 1 per 3,700 bases. Aside from PCR, Taq is also widely used in TA cloning due to its ability to leave 3’ overhangs.
* Some commercial modifications to consider include: “High Fidelity” with the recombinant addition of a 3’-5’ proofreading activity, and a “Hot Start” feature to reduce non-specific amplification.

 **Pfu Polymerase**

* Pfu Polymerase is a thermostable DNA polymerase isolated from the archaeal thermophile ***Pyrococcus furiosus***.
* The 90-kDa Pfu polymerase enzyme features a 3’-5’ exonuclease proofreading ability, allowing for higher fidelity than Taq.
* The error rate of Pfu has been reported to be at least 10-fold lower than that of Taq. However, Pfu amplifies at a slower rate than Taq.
* Thus, Pfu polymerase is suitable for DNA amplification applications requiring greater accuracy.

**Pfx Polymerase**

* Pfx Polymerase is a commercial recombinant thermostable DNA polymerase.
* It features a 3’-5’ exonuclease activity and thus a reported higher fidelity than traditional Taq.
* Some versions also feature a hot-start ability for improved PCR specificity. Pfx polymerase is recommended for PCR applications that require high accuracy including site-directed mutagenesis and PCR expression cloning.

**Bst Polymerase**

* Bst Polymerase, or Bst DNA Polymerase I, was isolated from ***Bacillus stearothermophilus***.
* This 97 kDa enzyme features a similar 5’-3’ polymerase activity of *E. coli*, but lacks the 3’-5’ exonuclease activity.
* Bst polymerase is recommended for applications such as Isothermal amplification (LAMP) and DNA sequencing through high GC regions.

 **Tfi Polymerase**

* Tfi Polymerase is a recombinant thermostable DNA polymerase originally isolated from ***Thermus filiformis***.
* Similar to Taq, this 92-kDa enzyme has a 5’-3’ polymerase activity and lacks the proofreading 3’-5’ exonuclease capability.
* The optimum pH lies between 8.4 and 9.0, and the temperature between 70 to 72.5 °C.
* The yield, specificity, and fidelity has been reported as comparable to Taq, and thus may serve as a less costly alternative.

**Tth Polymerase**

* Tth Polymerase is a thermostable DNA polymerase originally isolated from ***Thermus thermophilus***.
* It has a 5’-3’ polymerase activity, a 3’-5’ exonuclease activity, and, in the presence of Mn2+, an additional reverse transcriptase activity.
* It is active at 74°C and can withstand up to 95°C.
* Thus, in addition to amplifying DNA, its thermostable nature also makes it ideal in reverse transcription since higher temperatures can overcome secondary RNA structures. Thus, Tth Polymerase is ideal in applications such as PCR, RT-PCR, reverse transcription and cDNA synthesis.