PCR Optimization: Additives

- Glycerol (5-10%), formamide (1-5%), or DMSO (2-10%) can be added to the PCR for template DNA with high GC content (these lower the Tm of primer-template hybridization and help relax GC secondary structure.
- Betaine (0.5-2 M) is also useful for high GC content templates, but should not be used with *Pfu* or *Pfu* blends from Agilent. Perform a tritration to determine optimum concentration. When using betaine, reduce melting temperature (92-93°C) and annealing temperature (1-2°C lower).
- BSA (up to 0.8 µg/µl) may also improve the efficiencies of PCR reactions.