Rebecca Varney, various online sources 01-2018

**PCR: When it doesn't work, add more stuff!**

| Reagent | Concentration | Purpose | Why it works | Caveats | Downstream issues |
|---|---|---|---|---|---|
| **BSA** | Up to 0.8 mg/ml | MAGIC (no seriously, just add some and things just work). | MAGIC (coats inside walls of tubes which prevents DNA from binding there, and also takes up space in tubes which can increase Taq performance.) | | Too much can interfere with sequencing, but usually not. |
| **Betaine** | 1-1.7M | Reduces secondary structures | | | |
| **Formamide** | 1-5% | GC-rich regions, reduces secondary structures | | Yes, it will cross-link the DNA a bit | Can inhibit Taq- you can add a touch more taq to compensate |
| **DMSO** | 2-10% (start with 2%) | GC-rich regions, reduces secondary structures | | 10% will start killing off Taq | |
| **Triton X-100** | 0.1-1% | Reduces secondary structures | Will increase yield | Increases non-specific amplification | |
| **Tween-20/-40** | 9.5% | Reduces secondary structures Neutralizes SDS carryover | | Increases non-specific amplifications | |
| **MgCl2** | Already in master mix Can increase (or decrease) 1.0-4.0mM | Higher salt decreases Taq specificity. Lower salt increases specificity but eventually Taq won't function | Chelaters (EDTA, citrate) take up MgCl2; you might need to add more | Thaw and vortex- forms gradients in tubes over time. | Too much salt will carry over and inhibit subsequent sequencing reactions |

| **TMAC** | 15-100mM | Increases primer specificity and melting temperature | Eliminates non-specific binding, helps with RNA contamination. | Great for degenerate primers | Too much can interfere with sequencing reactions. |
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| **7-deaza-2'-deoxyguanosine (7-deaza dGTP)** | Replace all G's with 7-deaza-2'-deoxyguanosine | Combats GC rich regions. Reduces duplex stability. | | Can use 7-deanza dGTP:dGTP at 3:1 ratio. | Must be completely removed prior to sequencing reactions (sephadex or column purification recommended) |