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PCR AMPLIFICATION FROM GC-RICH TEMPLATES

Based on recommendations from the Qiagen HotStarTaq kit and using the kit's reagents where indicated(*)

For a reaction volume of 100 μL , assemble the following:

| | |
|---|---|
| QIAGEN PCR buffer* (10x): | 10 μL |
| Q-solution* (5x): | 20 μL |
| dNTP mix (10 mM each): | 2.0 μL (200 μM final) |
| (purchased or prepared by mixing equal amounts from 100 mM stocks of each dNTP) | |
| 5' primer (100 μM): | 1 μL (1 μM final) |
| 3' primer (100 μM): | 1 μL (1 μM final) |
| filtered milliQ water: | 64.5 μL |
| template** (<<1 $\mu\text{g}/\mu\text{L}$): | 1 μL |
| HotStarTaq*: | 0.5 μL (2.5 units final) |

When using the HotStarTaq reagents, the first step in the thermal cycler should be a 15 minute incubation at 95°C to activate the polymerase; following this, you may program any standard cycling conditions with denaturing times typically from 30 seconds to 1 minute, annealing times from 30 seconds to 2 minutes, and extension times from from 1-3 minutes; annealing temperatures should be a function of the primers' T_M 's, typically in the range of 50-60°C for GC-rich templates; remember to finish the cycling with an extra 10 minutes at the extension temperature (72°C); refer to the HotStarTaq manual for additional tips and considerations.

**Too much template is as bad as no template. Although the precise amount is not critical, shoot for something in the range of 10-50ng of template