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 uM 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 µg/µL):	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