Q5 PCR

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:
Primary Experimenter (contact):
Other Experimenters:

Reagent	Details	Quantity	
		Suggested:	Used:
ddH2O (nuclease-free)		*Var. µL	
dNTP mix (10 mM)		1 µL	
5X Q5 Reaction Buffer		10 µL	
Forward Primer (10 uM)	(ID)→	2.5 µL	
Reverse Primer (10 uM)	(ID)→	2.5 µL	
Template DNA	(Name)→	**Var. µL	
5X GC Enhancer (optional)	Use if primer GC content is >60%	10 µL	
Q5 DNA polymerase		0.5 µL	
		50 µL Total	

* (µL H2O) = Up to 50 uL total
**1 µl of 1 pg -1 ng/µl for plasmid or viral DNA; 1 µl of 50-250 ng/µL for genomic DNA

Critical Steps:
- Add Q5 last, minimize time out of freezer, keep on ice if needed for multiple tubes
- Program PCR machine before adding Q5, do a hot start
- Add each component in order listed above to a PCR tube, making sure to mix components

NOTE:
- Make a mastermix for number of PCRs + 1 if doing more than two PCRs (mastermix includes H2O, dNTPs, PCR buffer)

PCR Machine Settings:

		Rec.	Used:	Rec.	Used:
Step 1	Initial denaturing	98 °C		30 seconds	
Step 2 (25 – 30 cycles)	Denature	98 °C		10 seconds	
	Anneal	*Var.		30 seconds	
	Extend	72 °C		30 sec/kb	
Step 3	Final Extension	72 °C		2 minutes	
Step 4	Hold	4 °C		Indefinite	

* Annealing temperatures required for use with Q5 tend to be higher than with other PCR polymerases. The NEB Tm calculator should be used to determine the annealing temperature when using Q5. Typically, primers greater than 20 nucleotides in length anneal for 10–30 seconds at 3°C above the Tm of the lower Tm primer. If the primer length is less than 20 nucleotides, an annealing temperature equivalent to the Tm of the lower primer should be used. A temperature gradient can also be used to optimize the annealing temperature for each primer pair. For two-step cycling, the gradient can be set as high as the extension temperature. For high Tm primer pairs, two-step cycling without a separate annealing step can be used.