

Phusion PCR

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:

Source: [NEB](#)

Experiment Time:

Primary Experimenter (contact):

Assembled: 6/27/2012

Other Experimenters:

Reagent	Details	Quantity	
		Suggested:	Used:
ddH2O (nuclease-free)		*Var. μL	
dNTP mix (10 mM)		1 μL	
5X Phusion Buffer	(HF or GC?) \rightarrow	10 μL	
Forward Primer (10 μM)	(ID) \rightarrow	2.5 μL	
Reverse Primer (10 μM)	(ID) \rightarrow	2.5 μL	
Template DNA	(Name) \rightarrow	**Var. μL	
DMSO (optional)	Prevents primer secondary structure, alter H2O if	1.5 μL	
Phusion DNA polymerase		0.5 μL	
		50 μL Total	

* ($\mu\text{L H}_2\text{O}$) = (32.5 - $\mu\text{L DNA}$)

**1 μl of 1 pg -10 ng/ μl for plasmid or viral DNA; 1 μl of 50-250 ng/ μL for genomic DNA

Critical Steps:

- Add Phusion last, minimize time out of freezer
- Program PCR machine before adding Phusion
- Add each component in order listed above to a PCR tube

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		10 minutes	
Step 4	Hold	4 °C		Indefinite	

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