Vent PCR

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:

Source: NEB

Primary Experimenter (contact):
Other Experimenters:

Assembled: 6/27/2012

Reagent	Details	Quantity	
		Suggested:	Used:
ddH2O (nuclease-free)		*Var.	
dNTP mix (10 mM)		1 µL	
10X ThermoPol Reaction Buffer		5 µL	
Forward Primer (10 uM)	(ID)→	1 µL	
Reverse Primer (10 uM)	(ID)→	1 µL	
Template DNA	(Name)→	**1 µL	
DMSO (optional)	Prevents primer secondary structure, alter H2O if	3%	
Vent DNA polymerase		0.5 µL	
$MgSO_4$		***1-6mM	
		50 µL Total	

*H2O volume to use for non-DMSO rxn = (50 µL) – (9.5 µL) – (µL $MgSO_4$)
**1 µl of 1-10 ng/µl for vector DNA; 1 µl of 50-250 ng/µL for genomic DNA
*** Primer extensions longer than 2 kb almost always require magnesium levels higher than 2 mM, while for primer extensions shorter than 2 kb, there is no correlation between length and optimum magnesium concentration

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)
- See: NEB Vent PCR optimization if issues arise

PCR Machine Settings:

		Recommended: Used:	Recommended: Used:
Step 1	Initial denaturing	95 °C	5 minutes
Step 2 (25 – 30 cycles)	Denature	95 °C	30 seconds
	Anneal	*Variable	30 seconds
	Extend	72 °C	60 sec/kb
Step 3	Final Extension	72 °C	5 minutes
Step 4	Hold	4 °C	Indefinite

* Lowest primer Tm – 5 °C