PCR using OneTaq

Introduction

Adapted by Jacob Mejlsted from NEB's protocol

Materials

- Materials
  - 1 PCR tube per reaction
- Chemicals
  - OneTaq standard reaction buffer
  - dNTPs
  - Primers
  - DNA template
  - OneTaq DNA Polymease

Procedure

Method

1. To a tube add the following

PCR components

	A	B	C	D
1	**Component**	**25 µl reaction*** **	**50 µl reaction*** **	**Final Concentration**
2	5X OneTaq Standard Reaction Buffer*	5 µl	10 µl	1X
3	10 mM dNTPs (#N0447)	0.5 µl	1 µl	200 µM
4	10 µM Forward Primer	0.5 µl	1 µl	0.2 µM
5	10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM
6	OneTaq DNA Polymerase	0.125 µl	0.25 µl	1.25 units/50 µl PCR**
7	Template DNA**	variable	variable	< 1,000 ng
8	Nuclease-free water	to 25 µl	to 50 µl	

*One*Taq* GC Reaction Buffer and High GC Enhancer can be used for difficult amplicons

**For plasmids or viral DNA, use 1 pg–10 ng DNA for a 50 µl reaction.

***It can be advantagous to pool some of the parts into a master mix, as some labs cannot dispence 0.125 µl accurately.

2. Alternatively, a master mix can be prepared

Homemade master mix

	A	B	C
1	**Reactant**	**Per reaction (50uL) [µl]**	**Mastermix [µl]**
2	Number of reactions	1	10
3	5X OneTaq Standard Reaction Buffer*	10	100
4	10 mM dNTPs (#N0447)	1	10
5	10 µM Forward Primer	1	Added individually
6	10 µM Reverse Primer	1	Added individually
7	OneTaq DNA Polymerase	0.25	2.5
8	Template DNA	1	Added individually
9	Nuclease-free water	34.75	347.5

3. Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary.

4. Transfer PCR tubes to a PCR machine and begin thermocycling using the routine programme:

PCR Programme

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	94°C	30 seconds
3	30 Cycles	94°C	15-30 seconds
4		45-68°C	15-60 seconds
5		68°C	1 minute per kb
6	Final Extension	68°C	5 minutes
7	Hold	4-10°C	

5. PCR products can then by digested by DpnI, purified, stored at -20 C, or a combination.