PCR

Materials

- High-Fidelity PCR Master Mix phusion
- Forward primer
- Reverse primer
- DMSO
- Template DNA (plasmid DNA)
- Milli Q water or treated with DEPC
- eppendorf tubes 1.5 mL

Equipment

- Termocylcer

Procedure

Reaction Setup: We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler **preheated to the denaturation temperature (98°C).** All components should be mixed and centrifuged prior to use. It is important to add Phusion Master Mix last in order to prevent any primer degradation caused by the 3′→ 5′ exonuclease activity. **Please note that protocols with Phusion DNA Polymerase may differ from protocols with other standard polymerases. As such, conditions recommended below should be used for optimal performance.**

Procedure:

1. Clean the working area with 70% ethanol
2. Mix all the necessary components in a PCR tube on ice

Notes: Gently mix the reaction. Add first the bigger volumes. Do the substrate to see how much of water to add in order to have a final volume of 25 o4 50 uL

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

Component	25 µl Reaction	50 µl Reaction	Final Concentration
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM

10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
DMSO (optional)	(0.75 µl)	(1.5 µl)	(3%)
2X Phusion Master Mix	12.5 µl	25 µl	1X
Template DNA	0.5 pg- 5 ng	1 pg- 10 ng	< 250 ng
Nuclease-free water	to 25 µl	to 50 µl	

4. Transfer PCR tubes from ice to a PCR machine with the block preheated to 98°C and begin thermocycling:

Thermocycling conditions for a routine PCR:

STEP	**TEMP**	**TIME**
Initial Denaturation	98°C	30 seconds
25-35 Cycles	98°C 68°C 72°C	5-10 seconds 10-30 seconds 15-30 seconds per kb
Final Extension	72°C	5-10 minutes
Hold	4°C	

PCR product:
The PCR products generated using Phusion DNA Polymerase have blunt ends; if cloning is the next step, then blunt-end cloning is recommended

Annealing temperature:
http://tmcalculator.neb.com/#!/main

PCR 17-08-2018
Tubo 1- 2 ng DNA sin DMSO, 9.5uL de H2O libre de nucleasas
Tubo 2- 5 ng DNA sin DMSO, 9.5uL de H2O libre de nucleasas

Tubo 3- 5ng DNA sin DMSO, 8.75 uL de H2O libre de nucleasas

Nuevo stock de DNA 4 ng/uL en 10 uL

STEP	**TEMP**	**TIME**
Initial Denaturation	98°C	3 minutos
25 Cycles	98°C 68°C 72°C	20 segundo 30 segundos 90 segundos
Final Extension	72°C	8 minutos
Hold	4°C	

20-Agosto
For a 25µl reaction volume:
Component Volume Final Conc.
GoTaq® Green Master Mix, 2X 12.5µl 1X
upstream primer, 10µM 1. 25µl 0.1–1.0µM
downstream primer, 10µM 1. 25 µl 0.1–1.0µM
DNA template 1–5µl <250ng
Nuclease-Free Water to 25µl N.A. 10

30 rxn
375
300
37.5

Component	**25 µl Reaction**	**Final Concentration**
10 µM Forward Primer	1.25 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	0.5 µM

2x GOTAQ green mastermix	12.5 µl	1X
Template DNA	0.5 pg- 5 ng	< 250 ng
Nuclease-free water	to 25 µl	

STEP	**TEMP**	**TIME**
Initial Denaturation	95°C	8 minutos
30 Cycles	95°C 58°C 72°C	30 segundo 30 segundos 3 min
Final Extension	72°C	10 minutos
Hold	4°C	

https://www.promega.com/-/media/files/resources/protocols/product-information-sheets/g/gotaq-green-master-mix-protocol.pdf

PCR de colonia Zero blunt TOPO

Component	**25 µl Reaction**	**Final Concentration**
10 µM Forward Primer	0.5 µl	0.5 µM
10 µM Reverse Primer	0.5 µl	0.5 µM
2x GOTAQ green mastermix	12.5 µl	1X

Template DNA	COLONIA	< 250 ng
Nuclease-free water	11.5 uL	

STEP	**TEMP**	**TIME**
Initial Denaturation	95°C	8 minutos
30 Cycles	95°C 45°C 72°C	30 segundo 30 segundos 3 min
Final Extension	72°C	10 minutos
Hold	4°C	

PCR de colonia 25 Agosto
For a 25µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Green Master Mix, 2X	12.5µl	1X
upstream primer, 10µM	1. 25µl	0.1–1.0µM
downstream primer, 10µM	1. 25 µl	0.1–1.0µM
DNA template	COLONIA	<250ng
Nuclease-Free Water	10uL .	10

STEP PLAN A	**TEMP**	**TIME**

Initial Denaturation	95°C	8 minutos
30 Cycles	95°C 58°C 72°C	30 segundo 30 segundos 3 min
Final Extension	72°C	10 minutos
Hold	4°C	

https://www.promega.com/-/media/files/resources/protocols/product-information-sheets/g/gotaq-green-master-mix-protocol.pdf