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Version 1

Sub-Cloning Primer Design and PCR V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.zjcf4iw>

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External link:

https://www.embl.de/pepcore/pepcore_services/cloning/pcr_strategy/primer_design/amplification/index.html

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Protocol status: In development

We are still developing and optimizing this protocol

Created: March 27, 2019

Last Modified: March 27, 2019

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Keywords: pcr

Materials

MATERIALS

 dNTPs

 Taq DNA Polymerase with Standard Taq Buffer - 20,000 units **New England Biolabs Catalog #M0273E**

**1** Combine the following in a small PCR tube:

	1 uL	Forward Primer (10 uM)
	1 uL	Reverse Primer (10 uM)
	1uL	Template DNA (may need adjustment for at least 20 ng; remember to adjust water volume!)
	1 uL	dNTPs
	0.25 uL	Taq Polymerase
	5 uL	10X Thermopool Buffer
	41.7 5 uL	ddH ₂ O



2 PCR Conditions as programmed into thermocycler as BECKTAQT

Denaturation  00:00:30  95 °C

x30

Denaturation  00:00:15  95 °C

Annealing  00:00:45  55 °C Adjust to 5 less than primer T_m

Elongation  00:00:30  68 °C

Final extension  00:05:00  68 °C