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Sub-Cloning Primer Design and PCR V.1

DOI
https://dx.doi.org/10.17504/protocols.io.zjcf4iw

Abby Jurgensmeier[1], Moriah Beck[1]

[1]Wichita State University

Beck Lab

Abby Jurgensmeier

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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

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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 Thermopol Buffer
	41.75 uL	ddH20

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 Tm

Elongation 00:00:30 68 °C

Final extension 00:05:00 68 °C