

Oct 08, 2022

 PCR

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l694n1lqe/v1>

Ana Belem García González¹, Jair Alexis Gardea Sáenz¹, Irán Alessandra Chaparro Rodríguez¹, Georgina Diego¹

¹Tecnologico de Monterrey Campus Chihuahua



Elena Elizabeth Mercado Flores

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Protocol Citation: Ana Belem García González, Jair Alexis Gardea Sáenz, Irán Alessandra Chaparro Rodríguez, Georgina Diego 2022. PCR. protocols.io <https://dx.doi.org/10.17504/protocols.io.bp2l694n1lqe/v1>

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Protocol status: Working

We use this protocol and it's working

Created: September 08, 2022



Last Modified: October 08, 2022

Protocol Integer ID: 69748

Keywords: pcr, protocol

Abstract

50 μ L volume PCR protocol



RECOVERY IDT SEQUENCES

- 1 Centrifuge the tubes with the sequences for one minute at 3,000 g x m in the centrifuge
- 2 Centrifuge for 5 seconds in the microcentrifuge
- 3 Add nuclease-free water needed to have a final concentration of 10 ng/uL.
- 4 Vortex, followed by incubating for 20 minutes at 50°C.
- 5 Mix briefly by vortex and centrifuge for 8 seconds in mini centrifuge.

PCR

- 6 Add nuclease-free water to the first forward and reverse until a concentration of 100 uM is reached, centrifuged quickly in microcentrifuge.
- 7 Make dilutions with 10 uL of primers and 90 uL of nuclease-free water to reach a concentration of 10 uM
- 8 In 0.2 mL Eppendorf tubes make the following mixture.

9

	A	B
	Nuclease Free Water	17 uL
	Buffer 10X PCR	5 uL
	50 mM MgCl ₂	1.5 uL
	10 mM dNTP's	1 uL



A	B
10 uM Forward primer	2.5 uL
10 uM Reverse primer	2.5 uL
DNA (25 mg/uL)	20 uL
Taq Platinum Polymerase	0.5 uL
Final Volume	50 uL

10 Perform the next cycle in thermocycler

A	B	C	D
1 Cycle	Initial activation	2 minutes	94°C
35 Cycles	Denaturation	30 seconds	94°C
35 Cycles	Alignment	30 seconds	50°C
35 Cycles	Extension	2 minutes	72°C
1 Cycle	Final Extension	5 minutes	72°C
Maintenance	Maintenance	-	4°C