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High GC-content PCR

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Works for me

This protocol is published without a DOI.

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ABSTRACT

X7 is a Pfu-sso7d fusion DNA polymerase that is compatible with uracil-excision cloning and has been used with success to amplify our high GC-content sequences and it is a valuable tool for USER cloning.

Before starting

Please note that protocols with X7 may differ from protocols with other polymerases. Conditions recommended below were tested with our sequences, mostly high GC-content sequences from *Chlamydomonas reinhardtii* expression vectors.

Please refer to the original work that develop the fusion polymerase.

All components should be mixed prior to use.

Reference:

Nørholm, M. H. H. (2010). A mutant Pfu DNA polymerase designed for advanced uracil-excision DNA engineering. BMC Biotechnology, 10. <https://doi.org/10.1186/1472-6750-10-21>

PROTOCOL CITATION

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MATERIALS TEXT

- Phusion® GC Buffer (NEB) 5X
- Betaine 5M
- dNTPs 10 uM
- 10 µM Forward Primer
- 10 µM Forward Primer
- Template DNA
- X7 polymerase
- Nuclease-Free Water
- Ice bucket
- Thermocycler

DISCLAIMER:

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Reaction preparation

- 1 Set up the reaction using the following table on ice:

Reagent	Volume (uL)
Phusion® GC Buffer (NEB) 5X	10
Betaine 5M	10
dNTPs 10 uM	1
10 µM Forward Primer	2.5
10 µM Reverse Primer	2.5
Template DNA	Variable (~0.5-1)
X7	0.5
Nuclease-Free Water	23
Final Volume	50

X7 polymerase developed in Nørholm, M. H. H. (2010). A mutant Pfu DNA polymerase designed for advanced uracil-excision DNA engineering. BMC Biotechnology, 10. <https://doi.org/10.1186/1472-6750-10-21>

Mix the reaction gently and spin it down if necessary.

- 2 Transfer PCR tubes to a thermocycler and start the PCR program. Below it is presented a default setup which can be varied accordingly to the target amplicon. The recommended cycle setup and temperatures are shown below.

STEP	TEMP	TIME
Initial Denaturation	98 °C	30 seconds
35-40 Cycles	98 °C	10 seconds
	60 °C	20 seconds
	68 °C	1 minute/kb
Final Extension	72 °C	5 minutes
Hold	4 – 10 °C	

Melting temperature can be adjusted accordingly with the primers used.