Sub-Cloning Primer Design and PCR

Abby Jurgensmeier[1], Moriah Beck[1]

[1]Wichita State University

Mar 27, 2019

Working

dx.doi.org/10.17504/protocols.io.zjcf4iw

Beck Lab

Abby Jurgensmeier

EXTERNAL LINK

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

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
dNTPs		
Taq DNA Polymerase with Standard Taq Buffer - 20,000 units	M0273E	New England Biolabs

BEFORE STARTING

{"blocks":[{"key":"ef1qd","text":"Primer Design for subcloning a gene of interest into the MCS of a new vector","type":"unstyled","depth":0,"inlineStyleRanges":[{"offset":0,"length":76,"style":"bold"}],"entityRanges":[],"data":{}},{"key":"9pm8g","text":"Forward","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"8n6m7","text":"1. The forward primer overlaps with the 5'-end of the gene of interest.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"2cqq7","text":"2. Use a restriction site compatible with an available restriction enzyme near the beginning of the multiple cloning site in your vector. Add 2-10 nucleotides on the 5’ end of the site for optimal cleavage efficiency.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"7ipop","text":"3. For a C-terminal tag, include a start codon. The restriction site for the NdeI enzyme already has a start codon (CATATG), so it may be beneficial to use this site.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"1bb8e","text":"4. Overlap with the 5’ end of the gene of interest to achieve a primer of 18-30 bases total and a melting temperature (Tm) of 60 (calculation below). The primer should have a GC content of 40-60%.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"fpnj7","text":"","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"fpnd4","text":"Reverse","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"dsno5","text":"1. The reverse primer overlaps with the DNA strand complementary to the 3'-end of the gene of interest.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"e8von","text":"2. Use a restriction site compatible with an available restriction enzyme after (3’ to) the restriction site chosen above. Add 2-10 nucleotides on the 5’ end of the site for optimal cleavage efficiency.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"3epma","text":"3. For an N-terminal tag, include a stop codon. To increase termination efficiency, you may want to use 2-3 stop codons.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"809ua","text":"4. Overlap with the 3’ end of the gene of interest to achieve a primer of 18-30 bases total and a melting temperature (Tm) of 60 (calculation below). The primer should have a GC content of 40-60%.","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"d1g0m","text":"","type":"unstyled","depth":0,"inlineStyleRanges":[],"entityRanges":[],"data":{}},{"key":"5or2e","text":"Primer Melting Temperature:

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**