VERSION 1

MAR 27, 2019

OPEN ACCESS

DOI:

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

External link:

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

Protocol Citation: Abby Jurgensmeier, Moriah Beck 2019. Sub-Cloning Primer Design and PCR. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.zjcf4iw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development
We are still developing and optimizing this protocol

Created: Mar 27, 2019

Last Modified: Mar 27, 2019

Sub-Cloning Primer Design and PCR V.1

Abby Jurgensmeier¹, Moriah Beck¹

¹Wichita State University

Beck Lab

Abby Jurgensmeier

MATERIALS

MATERIALS

dNTPs **Contributed by users**

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

```
{
  "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": "2cq7",
      "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°C (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°C (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 adjust ment for at least 20 ng; reme mber to adjust water volum e!)
1 uL	dNTP s
0.25 uL	Taq Polym erase
5 uL	10X Ther mopol Buffer
41.75 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