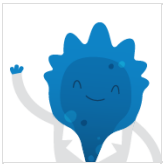


Version 2

Aug 14, 2020

How to Design a Primer V.2

Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

1 Works for me dx.doi.org/10.17504/protocols.io.bjq3kmyn

Addgene The Nonprofit Plasmid Repository
Addgene

ABSTRACT

This protocol describes how to design a primer. To see the full abstract and additional resources, visit https://www.addgene.org/protocols/primer-design/.

EXTERNAL LINK

https://www.addgene.org/protocols/primer-design/

DOI

dx.doi.org/10.17504/protocols.io.bjq3kmyn

PROTOCOL CITATION

Addgene The Nonprofit Plasmid Repository 2020. How to Design a Primer. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjq3kmyn

EXTERNAL LINK

https://www.addgene.org/protocols/primer-design/

CREATED

Aug 14, 2020

LAST MODIFIED

Aug 14, 2020

PROTOCOL INTEGER ID

40443

GUIDELINES

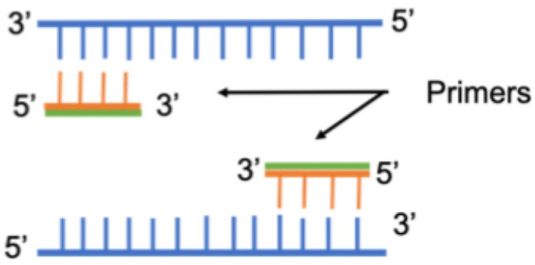
General Guidelines

The main property of primers is that they must correspond to sequences on the template molecule (must be complementary to template strand). However, primers do not need to correspond to the template strand completely; it is essential, however, that the 3’ end of the primer corresponds completely to the template DNA strand so elongation can proceed. Usually a guanine or cytosine is used at the 3’ end, and the 5’ end of the primer usually has stretches of several nucleotides. Also, both of the 3’ ends of the hybridized primers must point toward one another.

The size of the primer is very important as well. Short primers are mainly used for amplifying a small, simple fragment of DNA. On the other hand, a long primer is used to amplify a eukaryotic genomic DNA sample. However, a primer should not be too long (> 30-mer primers) or too short. Short primers produce inaccurate, nonspecific

DNA amplification product, and long primers result in a slower hybridizing rate. On average, the DNA fragment that needs to be amplified should be within 1-10 kB in size.

The structure of the primer should be relatively simple and contain no internal secondary structure to avoid internal folding. One also needs to avoid primer-primer annealing which creates primer dimers and disrupts the amplification process. When designing, if unsure about what nucleotide to put at a certain position within the primer, one can include more than one nucleotide at that position termed a mixed site. One can also use a nucleotide-based molecular insert (inosine) instead of a regular nucleotide for broader pairing capabilities.



Taking into consideration the information above, primers should generally have the following properties:

- Length of 18-24 bases
- 40-60% G/C content
- Start and end with 1-2 G/C pairs
- Melting temperature (Tm) of **50 °C** - **60 °C**
- Primer pairs should have a Tm within **5 °C** of each other
- Primer pairs should not have complementary regions

Note, if you will be including a restriction site at the 5' end of your primer, note that a 3-6 base pair "clamp" should be added upstream in order for the enzyme to cleave efficiently (e.g. GCGGCG-restriction site-your sequence).

Citation: Addgene The Nonprofit Plasmid Repository (08/14/2020). How to Design a Primer. https://dx.doi.org/10.17504/protocols.io.bjq3kmyn