Experiment design

Designing Life with AI

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1 Primer Design Guide

Primers (also: oligos or oligonucleotides) are short sequences of nucleotides used for PCR-based DNA amplification. We use them for strain genotyping, molecular cloning, and sequencing. There are several characteristics we seek when designing primers:

1. **Software tool to use**
   We usually design primers by loading a DNA sequence of interest into Snapgene and simply selecting a stretch of DNA with the computer mouse. Snapgene automatically computes the melting temperature of a selected DNA sequence and displays it near the top of the window.

2. **Melting temperature**
   Primers should have a melting temperature between 50°C and 60°C. Most of the primers we use in the lab have a melting temperature of Tm = 56°C. Aim for the same Tm for your primers.

3. **Choose at least one C or G at the 3' end**
   Because DNA polymerase adds nucleotides to the 3' end of the primer, it is common to choose C's and G's as the last nucleotide(s) of the primer because the C-G bond is stronger than the A-T bond, and may create a more stable end for the DNA polymerase to extend.

4. **GC content**
   Primers should have a GC content of around 50% (roughly the same amount of GC and AT nucleotides). Deviations from this of up to 20% (30% to 70%) are usually tolerated.

5. **Length**
   A primer should be between 15 bp and 25 bp long.

6. **Repeats**
   A primer should not contain more than three consecutive nucleotides that are the same.

7. **Uniqueness**
   A pair of primers should define a unique PCR product in the template DNA. Random DNA sequences are essentially unique but be careful when you are working with repetitive sequences.

8. **Secondary structure**
   A primer should not have a strong secondary structure (example: `..GATATA..TATATC..` can form a DNA hairpin). They can be hard to catch by just looking at the sequence and you can check for their presence using online tools. (In practice, most of us do not really check for secondary structure, although we should.)

9. **Primer-dimer**
   A primer-dimer is an undesired by-product of the PCR reaction which comes about when the two primers are partly complementary. To avoid it, you should not use pairs of primers that can bind to each other.

To verify the presence of secondary structures and primer dimers, you can use online tools like the one available at: https://www.sigmaaldrich.com/CH/en/configurators/tube?product=standar

- Secondary structure: < Moderate

- Primer Dimer: No

1.1 Gibson Primers

In Gibson cloning, the ends of the insert do not have to have restriction sites that match corresponding restriction sites in the vector backbone. Instead, the ends of the insert have to have about 30 bps of homology with the DNA flanking the insertion site in the backbone. Because one does not need to create matching restriction site DNA overhangs, Gibson cloning can be easier than restriction cloning. Furthermore, the protocol is faster, and more than two pieces of DNA can be assembled at the same time. Unfortunately, Gibson cloning rarely works with big pieces of DNA (>2 kbs) and can sometimes fail for no discernible reason.

Design primers for Gibson Assembly

Each of the two primers for amplifying the insert should contain a 3' part that anneals to the template DNA with Tm = 56°C and also a 5' tail that is homologous to the backbone around the cut site. Vojislav recommends at least 20 bps of homology; Sahand recommends 30 bps of homology. (optional) In Gibson assembly, you can introduce new restriction sites that are going to be unique in the final plasmid.
This website explains the details of designing gibson primers:

Gibson primer design guide

You should be careful when designing the PCR fragments to restriction fragments primers as there some specificity related to the type of restriction enzyme used.

2 Plasmid design

We will here talk about the case where a vector has to be edited in order to fit the need of your experiment. The general idea behind this approach is to take a plasmid that already fits some of the characteristics you need and to edit parts of it, most of the time you'll cut out a gene and replace it with your gene of interest and keep the promoter and terminator of the initial gene.

When desinging a plasmid this way you have to carefully chose your vector plasmid. Ideally you want to choose a vector with suitable anti-biotic markers. Those are essential for selection along the whole experiment. Usually your vector will have the Ampicillin marker so that you can grow your cell culture in medium containing ampicillin and be sure that no other bacteria grows (keep in mind that ampicillin is only effective against bacteria). If you plan on transforming your plasmid into other cells you need to add another marker to your plasmid in order to confirm successful transformation. When digesting your plasmid in order to insert your gene of interest you need to make sure that the restriction enzyme you're using does not cut in your gene of interest otherwise your plasmid wont be viable. If the only restriction enzymes that work for you cuts into your gene of interest you can use gibson assembly to avoid having to digest your insert.

3 Guide to Writing a Lab Notebook / ELN

1. Objective
2. Hypothesis
3. Methodology (protocol + notes during execution of protocol)
4. Results
5. Conclusion/Discussion
6. Next steps

4 Guide to Designing a Protocol

1. Define research question
2. Define objective of experiment
3. Write the hypothesis
4. Based on the research question, which conditions would you want to test?
5. Based on the research question, which samples & controls (+ive, -ive) should you include?
6. Which methods will be needed for the experiment?
7. What readout will be used to analyze the outcome of the experiment?
8. What is the expected result/outcome for all samples (including controls)?
9. Write Step-by-Step what the experiment procedure is.

5 Popular Palindromic Six-Cutter Restriction Enzymes

Enzyme	**Recognition Sequence**
AfeI	AGCGCT
AgeI	ACCGGT
ApaI	GGGCCC
BamHI	GGATCC
BglII	N_zAGATCT
ClaI	ATCGAT
DraI	`!TTAAA`
EagI	CGGCCG
EcoRI	GAATTC
EcoRV	GATATC
HindIII	AAGCTT
KpnI	GGTACC
NotI	GCGGCCGC
PmeI	GTTTAAAC
PmlI	CACGTG
PstI	CTGCAG
PvuII	CAGCTG
SacI	GAGCTC
SacII	CCGCGG
SalI	GTCGAC
SmaI	CCCGGG
SpeI	ACTAGT
StuI	AGGCCT
XbaI	TCTAGA
XhoI	CTCGAG
XmaI	CCCGGG