Creating an artificial DNA fragment (Dimer)

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This Protocol will guide you how to make a completely artificial DNA sequence and how to clone it into a vector. With this method sequences can be made up to 80 bp, it is only limited by the length of the primers you are able to order. It is a great method to create a multiple cloning site with the order of restriction enzymes as you want them!

The general concept is simple: A small artificial DNA fragment is cloned into a vector of choice. These vector needs to have two different restriction enzymes allowing to cut it leaving different sticky ends on both sides. For the artificial DNA fragment two primers are used, one for the forward strain and one for the reverse. They will be mixed and let bind to each other to form the double strain DNA. Because they are made with a just a little overhang, they can directly bind to the restriction sites. DNA fragments are synthesized in a way that they already digested!

- 1. Find a suitable vector you want to use and identify the two restriction enzymes to use
- 2. Primer design: Write down the sequence of the restriction sites
- 3. Alternatively, you can choose if you would like to destroy them. Therefore, delete one nucleotide closest to your artificial sequence of each restriction side. Take care that you artificial sequence does not start with it!
- 4. Make up your artificial sequence
- 5. Insert it between the two restriction sites
- 6. Reverse complement it
- 7. This are the two primers, trim them to simulate digestion
- 8. Test the result by aligning them in a cloning program
- 9. Order the primers
- 10. Digest the vector using the two restriction enzymes, do not use CIP or SIP to remove phoshates.
- 11. Purify the vector using a gel.
- 12. Dilute the primers to a 100 μ M concentration
- 13. Prepare in an Eppi the reaction mix

Forward Primer (100 µM)	20 µl
Reverse Primer (100 µM)	20 µl
5 M NaCl-Solution	20 µl
Water	20 µl

- 14. Heat to 95 °C for 10 min
- 15. Let slowly cool to room temperature
- 16. Dilute the solution 1:100 and 1:1000 and use it as Insert in a ligation reaction.
- 17. Sequence all clones to be sure there are no synthesis errors in your artificial sequence

Commented Protocol:

1. Find a suitable vector you want to use and identify the two restriction

enzymes to use

For example: Kpn I and Hind III

2. Primer design: Write down the sequence of the restriction sites

For example: GGTACC AAGCTT

3. Alternatively, you can choose if you would like to destroy them.

Therefore, delete one nucleotide closest to your artificial sequence of each restriction side. Take care that you artificial sequence does not start with it!

For example: GGTAC[ATG] [TCG]AGCTT

The sequence must start with one of the nucleotides in the brackets, otherwise add an additional nucleotide. In the following example we leave the sites intact!

4. Make up your artificial sequence

For example: GCGGCCGCCTGCAGGAATTC (some restriction sites)

5. Insert it between the two restriction sites

For example: GGTACCGCGGCCGCCTGCAGGAATTCAAGCTT

6. Reverse complement it

For example: AAGCTTGAATTCCTGCAGGCGGCCGCGGTACC

7. This are the two primers, trim them to simulate digestion

For example: Forward: CGCGGCCGCCTGCAGGAATTCA Reverse: AGCTTGAATTCCTGCAGGCGGCCGCGGTAC

8. Test the result by aligning them in a cloning programm

For example: CGCGGCCGCCTGCAGGAATTCA CATGGCGCCGGCGGACGTCCTTAAGTTCGT

9. Order the primers

Order high quality to have only few primers containing errors.

10. Digest the vector using the two restriction enzymes, do not use CIP or SIP to remove phoshates.

Primers do not have a 5' phosphate. If you cip the vector, the ligation will not work.

<u>11. Purify the vector using a gel.</u>

Important to get rid of undigested vector.

<u>12. Prepare the primers 100 µM concentration</u>

Usually they are delivered in this concentration.

13. Prepare in an Eppi the reaction mix

Forward Primer (100 µM)	20 µl
Reverse Primer (100 µM)	20 µl
5 M NaCl-Solution	20 µl
Water	20 µl

14. Heat to 95 °C for 10 min

Use a heat block or PCR machine.

15. Let slowly cool to room temperature

Can be done by packing the Eppi in aluminium foil and leave it to cool down for 15 min.

16. Dilute the solution 1:100 and 1:1000 and use it as Insert in a ligation reaction.

Usually I only make one dilution 1:200 and it always worked.

<u>17. Sequence all clones to be sure there are no synthesis errors in your artificial sequence</u>

Strangely I have never had an error, but it is better to check always.

References and Comments:

I learned this protocol in H. Schallers Lab and used it since then many times. It really works well.

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