A&A BIOTECHNOLOGY
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Manual

OverLap™ Assembly Cloning Kit

Kit for rapid cloning of multiple PCR products. DNA Ligase free procedure. Based on the Gibson method.

catalog #	size
1024-10	10 reactions
1024-50	50 reactions

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Applications

- PCR product ligase-independent cloning
- simultaneous fusion and cloning of multiple PCR products
- cloning and mutagenesis
- cloning and intron excision
- gene knockout

Contents

component	10 reactions	50 reactions	storage
OverLap™ enzyme mix	32 µl	140 µl	-20 °C
OverLap™ buffer (5x reaction buffer)	65 µl	270 µl	-20 °C
nucleotides	35 µl	140 µl	-20 °C
ultrapure water	1.5 ml	5 x 1.5 ml	-20 °C
DNA control	16 µl	25 µl	-20 °C
SOC medium	4 x 4 ml	18 x 4 ml	-20 °C

Notes

- Repeated freeze-thaw cycles do not influence the activity of this product.

Reference

1. Gibson D.G. et al (2009). Nature Methods, 343-345
2. Gibson, D.G. et al (2010). Nature Methods, 901-903

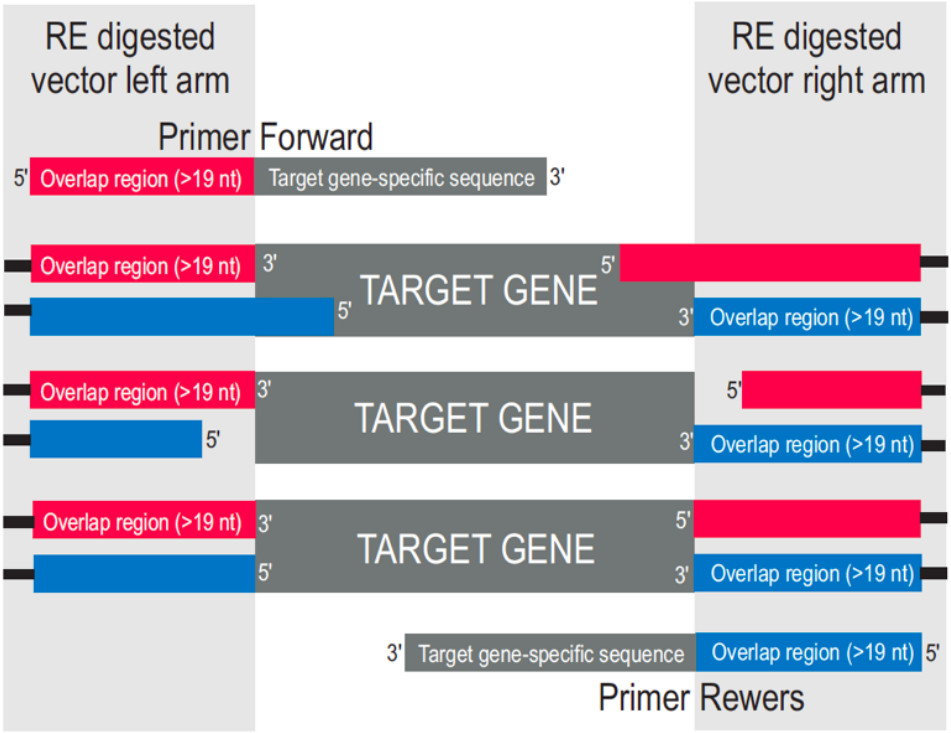
DNA concentration calculation formula

To calculate the number of pmoles of each fragment based on fragment length and weight, we recommend the following formula:

pmol = (weight in ng) x 1000 / (bp x 650 daltons)

length of DNA fragment (bp)	DNA fragment quantity (0.1 pmol)
200	6.5 ng
300	19.5 ng
500	32.5 ng
1000	65 ng
2000	130 ng
3000	195 ng
4000	260 ng
5000	325 ng

Primer design and cloning strategy



Protocol

1. Prepare purified DNA fragments* (vector and insert) suspended in nuclease-free water in appropriate concentration. DNA fragments should be mixed in equimolar ratio (concentration calculation formula, page 4).

2. Set up the following reaction on ice:

Recommended amount of DNA fragments (pmol)

reaction component	**2 DNA fragments***	**3-6 DNA fragments***	**control reaction**
total fragments amount	0.02-0.5 pmol	0.2-1.0 pmol	2 µl DNA control**
OverLap™ buffer	4 µl	4 µl	4 µl
nucleotides	2 µl	2 µl	2 µl
OverLap™ enzyme mix	2 µl	2 µl	2 µl
ultrapure water	up to 20 µl	up to 20 µl	10 µl

* one of these fragments is a vector
** DNA control is composed of pUC19/Sma I vector and DNA fragments from Lambda phage (1000 bp). The proper conduct of the cloning procedure results in the achievement at least 100 colonies for the control reaction

3. Incubate samples:
2 DNA fragments for **15 min** at **45 °C**
3-6 DNA fragments for **60 min** at **45 °C**

4. Place samples on ice or store at -20 °C for transformation at a later time (max 48 hours).

5. Transfer reaction mixture (20 µl of assembly reaction) to *E.coli* competent cells according to the standard transformation protocol.
We recommend using 950 µl of SOC medium for outgrowing *E.coli* cells after heat-shock or electroporation immediately. Incubate transformed cells in SOC medium for 60 min at 37 °C and vigorously shake.

We recommend the following products for use in OverLap™ Assembly strategy:

- PCR Mix Rapid (cat.# 2009-100, 2009-1000) for PCR products amplification
- Clean-Up Concentrator (cat.# 021-50C, 021-250C) / Gel-Out Concentrator (cat.# 023-50C, 023-250C) for DNA fragments purification
- E.coli Transformer Kit (cat.# 4020-240) / E.coli Transformer Express Kit (cat.# 4020-240E) for *E.coli* competent cells preparation and transformation

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