

DATA SHEET

Version: 03
Revision date: 15/06/2023

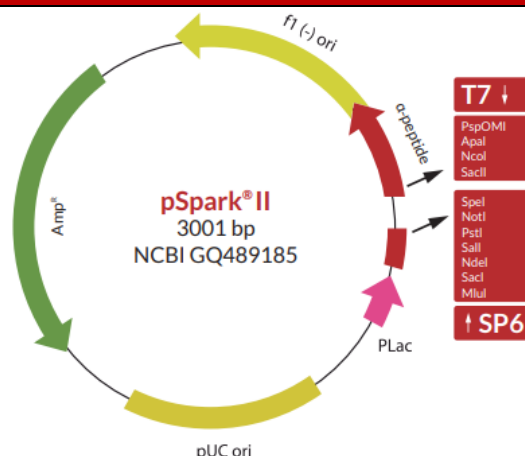
1. Identification

Product name	pSpark® II
	20ng/uL 20 reactions
Cat No	C0002

2. Description

pSpark® II is a highly efficient, accurate and easy-to-use DNA cloning system based on a breakthrough technology for cloning blunt ended DNA generated by PCR with a proofreading or High-Fidelity DNA Polymerases.

The vector is prepared by digestion of pSpark® II at EcoRV site before treating both ends to prevent vector self-ligation. The end treatment is supported by an exclusive know-how that guarantees a higher cloning efficiency than just dephosphorylated vector.



3. Composition

Item	Quantity
pSpark® II DNA cloning vector	20 rxn (20ng/μL)
10X PEG 6000 solution	150 μL
1kb control insert	5 μL
T4 DNA ligase (5 Weiss U/μL)	20 μL
10x T4 DNA ligase Buffer	100 μL

4. Features

- **Unprecedented high cloning efficiency:** > 2,500 positive colonies expected under optimal conditions.
- **Great sensitivity:** over hundreds positive colonies with few nanograms of insert.
- **High stability:** eliminates cloning bias or pitfalls.
- **Time-saving protocol:** no hidden steps such as phosphorylation, just ligation after PCR and transformation.
- **Powerful:** clone from < 1 ng/kb to up to 14 kb, obtain 4x more positive colonies using 3x less DNA insert.
- **Easy-to-use:** eliminate recombinant screening due to its < 1% background, avoiding "suicide" strategies from toxic genes.
- **Great versatility:** compatible with any protocol, proofreading polymerase, competent cells, ligation time or primers.
- **Flexible:** ligation time from 10 minutes to overnight.
- **Robust for every DNA size:** just 6.7 ng per kb of insert needed for optimal ligation.
- **High cost-saving:** reduces your cloning costs as no expensive phosphorylated primers are needed.



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- **Eliminates positive selection vector.**

5. Storage specifications

Store in a non-frost-free freezer at **-20°C**.

6. Applications

- General cloning.
- Clone PCR fragments included in a low amount.
- Cloning of PCR products amplified with High Fidelity Polymerases.
- Cloning of PCR fragments generated with blend polymerases.
- Production of ssDNA.
- Blue/white screening for recombinants.
- In vitro transcription from T7/SP6 dual-opposed promoters.

7. Recommended protocol

1. Amplification

Amplify DNA with any proofreading polymerase following suggested protocol. Gel purifies the insert or check by electrophoresis the quality of amplified DNA. If a single band appears on the gel and template DNA is not a vector with Ampicillin resistance, like the pSpark® II DNA cloning vector, then gel purification is not needed.

2. Ligation

Suggested ratio of insert to vector is 5:1. Use the equation **ng insert = 33ng x kb** (of insert, that is, for a 1kb insert only 33 ng are needed) or use **1 µL** of unpurified PCR product for directly ligation.

Mix:

1 µL of pSpark® II DNA cloning vector (20ng/µL)

1 µL 10x T4 DNA ligase buffer

1 µL 10x PEG 6000 solution

X µL of insert

1 µL of T4 DNA ligase (5 Weiss units)

H₂O to **10 µL**

Incubate the reaction 60 minutes at 22°C (a ligation time from 10min to overnight could be used)

3. Transformation

Transformation can be made with your regular protocol, competent cells and selective media. As pSpark® II DNA cloning vector has Ampicillin resistance, this antibiotic is needed. Also, as pSpark® II DNA cloning vector has blue/white screening capability, IPTG/XGal plates are recommended. A suitable transformation protocol is:

- Thaw competent cells on ice, take **50 µL** and put into a cool 1.5 mL tube.
- Add **10 µL of the ligation mixture**, mix by gently flicking with fingertips and incubate on ice for 30 minutes.
- Heat-shock the cells at **exactly 42°C for exactly 45 seconds** (do not shake) and then transfer to ice for 2 minutes.
- Plate the cells into media with antibiotic, IPTG and XGal and incubate overnight at 37°C.



4. Analysis of transformants

Either colony PCR or plasmid purification and digestion could be used for analysis of transformants. pSpark® II DNA cloning vector has pUC/M13 forward and reverse sequencing primers binding site for sequencing or amplification of insert. Insert size when amplified with pUC/M13 fw and rev primers is about 200bp longer than the real size of insert.

5. Expected results

Up to 2500 positive white colonies for a 60 min ligation of 33 ng of supplied control insert (1 kb) and less than 20 negative blue colonies when using cells with a transformation efficiency of 4×10^7 cfu/ug.

7. Further information

Product Use Limitations	This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
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