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DATA SHEET

Version: 03
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1. Identification

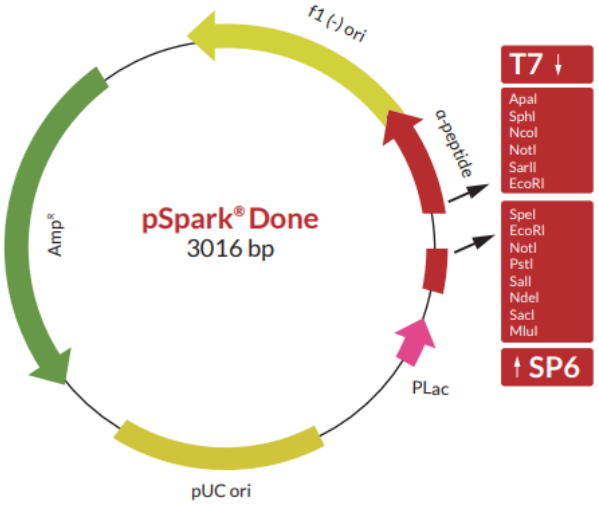
Product name **pSpark® Done**

(20ng/uL) 20 reactions

Cat No **C0006**

2. Description

pSpark® Done is a highly efficient, accurate and easy-to-use DNA cloning system designed for cloning of blunt ended DNA with very high efficiency. The MCS of the pSpark® Done vector incorporates sequences on either side of the insert that are recognized by the restriction enzymes NotI and EcoRI. This allows the insert DNA to be removed with a single restriction digest using either of these enzymes.



3. Composition

Item	Quantity
pSpark® Done 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

- **Optimized:** recognition sites for NotI and EcoRI either side of the insert of cloning point. Transcription-free.
- **Flexible:** allows removing the desired insert DNA with others restriction digestion.
- **Unprecedented efficiency:** > 2,500 positive colonies expected under optimal conditions.
- **Easy-to-use:** eliminate screening of recombinants due to its < 1% background.
- **Time-saving protocol:** avoids any step required after PCR, just 19 minutes from PCR to plating
- **Powerful:** obtain 5x more positive colonies using 10x less DNA insert.
- **High stability:** eliminates cloning bias or pitfalls.
- **Great versatility**: compatible with any protocol, proofreading polymerase, competent cells, ligation time or primers.
- **Sensitive:** clone from 50 bp insert to up to 14 kb with just 5ng per kb of insert.
- **Cost avoidance:** removes expensive primer phosphorylation use.
- **Eliminates positive selection vector.**
- **Robust for every DNA size:** just 6.7 ng per kb of insert needed for optimal ligation.

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5. Storage specifications

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

6. Applications

- Cloning of high-fidelity PCR amplified products.
- Production of ssDNA.
- Blue/white screening for recombinants.
- In vitro transcription from T7/SP6 dual-opposed promoters.
- One restriction enzyme allows gene fragment excision.

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® Done 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 **1µL** of unpurified PCR product for directly ligation.

Mix:

1 µL of pSpark® Done 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)
H_2O 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® Done DNA cloning vector has Ampicillin resistance, this antibiotic is needed. Also, as pSpark® Done DNA cloning vector has blue/white screening capability, IPTG/XGal plates are recommended.
A suitable transformation protocol is:

- Thaw competents 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.

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4. Analysis of transformants

Either colony PCR or plasmid purification and digestion could be used for analysis of transformants. pSpark® Done 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 x 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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