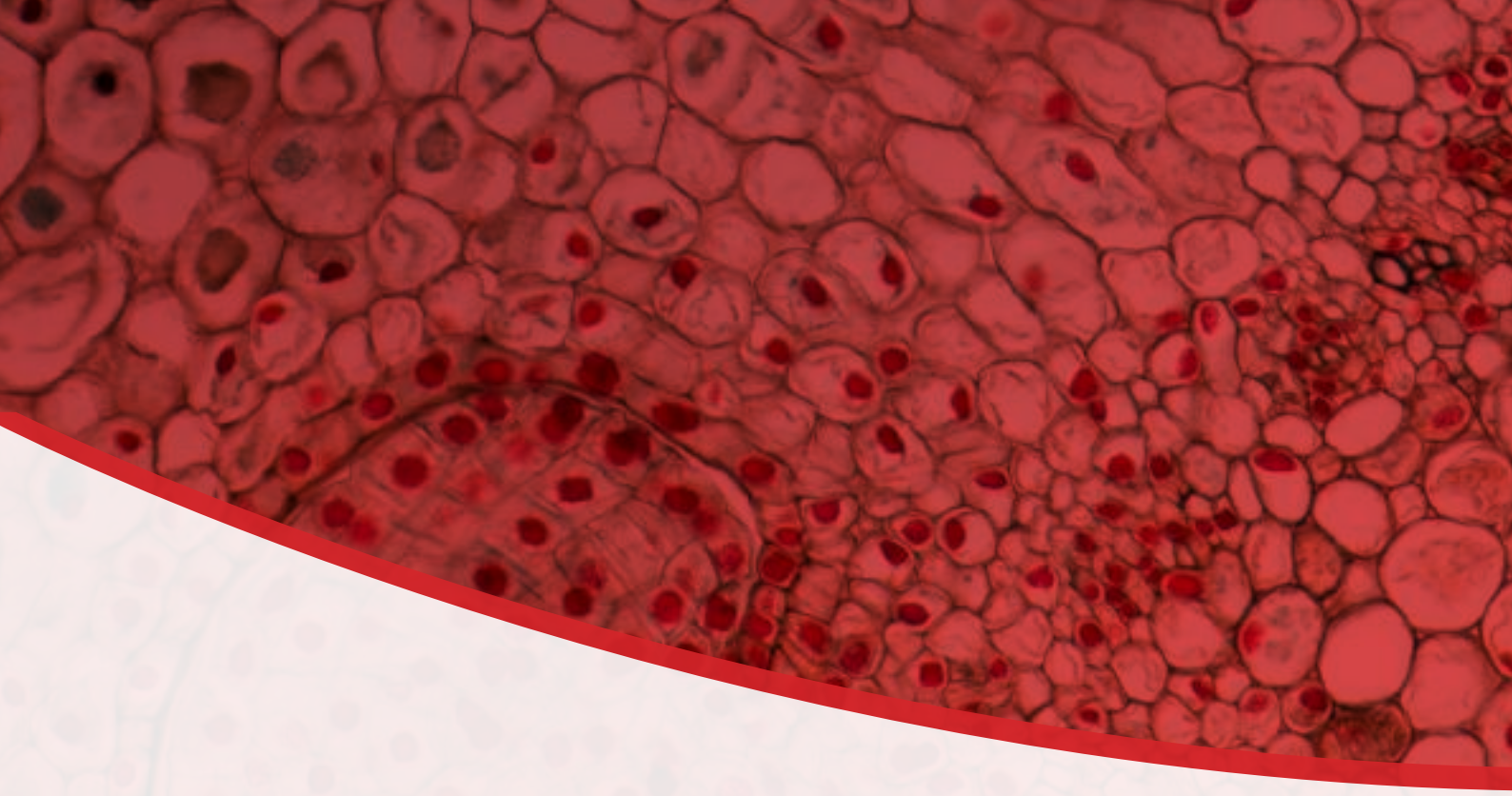


1. DNA Cloning

Blunt-end DNA Cloning Kits
TA DNA Cloning Kits
Universal DNA Cloning Kits
Chemically competent cells
Mutagenesis
Other compounds

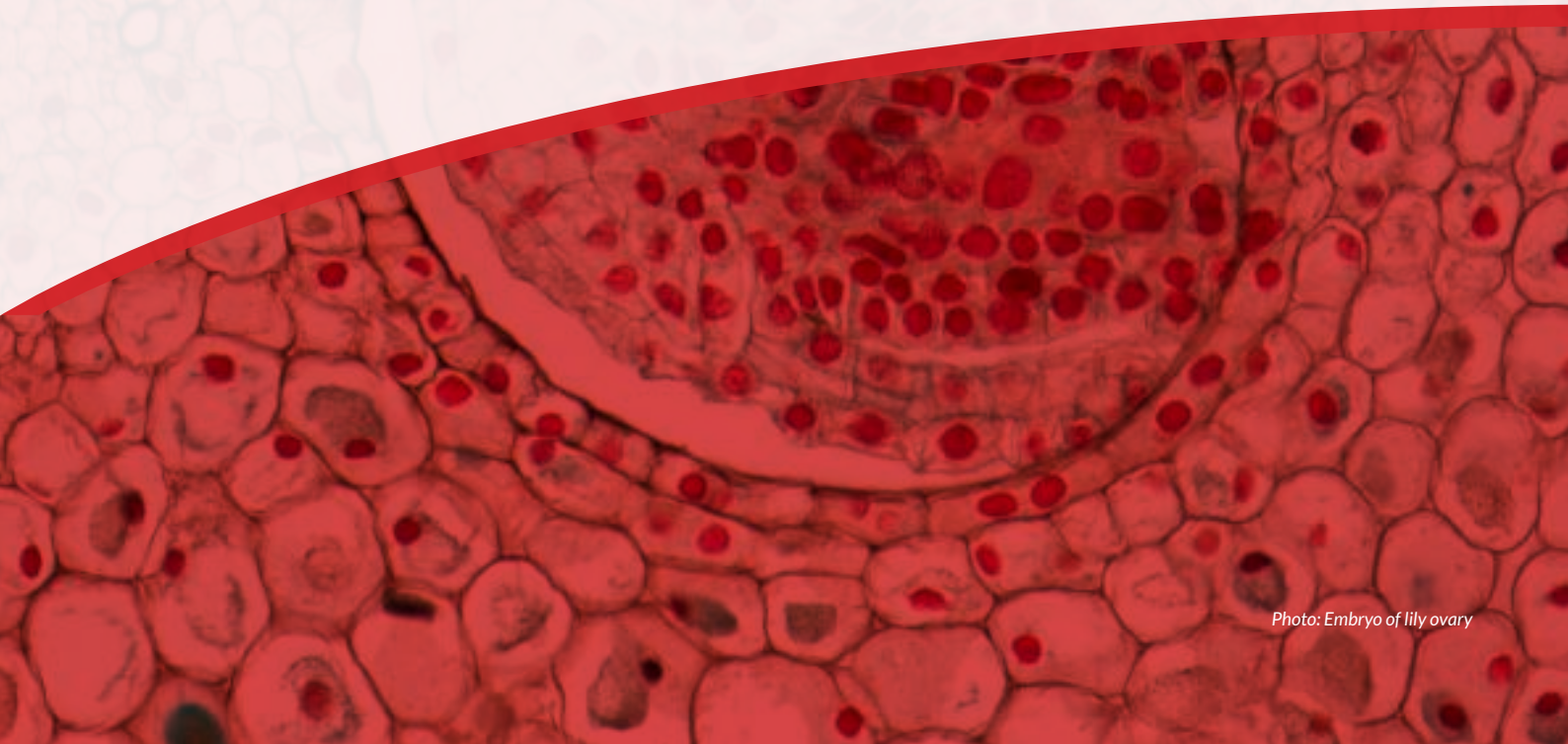


Photo: Embryo of lily ovary

DNA Cloning

pSpark® DNA Cloning Vectors Selection Guide:

	pSpark®							
Features	**I**	**II**	**III**	**IV**	**V**	**Done**	**TA**	**TA Done**
Catalog Number	**C0001**	**C0002**	**C0003**	**C0004**	**C0005**	**C0006**	**C0020**	**C0021**
Page	12	14	14	15	15	16	16	17
Blunt-End Cloning	✓	✓	✓	✓	✓	✓		
TA Cloning							✓	✓
Advanced MCS	✓		✓	✓	✓			
Classic MCS		✓					✓	
Done MCS						✓		✓
Ampicillin Resistance	✓	✓	✓	✓	✓	✓	✓	✓
Amp/Kanamycin Resistance			✓					
High copy number (pUC origin)	✓	✓	✓	✓		✓	✓	✓
Low copy number (pBR322 origin)					✓			
Advantages								
Cloning without Toxic genes	✓	✓	✓			✓	✓	✓
Cloning of unstable fragments				✓	✓			
kb cloning limit	✓	✓	✓	✓	✓	✓	✓	✓
Less initial insert amount needed	✓	✓	✓	✓	✓	✓	✓	✓
Extremely high cloning efficiency	✓	✓	✓	✓	✓	✓	✓	✓
Flexibility and free protocol	✓	✓	✓	✓	✓	✓	✓	✓
Very low background	✓	✓	✓	✓	✓	✓	✓	✓
High stability with no cloning bias	✓	✓	✓	✓	✓	✓	✓	✓

Blunt-end DNA Cloning Kits

pSpark® I

For highly efficient, accurate and robust general cloning from PCR High Fidelity fragments, without the use of toxic genes

Ordering info:

Cat No.	Size
C0001-S	10 rxn
C0001	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® I (20 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 150 µL PEG 6000 (10x)
- 5 µL Insert Control 1 kb (20 ng/µL)

Related Products:

- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- CVX5α™ Chemically Competent cells (p.18)
- Custom cloning services (p.140)
- CleanEasy™ PCR Purification Kit (p.91)
- PickMutant™ Site-directed Mutagenesis Kit (p.19)
- FastPANGEA™ High Fidelity DNA Pol. (p.105)
- Ampicillin (p.126)
- ITPG (p.19)
- X-Gal (p.19)

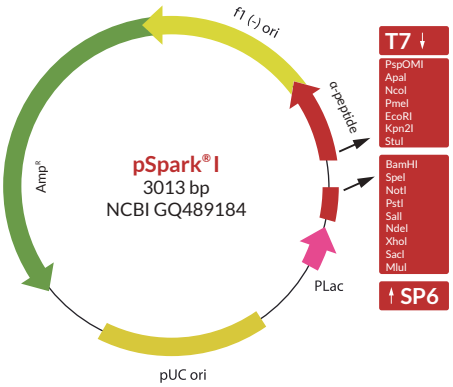
Description:

pSpark® I is a highly efficient, accurate and easy-to-use DNA cloning system based on a novel breakthrough technology to generate blunt vectors with a highly cloning efficiency.

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

Advantages & Features:

- ✔ **Unprecedented high cloning efficiency:** > 2,500 positive colonies expected under optimal conditions.
- ✔ **Easy-to-use:** eliminate recombinant screening due to its <1% background, avoiding "suicide" strategies from toxic genes.
- ✔ **Time-saving protocol:** no hidden steps such as phosphorylation, just ligation after PCR and transformation.
- ✔ **High stability:** eliminates cloning bias or pitfalls.
- ✔ **Powerful:** clone from < 1 ng/kb, obtain 5x more positive colonies using 10x less DNA insert.
- ✔ **Compatible with blue/white screening.**
- ✔ **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.
- ✔ **Eliminates positive selection vector.**
- ✔ **High cost-saving:** reduces your cloning costs as no expensive phosphorylated primers are needed.
- ✔ **Robust for every DNA size:** just 6.7 ng per kb of insert needed for optimal ligation.



Applications:

- ✔ General cloning.
- ✔ Cloning of High Fidelity PCR amplified products.
- ✔ Production of ssDNA.
- ✔ Blue/white screening for recombinants.
- ✔ *In vitro* transcription from T7/SP6 dual-opposed promoters.

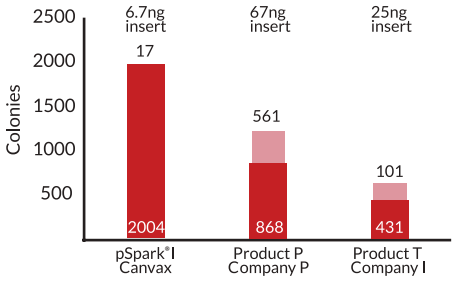
Quality control:

- ✔ Functionally test using 1.0 kb PCR fragment.

Comparison with other popular vectors:

In 2016, Canvax conducted a rigorous study where the efficiency of all pSpark® Blunt-end DNA Cloning systems were analyzed in comparison other popular cloning systems, developed almost two decades ago. In this catalog the results of pSpark® I compared to Product P and Product T are presented. If you want to review the full white paper, please visit pspark.canvaxbio.com

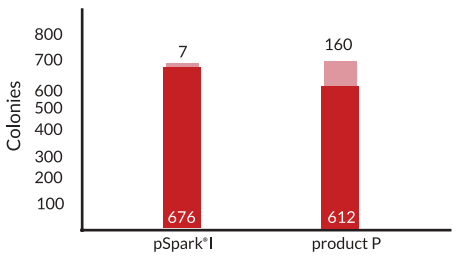
Figure 1.1: Efficiency and background



Cloning efficiency of pSpark® I over other popular cloning systems. The cells used had a cloning efficiency of 2 x 10^7 cfu/µg.

As shown in the previous figure, the background for pSpark® I is 0.8%, while in other cases, it is 40% and 20%, respectively. On the other hand, pSpark® I has an efficiency of 300 cfu/µg of DNA Insert, while other products have 13 cfu/µg and 17 cfu/µg of DNA, respectively.

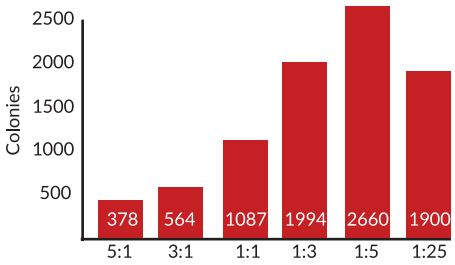
Figure 1.2: Robust and versatile



Cloning efficiency using pSpark® I with blend polymerase. The 1 kb-insert was amplified with FastPANGEA™ High Fidelity DNA Polymerase MasterMix for cloning with pSpark® I and with blend polymerase to clone with Company P. Competent cells had a cloning efficiency of 2 x 10^7 cfu/µg.

Despite the similarity of the results, it is important to highlight that PCR products, obtained with a mix of both DNA polymerases, contain a mixture of molecules with blunt ends and molecules with adenine at the 3´ends in a proportion of 30% and 70%, respectively. Therefore, pSpark® I is more robust and versatile than Product P.

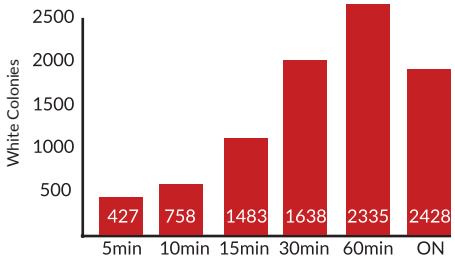
Figure 1.3: Insert amount



Number of positive white colonies obtained after ligation with different ratios of pSpark® I vector:insert. The amount of vector was the same in all cases, varying the amount of insert to achieve the vector: insert ratio identified. The background was less than 1%. Competent cells had an efficiency of 2 x 10^7 cfu/µg.

As is described, it allows obtaining a high number of colonies even using < 1 ng of insert as in the 5:1 vector: insert ratio.

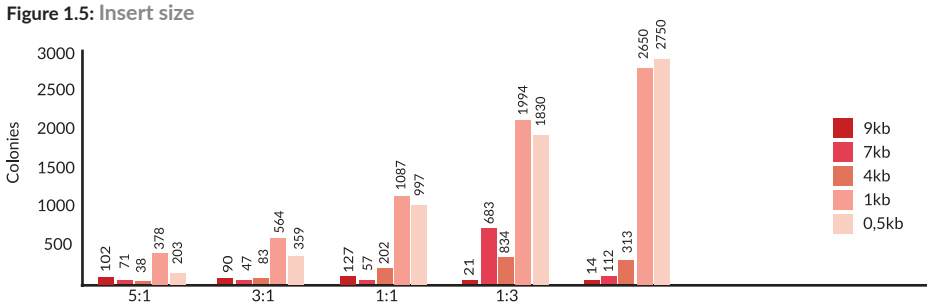
Figure 1.4: Ligation Time



pSpark® I ligation-determined efficiency in response to different ligation times. Competent cells used had an efficiency of 2 x 10^7 cfu/µg. Is possible to use pSpark® using almost any lab protocol, ligation temperature (example: 25°C-RT, 22°C, 16° or 4°C), and it could even tolerate some changes depending on the needs of each cloning task or laboratory resources.

It is necessary to emphasize that with only 5-10 minutes of ligation time, >400-700 positive colonies and a background <1% are obtained.

Figure 1.5: Insert size



Efficiency of cloning pSpark® I inserts of different sizes using different vector: insert ratios. Inserts were used 0.5 kb, 1kb, 4kb, 7kb and 9kb in the ratios indicated below. Competent cells were 2 x 10^7 cfu/µg DNA. Background was always below 1%.

As is shown, the vector: insert relationship 1:5 is the best with >2,000 positive colonies for inserts equal or < 1kb.

pSpark® II

For highly efficient, accurate and easy general cloning with classical MCS, without the use of toxic genes

Ordering info:

Cat No.	Size
C0002-S	10 rxn
C0002	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® II (20 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 150 µL PEG 6000 (10x)
- 5 µL Insert Control 1 kb (20 ng/µL)

Related Products:

- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- CVX5α™ Chemically Competent cells (p.18)
- CleanEasy™ PCR Purification kit (p.91)
- Custom Cloning services (p.140)
- BrightMAX™ DNA Ladders (p.116)
- Ampicillin (p.126)
- ITPG (p.19)
- X-Gal (p.19)

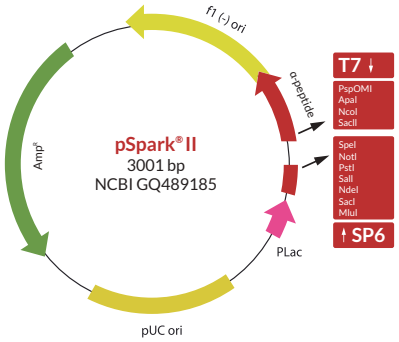
Description:

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 a exclusive know-how that guarantees a higher cloning efficiency than just dephosphorylated vector.

Advantages & 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.
- ✓ **Eliminates positive selection vector.**



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.

Quality control:

- ✓ Functionally test using 1.0 kb PCR fragment.

Comparison with other vectors:

- ✓ Please visit page 13 to review it.

pSpark® III

For highly efficient, accurate and easy cloning with Ampicillin and Kanamycin resistance cassettes, without the use of toxic genes

Ordering info:

Cat No.	Size
C0003-S	10 rxn
C0003	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® III (20 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 150 µL PEG 6000 (10x)
- 5 µL Insert Control 1 kb (20 ng/µL)

Related Products:

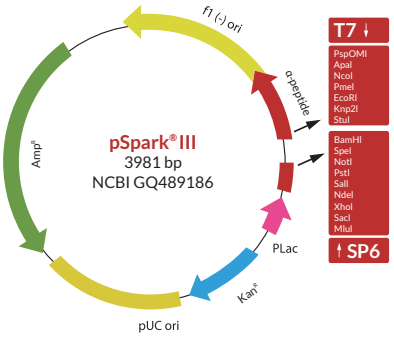
- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- CVX5α™ Chemically Competent cells (p.18)
- CleanEasy™ PCR Purification kit (p.91)
- Custom Cloning services (p.140)
- PickMutant™ Site-directed Mutagenesis Kit (p.19)
- FastPANGEA™ High Fidelity DNA Pol. (p.105)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)
- Kanamycin (p.126)

Description:

pSpark® III is a highly efficient, accurate and easy-to-use DNA cloning system that combines Ampicillin and Kanamycin resistance. Ideal for cloning PCR products amplified from any plasmid vector without the need to gel-purify bands to eliminate the background due to the template vector used for PCR.

Advantages & Features:

- ✓ **Unprecedented high cloning efficiency:** > 2,500 positive colonies expected under optimal conditions.
- ✓ **Time-saving protocol:** no hidden steps such as phosphorylation, just ligation after PCR and transformation.
- ✓ **Powerful:** obtain 5x more positive colonies using 10x less DNA insert.
- ✓ **Easy-to-use:** eliminate recombinant screening due to its <1% background, avoiding "suicide" strategies from toxic genes.
- ✓ **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.
- ✓ **High cost-saving:** reduces your cloning costs as no expensive phosphorylated primers are needed.
- ✓ **Eliminates positive selection vector.**



Applications:

- ✓ Cloning directly from PCR using plasmid cloned genes as template.
- ✓ Unpurified PCR cloning.
- ✓ Cloning of high fidelity PCR amplified products.
- ✓ Production of ssDNA.
- ✓ Blue/white screening for recombinants.
- ✓ *In vitro* transcription from T7/SP6 dual-opposed promoters.

Quality control:

- ✓ Functional test using a 1.0 kb PCR fragment.

Comparison with other vectors :

- ✓ Please visit page 13 to review it.

pSpark® IV

For highly efficient, stable and powerful cloning under transcription-free conditions

Ordering info:

Cat No.	Size
C0004-S	10 rxn
C0004	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® IV (20 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 150 µL PEG 6000 (10x)
- 5 µL Insert Control 1 kb (20 ng/µL)

Related Products:

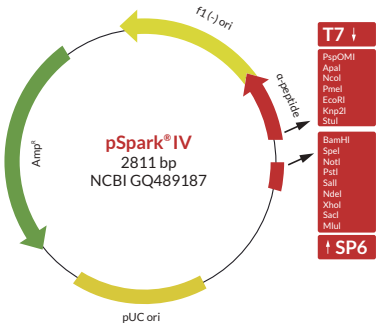
- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- CVX5α™ Chemically Competent cells (p.18)
- CleanEasy™ PCR Purification kit (p.91)
- Custom Cloning services (p.140)
- BrightMAX™ DNA Ladders (p.116)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)

Description:

pSpark® IV is a highly efficient, accurate and easy-to-use DNA cloning system that exploit its very low background feature for the expression of toxic genes under transcription-free conditions. In this vector, the *lac* promoter has been eliminated and therefore blue/white screening is not allowed (alpha-peptide coding region remains and you can find blue colony). The vector is ideal for cloning genes that produce toxic polypeptides by transcription/translation.

Advantages & Features:

- **Unprecedented high cloning efficiency:** > 2,500 positive colonies expected under optimal conditions.
- **Transcription-free.**
- **Easy-to-use:** eliminate screening of recombinants due to its <1% background.
- **High stability:** eliminates cloning bias or pitfalls.
- **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.
- **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.**



Applications:

- Cloning of high fidelity PCR amplified products.
- Production of ssDNA.
- *In vitro* transcription from T7/SP6 dual-opposed promoters.
- Cloning of toxic genes.

Quality control:

- Functional test using a 1.0 kb PCR fragment.

Comparison with other vectors :

- Please visit page 13 for a review.

pSpark® V

For highly efficient, accurate and easy cloning with pBR322 and transcription-free conditions

Ordering info:

Cat No.	Size
C0005-S	10 rxn
C0005	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® V (20 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 150 µL PEG 6000 (10x)
- 5 µL Insert Control 1 kb (20 ng/µL)

Related Products:

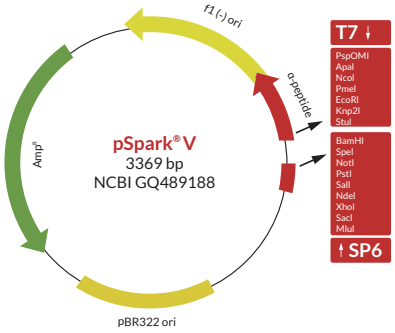
- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- CVX5α™ Chemically Competent cells (p.18)
- CleanEasy™ PCR Purification kit (p.91)
- Custom Cloning services (p.140)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)

Description:

pSpark® V is a highly efficient, accurate and easy-to-use DNA cloning system developed with low copy number, as a help for cloning of inserts with the highers kb. This low copy variant is also transcription-free, for the most demanding cloning tasks. In this vector, the *lac* promoter has been eliminated and therefore blue/white screening is not allowed (alpha-peptide coding region has been truncated).

Advantages & Features:

- **Unprecedented high cloning efficiency:** > 2,500 positive colonies expected under optimal conditions.
- **Transcription-free.**
- **Easy-to-use:** eliminate screening of recombinants due to its <1% background.
- **High stability:** eliminates cloning bias or pitfalls.
- **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.
- **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.
- **Optimized:** truncated alpha-peptide coding region.
- **Cost avoidance:** removes expensive primer phosphorylation use.
- **Eliminates positive selection vector.**



Applications:

- Cloning of toxic genes.
- Cloning of unstable genes, for example genes with repeated sequences.
- Cloning of high fidelity PCR amplified products.
- Production of ssDNA.
- Blue/white screening for recombinants.
- *In vitro* transcription from T7/SP6 dual-opposed promoters.

Quality control:

- Functional test using a 1.0 kb PCR fragment.

Comparison with other vectors :

- Please visit page 13 for a review.

pSpark® Done

For highly efficient, accurate and easy cloning of PCR fragments with EcoRI and NotI flanking the insertion site

Ordering info:

Cat No.	Size
C0006-S	10 rxn
C0006	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® Done (20 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 150 µL PEG 6000 (10x)
- 5 µL Insert Control 1 kb (20 ng/µL)

Related Products:

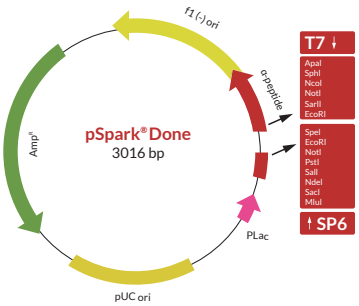
- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- CVX5α™ Chemically Competent cells (p.18)
- CleanEasy™ PCR Purification kit (p.91)
- Custom Cloning services (p.140)
- FastPANGEA™ High Fidelity DNA Polymerase (p.105)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)

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.

Advantages & Features:

- ✔ **Optimized:** recognition sites for NotI and EcoRI either side of the insert of cloning point.
- ✔ **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.
- ✔ **Eliminates positive selection vector.**
- ✔ **Cost avoidance:** removes expensive primer phosphorylation use.
- ✔ **Robust for every DNA size:** just 6.7 ng per kb of insert needed for optimal ligation.



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.

Quality control:

- ✔ Functionally test using 1.0 kb PCR fragment.

Comparison with other vectors :

- ✔ Please visit page 13 to review it.

TA DNA Cloning Kits

pSpark® TA

For efficient, stable and easy cloning of non-proofreading PCR fragments or PCR from blend enzymes

Ordering info:

Cat No.	Size
C0020-S	10 rxn
C0020	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® TA DNA Cloning vectors (50 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 5 µL Insert Control 600 bp (30 ng/µL)

Description:

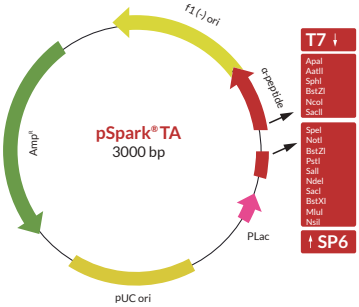
pSpark® TA is efficient, stable and easy-to-use DNA cloning vector based on an optimized TA technology for cloning single 3´-adenine overhanging DNA. The vectors are prepared by digestion of pSpark® TA at EcoRV site and the subsequent addition of a single thymidine at each 3´- end to allow cloning Taq DNA Polymerase amplified DNA fragments. Its exclusive procedure offers greater efficiency and less background of blue colonies than the others TA vectors.

Advantages & Features:

- ✔ **Efficient:** >600 white positive colonies expected under optimal conditions.
- ✔ **Easy-to-use:** eliminate screening of recombinants due to its <4% background.
- ✔ **High stability:** vector without cloning bias due to transcription of toxic genes.
- ✔ **Fast protocol:** ligation time from 60 minutes to overnight.
- ✔ **Compatible:** with direct cloning of PCR products.
- ✔ **Great versatility.**
- ✔ **Cost avoidance:** removes primer phosphorylation.

Applications:

- ✔ Cloning of non-proofreading PCR fragments.
- ✔ Production of ssDNA.
- ✔ Blue/white screening for recombinants.
- ✔ *In vitro* transcription from T7/SP6 dual-opposed promoters.



Quality control:

- ✔ Functional test using a 600 bpPCR fragment.

Related Products:

- TruePure™ dNTPs (p.115)
- Horse-Power™ Taq DNA Polymerase (p.102)
- CVX5α™ Chemically Competent cells (p.18)
- Horse-Power™ Red-Taq DNA Polymerase (p.107)
- Horse-Power™ Green-Taq DNA Polymerase (p.107)
- CleanEasy™ PCR Purification kit (p.91)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)

pSpark® TA Done

For efficient, stable and easy cloning of PCR fragments with EcoRI and NotI flanking the insertion site

Ordering info:

Cat No.	Size
C0021-S	10 rxn
C0021	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® TA Done (50 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 5 µL Insert Control 600 bp(30 ng/µL)

Related Products:

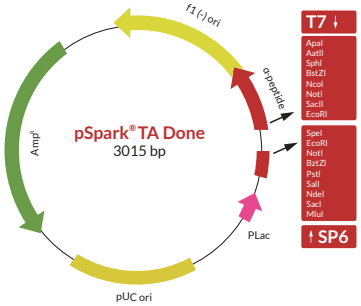
- Horse-Power™ Taq DNA Polymerase (p.102)
- CVX5α™ Chemically Competent cells (p.18)
- CleanEasy™ PCR Purification kit (p.91)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)
- Horse-Power™ Red-Taq DNA Polymerase (p.107)
- Horse-Power™ Green-Taq DNA Polymerase (p.107)

Description:

pSpark® TA Done is efficient, stable and easy-to-use DNA cloning vector based on an improved TA technology that offers all of the advantages of pSpark® TA with the added convenience of recognition sites for EcoRI and NotI flanking the insertion site. Thus, several options exist to remove the desired insert DNA with a single restriction digestion.

Advantages & Features:

- **Convenient:** recognition sites for EcoRI and NotI flanking the insertion site.
- **Flexible:** allows removing the desired insert DNA with other restriction digestion.
- **Efficient:** >600 white positive colonies expected under optimal conditions.
- **Stable:** without cloning bias due to transcription of toxic genes.
- **Easy-to-use:** eliminate screening of recombinants due to its <4% background.
- **Fast protocol:** ligation time from 60 minutes to overnight.
- **Compatible:** with direct cloning of PCR products.
- **Great versatility:** compatible with any competent cell or primer design.
- **Cost avoidance:** removes primer phosphorylation.



Applications:

- Cloning of non-proofreading PCR fragments.
- Production of ssDNA.
- Blue/white screening for recombinants.
- *In vitro* transcription from T7/SP6 dual-opposed promoters.

Quality control:

- Functional test using a 600 bp PCR fragment.

pMBL-T™ Vector

Efficient, convenient and fast cloning of DNA fragments with A overhangs

Ordering info:

Cat No.	Size
C0030	20 rxn

Includes for 20 rxn:

- 20 µL pMBL-T™ Vector (50 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 100 µL T4 DNA Ligase Buffer (10x)
- 5 µL Insert Control 600 bp (30 ng/µL)

Related Products:

- Horse-Power™ Taq DNA Polymerase (p.103)
- T4 DNA Ligase (p.111)
- CVX5α™ Chemically Competent cells (p.18)
- Horse-Power™ Red-Taq DNA Polymerase (p.107)
- Horse-Power™ Green-Taq DNA Polymerase (p.107)
- CleanEasy™ PCR Purification kit (p.91)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)

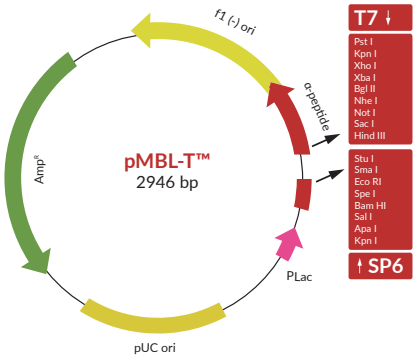
Description:

pMBL-T™ Vector DNA Cloning Kit is an efficient, convenient and fast system for the cloning of PCR products. The vector is prepared by cutting pMBL-T™ vector with EcoRV and adding a 3´ terminal thymidine to both ends. These single 3´-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product into the plasmids by preventing recircularization of the vector and providing a compatible overhang for PCR products generated by certain thermostable polymerases such as Horse-Power™ Taq DNA Polymerase.

These polymerases often add a single deoxyadenosine, in a template-independent fashion, to the 3´-ends of the amplified fragments.

Advantages & Features:

- **Highly efficient:** > 90% white colonies in a transformation with supplied insert control.
- **Proven performance:** > 1,000 recombinant colonies in optimal conditions.
- **Fast and easy protocol:** results from 15 min protocol.
- **Optimized:** improve efficiency of ligation of a PCR product into the plasmid.
- **Compatible:** overhang for ligation of PCR products preventing recircularization of the vector.
- **Designed** by cutting the vector with EcoRV and adding a 3´ terminal thymidine to both ends.



Applications:

- Cloning of PCR fragments into DNA.
- Cloning vector.
- Blue/white screening for recombinants.

Quality control:

- Functionally test using 600 bp PCR fragment.

Universal DNA Cloning Kit

pSpark® Universal DNA Cloning kit

Highly efficient, robust and easy-to-use system compatible with Blunt and TA DNA cloning

Ordering info:

Cat No.	Size
C0019	20 rxn

Includes for 20 rxn:

- 20 µL pSpark® II (20 ng/µL)
- 20 µL pSpark® TA DNA Cloning vector (50 ng/µL)
- 20 µL T4 DNA Ligase (5U/Weiss)
- 200 µL T4 DNA Ligase Buffer (5x)
- 150 µL PEG 6000 (10x)
- 5 µL Insert Control 1 kb (20 ng/µL)
- 5 µL Insert Control 600 bp (30 ng/µL)

Related Products:

- pSpark® II DNA Cloning vector (p.14)
- pSpark® TA DNA Cloning vector (p.17)
- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- Horse-Power™ Taq DNA Polymerase (p.103)
- CVX5α™ Chemically Competent cells (p.18)
- CleanEasy™ PCR Purification kit (p.91)
- ITPG (p.19)
- X-Gal (p.19)
- Ampicillin (p.126)

Description:

pSpark® Universal is a highly efficient, accurate and easy-to-use DNA cloning kit ideal for a broad range of PCR fragments cloning applications. There is a range of DNA polymerases available that do not generate PCR products with identical ends: proofreading DNA polymerases leave blunt ends while blends of polymerases and non-proofreading DNA polymerases leaves 3´A overhangs. Therefore, it is necessary to employ different vectors to clone both kinds of PCR fragments.

pSpark® Universal DNA cloning kit has been designed to save time, looking for a kit for several cloning scenarios. It is mainly composed of two cloning vectors which allow blunt or TA DNA cloning. For blunt DNA cloning and TA DNA cloning, pSpark® II DNA cloning vector and pSpark® TA DNA cloning vector, respectively, are included.

Advantages & Features:

- **Compatible with Blunt and TA DNA cloning:** it is composed by pSpark® II (p.14) and pSpark® TA DNA cloning vector (p.16).
- **Convenient:** ideal for a broad range of PCR fragments cloning applications.
- **Versatile:** compatible with any DNA polymerase.

Applications:

- Cloning of high fidelity PCR amplified products into pSpark® II Blunt DNA cloning vector.
- Cloning of non-proofreading PCR fragments into pSpark® TA DNA Cloning vector.
- Production of ssDNA.
- *In vitro* transcription from T7/SP6 dual-opposed promoters.

Quality control:

- Functionally test using 1.0 kb PCR fragment (pSpark® II) and 600 bp PCR fragment (pSpark® TA).

Chemically Competent Cells

CVX5α™ (1 x 10^7 CFU/µg)

Versatile, convenient and cost-effective solution for routine subcloning procedures

Ordering info:

Cat No.	Size
C0031	40 rxn (4 x 500 µl)
C0032	40 rxn (40 x 50 µl)
C0033	90 rxn (9 x 500 µl)

Includes for 40 rxn:

- 2,000 µl CVX5α™ (1 x 10^7 CFU/µg)
- 10 µl pUC18 Transformation Control Plasmid (10 ng / µl)
- 50 mL SOC Medium
- Dry ice

Description:

CVX5α™ Chemically competent cells are a versatile, convenient and cost-effective solution for routine subcloning procedures or any application where the starting DNA is not limiting.

CVX5α™ are calcium chloride-treated to facilitate attachment of the plasmid DNA to the competent cell membrane.

Advantages & Features:

- **Versatile:** proven performance for high-efficiency transformation in a wide variety of applications.
- **Convenient:** ideal for routine.
- **Compatible:** with blue/white screening of colonies on bacterial plates containing Bluo-gal or X-gal.
- **Cost avoidance:** dry ice free of charge.

CVX5α™ Genotype:

F - , gyrA96, recA1, endA1, thi1, hsdR17 (rK - mK +), deoR, supE44, Δ (*lacZ*YA-argF) U169 Φ80*lacZ*ΔM15.

Applications:

- Routine cloning and subcloning of genes into plasmid vectors.

Quality control:

- Each lot of competent cells is tested to verify transformation efficiencies using 100 pg pUC18 supercoiled DNA and the recommended protocol.
- Under these conditions, transformation efficiency will be ≥ 1 x 10^7 cfu/µg pUC18.
- Transformation efficiency is defined as the number of colony forming units (cfu) produced by transforming 1 µg of plasmid (3 kb) into a given volume of competent cells.

Note:
Optimal competence for cloning but it is not enough for the generation of cDNA libraries.

Related Products:

- pSpark® Blunt-end DNA Cloning vectors (p.12)
- pSpark® TA DNA Cloning vectors (p.16)
- pOnebyOne™ Mammalian Expression vectors (p.22)
- pColiExpress™ Glue Enzyme kits (p.34)
- Custom Cloning services (p.140)

Mutagenesis

PickMutant™

For a reliable, robust and highly efficient Site-directed Mutagenesis based in PCR

Ordering info:

Cat No.	Size
MT001	15 rxn

Includes for 15 rxn:

- 150 µl MasterMix Proofreading DNA Polymerase (2x)
- 300 U Glue enzyme (10 U/µl)
- 40 µl Glue enzyme Buffer (10x)
- 5 µl Insert Control DNA
- 15 µl pSpark® I (20 ng/µl)

Description:

PickMutant™ is a reliable, robust and highly efficient PCR-based mutagenesis kit. Extremely easy-to-use, the kit allows creating single or multiple point mutations, deletions or insertions using a rapid and easy protocol. All these mutation could be obtained by PCR using a FastPANGEA™ High Fidelity DNA Polymerase and well-designed mutagenesis primers. The assembled mutagenic PCR fragments is cloned into pSpark® cloning vector, specially designed to clone blunt PCR fragments with high efficiency or into other vector designing, in this case, an additional specific vector primer pair.

Advantages & Features:

- **Highly Effective** point mutations (single or multiple), deletions or insertions.
- **Easy and fast protocol:** it takes less than 3 hours in one step procedure.
- **Cost avoidance:** compatible with any bacterial strains or primers.
- **Versatile:** compatible with any cloning vector.
- **Efficient:** includes highly efficient pSpark® to clone blunted fragments.
- **Robust:** simultaneous assemble and clone of PCR fragments.

Applications:

- Site-directed Mutagenesis.
- Study protein function.
- Identify enzyme active sites.
- Design new proteins.

Quality control:

- The kit has been tested using the insert control DNA provided.

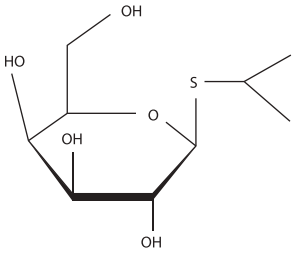
Related Products:

- Custom Mutagenesis services (p.140)
- pSpark® I DNA Cloning vector (p.12)
- FastPANGEA™ Long PCR DNA Polymerase (p.106)
- Molecular Microbiology services (p.140)
- ITPG (p19)
- X-Gal (p.19)

Related Compounds

IPTG

Isopropyl β-D-thiogalactopyranoside



Specifications:

CAS Number: 367-93-1
Chemical Formula: $C_9H_{18}O_5S$
Molecular Weight: 238.30
Purity (HPLC)(on dry basis): <99.0%
Melting point: 110 - 114°C
Identity (IR): conforms to structure
Solubility: soluble in water and methanol
Heavy metals (Pb): >5ppm
1,4-Dioxane: Not detected
pH(5% in water): 5.0 - 7.0
Water content (Karl Fischer): >1.0%

Ordering info:

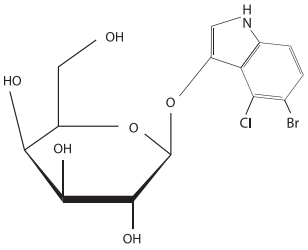
Cat No.	Size
C0040	5g
C0041	25g

Applications:

- Blue/white screening.
- Expression of genes under *lac* promoter control.

X-Gal

5-Bromo-4-chloro-3-indolyl β-D-Galactopyranoside



Specifications:

CAS Number: 7240-90-6
Chemical Formula: $C_{14}H_{15}BrClNO_6$
Molecular Weight: 408.63
Assay (HPLC): <98% w/w
Purity (HPLC): <99%
Purity (TLC): single spot
Water content (Karl Fischer): >1%
Identity (IR): conforms to structure
Solubility (5% w/v, DMF): soluble

Ordering info:

Cat No.	Size
C0043	1g
C0044	5g

Applications:

- Blue/white screening.
- Gene expression detection of β-galactosidase reporter.
- Detection of β-galactosidase activity in immunological and histochemical applications.