

Advantages of pSpark® over other popular DNA Cloning systems on the market: pGEM®-T and TOPO TA cloning®

This study compares the efficiency of the pSpark® I DNA Cloning system with other popular cloning systems, present already in the market since almost two decades: pGEM®-T of Promega Corporation and TOPO® TA cloning of Invitrogen (Life Technologies).

1. Introduction

Cloning vectors got their **importance** since the advent of recombinant DNA technology in biological research. Bolivar developed **pBR322** in 1977, the first cloning vector that paved the way for a vast array of other vectors with different attributes and functions.

Several years later, in **1993**, Promega developed **pGEM®-T**, an evolution of pBR322. This product introduced a new technique based on TA cloning. It avoided the use of restriction enzymes and made DNA cloning easier and quicker in comparison with the previous methods. PCR products amplified using Taq DNA polymerase have a single base (mostly adenine) to the 3' end overhang. Such PCR amplified inserts are cloned into linearized vectors that have complementary 3' thymine overhangs.

Four years later, in **1997**, Invitrogen developed TOPO® TA cloning and TOPO® Zero Blunt, still one of the most used technologies today on the market. This technique utilises the inherent biological activity of DNA topoisomerase I to cleave and rejoin supercoiled DNA ends to facilitate replication. During replication, the enzyme digests DNA specifically at this sequence, unwinds the DNA and re-ligates it again at the 3' phosphate group of the thymidine base. This topoisomerase based ligation is the basis of topoisomerase based cloning.

In **2009**, Clontech developed its **InFusion®** cloning system, a blunt-end DNA cloning technology. It takes advantages over TA cloning as easy manipulation, low cost, and lack of addition of a base to the amplified sequence owing to the 3'-overhangs produced by a polymerase like Taq, which is especially useful in site-directed mutagenesis analysis.

Recently, **Canvax Biotech** has developed a technology which makes cloning faster, easier, cheaper and with more efficient results in comparison with other Blunt-end technologies. **pSpark®** systems are based on a blunt ended DNA amplified by PCR with proofreading or high-fidelity DNA polymerases. The vector is prepared by digestion of pSpark® vectors at the EcoRV site before treating both ends to prevent vector self-ligation. This treatment results in a vector with a cloning efficiency about 50 times more efficient than TA based vectors, that is, up to 4 times more colonies are obtained using 12,5 times less DNA.

2. Objectives of the present study

- Evaluating the efficiency of pSpark® with different quantities and insert sizes, ligation time and types of DNA polymerase.
- Comparing pSpark® with two of the most popular cloning systems, in the market already decades ago, pGEM®-T of Promega and TOPO® TA of Invitrogen.

3. Method

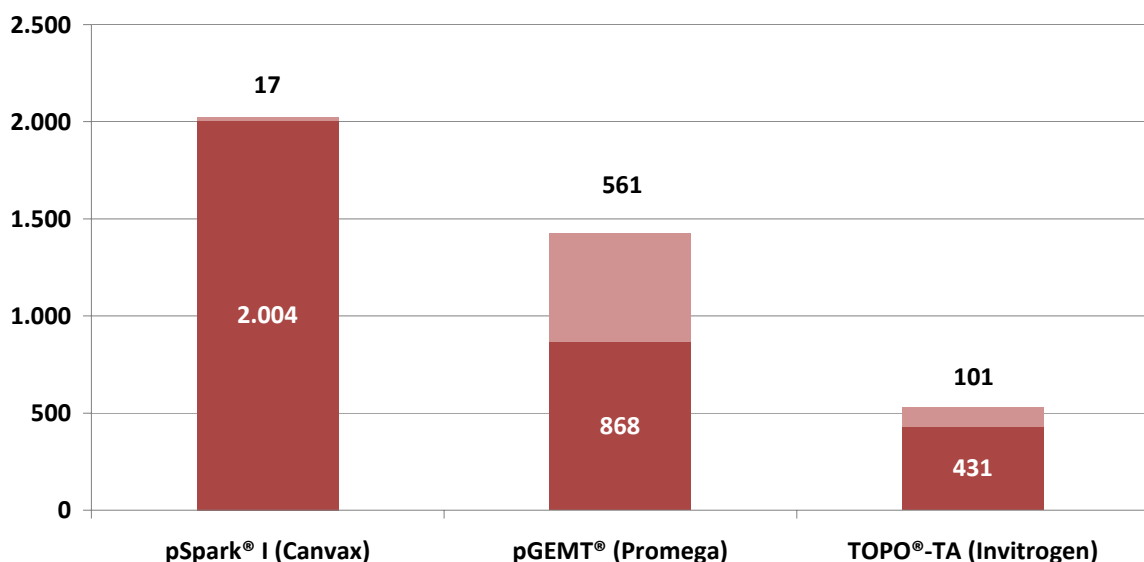
Every experiment of transformation was conducted with DH5α of Escherichia coli, LB culture medium, XGal and Ampicillin at 50 µg/mL.

For pSpark® based DNA cloning, inserts were amplified either with Taq DNA Polymerase (Canvax), with high fidelity polymerase Phusion™ (Thermo Fisher Scientific) or with Expand High Fidelity PCR System (Roche), a blend of Taq with a low amount of a high fidelity DNA Polymerase, following manufacturers recommendations. To check PCR yield and purify DNA fragments, an Agarose low EEO at 0,7% in TAE 1X was used. A DNA Ladder of a HindIII λ digestion was used as marker in gel electrophoresis.

For pGEM®-T and TOPO® TA, **experiments were conducted following the recommendations of Promega Corporation and Invitrogen**, respectively, described in their product manuals.

4. Results

Figure 1 shows the results of the first experiment, in which cloning efficiency and background of pSpark® were analysed. It is shown in comparison with pGEM®-T and TOPO® system.



Fragment	Cloning system	White colonies	Blue colonies	Background (%)
1 kb Phusion™	pSpark® I (Canvax)	2.004	17	0,80%
1 kb Taq	pGEM®-T (Promega)	868	561	64%
1 kb Taq	TOPO®-TA (Invitrogen)	431	101	23%

Figure 1. Cloning efficiency of pSpark® I over pGEM®-T and TOPO®. The cells used had a cloning efficiency of 2×10^7 cfu/ μ g.

Efficiency

Cloning efficiency was measured using different amounts and different insert sizes, ligation times and several types of DNA polymerases.

pSpark® I shows a relative cloning efficiency (ratio between the number of white colonies and ng of insert used) of 300 cfu/ng insert when 2×10^7 cfu/ μ g competent cells are used. The results of this analysis shows that pGEM®-T and TOPO®-TA, under their optimal conditions, had a cloning efficiency of just 17 and 8.6 cfu/ng insert, respectively. The above data shows that pSpark® I is **18 times more efficient than pGEM®-T and 35 times than TOPO® TA system**. This fact is really important for cloning PCR products where PCR yield has not been optimised or where there is a limited amount of PCR product.

pSpark® I cloning efficiency was analyzed **using different vector to insert ratios**. The results are shown in the following figure:

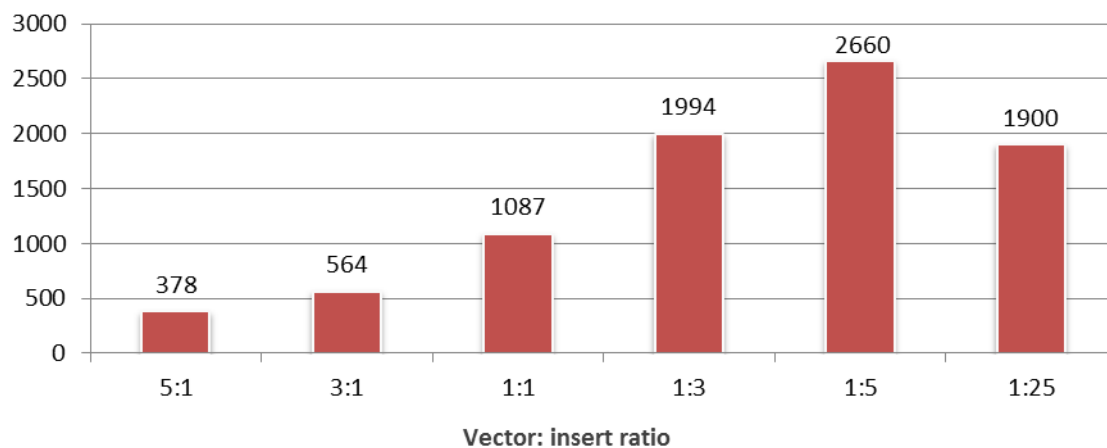


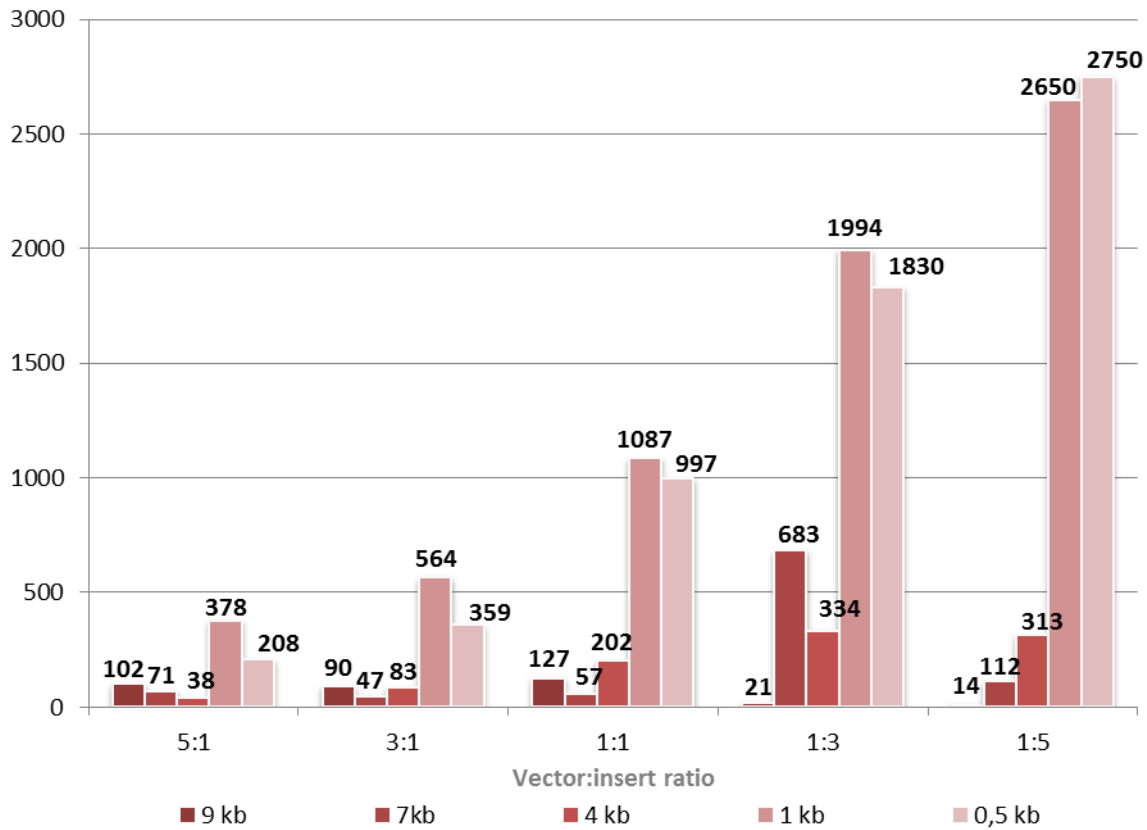
Figure 2. Number of positive white colonies obtained after ligation with different proportions of pSpark® vector-to-insert. The amount of vector was the same in all cases varying the amount of insert to obtain the ratio of vector to insert identified. The background was less than 1%. Competent cells had an efficiency of 2×10^7 cfu/ μ g.

As observed in Figure 2, the vector to insert ratio with the highest number of colonies obtained is 1:5, that is, 30 ng of a 1 Kb insert and 20 ng of pSpark® I to obtain 2.660 positive clones and less than 1% background of blue colonies.

The use of a vector to insert ratio of 5:1 allows analysis of the cloning efficiency when insert is present in very limited amounts in ligation mixture. In this case only 6 ng of insert used in the

ligation generated 378 positive clones. These results show that cloning into pSpark® I is **even possible with a very limited amount of insert**, for example, when insert availability is very limited in difficult amplifications or when non-optimised PCR is used as a source of insert.

Efficiency was also **tested using inserts of different sizes and vector to insert ratios**. The number of colonies obtained are shown in the next figure:



	5:1	3:1	1:1	1:3	1:5
9 kb	102	90	127	21	14
7kb	71	47	57	683	112
4 kb	38	83	202	334	313
1 kb	378	564	1.087	1.994	2.650
0,5 kb	208	359	997	1.830	2.750

Figure 3. Cloning efficiency of pSpark® fragments with different sizes. The pSpark® vector has a size of 3 kb. Cells had an efficiency of 2×10^7 cfu/ μ g.

As shown in Figure 3, for cloning inserts up to 7 Kb, the best vector-to-insert ratio to obtain the highest number of colonies is 1:5. However, for a 9 kb insert cloning, there is a higher number of colonies when a higher amount of vector than that of insert is used, that is, in the ratios 5:1, 3:1 and 1:1.

Flexibility

The **variable of ligation time** was chosen as another measure of kit flexibility, because it has previously been shown to be useful for researchers to adjust ligation time. The results obtained with an optimal vector-to-insert ratio of 1:5, are shown in Figure 5:

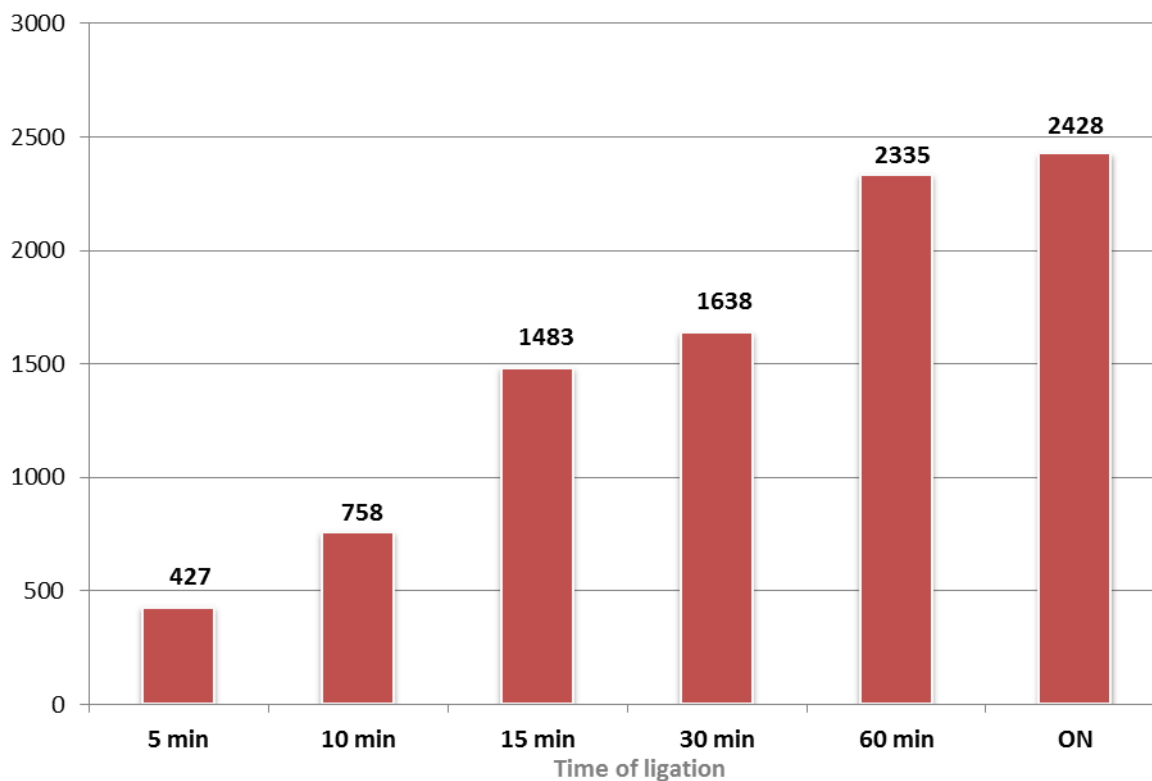


Figure 4. pSpark® I ligation determined efficiency in response to different ligation times. Negative colonies numbers are not shown, although in all cases it was less than 1%. O.N. = overnight ligation. Competent cells used had an efficiency of 2×10^7 cfu/ μ g.

As shown in Figure 4, the optimal ligation time to obtain the highest number of colonies ranges from 60 minutes to overnight, with outcomes of 2.335 and 2.428 colonies obtained. It is important to note that with only 5-10 minutes of ligation time more than 400-700 positive colonies were obtained, that normally is a sufficient number of colonies to get a recombinant clone in routine DNA cloning.

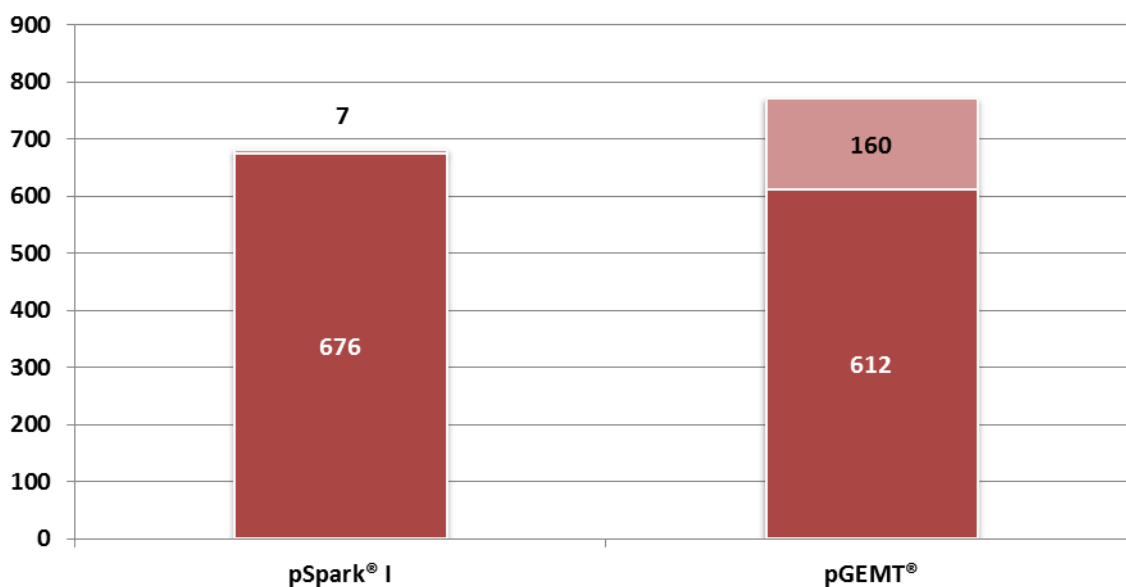
Therefore, in situations where there is not much time or not much insert available, it is possible to obtain hundreds of recombinant clones with pSpark® I, in cases in which using other systems with lower flexibility it is not normally possible. Furthermore, pSpark® I enables the use of phosphorylated or dephosphorylated primers, primers with any sequence at the end 5' and also allows the use of any bacterial strain for transformation. This information shows that pSpark® I **makes possible to obtain hundreds of positive recombinant clones following several DNA cloning protocols**, where amount of insert, insert to vector ratio, ligation time, *E. coli* strain and transformation protocol can be changed.

As well, experiments show that to work with pSpark® I **it is not necessary to follow a strict protocol, that could even tolerate some changes depending on the needs of each cloning or laboratory resources.**

Robustness

Another important feature shown by tests is the ability of pSpark® I to **clone amplified PCR fragments with different types of DNA polymerases**. This advantage facilitates the daily work of researchers.

Experiments were conducted in order to compare the cloning efficiency of an insert of 1 kb DNA amplified with a mixture of Expand™ High Fidelity polymerase PCR System from Roche in the system of pGEM®-T vs pSpark® I. The results are reflected in the following figure:



Cloning system	White Colonies	Blue Colonies	Background (%)
pSpark® I (Canvax)	676	7	1%
pGEM®-T (Promega)	612	160	26%

Figure 5. Efficiency comparison between amplified cloning fragments with a mixture of both Taq and a High Fidelity enzyme of pSpark® and pGEM®-T .

Despite the similarity of the results, it is important to highlight that PCR products obtained with a mix of both DNA polymerases like Taq and a high fidelity DNA polymerase as Pfu mixes of contain a mixture of molecules with blunt ends and molecules with adenine at the 3' ends in a proportion of 30% and 70%, respectively. It means that only 30% of amplified molecules may be cloned into pSpark® I. Considering the information shown above, its relative cloning efficiency was 338 cfu/ng for pSpark® DNA cloning while value for pGEM®-T was 18 cfu/ng of insert. Again, **it means that pSpark® efficiency is about 18 higher that of pGEM®-T.**

Background

As shown in Figures 1 and 5, pSpark® background is less than 1%, while in the same test with TOPO®-TA and pGEM®-T , the results are higher, 23% and 64% respectively, **showing that pSpark® background is at least 28 times lower in comparison with pGEM®-T .**

5. Conclusions

Experiments conducted show that pSpark®:

- Is **18 times** and **35 times more efficient** than pGEM®-T and the TOPO® TA cloning systems, respectively.
- Its background is up to **23 times lower than that of pGEM®-T**.
- Is **Easier and Faster**: It is not necessary to follow a strict protocol, but it could even be changed depending on the needs of each cloning or laboratory resources (specially time of ligation and amount of insert).
- Is **Flexible**: because it is able to clone amplified fragments of PCR with different types of DNA polymerases (that is, Hifi blends or High Fidelity polymerases).

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