canvax

Better tools for the best sciences

PickMutant® Site-directed Mutagenesis Kit

Manual for catalogue number MT001

(15 reactions)

Upon Receipt Store Kits at-20ºC

Product Manual

Version 3.0

Last updated: June 2014

www.canvaxbiotech.com

Table of contents

Materials provided (15 reactions)

Item	Quantity	Storage
Master Mix Proofreading DNA polymerase (*Includes: DNA Polymerases, dNTPs, $MgCl_2$*)	150µl	-20ºC
Glue enzyme (10 U/µl)	300 U	-20ºC
10x Glue enzyme Buffer	40 µl	-20ºC
Control Insert DNA	5 µl	-20ºC
pSpark®-I cloning vector (20ng/µl)	15µl	-20ºC

Expiration date: See on the label kit.

Additional material required

For cloning

- ✓ PCR Purification kit (Recommended: AN0063 from Canvax Biotech)
- ✓ Gel Extraction kit (Recommended: AN0070 from Canvax Biotech)
- ✓ High efficiency competent cells (Recommended: C0031 from Canvax Biotech)
- ✓ LB agar plates for ampicillin selection

For transformants analysis

- ✓ Taq DNA polymerase kit (Recommended Red-Taq DNA polymerase kit , Cat.Nº:P0027 from Canvax Biotech)
- ✓ Primer forward for colony PCR
- ✓ Primer reverse for colony PCR
- ✓ Restriction enzymes
- ✓ Sequencing primer

For plasmid purification

- ✓ Plasmid Purification kit (Mini format) (Recommended WideUSE Plasmid Purification Kit ,Cat.Nº:AN0068 from Canvax Biotech)

1. INTRODUCTION

1.1 Description

In vitro mutagenesis methods are extensively used in molecular biology to generate genetic variants from original DNA. Site-directed mutagenesis (SDM) includes several approaches that allow to create a mutation at a defined site for a great variety of applications. Over the last three decades, various SDM methods have been described and some commercial SDM kits based on these techniques are available. The PCR-based SDM methods are used more frequently than the non-PCR-based methods.

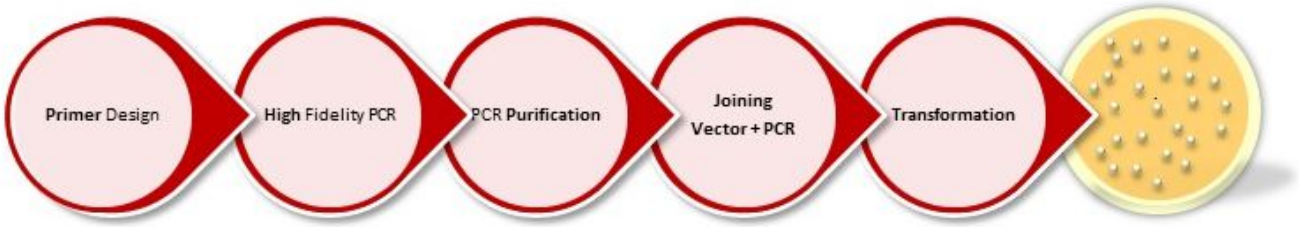
PickMutant® Site-directed Mutagenesis kit is a highly efficient PCR-based mutagenesis kit. Then it does not require extraordinary skills in molecular biology, allowing create single or multiple point mutations, deletions or insertions using a rapid and simple protocol. All these mutation could be obtained by PCR using a high fidelity DNA polymerase and well designed mutagenesis primers. The assembled mutagenic PCR fragments can be 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. The system is so robust that PCR fragments can be assembled and cloned simultaneously in an orientated manner into a selected vector in a unique step.

1.2 Advantages of the system

PickMutant® Site-directed Mutagenesis kit is a robust and efficient PCR-based mutagenesis kit with many advantages, such as:

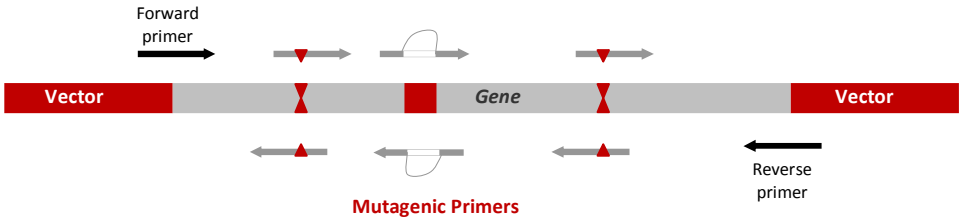
- Effective for point mutations (single or multiple), deletions or insertions.
- Easy and rapid one day protocol.
- Mutagenesis methylation independent.
- Use of standard primers.
- Restriction enzymes are just required to linearize your own vector.
- No require special bacterial strains.
- Any vector may be used to clone the mutagenic PCR fragment and besides the kit includes an efficient blunt cloning vector, pSpark® based in a novel technology to clone blunted fragments.

1.3 Schematic overview



2. PROCEDURES

PickMutant Site-directed Mutagenesis kit is a based-PCR mutagenesis method so the design of primers is essential to obtain the desired mutations. Your mutagenized PCR fragment could be cloned into pSpark® cloning vector without attend to special consideration but if you select your own vector to clone it, a specific primer pair (section 2.2) must be design to cloning your gene of interest in the correct orientation.



Considerations for forward and reverse primer are described in section **2.2.** while mutagenic primers are described in the following section.

2.1 Mutagenic Primers Design

2.1.1 Generating Point Mutations by PickMutant SDM

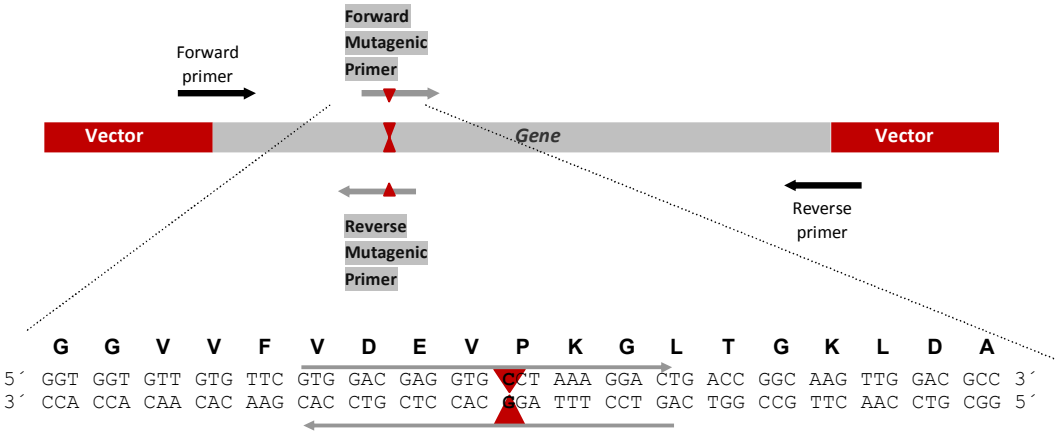
A point mutation is a type of mutation that causes a single base substitution with another nucleotide. Site-directed mutagenesis is directed to the obtention of nonsense (*a stop codon is created and a truncated protein is produced*) or missense mutations (*change of original aminoacid*). A beautiful biotech application of missense mutations has been the conversion of green fluorescent protein from *Aequorea victoria* in the yellow, cyan and blue variants of the protein.

Some important tips to consider in primer design are:

1. Mutagenic primers should contain at least 7 bases 5´of the mutation site. The Tm for primers must be calculated using only the template complementary bases.
2. The size of complementary sequence to target gene must be 18-30 bp for good target specificity.

3. Primers hairpins with a melting temperature higher than 46ºC should be avoided because of those structures decreasing PCR efficiency. You must include the point mutation in both primers. Take care to include the mutation and its complementary modification in each primer.

For example, to obtain a mutant gene in the indicated position modifying proline by threonine:



Forward Mutagenic Primer could be: ***5´ GTGGACGAGGTGACTAAAGGAC 3´*** *(Tm=66ºC)*

Reverse Mutagenic Primer could be: ***5´ GTCCTTTAGTCACCTCGTCCAC 3´*** *(Tm=66ºC)*

A single point mutation implies two PCR reactions. One of them use forward vector primer plus reverse mutagenic primer and a second reaction with forward mutagenic primer plus reverse vector primer. Forward or reverse vector primers could be on the gene of interest instead on the vector sequence (upstream or downstream mutation site).

Sometimes the term point mutation includes insertions or deletions of a single base pair. If you need to obtain mutants with insertions or deletions of a single nucleotide, proceeds like substitution point mutations but inserting an additional new base or deleting one, depending on the case. Keep in mind both mutations generate frameshifting mutation.

2.1.2 Generating Insertions by PickMutant SDM

Two strategies are used to obtain insertion mutants. These depend on the size of the fragment sequence to insert. For short insertion sequences (6-15 bp), the sequence to be inserted could be added to the 5´end of both mutagenic primers. For insertions of more than 15 nucleotides, the insertion sequence can be split between the primers although at least 15-20 bp of complementary region of 5´ end must be included in both.

One insertion mutation implies two PCR reactions. One of them use a forward gene primer or forward vector primer plus reverse mutagenic primer and a second reaction with forward mutagenic primer plus reverse gene primer or reverse vector primer.

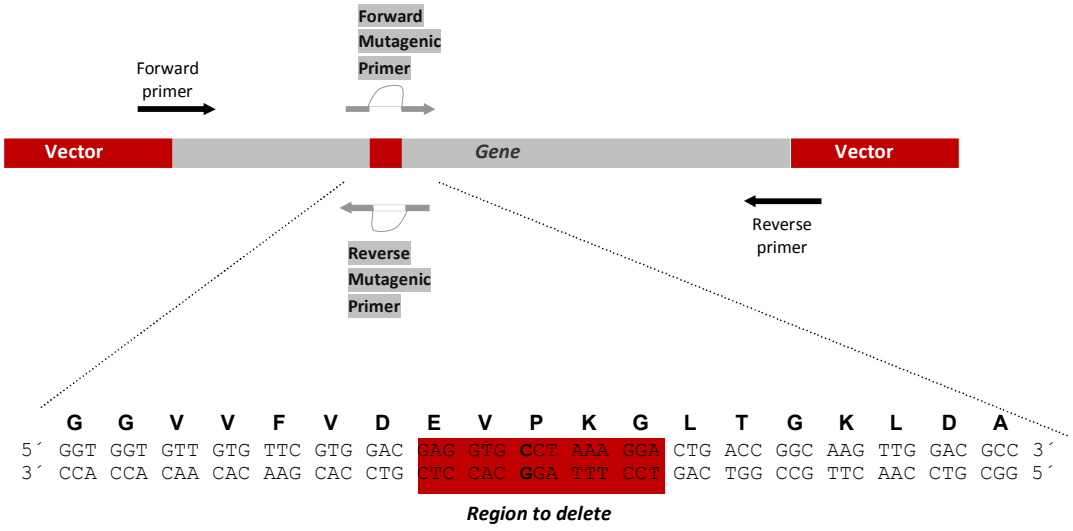
2.1.3 Generating Deletions by PickMutant SDM

Deletion is a kind of mutation in which a fragment of the gene sequence is removed. PickMutant Site-directed Mutagenesis kit can be used to obtained deletion mutants using mutagenic primers that flank the region to be deleted.

Some important tips to consider in primer design are:

1. The two primers should be designed in opposite directions with their 5´ends adjacent to the area to be deleted. Both primers should contain 10 bases 5´of the deletion area plus 18-30 bases 3´of the deletion area.
2. The primers could contain mismatches and/or insertions, taking care they are present in both mutagenic primers. The Tm for primers must be calculated using only the template complementary bases.
3. The size of complementary sequence to target gene must be 18-30 bp for good target specificity.
4. Primers hairpins with a melting temperature higher than 46ºC should be avoided because of those structures decreasing PCR efficiency. Take care to include the complementary modification in both primers.

For example, to delete a 15 bp fragment in the sequence shown below, the mutagenic primers could be:



Forward Mutagenic Primer: ***5´ GTTCGTGGAC-CTGACCGGCAAGTTGGACGCC 3´***

Reverse Mutagenic Primer: ***5´ TGCCGGTCAG-GTCCACAAGCACAACACCACC 3´***

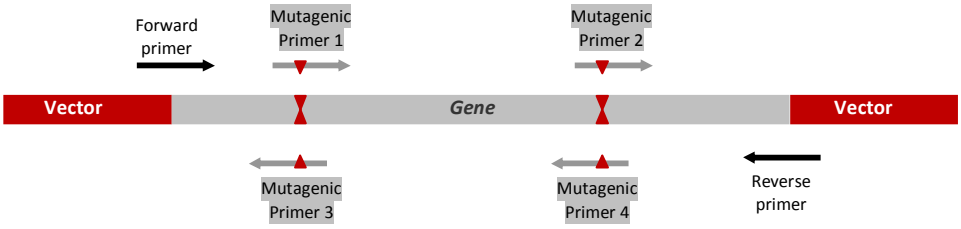
Although the example includes a 15 bp deletion area, deletion can be all higher than you need.

One deletion event implies two PCR reactions. One of them use a forward gene primer or forward vector primer plus reverse mutagenic primer and a second reaction with forward mutagenic primer plus reverse gene primer or reverse vector primer.

2.1.4 Generating Multiple Mutations by PickMutant SDM

The approach to generate multiple mutations is similar to one point mutations although the number of PCR reactions to use is higher. In a multiple mutation process, the number of PCR reactions is the number of mutations you need to create plus one. If you are generating a mutant with 2 mutations you must be set up 3 PCR reactions. Like in all standard PCR, each reaction should include a forward and reverse primer.

For example to create a double mutant you will need 4 mutagenic primers: the forwards mutagenic primers 1 and 2; the reverse mutagenic primers 3 and 4 and the no mutagenic primer pair. Design the pair 1/3 and 2/4 following the instructions indicated in Generating Point Mutations by PickMutant SDM (section 2.1.1).



PCR Reaction	Primer Forward	Primer Reverse
1	Non mutagenic Forward Primer	Mutagenic Primer 3
2	Mutagenic Primer 1	Mutagenic Primer 4
3	Mutagenic Primer 2	Non-mutagenic Reverse Primer
Non mutagenic reverse primers could be gene-specific or vector specific in order to guarantee directional cloning		

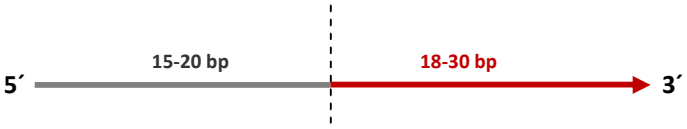
2.2 Vector Consideration

Non mutagenic primers indicated in all figures just as forward and reverse primer must be selected in order to your cloning objectives. You can clone assembled mutagenic fragment in a blunt cloning vector like pSpark®, included in this kit because of its exceptional high efficiency of blunt cloning or in your final vector in an oriented manner.

PickMutant Site-directed Mutagenesis kit allows you to clone in an oriented manner your assembled mutagenized fragment. So non mutagenic primers have two regions:

Forward Primer: 5´ Vector complementary region-Gene complementary region 3´ (positive strand)

Reverse Primer: 5´ Vector complementary region-Gene complementary region 3´ (negative strand)

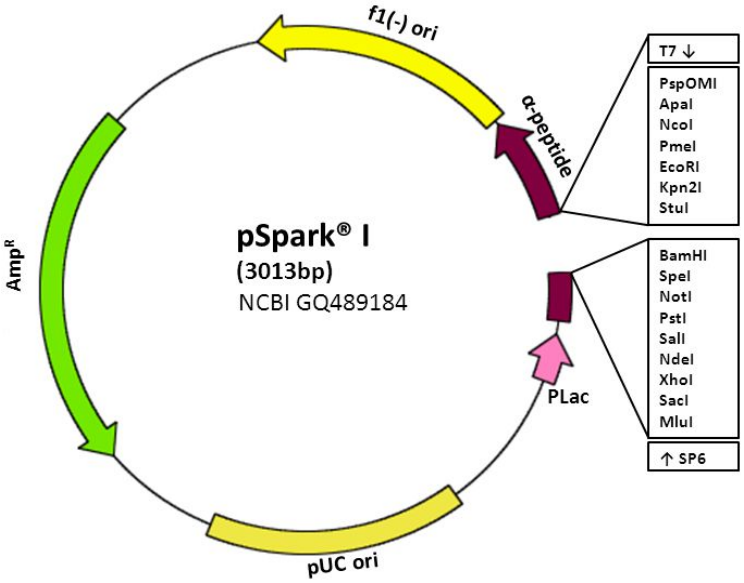


To clone in pSpark®-I, your non mutagenic primers must have the following sequences:

Forward Primer: 5´ CGG ATC AGG CCT GAT -Gene complementary region 3´ (positive strand)

Reverse Primer: 5´ CTA GTG GAT CCT GAT-Gene complementary region 3´ (negative strand)

If you required inverted orientation, design the primers interchanging vector complementary region. Although pSpark® vector includes some unique restriction enzymes at the multiple cloning site, we highly recommended include convenient restriction sites in these primers to subsequent cloning steps.



pSpark® is supplied as a linear vector. If you need any other vector, it should be linearized. To design the primers, vector complementary region of forward primer is on one end of the linear vector while reverse primer is on the other, in dependence direction you required. Gene complementary region must be designed attending general rules of a specific primer.

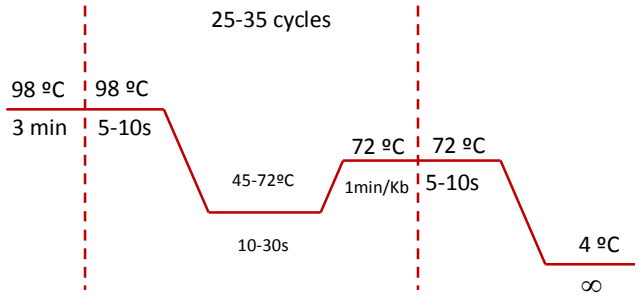
2.3. High Fidelity Amplification to Introduce Mutations

1. **Assemble the following reagents in a thin-walled PCR tube.**

Component	Volume	Final concentration
Master Mix Proofreading DNA polymerase	10 µL	1X
Primer A	X µL	0.75 µM[(1)]
Primer B	X µL	0.75 µM[(1)]
Template DNA	X µL	20-50 ng DNA[(2)]
DMSO (optional)	(X µL)	3%[(3)]
H_2O	Add to 20 µL	

[(1)]The recommendation for final primer concentration is 0.5 µM but it can be varied in a range of 0,2-1,0 µM if needed.
[(2)]For gDNA used 100-300 ng DNA.
[(3)]Addition of DMSO is recommended for GC-rich amplicons. **If DMSO is added in the PCR reaction, Tm must be decreased about 3º C.**

2. **Mix reagents completely, and then transfer to a thermocycler.**
3. **Perform the following cycling conditions:**



2.4. Cloning

2.4.1. Cloning into pSpark®-I

1. Spin pSpark® vector to collect content at the bottom of the tubes.

2. **<u>On ice</u>**, set up reaction as described below. If you thawed all kits components out of ice, you must pre-chill all them before use during 10 minutes.

Match Reaction	Cloning Reaction	Control Reaction	Background Reaction
pSpark® vector (20 ng/µL)	1 µL	1µL	1 µL
10x Glue-Enzyme Buffer	1.5 µL	1.5 µL	1.5 µL
PCR Product	X µL	-	-
Control Insert DNA	-	2 µL	-
Water (*Molecular Biology grade*)	up 13 µL	up 13 µL	up 13µL

3. Mix the reactions by pipetting.
4. Incubate 10 minutes on ice.
5. Add 2 µL Glue-Enzyme (10 U/µL) to each tube, mix gently and incubate 45 minutes on ice.
6. Proceed to transformation (see **Section 2.5**)

We strongly recommend:


Note

To prepare a match-reaction with the supplied control insert to check that match reaction and transformation process are working properly. This positive control MUST be prepared and transformed at the same time than your samples.

2.4.2 Cloning into your own vector

1. Linearize your vector with appropriate restriction enzymes. Dephosphorylate it if the ends are compatible. Purified the linearized vector.
2. **On ice**, set up reaction as described below. If you thawed all kits components out of ice, you must pre-chill all them before use during 10 minutes.

Match Reaction	Cloning Reaction	Background Reaction
Linearized vector (20 ng/µL)	1 µL	1 µL
10x Glue-Enzyme Buffer	1.5 µL	1.5 µL
PCR Product	X µL	-
Water (*Molecular Biology grade*)	up 13 µL	up 13µL

3. Mix the reactions by pipetting.
4. Incubate 10 minutes on ice.
5. Add 2 µL Glue-Enzyme (10 U/µL) to each tube, mix gently and incubate 45 minutes on ice.
6. Proceed to transformation (see **Section 2.5**)

2.5 Transformation using chemical competent cells

Use competent cells with a competence of at least $1x10^7$ colonies/ µg DNA following the protocol provided with the competent cells. Standard and fast protocol for transformation can be found below. In our hands the fast transformation protocol gives no less than 4-5 fold of the number of colonies obtained using the classical transformation protocol. Thus, for the most demanding cloning task the classical protocol is recommended but for routine cloning the fast transformation protocol is suggested as it saves 1.5 hours.

2.5.1 Standard protocol for transformation

1. Prepare one LB ampicillin plate for each match reaction, plus one plate for determining transformation efficiency and one plate for control transformation (vector without insert). Equilibrate the plates to room temperature prior to plating (**Step 8**).
2. Centrifuge the tubes containing the reactions to collect content at the bottom of the tube. Add 15 µL of each reaction to a sterile 1.5 mL microcentrifuge tube on ice. Set up another tube on ice with 50 pg uncut plasmid (*no supplied*) for determination of the transformation efficiency of the competent cells.
3. Remove a tube of frozen Competent Cells (*no supplied*) from storage at -80ºC and place in an ice bath until just thawed (about 10 to 15 minutes). Mix the cells by **gently** flicking the tube with your fingertips.

IMPORTANT: *Do not thaw competent cells with your hands. Keeping competent cells out of an ice bath even for extremely short times strongly affects the transformation efficiency of cells. Also avoid excessive pipetting, as the competent cells are extremely fragile and thus mixing of DNA with competent cells should be made by gently flicking and not by pipetting.*

4. **Carefully** transfer 50 µL of cells into each tube prepared in **Step 2**.
5. **Gently** flick the tubes to mix and place them on ice for 30 minutes.
6. Heat-shock the cells for exactly 45 seconds in a water bath at exactly 42°C (***Do not shake nor heat shock more than 45 seconds***).
7. Immediately return the tubes to ice for 2 minutes and plate all transformation mix onto pre-warmed LB ampicillin plates
8. Incubate the plates overnight (12–16 hours) at 37°C.

2.5.2 Fast transformation protocol

An alternative transformation protocol of only 5 minutes is recommended. The main difference between this protocol and the standard protocol is that this fast protocol avoids the heat shock step and instead this essential step occurs directly on the plate. If you feel unfamiliar with this protocol, please use the standard transformation protocol.

EXTREMELY IMPORTANT: *Before starting this protocol you must pre-warm LB Agar-antibiotic-X-Gal-IPTG plates at 37ºC for at least 1 hour. Heat shock occurs on the pre-warmed plates when using this protocol, thus it is essential that plates*

are pre-warmed before transformation and withdrawn from incubator to laminar flow cabinet only just before transformation.

1. Prepare one LB ampicillin plate for each match reaction, plus one plate for determining transformation efficiency and one plate for control transformation (vector without insert). Pre-warm plates at 37ºC in the incubator for at least one hour before transformation.
2. Centrifuge the tubes containing the match reactions and the control reaction to collect content at the bottom of the tube. Add 10 µL of each reaction to a sterile 1.5 mL microcentrifuge tube on ice. Set up another tube on ice with 25 pg uncut plasmid (*no supplied*) for determination of the transformation efficiency of the competent cells.
3. Remove a tube of frozen Competent Cells (*no supplied*) from storage at -80ºC and place in an ice bath until just thawed (about 10 to 15 minutes). Mix the cells by **gently** flicking the tube with your fingertips.

IMPORTANT: *Do not thaw competent cells with your hands. Keeping competent cells out of an ice bath even for extremely short times strongly affects the transformation efficiency of cells. Also avoid excessive pipetting, as the competent cells are extremely fragile and thus mixing of DNA with competent cells should be made by gently flicking and not by pipetting.*

4. **Carefully** transfer 50 µL of cells into each tube prepared in **Step 2**. For determination of transformation efficiency add 50 µL of competent cells to the tube prepared in **Step 2** containing 25 pg of uncut plasmid DNA.
5. **Gently** flick the tubes to mix and place them on ice for 5 minutes.
6. Plate all transformation mix onto pre-warmed LB ampicillin plates
7. Incubate the plates overnight (12–16 hours) at 37°C.

2.5.3 Analysis of transformants

Colony PCR is a suitable approach for screening colonies before isolation of plasmid DNA. The principle behind colony PCR is the lysis of plasmid bearing bacteria (after saving a portion of the bacterial colony since the sample is destroyed by colony PCR) and PCR using as template the crude unpurified plasmid DNA released from bacteria. The most common method for bacterial lysis is boiling at 100ºC for 10 minutes. Some protocols use the initial DNA denaturation step of PCR as the bacterial lysis step and thus in this protocol the bacterial colony is added directly to a master mix of polymerase, buffer, dNTPs and primers. The protocol below is one we have tested at Canvax but other protocols are also suitable.

Protocol for colony PCR

Previously, check the material required not supplied with kit. pUC/ M13 forward and reverse sequencing primers binding sites. Insert size amplified with these primers on empty pSpark® is about 200 bp.

1. For each bacterial colony to be screened prepare a 1.5 mL microcentrifuge tube with 30 µL of water.
2. Pick one colony with a sterile toothpick or a sterile pipet tip and resuspend the colony in the 1.5 mL microcentrifuge tube with water.
3. Streak the toothpick from **Step 2** in either a plate with antibiotic or liquid media with antibiotic (e.g. LB with antibiotic) for growing positive colonies. Discard the toothpick and repeat **Steps 2** and **3** for each colony to be screened.
4. Boil the tubes of **Step 2** in a water bath at 100ºC for 10 minutes to lyse the cells and inactivate nucleases. Please make sure that the tubes are tightly closed because by boiling the lids can pop open.
5. While tubes are boiling, prepare a PCR master mix follow the guidelines of your Taq polymerase supplier for a 50 µL final volume reaction. Prepare at least one reaction master mix more than the total number of colonies to be screened. Distribute 30 µL of the master mix into sterile PCR tubes.
6. Spin boiled tubes from **Step 4** at 14500 rpm during 5 minutes in a microcentrifuge.
7. Add 20 µL of cleared lysate from **Step 6** to each PCR tube with 30 µL of master mix prepared in **Step 5**.
8. Running PCR program at thermocycler machine.
9. Load the tube contents on agarose gel and visualize it.
10. Choose positive clones and grow it in an appropriate amount of LB-Amp Broth, and purify the construct

Note

You could analyse the recombinant plasmids by restriction analysis and by sequencing.

2.5.4 Sequencing

Confirm identity of your insert by sequence analysis of the construct. The sequence of pUC/ M13 forward and reverse primers are on the pSpark®-I cloning vector.

3. TROUBLESHOOTING

For questions not addressed here, please contact us at www.canvaxbiotech.com or alternatively contact your local Distributor.

PROBLEM	CAUSE	SOLUTION
No colonies (even in Control Insert Reaction)	Any component is missing in the match reaction	Repeat match reaction and transformation and don´t forget to include controls
	Competent cells are damaged or with very low efficiency	Check the transformation efficiency of *E. coli* competent cells. A transformation efficiency lower than $1x10^7$ cfu/µg is not recommended
Colonies only in the Control Insert Reaction	Any component is missing in the sample reaction	Repeat match reaction and transformation and don´t forget to include controls
	PCR insert is degraded or damaged	Check quality of insert by gel electrophoresis
	A very low amount or no PCR insert have been used for the reaction. Alternatively, a very high amount of insert was used	Check by gel electrophoresis the yield of PCR or agarose purification. Purified products can also be quantified by Abs at 260/280 nm (eg. by Nanodrop™). If needed increase/decrease amount of insert in a new match reaction.
	Salts and/or ethanol present in the purified PCR insert	Repeat PCR and purification from agarose for a new reaction and transformation.
	The reaction is not optimal	Optimise the reaction by trying other insert to vector ratios
	The PCR insert product has multiple bands and is used unpurified directly for cloning	- Gel purified your PCR insert or screen more colonies by colony PCR. - Also is possible that the template complexity is high (eg. genomic DNA) then is preferably synthetized two couple of primers: One, to obtain the gene of interest and the other pair, to clone in the expression vector. This fact avoids cloning artefacts due to the presence of additional long sequences for orientated cloning.
	PCR primers have a bad design	Check your primers designed according our recommendations. If the primers don´t anneal with the vector ends, no colonies are obtained.
	There is a negative correlation between the size of the fragments and the number of colonies after transformation (fewer colonies with increasing size insert)	Adjust the reaction in order to have a molar ratio vector: insert 1:5

Warranty: Canvax Biotech SL warrants that the product will meet the specifications stated on this Product Manual and agrees to replace the product free of charge if the product does not conform to the specifications but notice for replacement must be given within 30 days. In consideration of the above promises by Canvax Biotech SL, the purchaser agrees to and accepts the following conditions: (1) That this warranty is in lieu of all other warranties, expressed or implied; (2) That ALL WARRANTIES OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE ARE HEREBY EXCLUDED OR WAIVED; (3) That the purchaser's sole remedy shall be to obtain replacement of the product free of charge from Canvax Biotech SL; and (4) That this remedy is in lieu of all other remedies or claims for damages, consequential or otherwise, which the buyer may have against Canvax Biotech SL.

Guidelines for Safe Use of the Products: Canvax Biotech SL recommends that the purchaser or user of this product follow the Local Guidelines for Research involving Recombinant DNA Molecules. Canvax Biotech SL disclaims any and all responsibility for any injury or damage which may be caused by the failure of the purchaser or user to follow said local guidelines. See Material Safety Data Sheet (MSDS) on the web site (www.canvaxbiotech.com)

Notice to Purchaser: Information presented herein is accurate and reliable to the best of our knowledge and belief, but is not guaranteed to be so. Nothing herein is to be construed as recommending any practice or any product in violation of any patent or in violation of any law or regulation. It is the user's responsibility to determine for himself or herself the suitability of any material and/or procedure for a specific purpose and to adopt such safety precautions as may be necessary. Prices are subject to change without notice. Product claims are subject to change. Please access the Canvax Biotech SL web site (www.canvaxbiotech.com) for the most up-to-date version of this Product Manual.

Important licensing information: Canvax Biotech SL has the exclusive license of patents applications related to pSpark® DNA cloning systems. The purchase of products based on this technology conveys to the purchaser the non-transferable right to use the purchased product in research conducted by the purchaser (whether the purchaser is an academic or for-profit entity). The purchaser cannot transfer or sell (i) this product or its components or (ii) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The purchaser may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing to use such transferred materials or information only for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. In particular, the use of pSpark® DNA cloning systems for gene cloning to be used in antigens discovery is expressly prohibited. By use of this product the purchaser accepts the terms and conditions of this limited use statement. If the purchaser is not willing to accept the limitations of this limited use statement, Canvax Biotech SL is willing to accept return of the product with a full refund. For information about purchasing a license to use this product or the technology embedded in it for any use other than for research use please contact Canvax Biotech SL at (www.canvaxbiotech.com) or by e-mail: info@canvaxbiotech.com

Trademark information: Registered names and trademarks used in this document, even when not specifically marked as such, are not to be considered unprotected by law.
pSpark® and pOnebyOne® are registered trademark of Canvax Biotech SL; NanoDrop™ is a trademark of Thermo Fisher Scientific Inc.

This product is sold for **Research or Laboratory Use Only** and is not to be used for diagnostic, on humans or for any drug purposes.