



## Large scale purification of linear plasmid DNA for efficient high throughput cloning

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1 Technical Report

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3 **Large scale purification of linear plasmid DNA for**  
4 **efficient high throughput cloning**

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15 Keywords: linear vector, high throughput cloning, membrane protein, Mistic,  
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2 In this report we describe a rapid, simple, and efficient method for large  
3 scale purification of linear plasmid DNA to answer demand from high throughput  
4 gene cloning. The process is based on the separation of the linear vector from  
5 small DNA fragments by anion exchange chromatography. Gene cloning  
6 experiments by restriction/ligation or the In-Fusion™ technique confirmed the  
7 high quality of the linearized vector as 100% of the genes were successfully  
8 cloned.

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6 **1 Introduction**  
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8 Investigation of large numbers of proteins requires high throughput (HTP)  
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10 gene cloning approaches to prepare constructs that can be used for comprehensive  
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12 structural and functional analysis of proteins. Most methods do not require a  
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14 ligation step and these include Gateway cloning [1], Ligation Independent  
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16 Cloning (LIC) [2, 3] or In-Fusion™ [4], even though classical ligation-dependent  
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18 cloning has also been adapted to HTP [5].  
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21 Restriction/ligation and In-Fusion™ techniques are popular as they do not  
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23 require additional base pairs that convert into additional amino acids at the protein  
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25 extremities. The former method may be limited by the availability of unique  
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27 restriction sites. The latter one is more flexible as it allows in vitro assembly  
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29 through homologous recombination of fragments, providing that they include  
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31 extremities matching the sequences of the cloning site [4]. In that way, PCR  
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33 products can be cloned into any vector at any site of linearization. The success  
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35 rate of these techniques is strongly dependent on the quality of the linear vector.  
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37 For HTP approaches, the production of large batches of high quality linear vector  
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39 is of paramount importance. Small-scale purification is often based on gel  
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41 electrophoresis and spin column [6, 7], two steps that are incompatible with the  
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43 production of large amount of DNA.  
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47 Plasmid DNA purification [8] has been the subject of many studies,  
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49 allowing large scale and high grade plasmid preparation [9-13], including the use  
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51 of anion exchange chromatography [14-17]. The separation of multiple small  
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53 restriction fragments by anion exchange chromatography on FPLC columns was  
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1 previously described [18-20], showing a correlation between the size of linear  
2 DNA fragments and the salt concentration needed for elution. For a given size,  
3 substantial retardation of the d(A+T) rich fragments has been reported [19]. Anion  
4 exchange chromatography has also been used for plasmid adsorption, purification  
5 and quantification [9, 15, 21]. We report here the use of anion exchange  
6 chromatography for the large-scale purification of linear cloning vector and  
7 separation from small restriction fragments. This application was first suggested  
8 by Westman et al. [18], but to our knowledge, has never been described in the  
9 literature.

10 Our protocol has been developed using two expression vectors specifically  
11 constructed for an automated HTP approach to test the influence of purification  
12 tags on the protein behavior: That is the presence or absence of His-tag for  
13 soluble proteins, and of the His-Mistic-tag for membrane proteins. Membrane  
14 proteins constitute up to 30% of all proteins [22, 23] and are involved in  
15 numerous cellular processes. There are two bottlenecks in membrane protein  
16 structural analysis: high-yield protein production and crystallization. Recently, a  
17 novel membrane-associated protein discovered in *Bacillus subtilis*, Mistic, has  
18 been shown to enhance the expression of recombinant membrane proteins in *E.*  
19 *coli*, when fused to their N-terminus [24-26].

20 This article describes the purification of linearized expression vectors in  
21 sufficient quantity and quality to efficiently perform hundreds of gene cloning in  
22 parallel. This method offers a rapid and simple way to standard molecular  
23 biology laboratories to prepare linear plasmid DNA on a large scale, and is a good

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- 1 alternative to multiple small scale preparations or expensive preparation from
- 2 commercial suppliers.
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For Peer Review

## 2 Materials and Methods

### 2.1 pLIM13 and pLIM14 plasmids for high throughput cloning

The pLIM13 and pLIM14 vectors developed by the RoBioMol platform are derived from the commercial pET41c (Novagen) (Complete plasmid sequences are available upon request). For pLIM13, the toxic *ccdB* expression cassette [27] was PCR amplified from pDEST17 (Invitrogen) and inserted into pET41c as an *NdeI-BamHI* fragment (Figure 1). For pLIM14, a synthetic gene *mistic-ccdB* (Geneart) was first inserted as an *NdeI-BamHI* fragment into pET41c. The exchange of the *XbaI-NdeI* fragment of pET41c vector for one encoding a His6-tag resulted in pLIM14 (Figure 1).

These pLIM plasmids are designed so that the linearization with two restriction enzymes releases the toxic *ccdB* expression cassette (Figure 1). pLIM13 was designed to express proteins without tag, whereas pLIM14 was designed to express His6- or His6-Mistic-tagged proteins, depending on the restriction sites used to clone the corresponding genes (Figure 1).

### 2.2 Large scale plasmid purification

The pLIM13 and pLIM14 plasmids were prepared as previously described [10, 16] with some modifications. A 5 ml 2xTY + kanamycin (50 mg/L) overnight culture of *E. coli* DB3.1 strain (Invitrogen) containing pLIM13 or pLIM14 was used to inoculate 1 L of 2xTY + kanamycin (50 mg/L) into a 3 L flask. The culture was grown to saturation at 25°C for 36 hours, with 200 rpm shaking.



To improve yield, the culture was separated in 4 fractions (250 ml each) treated in parallel. Cells were harvested by centrifugation (6200 g, 15 min, 4°C) and each pellet was resuspended in 40 ml of GTE buffer (50 mM glucose, 25 mM Tris/HCl pH 8, 10 mM EDTA) and 4 ml of lysozyme (25 mg/ml). After 10 min at room temperature, 20 ml of a freshly prepared solution of 0.3M NaOH and 2% SDS were added. The mixture was gently mixed by inverting the tubes 5 times, and DNA denaturation was allowed to proceed 10 min on ice. Thirty ml of 3 M potassium acetate pH 5.5 were then added and mixed thoroughly by inverting the tubes 5 times, to allow the renaturation of plasmid DNA and the precipitation of chromosomal DNA, SDS-protein complexes and other cellular debris. This mixture was incubated 10 min on ice before centrifugation (19,000 g, 10 min). Supernatants containing plasmid DNA were pooled and passed through a funnel filter before the addition of RNase A to a final concentration of 50 mg/L and incubation at 37°C for 20 min. Plasmid DNA was precipitated with the addition of 0.6 volume of isopropanol and recovered after 20 min at room temperature by centrifugation (15,000 g, 10 min). DNA pellet was washed once with 2 ml of 70% ethanol, dried in a vertical laminar air-flow hood, and dissolved in 5 ml of 5 mM Tris/HCl pH 8.5.

**2.3 Large scale plasmid digestion**

One hundred micrograms of plasmid DNA were digested in a 5 ml final volume in the presence of 500 µL of buffer 4 (NEB), 50 µL of *Bam*HI HF (1000 U, NEB) and 100 µL of *Nde*I (2000 U, NEB) or 100µL of *Nco*I (1000 U, NEB), at

37°C for 5 hours. Prior the addition of NaCl to a final concentration of 600 mM, five µL of digestion product were analyzed by agarose gel electrophoresis.

#### 2.4 Linear vector isolation by Mono Q™ anion exchange chromatography

Chromatography was performed with an Äktabasic UPC apparatus (GE Helthcare) and monitored at 254 nm. The DNA sample was loaded at 1ml/min onto a Mono Q™ 5/50 GL, (GE Healthcare) column equilibrated with buffer A (10 mM Tris/HCl pH 8, 1 mM EDTA, 700 mM NaCl). After a wash at 0.5 ml/min with 10 ml of buffer A, a 64 ml linear gradient of 0 to 10% buffer B (10 mM Tris/HCl pH 8, 1 mM EDTA, 2 M NaCl) was applied at 0.15 ml/min. Peak fractions of 0.5 ml were precipitated individually with the addition of 0.6 volume of isopropanol. After 20 min room temperature, DNA pellets were recovered by centrifugation (21,000 g, 10 min, 4°C), washed with 70% ethanol and redissolved in 50 µL of 5 mM Tris/HCl pH 8.5. Two µL of these fractions were analyzed on 1% agarose gel. Fractions containing pure linear vector were pooled and assayed in gene cloning experiments. Homogeneity and the sequence extremities of the purified linear vector were checked by sequencing using the T7prom and T7term primers.

#### 2.5 Target genes amplification

Target genes were amplified by PCR using specific primers, designed with the Vector NTI software (Invitrogen), containing 5' extensions related to the vectors used for cloning. For pLIM13\_NdeI\_BamHI, the forward and the reverse

1 primer extensions were respectively 5'-AAGGAGATATACCATATG-3' and 5'-  
2 TACAGAATTTCGGATCCTTA-3', for pLIM14\_NdeI\_BamHI, 5'-  
3 ACCATCACCATCATATG-3' and 5'-TACAGAATTTCGGATCCTTA-3' and for  
4 pLIM14\_NcoI\_BamHI, 5'-  
5 CGGGCGGTAGCCATGGGGAAAATCTGTATTTTCAGGGC-3' (containing  
6 protease TEV site) and 5'-TACAGAATTTCGGATCCTTA-3'. These extensions  
7 allow both restriction/ligation (using the underlined restriction sites) and InFusion  
8 methods (relying on the extended sequence complementary to the vector).  
9 Primers were ordered at 20  $\mu$ M in a 96-wells microplate (MWG). Preparation of  
10 the 0.5  $\mu$ M primers mix and of the PCR reaction was fully automated on a  
11 Microlab Star robot (Hamilton). Reactions were performed with 100 ng of  
12 chromosomal DNA template, 10  $\mu$ L of primer mix 0.5  $\mu$ M, 4  $\mu$ L of dNTPs 2.5  
13 mM, 10  $\mu$ L of HF buffer 5X (Finnzyme), 0.5  $\mu$ L Phusion enzyme (2U/ $\mu$ L,  
14 Finnzyme) in a 50  $\mu$ L volume, using a Trobot PCR machine (Biometra) with the  
15 following parameters : 98°C, 1 min; 30 cycles of [98°C, 30 sec; 50°C, 30 sec;  
16 72°C, 1 min]; 72°C, 8 min.

17 Five  $\mu$ L of each PCR product were analyzed on 1% agarose gel. Satisfying  
18 PCR products (single or few multiple bands) were purified on a Nucleofast plate  
19 (Macherey-Nagel) and eluted with 40  $\mu$ L of water, using a Microlab Star  
20 automaton (Hamilton).

21  
22 **2.6 Cloning with the In-Fusion™ Advantage System**

Five  $\mu\text{L}$  of the PCR products were treated with Cloning Enhancer reagent (In-Fusion™, Clontech) according to the supplier's recommendations. The In-Fusion™ reactions were performed by mixing 100 ng of linear vector with 2  $\mu\text{L}$  of each treated insert (3  $\mu\text{L}$  for the 3501 bp-long insert) in 10  $\mu\text{L}$  final volume. After the addition of 40  $\mu\text{L}$  of TE, 2.5  $\mu\text{L}$  of each reaction were used to transform 50  $\mu\text{L}$  of NEB5 $\alpha$  competent *E. coli* cells (Biolabs). The transformed cells were pelleted by centrifugation (5200 g, 5 min) and spread onto LB (kanamycin 50 mg/L, glucose 10 mM) agar plates and incubated overnight at 37°C.

## 2.7 Cloning by restriction/ligation

Forty  $\mu\text{L}$  of PCR products were mixed with 5  $\mu\text{L}$  buffer 4 10X (NEB), 0.5  $\mu\text{L}$  *Nde*I (10 U) or *Nco*I (5 U) and *Bam*HI (5U) enzyme (NEB). After 2 h at 37°C, fragments were purified on a Nucleofast plate (Macherey-Nagel) and eluted with 40  $\mu\text{L}$  of water. Two  $\mu\text{L}$  of each insert were mixed with 100 ng of linear vector in the presence of 1  $\mu\text{L}$  of 10X T4 DNA ligation buffer, 1  $\mu\text{L}$  of T4 DNA ligase (3 U/ $\mu\text{L}$ , Promega), in a final volume of 10  $\mu\text{L}$ . After 3 hours at room temperature, ligation reactions were stopped at 65°C (10 min). Five  $\mu\text{L}$  were used to transform 50  $\mu\text{L}$  of DH5 $\alpha$  competent *E. coli* cells. The transformed cells were then pelleted before spreading on LB (kanamycin 50 mg/L, glucose 10 mM) agar plate and incubated overnight at 37°C.

## 2.8 Plasmid screening by restriction analysis

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1 Isolated transformants were used to inoculate 2 ml of 2xTY (50 mg/L  
2 kanamycin) medium in a 24-wells plates sealed with a porous film. Cultures were  
3 grown for 20 h at 37°C with agitation (200 rpm). Cells were pelleted and  
4 plasmids were prepared on a Qiagen BioRobot 8000 using the Nucleospin robot  
5 96 kit (Macherey-Nagel) and eluted with 125 µL of EB buffer. Eight µL were  
6 used for restriction analysis with 2.5 U of each enzyme in a final volume of 10  
7 µL; profiles were analyzed on 1% agarose gel. Two positive clones for each gene  
8 were sent for full length sequencing with the universal T7 prom and T7 term  
9 primers (Beckman Cogenics). Sequencing with internal primers was necessary  
10 for genes longer than 2 Kb.

11

### 3 Results and discussion

HTP cloning requires large quantities of high quality linearized vector. The commonly used method of purification is preparative gel electrophoresis followed by spin column extraction [6]. Both steps are poorly suited to large amounts of DNA. To overcome this limitation, we have scaled up the separation of restriction fragments by anion exchange chromatography [18].

#### 3.1 Construction of expression vectors for soluble or membrane proteins expression

Two expression plasmids were designed to express soluble or membrane proteins, with the same backbone vector. The pLIM13 vector allows expression of proteins without tag, whereas pLIM14 is for His6- or His6-Mistic-fusion for soluble or membrane proteins respectively. The Mistic protein from *B. subtilis* was shown to increase the yield by targeting fused proteins more efficiently to the membrane in *E. coli* [24], (F. Bernaudat, personal observations). The toxic *ccdB* gene was inserted between the restrictions sites used for linearization (Figure 1). The CcdB protein is a poison of the DNA-topoisomerase II complexes killing bacterial strains not expressing the antidote CcdA [28], or not containing the *gyrA462* mutation [28, 29]. The interest of *ccdB* is to select against the parental vector in *E. coli* sensitive strains.

Digestion of pLIM13 with *NdeI* and *BamHI* produces two fragments of 5082 bp and 311 bp. Digestion of pLIM14 with the same enzymes gives 5103 bp and 683 bp fragments, whereas digestion with *NcoI* and *BamHI* releases 5455 bp

1 and 331 bp fragments. The larger fragments are the linearized vectors, the small  
2 fragments contain the *ccdB* gene. These digestions allow the in frame insertion of  
3 any gene amplified with specific primers (see Materials and Methods and Figure  
4 1).

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18 **3.2 Large scale plasmid purification**

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20 The protocol for purification of large amount of pLIM13 and pLIM14 was  
21 derived from known procedures [10, 16], and yielded 1.8 mg of each low copy  
22 vector from 1 L of the CcdB resistant DB3.1 *E. coli* strain grown in 2xTY. The  
23 purified plasmid was mostly supercoiled, with the presence of a minor nicked  
24 DNA form and no detectable RNA or protein contamination. The yield was  
25 sufficient for the preparation of 18 batches of linear vector as described below.  
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37 **3.3 Large-scale linear plasmid purification by Mono Q™ anion exchange**  
38 **chromatography**  
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41 This procedure succeeded with the three linearized pLIM vectors. The  
42 purification of pLIM13 digested with *NdeI* and *BamHI* is presented in details  
43 below as an example.  
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48 One hundred micrograms of pLIM13 were digested and the NaCl  
49 concentration was brought to 600 mM prior to the chromatography to avoid  
50 adsorption of low charge density impurities on the Mono Q™ column [15]. The  
51 advantage of a Mono Q™ resin is the fast and strong binding of the DNA (Figure  
52 2) [21].  
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1 A salt gradient was employed to displace the different nucleic acids that  
2 should elute in order of increasing charge density [15]. The charge density of  
3 DNA is function of its length and conformation. Long fragments, being more  
4 folded, have higher charge densities and are eluted later. The separation was  
5 visualized by the detection of two peaks and confirmed by the agarose gel  
6 analysis (Figure 2). Fractions containing the linearized expression vector were  
7 pooled. A third early peak corresponded to the elution of restriction enzymes

### 9 **3.4 Quality control of linear plasmid**

10 One hundred nanograms of each vector (pET41c, pLIM13, and pLIM14)  
11 were transformed into NEB5 $\alpha$  and DH5 $\alpha$ . In both strains, approximately one  
12 thousand transformants were obtained with pET41c and none with pLIM13 as  
13 expected due to the presence of the *ccdB* gene in the latter plasmid. To our  
14 surprise, thousand transformants were also obtained with the pLIM14. As *ccdB* is  
15 still present, we hypothesize that the normally cytosolic CcdB protein is not  
16 functional when fused to Mystic and targeted to the membrane. However, this  
17 finding allowed us to evaluate the homogeneity of the purified linear vector, as we  
18 could compare the number of transformants obtained with the native and the  
19 linearized plasmid. Linearized pLIM14 yielded no transformant showing the  
20 absence of undigested parental vector.

21 The quality and quantity of the linear vectors isolated by Mono Q<sup>TM</sup>  
22 chromatography are suitable for high throughput cloning, even if yields vary with  
23 the vectors and the digestions. Yields after digestions and purification were 26%



(25µg), 43% (38µg) or 73% (69µg) for pLIM13\_ *NdeI*\_ *Bam*HI, pLIM14\_ *NdeI*\_ *Bam*HI and pLIM14\_ *NcoI*\_ *Bam*HI, respectively. Individual fraction precipitations and pooling are probably responsible for the loss of material.

**3.5 Efficient cloning with linear plasmid**

The suitability for high throughput cloning of the purified pLIM13\_ *NdeI*\_ *Bam*HI linearized vector was tested by the insertion 24 independent genes in parallel. The gene size varied between 387 bp and 3501 bp. Eleven genes were cloned by restriction/ligation and 13 using the In-Fusion™ technique. Genes containing restriction sites within their sequence were treated by the In-Fusion™ method.

The automated PCR amplification of the 24 genes was performed with the Phusion enzyme on a Hamilton robot. PCR products were analyzed on agarose gel (Figure 2) and showed the successful amplification of all the genes using the same PCR parameters. Purification on a Nucleofast plate eliminated primer dimers and allowed buffer exchange.

Genes to be treated by ligation were first digested with the two appropriate restriction enzymes, and purified again on Nucleofast to eliminate the short fragments released by the digestion. Genes destined to In-Fusion™ cloning were directly incubated with the Cloning Enhancer according the manufacturer's recommendations.

1 With both cloning methods, 100 ng of linear vector were mixed to 2-3  $\mu$ L of  
2 insert. We have deliberately chosen to standardize the volume of insert,  
3 independently of their size, in order to develop an efficient high throughput  
4 process for a wide range of gene sizes, as the amplification delivers PCR  
5 fragments in amounts that are not limiting. After ligation or In-Fusion™  
6 recombination, transformation suspensions were plated on agar containing  
7 kanamycine, and glucose to repress the expression leakage.

8 Between 20 and 100 transformants were obtained, without relationship  
9 between the size of the insert and the number of transformants. For the negative  
10 control (linear pLIM13 plasmid without insert), around 10 and 70 transformants  
11 were obtained. These transformants resulted from aberrant recombination events.  
12 To evaluate the cloning success rate, 2 to 8 transformants were analyzed for each  
13 of the 24 genes by plasmids miniprep followed by restriction mapping.

14 The 24 genes were successfully cloned in parallel into the expression vector  
15 indicating the good quality of the linear vector preparation (Table 1). Cloning  
16 efficiencies were not correlated to the size of the gene, although the most difficult  
17 gene to clone was the longest (3501 bp). Except in the latter case, 50% to 100%  
18 of positive clones were obtained for all other genes. The negative clones resulted  
19 apparently from aberrant recombination events, such as the clones obtained from  
20 the negative controls. The mean cloning efficiency by restriction/ligation was  
21 63%, whereas that obtained by In-Fusion™ was 80% including the lower yield  
22 obtained with the longest gene. The systematic full length gene sequencing

1 performed on two positive clones of each gene, showed the absence of mutation  
2 with a single exception likely originating from a PCR error.

3  
4 **4 Concluding remarks**

5 Large amount of linear expression vector is obtained by separating the  
6 vector from its insert by anion exchange chromatography. The cloning results  
7 indicate that the quality of the linear vector purified by Mono Q™ is compatible  
8 with different high throughput cloning techniques. However, the In-Fusion™  
9 technique is, in our view, better adapted to high throughput as this system  
10 catalyzes the joining of DNA pieces in a manner that is independent of the  
11 sequence of the cloned gene.

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19 The authors have declared no conflict of interest.

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## Figure legends

**Figure 1. pLIM vector maps.** Linearization of the vectors was performed using the restriction sites mentioned. PCR-amplified genes were inserted either by restriction ligation or by the In-Fusion™ method. (A) pLIM13 for the overexpression of untagged proteins; (B) pLIM14 for the overexpression of His-tagged (cloned between the *NdeI* and *BamHI* sites) or His-Mistic-tagged proteins (cloned between *NcoI* and *BamHI*). The T7 promoter induces expression in *E. coli* harbouring the  $\lambda$  (DE3) prophage.

**Figure 2. Linear plasmid purification by Mono Q™ anion exchange chromatography and PCR amplification of the 24 genes used in cloning experiments.** (A) Chromatogram showing the purification of pLIM13 digested with *NdeI* and *BamHI* monitored at 254 nm. Plasmids were loaded on a Mono Q™ 5/50 GL column. DNA was eluted with a 64 ml linear gradient of 700 mM (0% B) to 900 mM (10 % B) of NaCl at 0.15 ml/min. Arrows indicate the fractions analyzed. (B) 1% Agarose gel analysis of the fractions corresponding to the elution peaks shown in (A): M, DNA ladder; 1, loaded sample; 2, flow through (pooled fractions 2-4); 3 to 6, fractions corresponding to the *ccdB* insert; 7 to 15, fractions corresponding to the linear vector. (C) 1% Agarose gel analysis of the pooled elution fractions from the chromatographic separations of pLIM14\_ *NdeI*\_ *BamHI*: 1, Mistic-*ccdB* insert; 2, linear vector; and pLIM14\_ *NcoI*\_ *BamHI*: 3, *ccdB* insert; 4, linear vector. (D) 1% Agarose gel analysis of the PCR amplification of 24 independent genes. M, DNA ladder; 1 to



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- 1 24, PCR amplification obtained in the conditions described in Materials and
- 2 Methods section and successfully cloned into pLIM13\_ *NdeI*\_ *Bam*HI:

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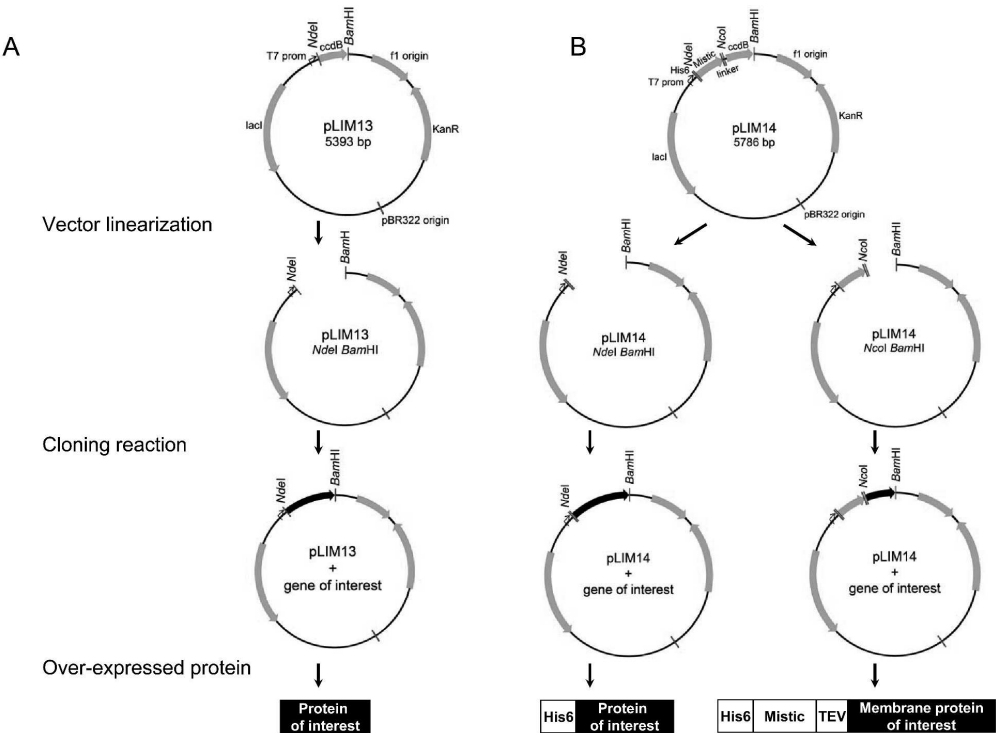
**Table 1. Gene cloning using pLIM13\_***NdeI-Bam***HI linear vector by restriction/ligation or InFusion techniques**

| Gene number | Plasmid | Technique used | Insert size (bp) | % of positive clones (Nb clones tested) |
|-------------|---------|----------------|------------------|---|
| 1           | pLIM13  | Ligation       | 1233             | 50% (4)                                 |
| 2           | pLIM13  | Ligation       | 1131             | 50% (4)                                 |
| 3           | pLIM13  | Ligation       | 1683             | 75% (4)                                 |
| 4           | pLIM13  | Ligation       | 816              | 50% (4)                                 |
| 5           | pLIM13  | Ligation       | 657              | 50% (4)                                 |
| 6           | pLIM13  | InFusion       | 627              | 87% (8)                                 |
| 7           | pLIM13  | InFusion       | 600              | 100% (8)                                |
| 8           | pLIM13  | Ligation       | 672              | 75% (4)                                 |
| 9           | pLIM13  | InFusion       | 3501             | 37% (8)                                 |
| 10          | pLIM13  | InFusion       | 603              | 100% (8)                                |
| 11          | pLIM13  | InFusion       | 1950             | 50% (8)                                 |
| 12          | pLIM13  | InFusion       | 1887             | 75% (8)                                 |
| 13          | pLIM13  | Ligation       | 1962             | 100% (4)                                |
| 14          | pLIM13  | InFusion       | 1557             | 75% (8)                                 |
| 15          | pLIM13  | Ligation       | 456              | 100% (4)                                |
| 16          | pLIM13  | InFusion       | 1119             | 55% (9)                                 |
| 17          | pLIM13  | InFusion       | 843              | 100% (3)                                |
| 18          | pLIM13  | InFusion       | 837              | 87% (8)                                 |

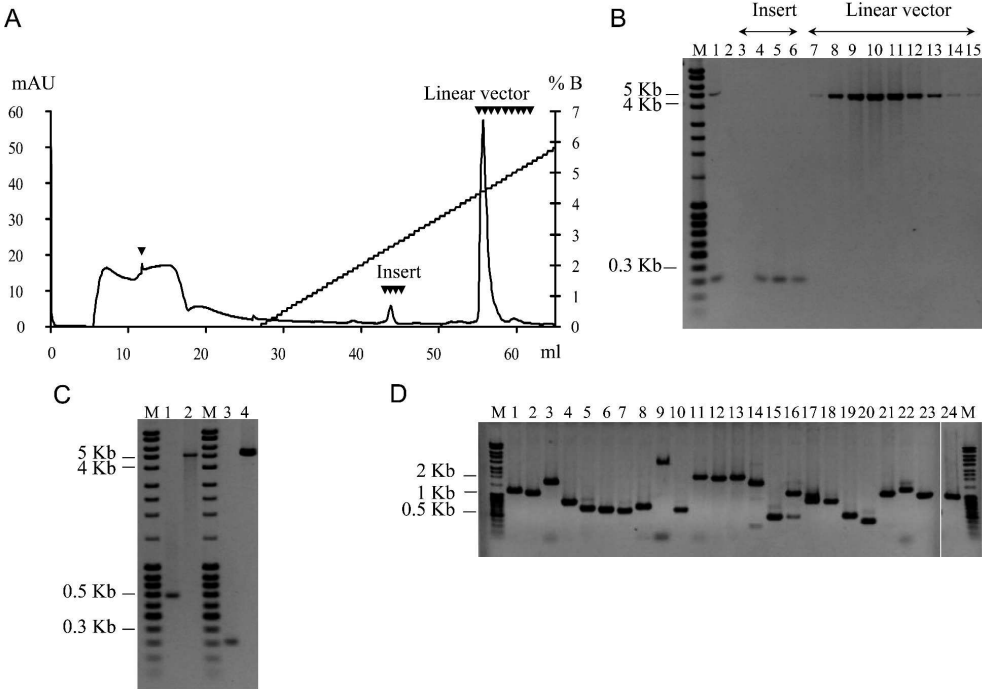
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|----|--------|----------|------|----------|
| 19 | pLIM13 | Ligation | 489  | 50% (4)  |
| 20 | pLIM13 | Ligation | 387  | 50% (2)  |
| 21 | pLIM13 | InFusion | 1116 | 100% (3) |
| 22 | pLIM13 | InFusion | 1302 | 100% (8) |
| 23 | pLIM13 | Ligation | 1038 | 50% (2)  |
| 24 | pLIM13 | InFusion | 1059 | 100% (8) |



245x180mm (600 x 600 DPI)



250x179mm (600 x 600 DPI)