KOR. JOUR. MICROBIOL April, 1992 p. 134~140 Vol. 30, No. 2

Transduction of the Wild-Type *polA* Gene of *Escherichia coli* K-12 in a ColE1-Derived Mini-Mu Plasmid

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The *polA*$^+$ gene can be transducted in a multicopy mini-Mu plasmid, but not cloned because the product of this gene is lethal when overproduced. Although, we obtained one surviving cell, in which the ColEl-derived mini-Mu plasmid suffered a spontaneous deletion exactly at the region where the *polA*$^+$ gene was cloned. The *PolA*$^+$ upstream flanking sequence containing the promoter and Pribnow-box was deleted *in vivo*; consequently this gene is not able to be expressed.

KEY WORDS ☐ ***Escherichia coli*, *polA*$^+$ gene, ColEl plasmid, *in vivo* cloning.**

It is known that the mini-Mu plasmid can be used for both generalized transduction of *Escherichia coli* genes and *in vivo* cloning in the same experiment (2, 8, 9, 27, 32). The *polA*$^+$ gene of *E. coli* is a structural gene of DNA polymerase I (21). It is known that the coloning of *polA*$^+$ gene in a plasmid ColEl was unsuccessful because the presence of this gene in multiple copies in a cell, resulting the overproduction of DNA pol I, which is lethal to the cell (18). However, this cloning was possible only by placing the expression of *polA*$^+$ gene under the inducible control of the bacteriophage lambda p_L promoter (24). It is known also, that the *polA1* cell (25, 26, 28) cannot be transformed by plasmid ColEl (20) because the maintaining of this plasmid depends the polymerase and 3′-5′ exonuclease activities of DNA pol I (14, 19). However, if the plasmid ColEl contains a DNA insert which is homologous with one part of the chromosome of the *polA*1 strain, then this plamid can integrate by homologous recombination at that site (6, 10).

We tried to clone *in vivo* the *polA*$^+$ gene in a ColEl-derived mini-Mu plasmid, the examine whether this plasmid can be maintained in *polA*1 cell by homologous recombination.

MATERIALS AND METHODS

Bacterial strains, plasmids and phages

E. coli K-12 strains are listed in Table 1. All plasmids are described in Table 2. Mu-1 temperate (13, 29) and Mu*cts*62 thermoinductible (12, 13) bacteriophages were used. Cultures containing mini-Mu or Mu*cts*62 were grown at 30℃. Other cultures were grown at 37℃.

Media

L. broth contained 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 5 g of NaCl per liter. The VB minimal medium for *in vivo* cloning possed the Vogel and Bonner salts (31) and supplemented with 0.5% glucose, thiamine, and the required amino acids. The Casamino Acids medium was used for the test of loss of plasmid which contained the same ingredients as VB minimal medium plus 0.25% Casamino Acids (Difco). The Davis Mingioli (DM) minimal medium (5) was used for selectrion of the transductants which was supplemented with

Table 1. *Escherichia coli K-12 strains.*

Strain	Genotype	Source
HB101	*hsdS*20 *supE*44 *ara*14 *galK*2 *lacY*1 *proA*2 *rpsL*20 *xyl*5 *mtl*1 *recA*3	B.J. Bachmann
M5004	*trpA*9825 *glnA*3 IN(*rrnD-rrnE*) 1	B.J. Bachmann
NPC5	*trpA*9825 *polA*1 IN (*rrnD-rrnE*) 1 mini-Mu::*polA spf*$^-$	This study (P3478 Mu*cts*62× M5004 Mu)
P3478	*thyA*36 *deoC*2 *polA*1, IN (*rrnD-rrnE*)1	B.J. Bachmann

polA : non-expressed *polA*$^+$ gene.

Table 2. *Plasmids used.*

Plasmid	Replicator	Relevant Characters	Chromosomal DNA Insert Size (kb)	Origine and Reference
pRRA101	ColE1, pSC101	Ap^{r}Km^{r}Tc^r	None	G.R. Drapeau
pNPC5	ColE1	Ap^{r}Km^{r}Tc^r*polA spf*$^-$	3.10	This study
pNPC4	None	Ap^{r}Km^{r}Tc^r*polA glnA/3*	*Yes*[a]	This study

Ap: ampicillin, kb: kilobase-pair, Km: kanamycin, *polA*$^-$: non-expressed *polA*$^+$ gene, ^{r}: resistant, ^{s}: sensitive, Tc: tetracycline. [a]: size of the chromosomal DNA insert is not determined. However, the genes of DNA insert were defined by the result at of the complementation test.

the required amino acids. Agar was added to 1.5% for plates. The appropriate antibiotics were added to sterile medium in the following concentrations: ampicillin, 25 μg/ml; chloramphenicol, 170 μg/ml and tetracycline, 15 μg/ml. Restriction endonucleases were obtained from Bethesda Research Laboratories Inc.

Methods

Lysogenisation by bacteriophages Mu-1 and Muc*ts*62 was as described Bukhari and Ljungquist (4). Plasmid DNAs were isolated by the method of Birnboim and Doly (3), purified by centrifugation to equilibrium in a CaCl gradient (23). Transformation of $CaCl_2$-treated cell was effected by the method of Mandel and Higa (22). Procedure for *in vivo* cloning was occured as described by Groisman *et al.* (9), Noël and Drapeau (27), Wang *et al.* (32). Digestion by restriction endonuclease were taken from Maniatis *et al.* (23).

RESULTS

In vivo cloning of the *polA*$^+$ gene in a ColE1-derived mini-Mu plasmid

Ampicillin resistant transductants can be divided in three classes: A. Reverted transductants contain the original plasmid pRRA101 (Table 2), therefore they are resistance to tetracycline. B. Transductants have a multicopy mini-Mu plamid harbouring a segment of chromosomal DNA, which is composed of other gene than *polA*$^+$, *glnA* 3. It is known that the chromosomal DNA insert replaced the pSC101 sequence of plasmid pRRA 101, consequently these transductants are sensitive to tetracycline. C. The same as class B, except the segment of chromosomal DNA consist of *polA*$^+$ and *glnA*3 genes.

We obtained a total of 120 Ap^r transductant: 42 (35%) of them belong to class A and 78 to class B and C. The remaining 78 Ap^r Tc^s transductants were grew in an ampicillin-free Casamino acids minimal medium overnight at 30 C, in order to lose their mini-Mu palsmid. After this process, it is possible to differentiate the type C from the B by selection. Out of 78 transductants 48 strains (61%) lost their plasmid in this way, so these strains can be selected for the *polA*$^-$ and *glnA*$^+$ genes (Table 3). We obtained eight strains (NPC1 to 8) which have the *polA*1-*glnA*$^+$ genotype, and they were found in the suspension stock (all of them posses their plasmid), consequently their mini-Mu plasmid have to contain the *polA*$^+$, *gln*3 genes. Afterwards, these NPC1 to 8 (mini-Mu::) strains were submitted to the complementation test, to confirm the genotype of their plasmid. This test had to show that the NPC1 to 8 (mini-Mu::) strains are resistant to MMS and can grow on a DM minimal medium which is not contains glutamin. Our result indicated that the NPC1 to 8 (mini-Mu::) strains could grow on a glutamin-free minimal medium, but they did not show the activity of *polA*$^+$ gene (they were sensitive to MMS). Consequently, the mini-Mu plasmid of NPC1 to 8 strains contains the non-expressed *polA*$^-$ gene beside the *glnA*3 (Table 3).

Table 3. *Genotypic determination of the transductants NPC1 to 8 by selection*[a].

Strain	Genotype	Genotype of mini-Mu plasmid pNPC1 to 8
D: P3478	*polA*1 glnA$^-$	
R: M5004	*polA*$^+$ *glnA*3	
Tr$^-$: NPC1 to 8	*polA*1 *glnA*$^+$	
Tr$^+$: NPC1 to 8*	*polA*1 *glnA*$^+$ (mini-Mu::)	*polA*$^-$ *glnA*3

[a]Selection for *polA*$^+$ gene by the test of resistance to MMS (DM minimal medium was supplemented with 0.05% MMS) and for the *glnA*$^+$ gene by streaking out on a glutamin-free DM medium. D: Donor, R: Recipient, Tr$^-$: Transductants are deprived of their plasmid (by the test of loss of plasmid), Tr$^+$: Transductants with their palsmid, *: Genetic determination by complementation test (between the chromosomal and plasmid-borne genes), *polA*$^-$: non-expressed *polA*$^+$ gene.

Caracterization of the mini-Mu plasmids pNPC1 to 8

Extracting of the mini-Mu plasmid to NPC1 to 8 strains, a big difference was remarqued in the yield of plasmids: Seven transductants always

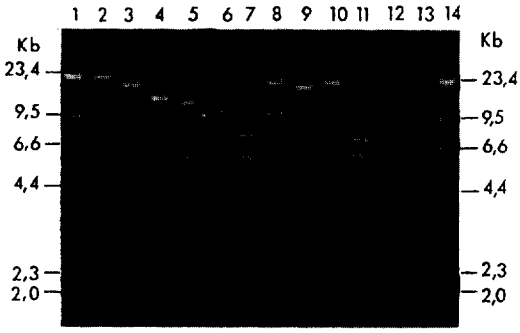

Fig. 1. *Determination of location of the chromosomal DNA insert on ColE1-derived mini-Mu plasmid pNPC5 by enzymatic digestion. Ethidium bromide-stained agarose gel.*
Lanes contain the following DNA: 1 and 14, *Hind*III-cut lambda phage (Standards, in kilobases, are shown at the two sides); 2, *Eco*RI-cut pRRA101; 3, *Eco*RI-cut pNPC5; *Pst*I-cut pRRA101; 5, *Pst*I-cut pNPC5; 6, *Eco*RI-*Pst*I-cut pRRA101; 7, *Eco*RI-*Pst*I-cut pNPC5; 8, *Hind*III-cut pRRA101; 9, *Hind*III-cut pNPC5; 10, *Kpn*I-cut pRRA1011; 11, *Kpn*I-cut pNPC5; 12, *Hind*III-*Kpn*I-cut pRRA101; 13, *Hind*III-*Kpn*I-cut pNPC5.

gave a faible concentration of their plasmid. We chose one strain (NPC4) among the seven and its plasmid (pNPC4) was transformed in *polA* $^{+}$ strain (HB101), to seek whether this plasmid can replicate when the DNA pol I is present in cell. (Chloramphenicol was added to the log. culture). Our result showed that the mini-Mu plasmid pNPC4 could not replicate in *polA* $^{+}$ cell, consequently it is not ColEl-type. The six other plasmids are similar to pNPC4. However, one transductant (NPC5) gave a big yield of its plasmid (nNPC5) all the time, because this plasmid is ColEl-type.

Restriction analysis of the multicopy mini-Mu plasmid pNPC5

We determined the place of the chromosomal DNA insert on pNPC5 by enzymatic digestions (Fig. 1, Table 4 and Fig. 2). Our results showed that the cleavage of pNPC5 by *Eco*RI and *Pst*I (Fig. 1, lane 7), gave three fragments: 7.5 kb, 6.0 kb and 4.3 kb. The 7.5 kb and 6.0 kb fragments can be found also at the *Eco*RI-*Pst*I-cut pRRA101 (lane 6; Table 4). These fragments represent some parts of the mini-Mu of plasmids pNPC5 and pRRA101 (Fig. 2). The 4.3 kb fragment includes the chromosomal DNA segment (Table 4). The digestion of pNPC5 by *Kpn*I (Fig. 1, lane 11) resulted three fragments: 7.0 kb, 6.5 kb and 4.3 kb. The 7.0 kb fragment contains the chromosomal DNA insert (Table 4). The 6.5 kb and 4.3 kb fragments can be found also at the *Kpn*I-cleaved

Table 4. *Analysis of the products of enzymatic digestion of plasmid pRRA101 and its derivative pNPC5.*

Restriction endonuclease	Plasmid pRRA101 (32.30 kb)	Plasmid pNPC5 (17.80 kb)
*Eco*RI	23.80 kb	17.80 kb[a]
	8.50 kb	
*Pst*I	13.15 kb	11.80 kb[a]
	13.15 kb	6.00 kb[b]
	6.00 kb[b]	
*Eco*RI-*Pst*I	10.30 kb	7.50 kb[b]
	7.50 kb[b]	6.00 kb[b]
	6.00 kb[b]	4.30 kb[a]
	5.65 kb	
	2.85 kb	
*Hind*III	20.30 kb	17.80 kb[a]
	9.80 kb	17.80 kb[a]
	9.80 kb	
	2.20 kb	
*Kpn*I	25.50 kb	7.00 kb[a]
	6..50 kb[b]	6.50 kb[b]
	4.30 kb[b]	4.30 kb[b]
HindIII-*Kph*I	12.50 kb	7.00 kb[a]
	6.80 kb	4.30 kb[b]
	4.30 kb[b]	3.50 kb[b]
	3.50 kb[b]	3.00 kb[b]
	3.00 kb[b]	
	2.20 kb	

[a]: restriction fragment containing the chromosomal DNA segement. [b]: sequence of mini-Mu.

pRRA101 (Fig. 1, lane 10; Table 4). These fragments came from the section of mini-Mu of plasmids pNPC5 and pRRA101 (Fig. 2). The 17.8 kb of molecular weight of pNPC5 indicates that a big deletion was occured on this plasmid (Fig. 2). The two extremities of mini-Mu: the C and (6.0 kb fragment) and S end (1.8 kb) were deleted and some parts of the chromosomal DNA insert was eliminated also. Since, the 4.3-kb *Eco*RI-*Pst*I fragment (Fig. 1, lane 7) contains the chromosomal DNA segment with less sequence of mini-Mu comparing to the 7.0 kb *Kpn*I fragment (lane 11; Fig. 2), therefore the 4.3-kb *Eco*RI-*Pst*I gragment was extracted from agarose gel.

Restriction analysis of the 4.3 kb *Eco*RI-*Pst*I fragment of plasmid pNPC5

Our result of digestion of *Eco*lRI-*Pst*I fragment by *Pvu*II enzyme (Fig. 3), gave three fragments: 2.45 kb (*Pvu*II-*Pst*I), 1.50 kb (*Eco*RI-*Pvu*Ii) and 0.35 kb (*Pvu*II-*Pvu*II). Consequently, there are two sites of *Pvu*II, which can be found in the *polA* $^{+}$ gene (exactly in the small fragment of DNA pol I). Furthermore, the 4.3 kb *Eco*RI-*Pst*I fragment has five restrictions sites of *hae*III: one is between

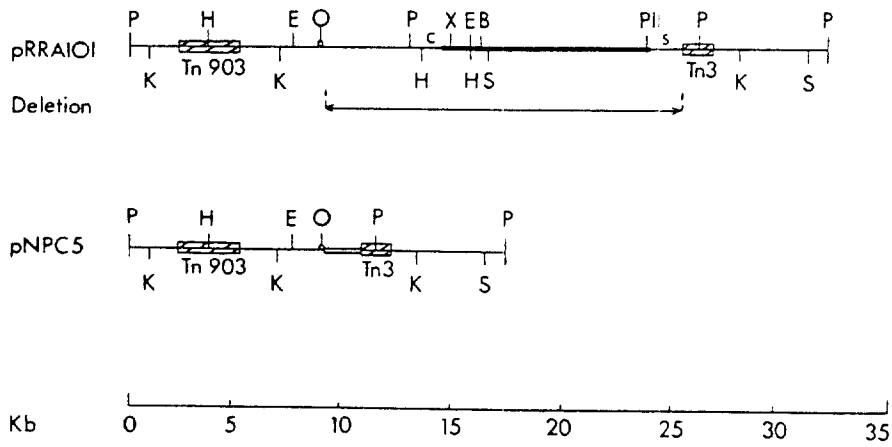

Fig. 2. *Restriction maps of plasmid pRRA101 and its derivative pNPC5 containing the chromosomal DNA insert.*
Restriction sites: B, *Bam*Hi; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; P, *Pst*I; PII, *Pvu*II; S, *Sal*I; X, *Xho*I. O, origine of replication of plasmid ColE1. Mini-Mu plasmid DNA is represented by thin line, pSC101 plasmid DNA by heavy line, and the chromosomal DNA by double line. Mini-Mu sequences contain the *aphA* gene which confers kanamycin resistance (Km^r) (hatched box) Tn903 and then *B-Lactamase* gene which confers ampicillin resistance (Ap^r) (hatched box) Tn3. The C and S indicate the repressor gene and the left end of Mu. Mote that the pRRA101 is a hybrid plasmid consists of mini-Mu section (ColE1 replicon) and pSC101 plasmid (replicon). Its derivative pNPC5 is a high-copy-number mini-Mu plasmid (ColE1-replicon), because the chromosomal DNA segement (double line) replaced the pSC101 sequence (heavy line) of plasmid pRRA10.

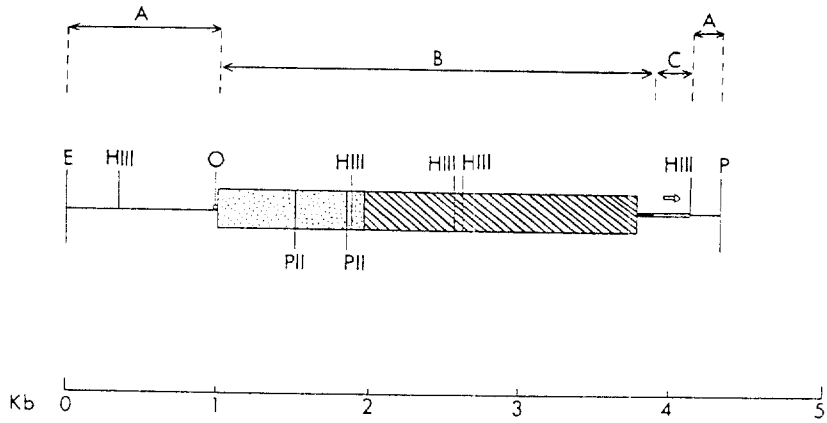

Fig. 3. *Restriction map of the 4.3 kb EcoRI-PstI fragment of plasmid pNPC5.*
Restriction sites: E, *Eco*RI; HIII, *Hae*III; P, *Pst*I; PII, *Pvu*II. O, origine of replication of plasmid ColE1. A: thin lines represent the parts of mini-Mu plasmid DNA. B and C: indicate the chromosomal DNA insert. B: sequence coding for DNA polymerase I: boxed area corresponding to the small (▨) and large (Klenow) (▩) proteolytic fragments, and the heavy line represents the downstream flanking region. C: double line indicates the sequence coding for spot 42 RNA. Open arrow shows the direction of transcription of *spf*$^+$ gene.

*Eco*RI and *ori*, three are in the *polA*$^+$ gene (one is in the small fragment and two are in the Klenow fragment of DNA pol I) and one site is downstream from the *spf*$^+$ gene (Fig. 3). Our results of digestions showed that the 3.1 kb chromosomal DNA insert is composed of a 2.8 kb sequence of *polA*$^+$ gene (which corresponds to the two proteolytic fragments of DNA pol I), its downstream flanking region (0.1 kb) and a 0.2 kb sequence incldluing the gene for Spot 42 RNA. Moreover, the 4.3 kb *Eco*RI-*Pst*I fragment contains 1.2 kb parts of mini-Mu (Fig. 3), which consists

of a 1.0 kb sequence between *Eco*RI and *ori*, (which localizes upstream from the *polA*$^+$ gene) and a 0.2 kb sequence representing the half of Tn3 (which is situated downstream from the *polA*$^+$ gene, after the *spf*$^+$) (Fig. 2, 3). Since, there was no restriction site of *Bgl*II, this shows that the *polA*$^+$ upstream flanking sequence (0.3 kb) was deleted, which included the promoter and Pribnow box. Moreover, there was no restriction site of *Bam*HI and *Bgl*II, that indicates the *glnA*3 gene was eliminated entirely (which was situated downstream from the *spf*$^+$ gene).

DISCUSSION

It is known that a *polA*1 mutant cannot be transformed by plasmid ColEl because the maintainance of this plasmid requires the polymerase and 3′-5′ exonuclease activities of DNA pol I (14, 19) and the *polA*1 strain is deficient in these activities (25, 26, 28). Our results of *in vivo* cloning showed when the *polA*$^+$ and *glnA*3 genes were transduced in the ColEl-derived mini-Mu plasmid, then this plasmid was maintained in *polA*1 cell by homologous recombinations. But this maintaining lasted only a short period of time until the multiplication cellular. Meanwhile, this ColEl-type plasmid (containing *polA*$^+$, *glnA*3 genes) could replicate in *polA*1 cell by the plasmid-encoded DNA pol I. This process resulted the overproduction of DNA pol I, which was lethal to the transductants. This result is according to Kelley *et al.* (18). Although, we obtained eight survivings cells (NPC1 to 8), where in the ColE1-derived mini-Mu plasmid had suffered a spontaneous deletion, exactly at the region where the *polA*$^+$ gene was cloned. We suppose that this deletion was occured in order to damage *in vivo* the *polA*$^+$ gene to prevent its expression during the multiplication cellular. In seven cells, the *ori* of mini-Mu plasmid was eliminated during the truncate of *polA*$^+$ gene. Consequently, these plasmids are not ColEl-type (see pNPC4 in Table 2). Moreover, we obtained one strain (NPC5), its plasmid pNPC5 remains multicopy, because the deletion did not affect *ori* while one part of the *polA*$^+$ gene was eliminated (Table 2). However, this ColE1-derived mini-Mu plasmid could not replicate any more in NPC5 strain because the plasmid-encoded DNA pol I is deficient in cell (Table 1).

Thereafter, the restriction map of plasmid pNPC5 was designed by the results of enzymatic digestions (Fig. 1, 2). Our results indicated that the 4.3 kb *Eco*RI-*Pst*I fragment contains the chromosomal DNA insert with the smallest part of mini-Mu, therefore this fragment was analysed more by restriction enzymes to investigate how the *polA*$^+$ gene was cloned (Fig. 3). Our results were compared with the known-restriction maps of the *polA*$^+$ gene (15-17), the *spf*$^+$ gene (11, 15, 16), the *glnA*$^+$ gene (1, 30) and the mini-Mu section of plasmid pRRA101 (Fig. 2) (27). Our results showed that the 3.1 kb chromosomal DNA insert contains the *polA*$^+$ gene (2.8 kb *polA*$^+$ sequence is intact with its downstream flanking region (0.1 kb)), and the *spf*$^+$ gene (total 0.2 kb sequence) (Fig. 3). However, the 17.8 kb of molecular weight of plasmid pNPC5 (Fig. 2) indicates a big deletion which was occured spontaneously in order to truncate the *polA*$^+$ gene to prevent its expression during the multiplication cellular. The restriction analysis showed that this deletion affected the two extremities of the chromosomal DNA insert (Fig. 3). Thus, the *polA*$^+$ upstream flanking region (0.3 kb) and the entire sequence of *glnA*3 gene (1.36 kb) (which was situated downstream from the as *spf*$^+$ gene). Consequently, the *polA*$^+$ gene cannot be expressed, because its deleted upstream flanking sequence contained the promoter and Pribnow-box (7, 17), which are essential for the regulation of the gene. However, the *spf*$^+$ gene can be expressed because its transcription starts at 0.15 kb beyond the end of *polA*$^+$ downstream flanking sequence (11, 15, 16). Furthermore, this deletion includes the two extremities of mini-Mu, which are bordered with the cloned DNA insert: the 6.0 kb sequence of C end and the 1.8 kb fragment of S end (Fig. 2).

The presented work showed that the *polA*$^+$ gene was transduced in a ColEl-derived mini-Mu plasmid, but not cloned because the product of this gene was lethnal when overproduced.

ACKNOWLEDGEMENTS

We wish to thank Dr. B. J. Bachmann of the *E. coli* Genetic Stock Center for providing us with some bacterial strains. We are also grateful to Dr. G. R. Drapeau from the Universite de Montréal for his gifts of plasmid pRRA101 and phages Mu-1, Muc*ts*62 and for helpful advices in the procedures for *in vivo* cloning. This work was supported by a grant (to Y.S.C.) from the Natural Sciences and Engineering Research Council of Canada.

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(Received February 12, 1992)
(Accepted March 3, 1992)

초 록: *Escherichia coli* K-12의 야생형 *polA*$^+$ 유전자의 ColEl 계열 Mini-Mu Plasmid에의 형질도입

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polA$^+$ 유전자는 다중 복제 mini-Mu plasmid로의 형질도입이 가능하나 이 유전자 산물은 과다생산시 치사효과를 보이기 때문에 이 유전자를 클로닝시킬 수 없었다. 그럼에도 본 실험에서 저자들은 생존하는 세포를 분리하였는 바 이 세포에서는 ColEl-유래 mini-Mu plasmid가 정확히 *polA*$^+$ 유전자의 클로닝 부위에서 자연적으로 결실이 일어났다. 이는 *in vivo* 상태에서, promoter와 Pribnow-box를 포함하는 *polA*$^+$ 유전자의 upstream flanking sequence가 결실되어 그 결과 이 유전자는 발현 될 수 없었던 것으로 확인되었다.