TABLE 3

cDNA Trapping of PI-4-Phosphate 5-Kinase and Inositol 1,3,4-Triphosphate 5/6-Kinase

Enzyme	Number of positive clones	% of clones sequenced
Mouse PI-4-phosphate 5-kinase α[a]	2	6
Mouse PI-4-phosphate 5-kinase β	6	20
Mouse PI-4-phosphate 5-kinase γ	4	13
Inositol 1,3,4-triphosphate 5/6-kinase	7	23
Others	12	38

[a] PI, phosphatidylinositol.

form β in the region where the oligonucleotide was derived (9). Thus, the cDNA encoding form γ was probably captured by the oligonucleotide for form β. It should be noted that the human liver and mouse brain libraries were constructed with vectors that have different flanking sequences at the polylinker region, which was used to establish by the polymerase chain reaction which library was the source from which a cDNA was isolated (4).

Among the cDNAs encoding other proteins in the kinase group, half coded for vitamin D-binding protein (Table 3), and the rest apparently coded for yet unidentified proteins based on a BLAST search in GenBank. These unidentified proteins may actually share a significant sequence identity with the oligonucleotides used for the trapping experiment (only a ~350-bp sequence in the 5′ end was determined), which probably contributed to their isolated. In contrast, the cDNA encoding vitamin D-binding protein shows no significant sequence identity with the oligonucleotides; the reason for its isolation remains to be determined. It was likely that the abundance of the cDNAs coding for the kinases to be isolated in these libraries was so low that an excess of the labeled oligonucleotides was available to hybridize with unrelated cDNAs and yielded false positives. This notion is supported by the fact that all of the sequenced clones in the P450 trapping experiment resulted in the isolation of target cDNAs, because P450 enzymes, the primary phase I drug-metabolizing enzymes, are very abundant in the liver (5, 7, 11, 12).

We report here a modified cDNA trapping technique that can accommodate multiple oligonucleotides and more than one library at the same time. This method has all the features of the original method such as screening of a large number of clones within a week, rapid isolation of full-length cDNA, and identification of related sequences. This modified method, compared with the original method, saves time and significantly reduces the cost, as the reagents used for cDNA trapping are expensive. This is particularly effective for isolating multiple forms of cDNAs.

Acknowledgment. The authors thank Dr. Chichester for reviewing the manuscript.

REFERENCES

1. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *in* Molecular Cloning: A Laboratory Manual, 2nd ed., pp. 8.3–8.82, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. Li, W. B., Gruber, C. E., Noon, M. C., Polayes, D., Schmidt, B., and Jessee, J. (1995) *Focus* **17,** 15.
3. Vorndam, A. V., and Kerschner, J. (1986) *Anal. Biochem.* **152,** 221–225.
4. Yan, B., Yang, D., Bullock, P., and Parkinson, A. (1995) *J. Biol. Chem.* **270,** 19128–19134.
5. Ged, C., Umbenhauer, D. R., Bellew, T. M., Bork, R. W., Srivastava, P. K., Shinriki, N., Lloyd, R. S., and Guengerich, F. P. (1988) *Biochemistry* **27,** 6929–6940.
6. Romkes, M., Faletto, M. B., Blaisdell, J. A., Raucy, J. L., and Goldstein, J. A. (1991) *Biochemistry* **27,** 3247–3255.
7. Lieber, C. S. (1990) *Pharmacol. Ther.* **46,** 1–41.
8. Ishihara, H., Shibasaki, Y., Kizuki, N., Katagiri, H., Yazaki, Y., Asano, T., and Oka, Y. (1996) *J. Biol. Chem.* **271,** 23611–23614.
9. Ishihara, H., Shibasaki, Y., Kizuki, N., Wada, T., Katagiri, H., Yazaki, Y., Asano, T., and Oka, Y. (1996) *J. Biol. Chem.* **271,** 8741–8746.
10. Wilson, M., and Majerus, P. W. (1996) *J. Biol. Chem.* **271,** 11904–11910.
11. Song, B. J., Gelboin, H. V., Park, S. S., Yang, C. S., and Gonzalez, F. J. (1986) *J. Biol. Chem.* **261,** 16689–16697.
12. Shephard, A. E., Palmer, C. N. A., Segall, H. J., and Philips, I. R. (1992) *Arch. Biochem. Biophys.* **294,** 168–172.

A Positive Selection Cloning System Based on the *gltS* Gene of *Escherichia coli*

József Gál,*,†,[1] Szilvia Szekeres,*,‡
Róbert Schnell,*,‡ Sándor Pongor,†
András Simoncsits,† and Miklós Kálmán*,‡

**Institute for Biotechnology, Bay Zoltán Foundation for Applied Research, Derkovits fasor 2, Szeged, 6726, Hungary; †International Centre for Genetic Engineering and Biotechnology (ICGEB), Area Science Park, Padriciano 99, I-34012 Trieste, Italy; and ‡Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Temesvári krt. 62, Szeged, 6726, Hungary*

Received September 25, 1998

Identification of the correct recombinant clones is often laborious despite the availability of the blue/white screening and positive selection cloning systems. Most of these

[1] To whom correspondence should be addressed at Institute for Biotechnology, Bay Zoltán Foundation for Applied Research, P.O. Box 2337, Szeged, 6701, Hungary. Fax: + 36 62 432250. E-mail: gal@bay.u-szeged.hu.

Analytical Biochemistry **266,** 235–238 (1999)
Article ID abio.1998.2957
0003-2697/99 $30.00

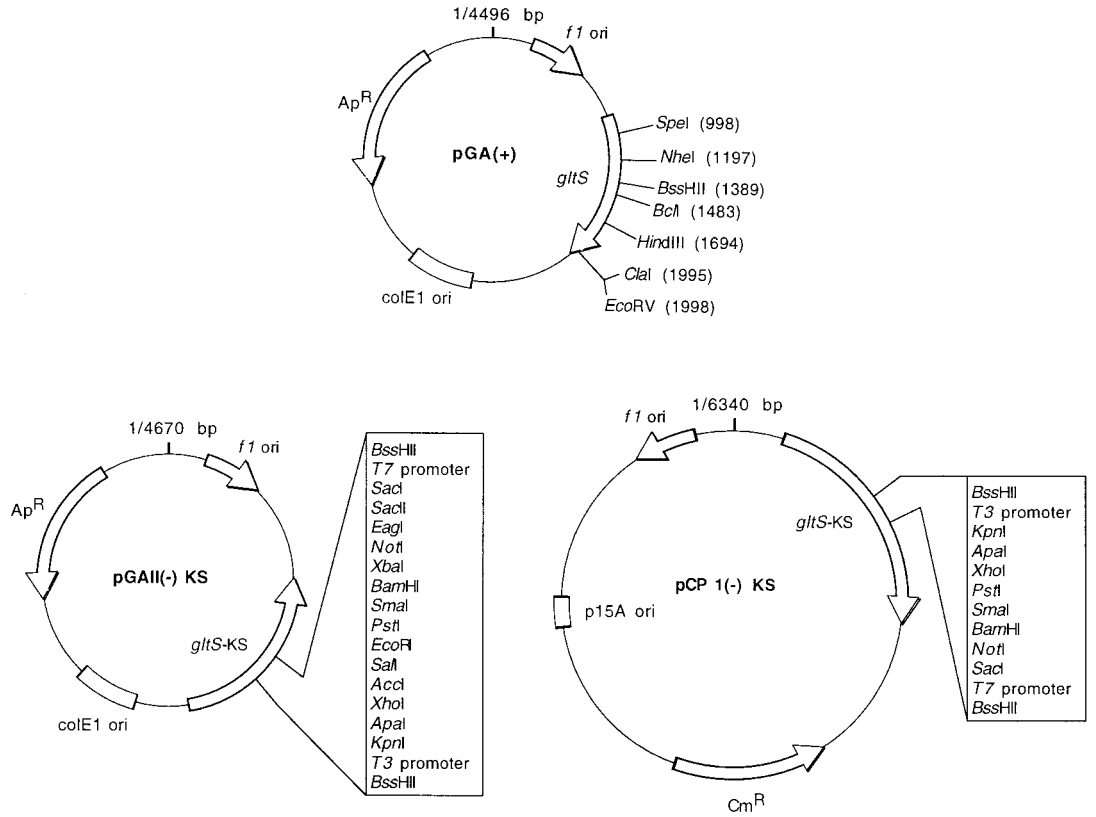

FIG. 1. The structure of *gltS*-based cloning vectors. *gltS*–KS: *gltS* gene containing the KS polycloning region. All indicated restriction sites, except for the *Bss*HII sites in the polycloning site-containing vectors are unique. In the SK derivatives, the order of restriction sites within the polycloning site is reversed.

systems are not free from particular problems which limit their general applicability (see Refs. 1–5 and 12 for overview). Here we describe a set of positive selection cloning vectors which utilize a novel selection principle, namely the conditional inhibitory effect of the *gltS* gene from *Escherichia coli* K12 on the growth of *E. coli* cells.

The *gltS* gene product GltS is a glutamate-specific permease (6) which is also responsible for the transport of the glutamate analog α-methyl glutamate (αMG)[2] (7). This analog interferes with the conversion of glutamate to glutamine and inhibits the growth of *E. coli* on glucose minimal medium (7). Inactivation of the *gltS* gene, for example, by insertion of foreign DNA, should abolish the αMG sensitivity, allowing recombinant clones to grow. This abolishment was indeed observed when a translational frameshift was introduced at the unique *Nhe*I site of *gltS* (6) and when the same site was used to clone a 2-kb *Nhe*I–*Nhe*I *Pichia pastoris* genomic fragment (not shown). Translational frameshifts introduced at the *Hin*dIII or at the *Cla*I site also abolished the αMG sensitivity, showing that a substantial part of the coding region could be used for insertional inactivation (see Fig. 1 for restriction map).

The selection vectors were constructed as follows. The *gltS* gene fused to a weak, constitutive derivative of the *E. coli lac* promoter was cloned between the *Hin*dIII and *Bam*HI sites of pTC01, replacing the antibody cloning/expression cassette of the vector (8). The promoter region lacking both the CAP binding site and the *lac* operator was derived from the pRIZ′O(−) vector (9). It was fused to the Shine–Dalgarno sequence of the *gltS* in the arrangement *Hin*dIII–TAATGTAAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGCATATG**AAGGAG**–*gltS*–*Bgl*II, where the −35 and the −10 regions are underlined and the Shine–Dalgarno sequence is in bold. The resulting vector pCP1(−) contains the p15A origin of replication and the chloramphenicol resistance gene. The promoter–*gltS* cassette was transferred to the smaller pBS(−) phagemid (Stratagene, La Jolla, CA) by using the *Pvu*II large fragment of the vector, resulting in pGA(+) and pGA(−), where (+) and (−) refer to the direction of the promoter–*gltS* fusion with respect to that of the f1 origin of replication: (+) means identical, while (−) divergent orientations. In

[2] Abbreviation used: αMG, α-methyl glutamate.

TABLE 1

Examples of Cloning and Selection Experiments Using the Positive Selection Vectors

Vector	Cloning sites used	Insert[a] size (kb)	Colony number on		Recombinant clone[c] per tested clone
			Nonselective[b] plate	Selective plate	
pGA(+)	*Hin*dIII	1.43	1480	154	10/10
pGAII(−) KS	*Pst*I	1.08	2090	111	9/10
pGAII(−) SK	*Pst*I	1.08	1425	67	10/10
pGAII(−) KS	*Eco*RI–*Bam*HI[d]	1.5	765	723	9/9
pGAII(−) SK	*Eco*RI–*Bam*HI[d]	1.5	795	730	10/10
pCP1(−) KS	*Bam*HI	0.24–1.42	1650	176	9/9

[a] The inserts were obtained by the same cleavage as indicated for the corresponding cloning vectors, except for in the pCP1(−) KS cloning. In this example a mixture of fragments ranging from 0.24 to 1.42 kb was obtained by partial *Sau*3AI cleavage.

[b] Estimated by plating aliquots of the transformation mixture onto LB-Amp or LB-Cm plates.

[c] Plasmid preparations were analyzed by appropriate restriction cleavage(s) and agarose gel electrophoresis.

[d] The double-cleaved vectors were purified by using the Qiaquick gel extraction kit (Qiagen GmbH, Germany) so that the cleavage mixture was not subjected to gel purification but mixed directly with solution QX1 of the kit.

the (+) vectors the sense, whereas in the (−) vectors the antisense strand of *gltS* is recovered after infection with helper phage. These vectors offer a limited set of restriction sites for positive selection cloning (*Spe*I, *Bss*HII, and *Cla*I in pCP1(−); *Spe*I, *Nhe*I, *Bss*HII, *Bcl*I, *Hin*dIII, *Cla*I, and *Eco*RV in the pGA vectors). To increase the cloning flexibility, we introduced the polycloning regions of the pBC KS and SK vectors (Stratagene) into the *gltS* coding region so that the permease function was maintained. The polycloning regions were PCR amplified by using the *M13* (−20) and the GCCAAGCGCGC**C**AATTAACCCTCAC primers (*Bss*HII site is underlined and an extra C residue used to maintain the reading frame of *gltS* is shown in bold), and the *Bss*HII-cleaved PCR products were cloned into the *Bss*HII site of the *gltS* gene of pGA(−) to obtain pGAII(−) KS and SK. The pGAII(+) KS and SK and the pCP1(−) KS and SK were also constructed (Fig. 1). XL1-blue cells (Stratagene) transformed with these vectors did not grow on plates of M9 glucose minimal medium (10) supplemented with thiamine, the appropriate antibiotic, and 40 μg/ml αMG (up to 6 days) at 37°C. Colonies were obtained, however, when αMG was omitted (2 days) or when LB-based plates were used (12–16 h, with or without αMG). The sequences of these *gltS*-based cloning vectors, assembled from known sequence elements, were deposited in the EMBL Nucleotide Sequence Database under Accession Nos. AJ005323–AJ005330 and AJ005339.

The applicability of the positive selection system is demonstrated here by several examples in which different restriction fragments of the HDHII/Ku autoantigen subunits were used (11). In the ligation reactions, the cleaved vectors were used without phosphatase treatment and gel purification. The amounts of the inserts and the vectors were not determined. XL1-blue cells transformed with the ligation mixtures were washed with 150 mM NaCl and plated on M9 glucose minimal plates containing 1 μg/ml thiamine, 100 μg/ml αMG (Sigma), 100 μg/ml carbenicillin (for pGA vectors), or 25 μg/ml chloramphenicol (for pCP1(−) KS) and incubated at 37°C for 2–3 days. The results (see Table 1) show that the selection is highly efficient. Even when the efficiency of the cloning (colony number on selective versus nonselective plate) was very low, all or nearly all selected and tested clones contained the expected insert. The only false positive detected in six different experiments contained vector-size DNA with probably a short deletion causing frameshift in *gltS*. Such cloning background is common in other systems and could be minimized by carefully performed enzymatic manipulations (vector cleavage and ligation).

The vectors described here combine the versatility of the Bluescript vectors with the possibility of positive selection. A slight disadvantage of our system is the use of the minimal medium which causes generally slow cell growth (2–3 days). However, special additives in the medium or special host strains are not required. The αMG is commercially available and inexpensive (about three times cheaper than the carbenicillin). Since the conditionally toxic *gltS* gene is expressed from a weak, constitutive promoter, there is no need to use an inducer in combination with a host strain overproducing the corresponding repressor (e.g., IPTG and *lacI*^{q} host). No special strain is required with respect to the chromosomal *gltS* allele either, since the latter does not interfere with the selection. There are no limitations as to the propagation of the empty cloning vectors. Although overproduction of GltS is toxic to the cells even without added αMG (6), the low-level synthesis in our system allows stable propagation of the vectors in commonly used laboratory strains. These

features, together with the highly efficient selection mechanism, should make our system an attractive alternative to other positive selection cloning systems.

Acknowledgments. The pTC01 vector was obtained from R. A. Lerner. J.G. was a recipient of an ICGEB fellowship. A.S. is on leave from the Department of Neurochemistry and Neurotoxicology, The Arrhenius Laboratories for Natural Sciences, Stockholm University, Sweden. This work was supported by OTKA Grant T016705.

REFERENCES

1. Messing, J. (1991) *Gene* **100,** 3–12.
2. Bernard, P., Gabant, P., Bahassi, E. M., and Couturier, M. (1994) *Gene* **148,** 71–74.
3. Bernard, P. (1996) *BioTechniques* **21,** 320–323.
4. Hengen, P. N. (1997) *Trends Biochem. Sci.* **22,** 105–106.
5. Kast, P. (1994) *Gene* **138,** 109–114.
6. Kalman, M., Gentry, D. R., and Cashel, M. (1991) *Mol. Gen. Genet.* **225,** 379–386.
7. Halpern, Y. S., and Umbarger, H. E. (1961) *J. Gen. Microbiol.* **26,** 175–183.
8. Collet, T. A., Roben, P., O'Kennedy, R., Barbas, C. F., III, Burton, D. R., and Lerner, R. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10026–10030.
9. Simoncsits, A., Chen, J., Percipalle, P., Wang, S., Törö, I., and Pongor, S. (1997) *J. Mol. Biol.* **267,** 118–131.
10. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
11. Tuteja, N., Tuteja, R., Ochem, A., Taneja, P., Huang, N. W., Simoncsits, A., Susic, S., Rahman, K., Marusic, L., Chen, J., Zhang, J., Wang, S., Pongor, S., and Falaschi, A. (1994) *EMBO J.* **13,** 4991–5001.
12. Schlieper, D., von Wilcken-Bergmann, B., Schmidt, M., Sobek, H., and Müller-Hill, B. (1998) *Anal. Biochem.* **257,** 203–209.