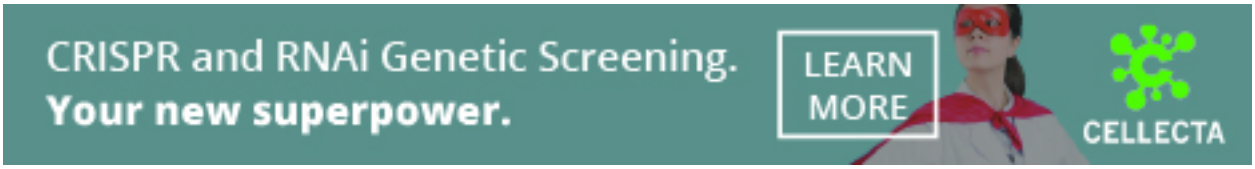
Rapid mapping of Escherichia coli::Tn5 insertion mutations by REP-Tn5 PCR.

P S Subramanian, J Versalovic, E R McCabe, et al.

Genome Res. 1992 1: 187-192
Access the most recent version at doi:10.1101/gr.1.3.187



Rapid Mapping of *Escherichia coli*::Tn*5* Insertion Mutations by REP-Tn*5* PCR

Prem S. Subramanian,[1] James Versalovic,[1]
Edward R.B. McCabe,[1,2] and James R. Lupski[1,2]

[1]Institute for Molecular Genetics and [2]Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030

We describe a novel method to map chromosomal *Escherichia coli*::Tn*5* insertion mutations rapidly. This method utilizes the ends of Tn*5* and the *E. coli* REP sequence as primer binding sites for the polymerase chain reaction (PCR). The unique *E. coli* chromosomal sequence located between these primer binding sites is amplified by PCR and used as a probe to identify the recombinant clones from the Kohara phage ordered *E. coli* miniset bank that contains the Tn*5* mutated loci. We used this approach to map two Tn*5* insertion mutations previously identified by their effect on glycerol metabolism. The insertion mutations mapped to *glpD*, the aerobic *sn*-glycerol-3-phosphate dehydrogenase gene. Phenotypic analysis of the mutant strains revealed one with partial GlpD activity, suggesting transposon-mediated alteration of promoter activity. This mapping method should be applicable to the rapid physical mapping of any insertion mutation in the *E. coli* chromosome.

Transposon Tn*5* mutagenesis is a powerful tool for generating random mutations in the *Escherichia coli* chromosome. The desired mutant colonies can be recovered readily by employing an appropriate genetic screen[1,2]; however, physical mapping of the transposon insertion mutation can be laborious, particularly if mutations in a number of genes are capable of generating the same phenotype. In a previous study,[3] we isolated 21 independent Tn*5* insertion mutations into the *E. coli* K-12 strain HB101[4] chromosome which diminished the cell's ability to ferment glycerol, as evidenced by an altered colony color phenotype on MacConkey-glycerol plates. Although 16 of the 21 mutations mapped to the *glpFK* operon, the remaining five did not. Two of these five, Tn*5*-5 and Tn*5*-14, showed normal GlpK enzymatic activity but were nonviable when transformed with a plasmid containing the *glpK* gene. A lethal effect caused by an increased copy number of the *glpK* gene was hypothesized to explain this observation, suggesting that Tn*5* insertions in a gene or genes downstream in the biochemical pathway resulted in the buildup of a toxic intermediate.[5,6]

To map these two interesting insertion mutations rapidly, we developed a strategy, based on the polymerase chain reaction (PCR),[7] that does not require prior knowledge of host chromosomal DNA sequences at the insertion site. This method is similar to a technique developed in eukaryotes that uses primers to human *Alu* repeat elements to amplify human specific sequences in rodent–human somatic cell hybrids (*Alu*-PCR),[8] and methods recently developed in prokaryotes to amplify unique *E. coli* chromosomal DNA sequences between REP (repetitive extragenic palindrome) elements, called REP-PCR, and ERIC (enterobacterial repetitive intergenic consensus) sequences, called ERIC-PCR.[9] The REP element has been predicted to comprise up to 1% of the genome of *E. coli*.[10] This ~35-bp palindromic sequence has been identified in the intercistronic regions of numerous operons, and its putative stem–loop structure has been proposed to decrease expression of downstream genes[11] while stabilizing transcripts of upstream sequences.[12] It has also been proposed to play a role in determining chromosome structure and to bind DNA gyrase and the histone-like protein HU.[13,14]

When an outwardly facing primer corresponding to either half of the REP palindrome and a Tn*5*-specific primer are used with chromosomal DNA from a Tn*5* insertion mutant strain as the template, PCR amplification products contain unique sequences that lie between the transposon and adjacent REP site(s) as well as between adjacent REP sequences themselves. Using PCR conditions to favor amplification from Tn*5* to REP, and by comparison to an amplification using REP primers alone, the resulting Tn*5*-REP amplification products can be identified readily. These DNA fragments are then used as probes for the mutated locus by screening an ordered *E. coli* genomic library.[15] In this manner, the insertion can be mapped quickly to a specific

1:187–194©1992 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/92 $3.00

region of the chromosome. Standard genetic mapping techniques using conjugation and transduction[16] can be time consuming and are accurate to about 1 min, or 47 kb,[17] although this can be improved with refined genetic mapping methods. In contrast, this PCR-based mapping strategy allows positive assignment of the mutation to ±50 bp (the limit of resolution on an agarose gel), when used in conjunction with the Kohara[18] physical map. In addition, the method provides physical material of a manageable size, in the form of the PCR product, for additional characterization of the insertion mutation site.

METHODS

Phenotypic Analysis

Strains HB101::Tn*5*-5 and HB101::Tn*5*-14 were grown overnight in rich media (LB) containing 40 μg/ml kanamycin, pelleted by centrifugation, and resuspended in M9 media containing no carbon source. The OD_{600} of each culture was measured, and 1×10^6 cells were inoculated into 5 ml of M9 media containing 10 mg/liter Leu, Pro (the HB101 auxotrophic markers), and thiamine in addition to one of the following carbon source(s): 10 mM glucose, 10 mM glucose/25 mM glycerol, 25 mM glycerol, 40 mM succinate, or 40 mM succinate/25 mM glycerol.[19] Cultures were incubated at 37°C with shaking, and the OD_{600} of each was measured at specific time intervals. Strain HB101 was cultured under similar conditions with the omission of kanamycin during initial growth.

REP-Tn*5* PCR

PCR amplification primers were prepared with an Applied Biosystems oligonucleotide synthesizer and DNA sequence information from the published sequence of Tn*5*,[3] and the REP consensus sequence.[10] The Tn*5* primer 5′-CTGGAAAACGGGAAAGGTTCCG-3′, primer E,[3] matches a sequence 30 bp from the end of the transposon; the REP1R-I primer 5′-IIIICGICGICATCIGGC-3′[9] (I = inosine, where I is a degenerate position in the sequence) is the reverse complement of the left side of the stem of the REP consensus palin-drome,[10] and the REP2-I primer 5′-ICGICTTATCIGGCCTAC-3′ corresponds to the right side of the stem of the REP palindrome.[10] Amplification reactions were performed in 50-μl reaction volumes using buffer and reagent concentrations (primers, deoxyribonucleotide triphosphates, template DNA, enzyme) as recommended by Perkin-Elmer Cetus. Amplification was conducted in a Perkin-Elmer Cetus first-generation thermal cycler as follows: denaturation at 94°C for 4 min, 25 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 1.5 min, and polymerase extension at 72°C for 2.5 min. PCR products were analyzed by electrophoresis through a 1% agarose gel and visualized with ethidium bromide staining. Products were purified using the Gene-clean II kit (Bio101), radiolabeled with [α-^{32}P]dCTP using a random hexanucleotide priming kit (Pharmacia), and hybridized to a commercially available membrane (Takara Shuzo Co., Ltd.) containing the Kohara et al.[18] ordered miniset λ phage library of the *E. coli* chromosome using published methods.[15] The background λ phage clones were visualized by addition of 0.1 ng of ^{32}P-labeled *Hin*dIII-digested λ DNA to the hybridization solution. The blots were exposed to X-ray film for 24–72 hr (with an intensifying screen) at −70°C.

Tn*5*-*glpD* PCR

The *glpD* primer 5′-AACTTCTGGTGCGCGTCGGC-3′, which corresponds to nucleotides 272–291 of the sense strand,[20] was used with the Tn*5* primer. Fifty nanograms of template DNA were added to the PCR reaction tubes containing 10% DMSO.[21] Reaction mixtures contained 50 pmoles of each primer, 62.5 nmoles of each of four dNTPs, and 2 units of AmpliTaq (Cetus) DNA polymerase. The amplification conditions consisted of an initial denaturation of 94°C (6 min); amplification proceeded with 30 cycles of denaturation at 90°C for 1 min, annealing at 62°C for 1 min, and polymerase extension at 70°C for 5 min. Products were analyzed by electrophoresis through a 0.8% agarose gel and visualized with ethidium bromide staining.

RESULTS

Phenotypic Characterization of Mutants

Isolation of the Tn*5* insertion mutants affecting glycerol metabolism has been described previously.[3] The glycolytic defect of the two insertion mutations, Tn*5*-5 and Tn*5*-14, was assessed by examining growth in defined media prepared from minimal media supplemented with specific carbon sources.[19] A *glpD* mutation will allow normal growth on substrates other than glycerol. In addition, normal growth will be seen if glycerol is given with a carbon source that represses glycerol metabolism (i.e., glucose). However, if glycerol is present alone or with a noninhibitory substance (i.e., succinate), abortive glycerol metabolism will lead to growth stasis.[22] This effect has been attributed to the accumulation of L-α-glycerophosphate within the cell.[5] When cultured in these defined media, strain HB101::Tn*5*-5 displayed a classic *glpD* phenotype: It was unable to grow in glycerol and grew very poorly on succinate with glycerol, but if glucose was present, growth was normal. However, strain HB101::Tn*5*-14 differed phenotypically: It evidenced poor growth on glycerol alone, but on succinate with glycerol, growth approached that of strain HB101 (Table 1).

TABLE 1 Growth of Tn*5* Insertion Mutants on Various Carbon Sources

	Sugar(s) added to M9 minimal broth				
Bacterial strain	Glucose	Glycerol	Glucose and glycerol	Succinate and glycerol	Succinate
HB101::Tn*5*-5	+++	−	+++	±	++
HB101::Tn*5*-14	+++	+	+++	++	++
HB101	+++	++	+++	++	++

(−) No growth; (±) $OD_{600} \leq 0.05$; (+) $0.05 \leq OD_{600} \leq 0.1$; (++) $0.3 \leq OD_{600} \leq 0.4$; (+++) $OD_{600} \geq 0.5$.

PCR Analysis of Mutated Chromosomal DNA

Concurrent with the phenotypic characterization, PCR amplification using Tn*5* and REP-specific primers was pursued. A schematic portrayal of the reaction is shown in Figure 1C. The REP to Tn*5* amplification occurs independent of the orientation of the Tn*5* insertion relative to the REP sequence. The transposon has a twofold axis of symmetry, and amplification may proceed in both directions because the Tn*5* primer has been designed for the inverted repeat.[3] The use of outwardly directed primers complementary to each half of the REP palindrome (REP1R-I and REP2-I) ensures that the orientation of the REP sequence will not preclude amplification. The PCR was conducted on chromosomal DNA from HB101::Tn*5*-5 and HB101::Tn*5*-14 in separate reactions using the Tn*5* primer with either the REP1R-I or REP2-I primers. Although purified chromosomal DNA was used as template DNA in these experiments, whole cells have also been used as sources of template DNA for amplification by picking colonies and transferring cells directly to PCR tubes (data not shown). Presumably these cells are lysed during the initial denaturation step of the PCR and no pretreatment of the cells is necessary. No REP-Tn*5* PCR products were obtained when the Tn*5* and REP1R-I primers were used together (data not shown). Use of Tn*5* with the REP2-I primer resulted in a single unique product being amplified from each mutant template chromosome that was not seen when wild-type HB101 chromosomal DNA was used as the template (Fig. 1A), nor when amplification using either primer alone was attempted (data not shown). Inter-REP amplification products were not observed under the conditions employed, which were empirically chosen (by raising the annealing temperature) to favor REP-Tn*5* products. The amplification of chromosomal DNA from HB101::Tn*5*-5 yielded a 390-bp fragment, and that from HB101::Tn*5*-14 chromosomal DNA gave an 890-bp product using the Tn*5* and REP2-I primers (Fig. 1A). These REP-Tn*5* PCR products were radiolabeled and used to screen an *E. coli* genomic library (Fig. 2A,B). Both probes hybridized to the same three phages, designated 616, 617, and 618 in the Kohara[18] miniset (Fig. 2C). The region of overlap mapped at 75 min on the *E. coli* genetic map,[17] and corresponded to the *glpD* locus.

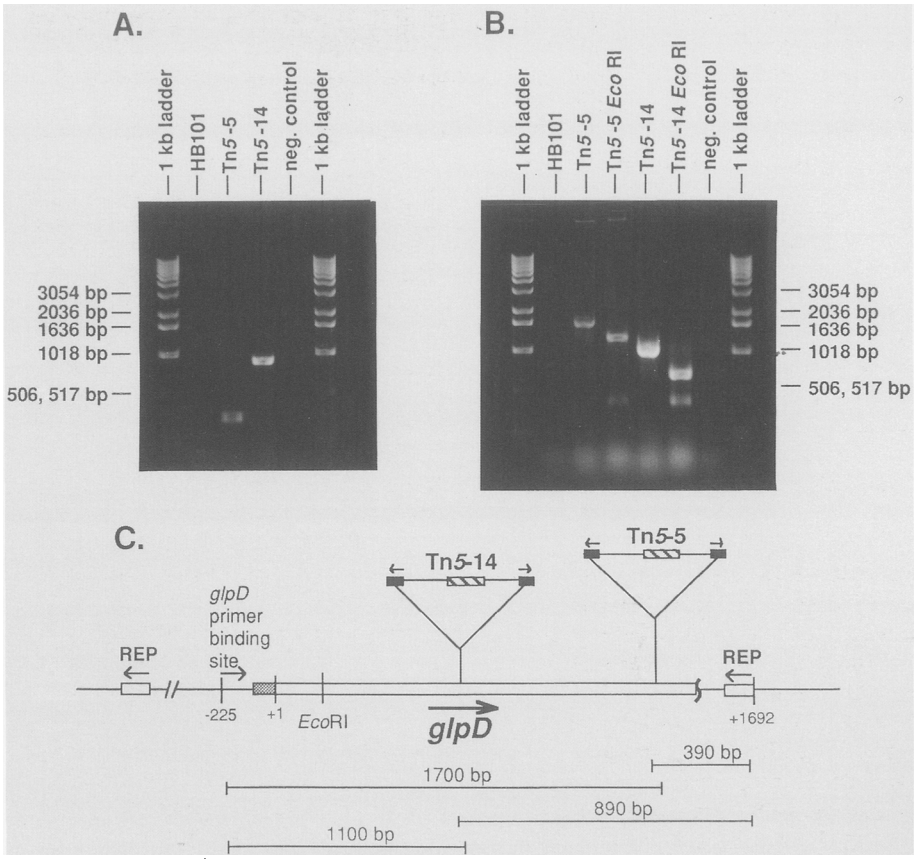


FIGURE 1 Analysis of insertion mutations by PCR. (*A*) PCR products generated by using chromosomal DNA from HB101, HB101::Tn*5*-5, HB101::Tn*5*-14 with REP and Tn*5* primers as described in the text. No template DNA was added to the negative control reaction. (*B*) PCR products generated by using chromosomal DNA and primers to the ends of Tn*5* and the *glpD* regulatory region. Note the decrease in size of the upper bands and the appearance of the band at ≡400 bp upon *Eco*RI digestion. (*C*) Schematic representation of the PCR amplification strategy. The break indicates that the REP site to its left is putative, not observed. The direction of transcription is shown by the arrow above *glpD*. The shaded box indicates the control region.[22] Size bars indicate the span of the PCR products obtained from the reactions shown above. Distance from the *glpD* primer binding site to the REP sequence can be determined by adding the sizes of the Tn*5*-REP and Tn*5*-*glpD* products after subtraction of transposon sequence. Each product contains 55 bp of Tn*5* sequence; in addition, 9 bp is subtracted from the sum of the products to account for the 9-bp duplication that results upon Tn*5* insertion.[1] The Tn*5*-5 data suggest a distance of 1971 ± 50 bp; the Tn*5*-14 data, 1871 ± 50 bp. The calculated distance from the *glpD* primer binding site to the REP primer binding site is 1917 bp.[23] Arrows indicate the location and directionality of the PCR primers.

To confirm these initial results and to map the sites of the Tn*5* insertions with more precision, PCR amplification between the *glpD* regulatory region and Tn*5* was performed (Fig. 1B). Each mutant DNA template generated a single product. HB101::Tn*5*-5 DNA yielded a single 1700-bp fragment, and HB101::Tn*5*-14 yielded an 1100-bp product (Fig. 1B). To establish further the identity of these products, the presence of an *Eco*RI site, located 415 bp from the 5′ end of the *glpD* primer,[20] was assayed. When digested with *Eco*RI, the length of both PCR products was indeed reduced by approximately 400 bp (Fig. 1B). Thus, the mutations Tn*5*-5 and Tn*5*-14 were localized to the *glpD* locus with the Tn*5*-5 insertion 1420 ± 50 bp and the Tn*5*-14 insertion 820 ± 50 bp downstream of the translation start site.[20] Nucleotide sequence data for the *glpD* locus[23] became available after com-

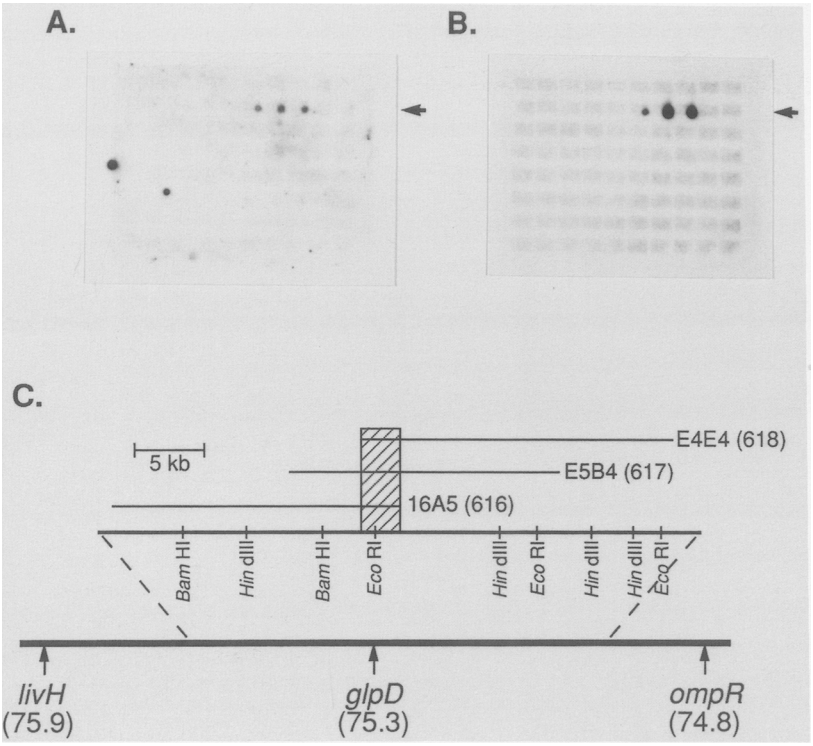


FIGURE 2 Correlation of the genetic and physical maps. (*A*) Autoradiogram of a filter containing the Kohara phage ordered miniset library in a dot blot array. The fragment obtained from amplification of HB101::Tn*5*-5 DNA was labeled and used as the probe. Exposure time was 72 hr at –70°C. The two extraneous nonspecific signals do not align with phages. (*B*) Autoradiogram of the same filter using the labeled fragment from the amplification of Tn*5*-14 DNA. Exposure time was 24 hr at –70°C. (*C*) Alignment of the Kohara[18] physical map and the Bachmann[17] genetic map. The hatched box indicates the region of overlap of all three phages identified from the autoradiograms.

pletion of this work. The authors reported two REP sequences beginning 60 bp downstream of the putative translational stop site, lying in opposite orientations. The REP2-I primer was localized to nucleotides 1676–1692 with identity of all bases other than inosine to the antisense strand. Calculation of the position of the two Tn*5* insertions using the known REP location yielded results consistent with those derived from the data presented here.

DISCUSSION

We have described a physical mapping technique that takes advantage of the presence of repetitive sequence elements in prokaryotic genomes. The method enables one to map insertion mutations quickly, by using the insertion element as one primer binding site and the REP sequence as the other genomically anchored, primer binding site for PCR amplification. The unique sequence spanned by the amplification product can be used as a probe to identify genomic regions corresponding to the insertion site. The application of the REP-Tn*5* PCR-based physical mapping technique was demonstrated by rapidly identifying the location within *glpD* of two Tn*5* insertion mutations affecting glycerol metabolism.

The locations of these insertions within the *glpD* locus suggest a functional explanation for the phenotypic differences observed between the two mutants. Tn*5*-14 appears to retain some *glpD* function; although it is unable to grow on glycerol alone, it can tolerate its presence in the media. Tn*5* IS*50* sequences have been shown to possess an outwardly directed promoter activity.[24–26] A partial *glpD* transcript initiated from within the transposon could be synthesized and translated to yield a functional, though incomplete, gene product lacking the amino-terminal region of GlpD. Such a mechanism does not appear to apply to the Tn*5*-5 insertion mutation, which is located more distally in the *glpD* coding sequence. REP sequences have not been found in coding regions,[10] and the proximity of the Tn*5*-5 insertion to the REP sequence suggests that it interrupts the distal coding sequence of the *glpD* gene, leading to insertional inactivation. In this position, outwardly directed promoter activity from Tn*5* into *glpD* would be quite unlikely to lead to gene function. Partial activity of a truncated GlpD protein cannot solely account for these phenotypes since the insertion at the 3′ end of *glpD*, Tn*5*-5, completely abolished GlpD function. Because the mutant with an insertion in the 5′ half of *glpD*, Tn*5*-14, contains partial activity, an outwardly directed promoter activity from the end of the transposon is likely.

Furthermore, these results suggest regions of GlpD that are critical for its activity. Assuming transposon-mediated promoter activity, a partial Tn*5*-14 *glpD* transcript would contain only the latter half of the coding sequence. It is this part of the gene that the Tn*5*-5 insertion disrupts, and transcripts from this mutant that originate at the normal promoter (if they occur) would encode a GlpD protein that lacks the carboxy-terminal coding information. Nucleotide sequence data[23] suggest that the amino-terminal portion of the protein contains a flavin-binding domain, while a putative glycerol-3-phosphate binding site has been found in the carboxy-terminal half. Expression of the portion of *glpD* distal to the Tn*5*-14 insertion appears to be sufficient for production of a protein that provides dehydrogenase activity. Alternatively, the incomplete protein might associate with a moiety (protein or otherwise) capable of providing the necessary electron transfer function that a non-flavin-containing enzyme would lack. In either case, the protein is not entirely normal, as evidenced by the partial mutant phenotype it conveys upon the cell. These results confirm that insertion mutations are not always null mutations.

This REP-Tn*5* PCR-based technique for rapid mapping and characterization of insertion mutations can be applied to a wide range of insertion mutation studies in *E. coli*, *S. typhimurium*, and other organisms for which repeat ele-

ments (such as REP sequences) have been defined. As new repeat sequences are discovered in diverse bacterial species, the utility of this technique will likely increase. REP-like and ERIC-like sequences are conserved in many diverse eubacterial species.[9] For any transposable element for which the sequence is known, a primer specific to its end may be synthesized and used in an initial REP-Tn PCR screen. Caution must be exercised when designing primers to the ends of the transposable element. The Tn*5* primer used in these experiments was later found to contain similarity to an *E. coli* chromosomal DNA sequence by performing a computer-aided search of the GenBank and EMBL databases with the FastA algorithm.[27] In addition, it is important to be sure that the strain utilized for mapping does not contain endogenous IS sequences with similarity to the primer binding site. If repetitive sequences are indeed present in the genome of interest, the principal limitation is the need for proximity of the insertion to the repetitive sequence. The fact that most transposons have a twofold axis of symmetry enables detection of a nearby repetitive sequence on either side of the transposon insertion. Presently maximum amplifiable distances using REP primers alone are in the range of 5 kb of DNA, but this limit appears to be a function of the processivity of the DNA polymerase under specific conditions.

Probing a library blot with the PCR products quickly enables one to obtain clones covering a small, defined portion of the genome. The mutation then can be mapped as described here if a known locus is found in the interval covered by the Kohara clones or by using the PCR product as a probe to identify restriction fragments of interest within the λ clones. In this manner, several new insertions in known loci can be identified, and new genes may be isolated and characterized within a period normally required to define a single mutation by traditional mapping methods.

ACKNOWLEDGMENTS

We thank Frans J. de Bruijn and George M. Weinstock for critical reviews and Linda G. Haway for manuscript preparation. This work was supported in part by Public Health Service grants to J.R.L. (RR-05425) and E.R.B.M. (5 R01 HD22563 and 1 P30 HD 27823). J.R.L. acknowledges support from the PEW Scholars Program in Biomedical Sciences. P.S.S. and J.V. are supported by the National Institutes of Health Medical Scientist Training Program at Baylor College of Medicine.

REFERENCES

1. de Bruijn, F.J. and J.R. Lupski. 1984. The use of transposon Tn*5* mutagenesis in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids—A review. *Gene* **27:** 131–149.
2. Berg, D.E. 1989. Transposon Tn*5*. In *Mobile DNA* (ed. D.E. Berg and M.M. Howe), pp. 185–210. American Society for Microbiology, Washington, DC.
3. Lupski, J.R., Y.H. Zhang, M. Rieger, M. Minter, B. Hsu, B.G. Ooi, T. Koeuth, and E.R.B. McCabe. 1990. Mutational analysis of the *Escherichia coli glpFK* region with Tn*5* mutagenesis and the polymerase chain reaction. *J. Bacteriol.* **172:** 6129–6134.
4. Boyer, H.W. and D. Roulland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* **41:** 459–472.
5. Cozzarelli, N.R., J.P. Koch, S. Hayashi, and E.C.C. Lin. 1965. Growth stasis by accumulated L-α-glycerophosphate in *Escherichia coli*. *J. Bacteriol.* **90:** 1325–1329.
6. Lin, E.C.C. 1987. Dissimilatory pathways for sugars, polyols, and carboxylates. In Escherichia coli *and* Salmonella typhimurium*: Cellular and molecular biology* (ed. F.C. Neidhardt, J.L. Ingraham, K.B. Low, B. Magasanik, M. Schaechter, and H.E. Umbarger), pp. 244–284. American Society for Microbiology, Washington, DC.
7. Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich, and N. Arnheim. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230:** 1350–1354.
8. Nelson, D.L., S.A. Ledbetter, L. Corbo, M.F. Victoria, R. Ramirez-Solis, T.D. Webster, D.H. Ledbetter, and C.T. Caskey. 1989. *Alu* polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources. *Proc. Natl. Acad. Sci.* **86:** 6686–6690.
9. Versalovic, J., T. Koeuth, and J.R. Lupski. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res.* **19:** 6823–6831.
10. Stern, M.J., G.F.-L. Ames, N.H. Smith, E.C. Robinson, and C.F. Higgins. 1984. Repetitive extragenic palindromic sequences: A major component of the bacterial genome. *Cell* **37:** 1015–1026.
11. Higgins, C.F., G.F.-L. Ames, W.M. Barnes, J.M. Clement, and M. Hofnung. 1982. A novel intercistronic regulatory element of prokaryotic operons. *Nature* **298:** 760–762.
12. Newbury, S.F., N.H. Smith, E.C. Robinson, I.D. Hiles, and C.F. Higgins. 1987. Stabilization of translationally active mRNA by prokaryotic REP sequences. *Cell* **48:** 297–310.
13. Shyamala, V., E. Schneider, and G.F.-L. Ames. 1990. Tandem chromosomal duplications: Role of REP sequences in the recombination event at the join-point. *EMBO J.* **9:** 939–946.
14. Yang, Y. and G.F.-L. Ames. 1990. The family of repetitive extragenic palindromic sequences: Interaction with DNA gyrase and histonelike protein HU. In *The bacterial chromosome* (ed. K. Drlica and M. Riley), pp. 211–225. American Society for Microbiology, Washington, DC.
15. Noda, A., J.B. Courtwright, P.F. Denor, G. Webb, Y. Kohara, and A. Ishihama. 1991. Rapid identification of specific genes in *E. coli* by hybridization to membranes containing the ordered set of phage clones. *BioTechniques* **10:** 474–477.
16. Singer, M., T.A. Baker, C. Schnitzler, S.M. Deischel, M. Goel, W. Dove, K.J. Jaacks, A.D. Grossman, J.W. Erickson, and C.A. Gross. 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping in *Escherichia coli*. *Microbiol. Rev.* **53:** 1–24.
17. Bachmann, B.J. 1990. Linkage map of

Escherichia coli K-12, Edition 8. *Microbiol. Rev.* **54:** 130–197.

18. Kohara, Y., K. Akiyama, and K. Isono. 1987. The physical map of the whole *E. coli* chromosome: Application of a new strategy for rapid analysis and sorting of a large genomic library. *Cell* **50:** 495–508.
19. Irani, M.H. and P.K. Maitra. 1977. Properties of *Escherichia coli* mutants deficient in enzymes of glycolysis. *J. Bacteriol.* **132:** 398–410.
20. Ye, S. and T.J. Larson. 1988. Structures of the promoter and operator of the *glpD* gene encoding aerobic *sn*-glycerol-3-phosphate dehydrogenase of *Escherichia coli* K-12. *J. Bacteriol.* **170:** 4209–4215.
21. Kogan, S., M. Doherty, and J. Gitschier. 1987. An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. *N. Engl. J. Med.* **317:** 985–990.
22. Zwaig, N., W.S. Kistler, and E.C.C. Lin. 1970. Glycerol kinase, the pacemaker for the dissimilation of glycerol in *Escherichia coli*. *J. Bacteriol.* **102:** 753–759.
23. Austin, D. and T.J. Larson. 1991. Nucleotide sequence of the glpD gene encoding aerobic *sn*-glycerol phosphate dehydrogenase of *Escherichia coli* K-12. *J. Bacteriol.* **173:** 101–107.
24. Berg, D.E., A. Weiss, and L. Crossland. 1980. Polarity of Tn*5* insertion mutations in *Escherichia coli*. *J. Bacteriol.* **142:** 439–446.
25. Lupski, J.R., S.J. Projan, L.S. Ozaki, and G.N. Godson. 1986. A temperature-dependent pBR322 copy number mutant resulting from a Tn*5* position effect. *Proc. Natl. Acad. Sci.* **83:** 7381–7385.
26. Nesin, M., J.R. Lupski, and G.N. Godson. 1988. Role of the 5′ upstream sequence and tandem promoters in regulation of the *rpsU-dnaG-rpoD* macromolecular synthesis operon. *J. Bacteriol.* **170:** 5759–5764.
27. Pearson, W.R. and D.J. Lipman. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci.* **85:** 2444–2448.

Received August 8, 1991; accepted in revised form October 31, 1991.