

Transcriptionally Active Polymerase Chain Reaction (TAP)

HIGH THROUGHPUT GENE EXPRESSION USING GENOME SEQUENCE DATA*

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An approach is described for making transcriptionally active PCR (TAP) fragments that were used directly in *in vitro* and *in vivo* expression experiments. TAP fragments encoding reporter genes were amplified in 1 day using typical PCR methodology and were expressed in cultured cells and in mice at levels comparable with a widely used cytomegalovirus promoter-based plasmid expression vector. Following intramuscular injection, a TAP fragment encoding hepatitis B surface antigen (HBsAg) induced anti-HBsAg antibody titers comparable with those induced by supercoiled plasmid encoding the same antigen. Epitope-tagged TAP fragments were generated and transfected into cells for rapid, high throughput immunocytochemical analysis of the tagged gene products. TAP fragments were also transferred directly into expression vectors by *in vivo* homologous recombination without conventional cloning, affording a high throughput cloning approach that does not require restriction enzyme digestion, ligations, or thymidine adenine complementation cloning. The methodology has been adapted to a robotic work station enabling the high throughput generation of transcriptionally active genes at the rate of more than 400 different genes per day. This technology offers a practical approach to directly utilize genome sequence data to generate functional proteomes.

Worldwide genome sequencing projects have now yielded complete DNA sequence data for microorganisms, insects, plants, and human and other mammalian genomes. Presently, the exponential increase in genome sequence information has not led to a similar increase in the availability of functional genes or proteins that are encoded by these sequenced genes, and consequently, the biomedical and pharmaceutical research communities have not yet been able to take full advantage of all the sequence data. Today, functional genomics research demands an increasing number of sequenced genes with unknown function to be expressed.

By far the most widely used approach to producing transcriptionally active genes is to clone them into a plasmid expression vector, transform and grow bacteria, and purify the plasmids. This tried and true approach has worked well for many years, but it is time- and labor-intensive, particularly when a large

number of genes needs to be simultaneously cloned in a transcriptionally active form. Large numbers of PCR fragments can be amplified on the required scale, but they are not transcriptionally active. The concept of developing an approach to making transcriptionally active PCR fragments has been published previously (1, 2), but this method has not yet been put widely into practice.

Here we describe a robust and practical approach for producing potent transcriptionally active PCR (TAP)¹ fragments in two sequential PCR reactions. The first step uses gene-specific primers to amplify the gene of interest. The second “nested” PCR step uses a mixture of DNA fragments to append promoter and terminator elements to this fragment. TAP fragments are as active as supercoiled pcDNA 3.1 plasmid in *in vitro* and *in vivo* transfection assays, and they can be used as DNA vaccines to induce *in vivo* immune responses in mice against the encoded antigen. TAP fragments can also be transferred rapidly into plasmid vectors by homologous recombination (3, 4), affording a high throughput cloning method that does not require the use of restriction enzymes or ligations. Both of these procedures can be applied to generate hundreds or thousands of transcriptionally active genes on a scale that is required today for functional genomics research.

EXPERIMENTAL PROCEDURES

Oligonucleotides and Reagents—All of the oligonucleotides used in this study were synthesized by Genset Corp. (La Jolla, CA). The PCR reactions were carried out using the Advantage cDNA PCR kit from CLONTECH Laboratories Inc. (Palo Alto, CA). Fluorescein anti-HA antibody was purchased from Roche Molecular Biochemicals. GenePORTER transfection reagent was from Gene Therapy Systems, Inc. (San Diego, CA).

Generation of Transcriptionally Active PCR Fragments—This method is comprised of two sequential PCR steps. The first step was carried out using primers (0.4 μ g each) containing universal TAP ends and sequences specific to the target gene. The 5' universal end sequences were complementary to the DNA fragment containing the CMV immediate early gene promoter and a shortened intron A with or without N-terminal HA tag. The fragments were derived from plasmids pCMV_m and pCMVmHA (Gene Therapy Systems, Inc.) and used in the second PCR step (see below) to attach the CMV promoter and epitope tag to the amplified gene. The 3' universal end overlapped with a DNA fragment that contained the SV40 early gene transcription terminator and was also used in the second-step PCR to attach the transcription terminator sequence to the amplified gene. To successfully generate the final TAP fragment, it is crucial for the design of the overlapping sequence to take into account that some thermal stable DNA polymerases (*e.g.* *Taq*) tend to add an extra “A” to the end of (first) PCR product. This was achieved by always having a “T” as the base immediately up stream of the overlapping region. To complete the synthesis

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¹ The abbreviations used are: TAP, transcriptionally active PCR; HA, hemagglutinin; CMV, cytomegalovirus; oligo, oligonucleotide; CAT, chloramphenicol acetyltransferase; ELISA, enzyme-linked immunosorbent assay; GFP, green fluorescent protein; CHO, Chinese hamster ovary; HBsAg, hepatitis B surface antigen.

of each oligo, 15–20 bp from the 5' end of the gene-specific sequence starting with ATG was added to the 5'-TAP oligos and 15–20 bp from the 3' end of gene specific sequence starting from the stop codon was added to the 3'-TAP oligo.

The 5'- and 3'-TAP universal sequences were as follows: 5'-TAP oligo = 5'-CTGCAGGCACCGTCGCTCGACTTAACA- (15–20 bp gene-specific sequence starting from ATG as described above); 5'-TAP oligo for HA tagging = 5'-ACGATGTTCCGGATTACGCTAGCCTCCCAGTT- (15–20 bp gene-specific sequence starting from ATG as described above); 3'-TAP oligo = 5'-CATCAATGTATCTTATCATGTCTGA- (15–20 bp gene-specific sequence starting from stop codon complementary sequence as described above).

The conditions for the first PCR step were denaturation at 94 °C for 1 min followed by 28 cycles of two-temperature PCR (30 s at 94 °C and 3 min at 68 °C). The PCR product was verified by 0.8% agarose gel electrophoresis and used as a DNA template for the subsequent second PCR step. The second PCR step was performed for 30 cycles with 30 s of denaturing at 94 °C, 30 s annealing at 60 °C, and 3 min extension at 68 °C. After verification by gel electrophoresis, the PCR product was used directly for transfection into cultured cells *in vitro*, or it was ethanol-precipitated and resuspended in saline to a final concentration of 1 mg/ml for injection into animals.

Plasmid Constructs—The plasmid pcDNA3.1 was purchased from Invitrogen. The CAT reporter gene was transferred into pcDNA3.1 from gWIZ-CAT (Gene Therapy Systems, Inc.). The TAP cloning vector was constructed by cloning TAP promoter and TAP terminator sequences into *BalI*- and *XmnI*-restricted gWIZ blank vector with a single *BglII* site separating the two. The linear TAP cloning vector was prepared by cutting with *BglII* followed by treatment with calf intestine phosphatase.

Gene Delivery and Expression—*In vitro* transfection was done using GenePORTER (Gene Therapy Systems, Inc.) following the procedures recommended by the manufacturer. For intramuscular injection, 30 μ l of DNA solution was injected into the tibialis muscle of restrained, awake mice using a disposable, plastic insulin syringe (Becton Dickinson, Franklin Lakes, NJ). Muscles were collected at different time points post-injection, immediately frozen, and stored at –80 °C until use. Animal care throughout the study was in compliance with the “Guide for the Use and Care of Laboratory Animals” National Research Council (19). CAT reporter gene expression was assayed using a CAT ELISA kit from Roche Molecular Biochemicals (catalog No. 1-363-727). Expression of GFP was detected by epifluorescence microscopy.

RESULTS

The promoter and terminator sequences used for this application were derived from an optimized, potent CMV IE gene promoter/enhancer plus a shortened and modified intron from the same gene and a transcription termination element from SV40, respectively. This very potent promoter/terminator combination was able to produce PCR fragments that retained substantial potency, comparing favorably with the widely used supercoiled CMV vector, pcDNA 3.1. Using these elements as a starting point, a universal method for creating TAP fragments encoding any gene-of-interest was devised having three steps: (i) custom oligo design; (ii) TAP primary fragment amplification; and (iii) TAP expression fragment amplification (Fig. 1A). A gel presenting typical examples of the TAP primary and TAP expression fragments is shown in Fig. 1B. Genes encoding hepatitis B surface antigen, CAT, and GFP were amplified with custom oligos to produce the corresponding primary TAP fragments for each gene. This amplification step yielded a single band with the expected molecular weight for each gene. The primary TAP fragments were amplified in the presence of the promoter and terminator sequences to produce the TAP expression fragments. This second amplification added 1050 nucleotides to the TAP primary fragment yielding a single band on the gel (Fig. 1B).

The *in vitro* transfection activities of the TAP fragments shown in Fig. 1B were compared with supercoiled plasmids expressing the same genes (Fig. 2). The plasmids were derived from pcDNA 3.1, a widely used commercial expression vector based on CMV IE gene promoter. The appearance and intensity of CHO-K1 cells transfected with either the TAP-GFP fragment

or supercoiled pcDNA3.1-GFP were identical (Fig. 2, A and B), and similar results were obtained with COS-7 cells (Fig. 2, C and D). COS-7 cells transfected with either the TAP-CAT fragment or supercoiled pcDNA3.1-CAT gave similar levels of CAT reporter gene product (Fig. 2E). Similarly, COS-7 cells transfected with a TAP fragment encoding hepatitis B surface antigen (HBsAg) gave transfection levels comparable with supercoiled pcDNA3.1-HBsAg plasmid (Fig. 2F). These results show no significant difference in the level of *in vitro* transfection activity between TAP fragments and supercoiled pcDNA 3.1 plasmid.

To determine whether the TAP fragments could be used *in vivo*, the TAP-CAT fragment or the supercoiled pcDNA3.1-CAT plasmid was injected into mouse tibialis muscles, and the muscles were assayed for CAT activity 8 days post-injection. The CAT activity recovered from the animals injected with pcDNA3.1-CAT plasmid was similar to that obtained from the TAP-CAT injected animals (Fig. 2G). To determine whether TAP fragments could be used in the context of a DNA vaccine, a TAP-HBsAg fragment was injected into mouse tibialis muscle, and the sera were analyzed for HBsAg antibodies 8 weeks post-injection. The TAP-HBsAg fragment produced antibody titers similar to pcDNA3.1 plasmid (Fig. 2H).

The TAP system makes it possible to rapidly express a large number of different proteins from sequenced genomes even when the identities and function of the proteins are unknown. In most of these cases antibodies will not be available for identification and purification. To circumvent this problem, recombinant proteins containing oligopeptide epitopes can be expressed. The HA epitope tag (YPYDVPDYA) is well characterized and highly immunoreactive (5). After transfection of this epitope-tagged TAP fragment into cells, the resulting HA-tagged proteins can be identified with commercially available anti-HA antibodies. The epitope tag is also useful for facilitating purification of the protein, identifying associated proteins, characterizing new proteins by immunoprecipitation, and determining subcellular localization (6–8). An HA epitope-CAT fusion gene was generated using the TAP system with a modified 5' promoter fragment encoding the HA epitope. The results are shown in Fig. 3. The HA-CAT fusion protein could be detected in cultured cells by using an anti-HA fluorescent antibody, and fusion of HA to CAT did not adversely effect its ability to be expressed in transfected cells, as indicated by the CAT ELISA (Fig. 3, A–C).

Once a gene with a specified function is identified using TAP technology, cloning it into a plasmid vector may make further characterization and manipulation more convenient. The cloning of TAP fragments can be achieved readily using an approach that is more efficient and simplified than the conventional cut-and-ligate or thymidine adenine complementation cloning methods. This is because the final PCR fragments generated by the TAP protocol contain universal 5' (TAP promoter) and 3' (TAP terminator) sequences. When co-transformed into bacteria with a linearized plasmid vector containing complementary ends, the TAP PCR fragment can be inserted into the vector catalyzed by endogenous *Escherichia coli* recombinases (3). We tested the efficiency of this *in vivo* recombination reaction by making a linear plasmid with TAP promoter and terminator sequences at each end and co-transfecting the vector together with a TAP fragment encoding CAT reporter gene into *E. coli* by electroporation. Restriction analysis of DNA preparations showed that more than 80% of antibiotic resistant colonies carried the recombinant plasmid with the CAT-encoding TAP fragment inserted in the correct orientation (Fig. 3D). Further functional analysis indicated that

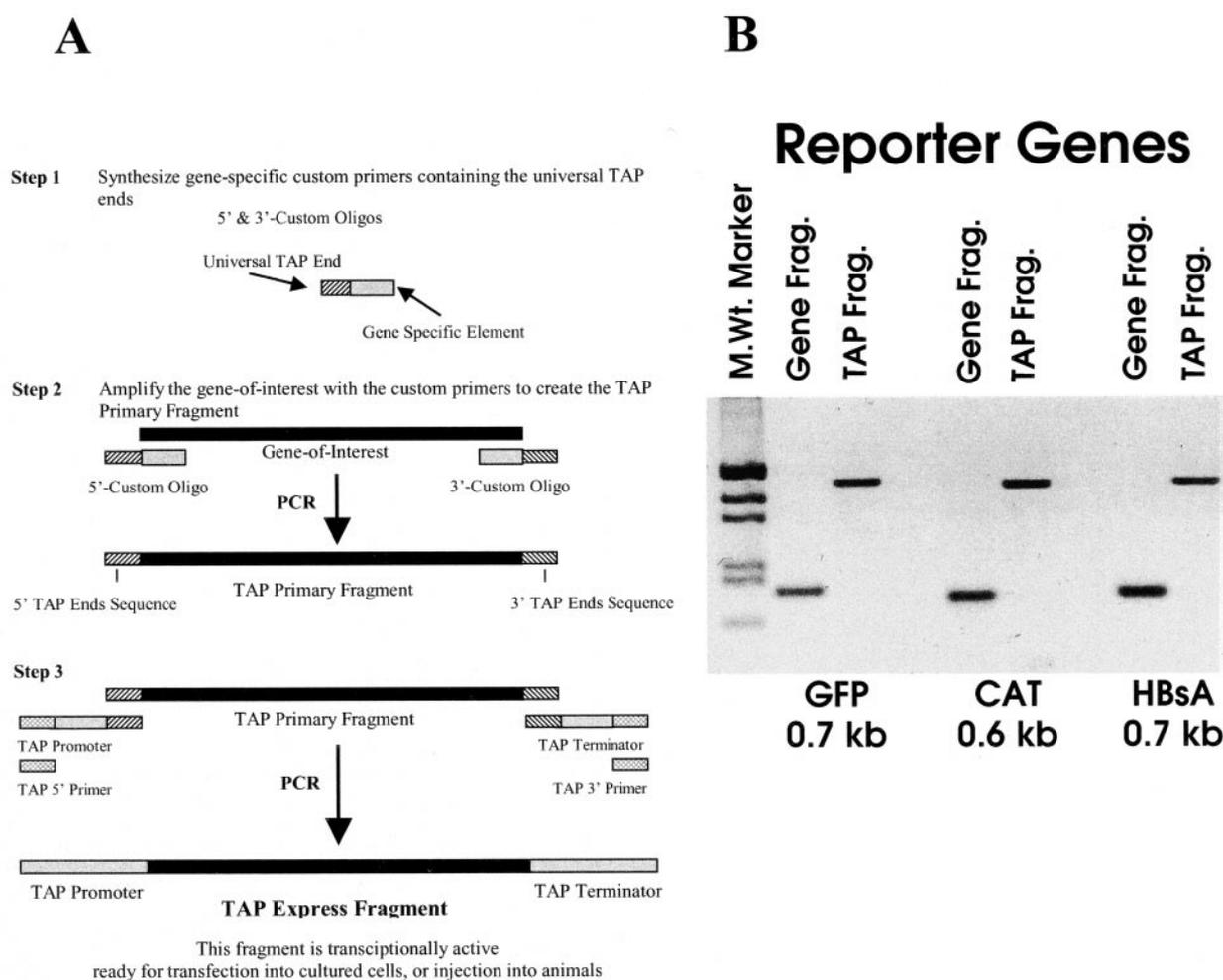


FIG. 1. A, summary of the TAP process. *Step 1*, obtain oligonucleotide primers to amplify the gene-of-interest. All of the oligonucleotides used in this study were synthesized by Genset Corp. Purpose: to design and construct gene-specific 5'- and 3'-custom oligos that also contain the 5'- and 3'-TAP ends. Primers complementary to the 5' and 3' ends of the gene of interest are synthesized. The 5'-custom oligo contains between 41 and 46 nucleotides; of these, 26 nucleotides comprise the 5'-TAP end universal sequence, and the other 15–20 nucleotides make up the gene-specific sequence. The 5'-oligo also incorporates the Kozak consensus sequence (A/GCCAUG) around the start codon for more efficient translation of mRNA (18). The 3'-custom oligo contains a minimum of 40 nucleotides; of these, 20 comprise the 3'-TAP end universal sequence, and the other 20 nucleotides are specific to the gene-of-interest. A complementary stop codon sequence, such as TCA or TTA, is added to the end of the gene sequence to achieve proper translational termination of the expressed gene. *Step 2*, amplify the gene-of-interest with the custom oligos to produce the TAP primary fragment. Purpose: to add the 5'- and 3'-TAP end sequences to the gene-of-interest. This step generates a DNA fragment that contains the gene-of-interest with the added 5'- and 3'-TAP universal end sequences. These 5'- and 3'-TAP end sequences are necessary for adding the TAP promoter and terminator fragments in the final step. *Step 3*, mix the promoter and terminator fragments with the TAP primary fragment and amplify using the universal primers. Purpose: to append TAP promoter and TAP terminator sequences to the gene-of-interest by nested PCR. This step generates a DNA fragment that contains the gene-of-interest flanked by promoter and terminator sequences, which is transcriptionally active and can be used directly for *in vitro* or *in vivo* expression studies. B, generation of TAP fragments encoding reporter genes or various antigens. DNA templates encoding GFP, CAT, or hepatitis B surface antigen were amplified with custom oligos specific to each gene, yielding TAP primary fragments containing the 5'- and 3'-TAP ends followed by secondary PCR to add TAP promoter and terminator elements to generate final transcriptionally active PCR fragments expressing each gene. The promoter fragment adds 850 bp, and the terminator fragment adds 200 bp, and therefore the final product is 1050 bp larger than the primary fragment. Primary and corresponding final TAP express fragments for each gene were run next to each other on agarose gels to verify the appropriate size.

those recombinant plasmids were able to express CAT activity after transfection into COS-7 cells (data not shown).

DISCUSSION

Worldwide genome sequencing projects have yielded complete DNA sequence data for microorganisms, insects, plants, and human and other mammalian genomes, but the functions of the majority of proteins encoded by these sequenced genes are unknown. The exponential increase in genome sequence information has not yet led to a similar increase in the availability of new proteins that are encoded by these sequenced genes, and consequently, the biomedical and pharmaceutical research communities have not yet been able to fully take practical advantage of all the sequence data. Usually the first step toward understanding gene function is to get it into a

transcriptionally active form so that the gene product can be expressed. Conventional cloning methodology is the standard way to accomplish this objective, but these established methods cannot keep pace with the sequencing activities. With the PCR-based approach described here, it is feasible to generate hundreds or thousands of transcriptionally active genes in a single day, a pace that is appropriate to the task.

Several papers have recently appeared showing that PCR fragments containing promoter and terminator elements are transcriptionally active (1, 2, 9, 10). The observation that linear DNA fragments can be transcriptionally active was surprising to some investigators because the literature contains references reporting that supercoiling is required for efficient expression from plasmids (11–15). If supercoiling is indeed re-

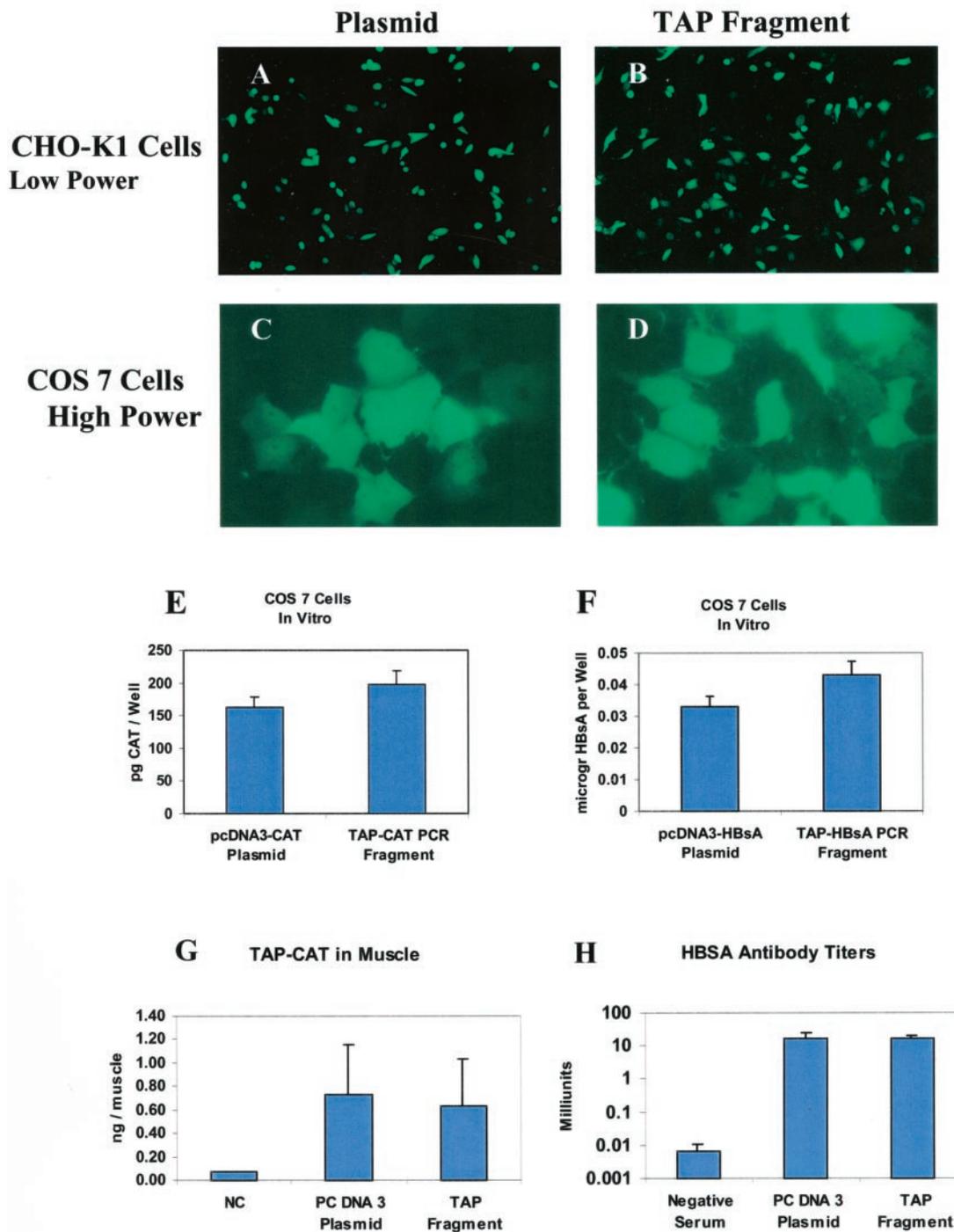
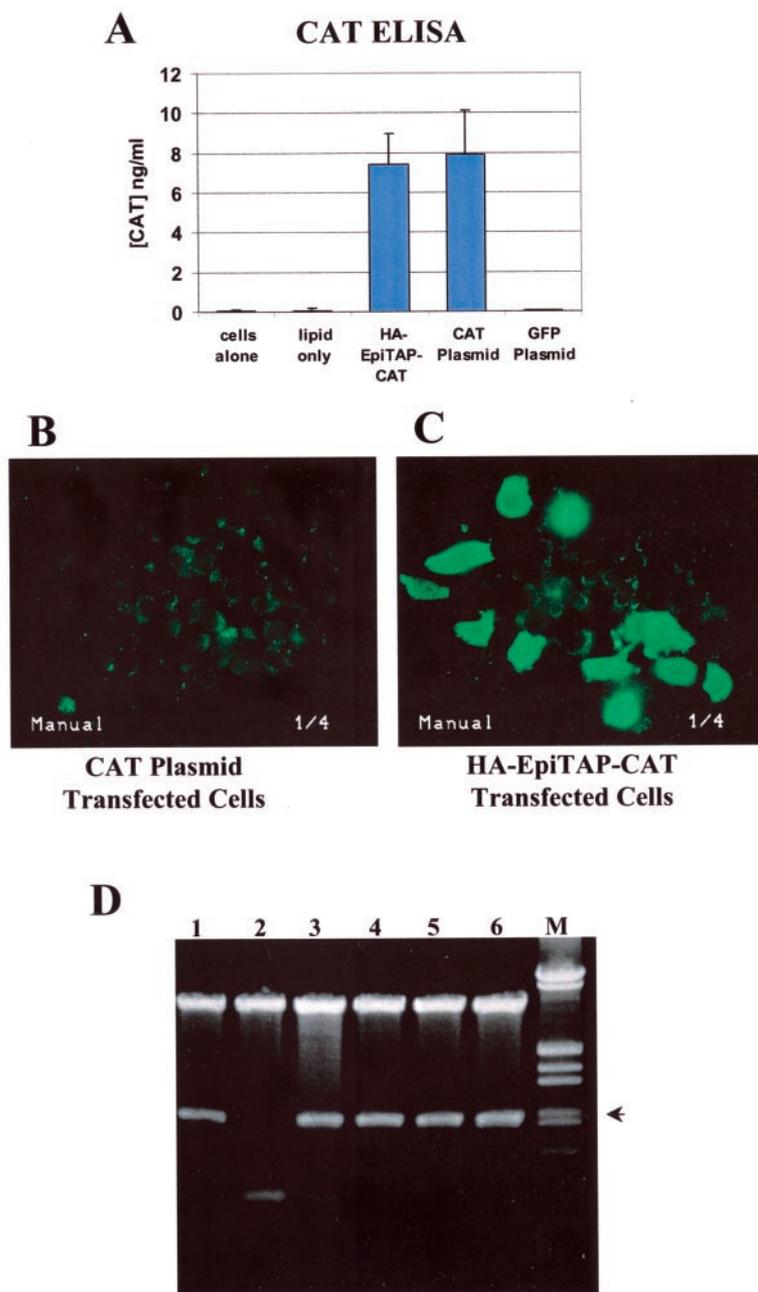


FIG. 2. *In vitro* and *in vivo* expression of TAP fragments compared with supercoiled plasmid constructs. TAP fragments encoding GFP, CAT, and HBsA was amplified from plasmids encoding the corresponding reporter genes as described in Fig. 1. *A–D*, 60,000 CHO-K1 cells were plated 1 day before transfection onto 22-mm coverslips in a 35-mm-diameter dish. The cells were transfected in serum-free OptiMEM with 1 μ g of TAP-GFP DNA and 5 μ l of GenePORTER transfection reagent. GenePORTER transfection reagent was from Gene Therapy Systems Inc. At 4 h post-transfection, 10% serum was added, and 2 days later the coverslips were inverted onto hanging-drop slides and examined by fluorescence microscopy. The images compare the transfection efficiency of the TAP express fragment and supercoiled pcDNA 3.1 plasmid. The percentage of transfected cells in the TAP express transfected cells was identical to that of the plasmid transfected cells. *E* and *F*, COS-7 cells were transfected with GenePORTER using either TAP express fragments or supercoiled pcDNA 3.1 plasmids encoding CAT or HBsAg. The expression levels obtained with the TAP express fragments were comparable with those obtained with the pcDNA3-CAT plasmid. *G*, groups of five mice were injected in the tibialis muscle with negative control saline (NC), 30 μ g of TAP-CAT express fragment, or 30 μ g of pcDNA3.1-CAT in saline. Eight days post-injection the animals were sacrificed, and the injected muscles were homogenized and assayed for CAT activity. The data show that the level of CAT enzyme activity recovered from the muscles injected with the TAP-CAT fragment was comparable with that obtained with the pcDNA3-CAT plasmid. *H*, animals were injected with saline only and 30 μ g of DNA encoding the hepatitis B surface antigen in the pcDNA3 plasmid or as a TAP fragment. Animals were boosted at 2 weeks, and sera were analyzed for antibody at 8 weeks.

quired for transcription, then one would not expect linear PCR fragments to be active. In fact the results from all of these older references show that although expression from linearized plas-

mid is lower than supercoiled plasmid, it is far from completely inhibited. Expression is usually only reduced to about 30% of the supercoiled plasmid level. Some of the papers that make

FIG. 3. Expression in CHO-K1 cells of HA epitope-tagged TAP-CAT fragments and cloning of TAP fragments by *in vivo* recombination in *E. coli*. *A*, shown are the results of a CAT ELISA performed in duplicate on CHO-K1 cell lysates. Cells were transfected with either HA-epiTAP-CAT fragments, CAT plasmid, or GFP plasmid genes. CAT ELISAs were performed 48 h post-transfection. Only the cells that were transfected with the CAT gene were positive in this assay, and there was no difference between the cells transfected with the CAT plasmid or HA-epiTAP-CAT fragments. *B* and *C*, for HA immunocytochemistry, CHO-K1 cells were transfected with either the regular CAT plasmid or the epiTAP-CAT (HA) fragments and incubated for 48 h. Cells were probed with anti-HA-fluorescein, and images were taken on a fluorescence microscope. Lipid-treated and untreated cells, included as controls, showed only background fluorescence similar to the CAT plasmid transfected cells (data not shown). Fluorescein anti-HA antibody was purchased from Roche Molecular Biochemicals. *D*, PCR-amplified TAP fragment encoding CAT reporter gene (1 μ g) was mixed directly with a linear cloning vector (0.2 μ g) containing identical ends and electroporated into the electrocompetent *E. coli* strain JC8679 followed by selection on LB agar plate containing 100 μ g/ml kanamycin. Miniprep DNA samples were isolated from six randomly selected colonies, restricted using *Pst*I, and analyzed by agarose gel electrophoresis. Clones containing the TAP insert yielded a band around 800 bp (lanes 1 and 3–6 indicated by the arrow), whereas the background blank vector showed a 200-bp fragment (lane 2).



this observation attribute the differences in transfection activity to torsional effects on the supercoiled plasmid, which open up the duplex and enable proteins of the transcription complex to better interact with the promoter/enhancer sequences, leading to higher levels of expression. Another interpretation is that the supercoiled plasmid is more resistant to nuclease digestion. Regardless of the actual mechanism by which linear plasmid is less active than supercoiled plasmid, a level of expression equaling $\sim 30\%$ of supercoiled plasmid is sufficient to be useful in functional expression assays. A paper by Sykes and Johnston (1) described an approach for producing transcriptionally active linear DNA fragments. This approach is based on their finding that amplified PCR fragments can be rendered transcriptionally active by hybridizing promoter and terminator sequences to them. PCR primers were chosen so that their termini would be complementary with two additional PCR fragments encoding promoter and terminator sequences. When the complementary promoter, coding, and terminator

fragments were mixed, they spontaneously hybridized to form "linear expression elements" that could be transfected into cultured cells or injected into animals leading to expression of the coding sequence. More recent publications have shown that PCR fragments can be generated that are nearly as transcriptionally active as supercoiled plasmid (1, 2, 9, 10).

The approach described here to making linear PCR fragments that are transcriptionally active at levels comparable with supercoiled plasmid will be a practical tool to facilitate functional screening of genomic or cDNA libraries and it can be readily adapted to a robotic workstation. There are many ways to apply the TAP system in functional genomics research. Genes encoding all of the antigens from complex microorganisms such as tuberculosis (*Mycobacterium tuberculosis*), anthrax (*Bacillus anthracis*), and malaria (*Plasmodium falciparum*) can be amplified and expressed. The TAP express fragments can be used directly *in vivo* to evaluate their ability to stimulate protective immunity, or the gene products can be

used to screen for the presence of antigen-specific antibody in immunized individuals. The epitope-tagged TAP fragments encoding large numbers of different genes can be transfected individually into cultured cells and their intracellular localizations determined even without having any knowledge of their function. For example, from a family of sequenced genes, it would be straightforward to determine which gene products are localized in the plasma membrane, the mitochondria, or the nucleus. TAP fragments can be generated that contain the T7 promoter so that either messenger RNA or protein can be produced in a cell-free system. TAP cloning also enables hundreds of amplified genes to be transferred rapidly into conventional plasmid expression vectors in the correct orientation. For example, with one 96-well PCR machine, 96 TAP fragments encoding different genes can be amplified in a single day, and the fragments can be mixed with bacteria containing the complementary vector. The next day, colonies can be selected and analyzed for insertion of the genes-of-interest. In this way a high throughput cloning activity involving hundreds of different genes, which would have previously taken many months to complete, can be reduced to a project taking a few days. TAP will also enable the effect of polymorphisms to be compared readily in functional assays so that the function of different variants can be rapidly compared with each other. In addition to pointing the way toward an improved method for identifying immunologically active antigens in complex organisms, an approach using transcriptionally active PCR fragments may find broader uses as a genomics tool to help elucidate the function of uncharacterized genes. It can be used to produce antibodies against partial or full-length proteins without the need for cloning, expression, and purification, and it allows the con-

struction of peptide or epitope fusions for rapid characterization of genes according to their subcellular locations. Ultimately, chemically modified PCR fragments may replace plasmids in synthetic gene delivery systems for gene therapy applications (16, 17).

REFERENCES

1. Sykes, K. F., and Johnston, S. A. (1999) *Nat. Biotechnol.* **17**, 355–359
2. Felgner, P. L., and Liang, X. (1999) *Nat. Biotechnol.* **17**, 329–330
3. Oliner, J. D., Kinzler, K. W., and Vogelstein, B. (1993) *Nucleic Acids Res.* **21**, 5192–5197
4. Zhang, Y., Muyrers, J. P., Testa, G., and Stewart, A. F. (2000) *Nat. Biotechnol.* **18**, 1314–1317
5. Niman, H. L., Thompson, A. M., Yu, A., Markman, M., Willems, J. J., Herwig, K. R., Habib, N. A., Wood, C. B., Houghten, R. A., and Lerner, R. A. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 7924–7928
6. Field, J., Nikawa, J., Broek, D., MacDonald, B., Rodgers, L., Wilson, I. A., Lerner, R. A., and Wigler, M. (1988) *Mol. Cell. Biol.* **8**, 2159–2165
7. Laminet, A. A., Apell, G., Conroy, L., and Kavanaugh, W. M. (1996) *J. Biol. Chem.* **271**, 264–269
8. Smith, S., and Blobel, G. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 10124–10128
9. Li, S., Brisson, M., He, Y., and Huang, L. (1997) *Gene Ther.* **4**, 449–454
10. Hoffman, C. R., Dileo, J. P., Li, Z., and Huang, L. (2001) *Gene Ther.* **8**, 71–74
11. Cherng, J., Schuurmans-Nieuwenbroek, N. M., Jiskoot, W., Talsma, H., Zuidam, N. J., Hennink, W. E., and Crommelin, D. J. (1999) *J. Controlled Release* **60**, 343–353
12. Xie, T. D., and Tsong, T. Y. (1993) *Biophys. J.* **65**, 1684–1689
13. Weintraub, H., Cheng, P. F., and Conrad, K. (1986) *Cell* **46**, 115–122
14. Ehrlich, R., Larousse, A., Jacquet, M. A., Marin, M., and Reiss, C. (1985) *Eur. J. Biochem.* **148**, 293–298
15. Hanahan, D., Lane, D., Lipsich, L., Wigler, M., and Botchan, M. (1980) *Cell* **21**, 127–139
16. Zelphati, O., Liang, X., Nguyen, C., Barlow, S., Sheng, S., Shao, Z., and Felgner, P. L. (2000) *BioTechniques* **28**, 304–316
17. Zanta, M. A., Belguise-Valladier, P., and Behr, J. P. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 91–96
18. Kozak, M. (1987) *Nucleic Acids Res.* **15**, 8125–8148
19. Institute of Laboratory Animal Resources, National Research Council (1996) *Guide for the Use and Care of Laboratory Animals*, National Academy Press, Washington, DC

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