Benchmarks

Vectors for Dual Expression of Target Genes in Bacterial and Mammalian Cells

BioTechniques 29:476-480 (September 2000)

For protein production and purification, many different cell systems ranging from bacteria to mammalian cell lines are commonly used. High-level expression of recombinant proteins in *E. coli* is often achieved using the strong yet tightly regulatable T7 promoter system (23). Many convenient T7 promoter-based bacterial expression vectors are commercially available from different sources and are widely used. For the expression of genes in mammalian cells, many different expression systems also exist, with the cytomegalovirus (CMV) immediate early enhancer/promoter-based expression vectors (4) being among the most common. Some of these mammalian expression vectors also carry a T7 promoter to facilitate the in vitro transcription/translation of inserted genes.

To generate vectors with the capacity to overcome the species barrier and to function in bacterial and mammalian cells, expression systems have been developed that use a T7 promoter in combination with a modified T7 RNA polymerase that is targeted to the nucleus of mammalian cells (2,12). Besides bacterial expression of target genes from these vectors, co-transfection of the expression vector and the vector carrying the modified T7 RNA polymerase gene leads to a strong expression of target genes in mammalian cells. A related strategy that relies on a combination of a phage promoter and an internal ribosomal binding site (IRES) derived from the encephalomyocarditis virus (EMCV) has also been used to express target genes in *E. coli* and mammalian cells using a single vector (8). Forman and Samuels (5) have combined the Rous sarcoma virus long terminal repeat (RSV-LTR) and the T7 promoter to generate a family of expression vectors (pEXPRESS) that allows expression of target genes in both eukaryotic and prokaryotic cells (independent of a modified T7 RNA polymerase), as well as translation of these genes in a cell-free system. Addi-

tionally, a single expression plasmid has been developed that allows protein expression in mammalian cell lines and in the yeast *Pichia pastoris* (16).

To combine the T7 promoter-based bacterial expression system with one of the strongest and most widely used CMV promoter/enhancer-based mammalian expression systems (15), we have established an easy procedure for the generation of expression vectors with the capacity to function in both bacterial and mammalian cells. Our strategy relies on the generation of a strong bacterial ribosomal binding site (*rbs*) downstream of the T7 promoter of a mammalian expression vector, in combination with a Kozak consensus sequence (9–11) for optimal translation initiation in mammalian cells. To illustrate this strategy, we have established a dual expression system amplifying the EGFP gene (Clontech Laboratories, Palo Alto, CA, USA) using PCR (1) and inserted it into the *NheI* and *XhoI* sites of the CMV promoter-based mammalian expression vector pCI (Promega, Madison, WI, USA) downstream of its T7 promoter (Figure 1B). pCI also contains an intron sequence for increased expression levels in mam-

Figure 1. Illustration of primers and plasmid control regions. (A) Primers used for the amplification of the EGFP coding region introduce the translational control regions and additional restriction sites for the generation of a dual-system expression vector. (B) Schematic representation of the transcription/translation control region of pCI-EGFP-dual and pCI-HisDFF40. All constructs carry an *rbs* in combination with a Kozak consensus sequence. The control region of pCI-EGFP-dual was constructed by PCR using primers that introduce the prokaryotic *rbs* and the Kozak consensus sequence during amplification of the EGFP gene. A coding region for a C-terminal His₆ tag was also introduced by PCR to allow facilitated purification of EGFP. pCI-HisDFF40 was constructed by subcloning an *XbaI/NotI* fragment from pET28-HisDFF40 (G. Meiss, unpublished data) into the eukaryotic expression vector pCI as described in the text.

Benchmarks

malian cells (17). The EGFP gene was amplified using *Taq* DNA polymerase (Promega) under standard PCR conditions (94°C for 2 min, 25 cycles of 94°C for 15 s, 58°C for 30 s, 72°C for 45 s and 72°C for 5 min) using the forward primer 5'-EGFPdual (Figure 1A) such that a bacterial *rbs* and a Kozak consensus sequence were created upstream of the EGFP coding region.

To facilitate the purification of EGFP, a coding sequence for a His₆ affinity tag was engineered downstream of the EGFP gene using the reverse primer 3'-EGFPdual (Figure 1A). This strategy is of general applicability, as shown in Figure 1B, in which we have transferred the DFF40/CAD-gene (3,7,13,14) previously cloned into the *Nde*I and *Sal*I sites of pET28a(+) (Novagen,

Madison, WI, USA) (G. Meiss, unpublished data), using the *Xba*I and *Not*I sites of this vector, into the mammalian expression vector pCI, resulting in pCI-HisDFF40 (Figure 1B). In addition to the bacterial *rbs*, which is needed for bacterial expression of target genes, pET28a(+) also provides a core Kozak consensus sequence (ACCATGG) for efficient translation initiation in mammalian cells, arising from an adenine directly upstream of the *Nco*I site providing the ATG start codon (Figure 1B).

For bacterial expression, *E. coli* BL21Gold(DE3) cells, which contain the T7 RNA polymerase gene under control of the lacUV promoter, were transformed with pCI-EGFPdual or pCI-HisDFF40, (Figure 1B), respectively, and cultured in LB broth at 37°C

to an OD₆₀₀ of 0.8. Because the DFF40 gene can only be expressed in the presence of DFF45 (3,6,14,18–20,22), the BL21Gold(DE3) cells also contained a second compatible plasmid, pACET-DFF45 (G. Meiss, unpublished data), a derivative of pACYC that provides chloramphenicol resistance and bears a pA15 origin of replication, coding for the DFF45 gene. Induction was achieved by adding isopropyl-β-D-thiogalactoside (IPTG) to a final concentration of 1 mM and culturing cells at 30°C overnight. The bacterial pellet of a 3-mL culture was washed with 1× STE buffer (10 mM Tris-HCl, pH 8.0, 50 mM NaCl and 1 mM EDTA), resuspended in 0.5 mL buffer A (20 mM HEPES, pH 7.4, 100 mM NaCl, 1 mM EDTA and 0.01% CHAPS) and lysed by sonication. After centrifugation at 30 000× *g*, the supernatant was applied to a spin column containing 0.2 mL Ni²⁺-NTA resin. The resin-bound protein was washed three times with 1 mL buffer A containing 20 mM imidazole. Elution was performed using 200 μL buffer A supplemented with 200 mM imidazole. For the expression of the new constructs in a mammalian system, 7.5 × 10⁵ human embryonic kidney 293 cells were transfected with pCI-EGFPdual or co-transfected with pCI-HisDFF40 and pCI-c-mycDFF45 (G. Meiss, unpublished data) using TransFast™ transfection reagent (Promega) according to the manufacturer's instructions. Twenty-four to forty-eight hours after transfection, cells were resuspended in buffer B (20 mM HEPES, pH 8.0, 400 mM KCl, 2 mM DTT and 20% glycerol) and lysed by four freeze/thaw cycles in liquid nitrogen. After centrifugation at 30 000× *g*, the supernatant was applied to a Ni²⁺-NTA spin column and purified as described above.

Using these constructs, EGFP and DFF40/CAD were expressed in 293 cells and in *E. coli* BL21Gold(DE3) cells (Stratagene, La Jolla, CA, USA) (Figure 2, A and B). Approximately 150 μg purified EGFP were obtained from 3 × 10⁶ 293 cells. Although the EGFP gene sequence is optimized for expression in cell lines of human origin (24), approximately 2 mg EGFP could be purified from 1 L *E. coli* BL21 Gold (DE3) cells transformed with pCI-EGFP-dual. Because the constructs

Figure 2. Expression of DFF40 and EGFP in bacteria and 293 cells. (A) Coomassie-stained SDS-polyacrylamide gel that demonstrates expression of DFF40 and EGFP in *E. coli* BL21Gold(DE3), transformed with pACET-DFF45 and the dual expression vectors pCI-HisDFF40, as well as pCI-EGFP-dual. M, marker; 1, fraction corresponding to 15 μL whole cell lysate of a 3-mL culture of BL21Gold(DE3) carrying pACET-DFF45 and pCI-HisDFF40 before induction; 2, fraction corresponding to 15 μL whole cell lysate after induction with 1 mM final concentration of IPTG; 3, fraction corresponding to 40 μL purified DFF40; 4, fraction corresponding to 15 μL whole cell lysate of a 3-mL culture of BL21Gold(DE3) carrying pACET-DFF45 and pCI-EGFP-dual before induction; 5, fraction corresponding to 15 μL whole cell lysate after induction with 1 mM final concentration of IPTG; 6, fraction corresponding to 40 μL purified EGFP. (B) Western analysis using Anti-His antibody (Amersham Pharmacia Biotech, Piscataway, NJ, USA) to demonstrate the expression of DFF40 and EGFP in 293 cells transfected with pCI-HisDFF40 + pCI-c-mycDFF45 and pCI-EGFP-dual, respectively. Fifteen microliters purified EGFP or DFF40, respectively, were loaded onto the gel.

Benchmarks

used lack the T7 terminator, our results demonstrate that a prokaryotic transcriptional terminator region is not compulsory for the expression of the EGFP gene, although its presence may improve mRNA stability and would probably further enhance the expression level.

The strategy presented here allows the easy generation of expression vectors with the capacity to express genes in both bacterial and mammalian systems that contains all convenient features such as affinity tags or protease cleavage sites, either introduced by PCR or provided by several commercially available prokaryotic expression vectors. Only a few commercial vectors for dual- or even triple-system (21) expression are available, but they do not have as many features as can be incorporated into dual expression vectors by the strategy used here. The dual expression vectors described here combine two of the most powerful bacterial and mammalian expression systems and allow for biochemical characterization of recombinant proteins produced in both cell systems, using a single construct. Our method eliminates the need for subcloning genes into different vectors and thus helps to save time and costs for the researcher.

REFERENCES

1. **Cormack, B.P., R. Valdivia and S. Falkow.** 1996. FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* 173:33-38.
2. **Dunn, J.J., B. Krippel, K.E. Bernstein, H. Westphal and F.W. Studier.** 1988. Targeting bacteriophage T7 RNA polymerase to the mammalian cell nucleus. *Gene* 68:259-266.
3. **Enari, M., H. Sakahira, H. Yokoyama, K. Okawa, A. Iwamatsu and S. Nagata.** 1998. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 391:43-50.
4. **Foecking, M.K. and H. Hofstetter.** 1986. Powerful and versatile enhancer-promoter unit for mammalian expression vectors. *Gene* 45:101-105.
5. **Forman, B.M. and H.H. Samuels.** 1991. pEXPRESS: a family of expression vectors containing a single transcription unit active in prokaryotes, eukaryotes and in vitro. *Gene* 105:9-15.
6. **Gu, J., R.P. Dong, C. Zhang, D.F. McLaughlin, X.W. Mei and S.F. Schlossman.** 1999. Functional interaction of DFF35 and DFF45 with caspase-activated DNA fragmentation nuclease DFF40. *J. Biol. Chem.* 274:20759-20762.
7. **Halenbeck, R., H. MacDonald, A. Roulston, T.T. Chen, L. Conroy and L.T. Williams.** 1998. CPAN, a human nuclease regulated by the caspase-sensitive inhibitor DFF45. *Curr. Biol.* 8:537-540.
8. **He, B., W.T. McAllister and R.K. Durbin.** 1995. Phage RNA polymerase vectors that allow efficient gene expression in both prokaryotic and eukaryotic cells. *Gene* 164:75-79.
9. **Kozak, M.** 1981. Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. *Nucleic Acids Res.* 9:5233-5262.
10. **Kozak, M.** 1987. At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. *J. Mol. Biol.* 196:947-950.
11. **Kozak, M.** 1997. Recognition of AUG and alternative initiator codons is augmented by G in position +4 but is not generally affected by the nucleotides in positions +5 and +6. *EMBO J.* 16:2482-2492.
12. **Lieber, A., U. Kiessling and M. Strauss.** 1989. High level gene expression in mammalian cells by a nuclear T7-phase RNA polymerase. *Nucleic Acids Res.* 17:8485-8493.
13. **Liu, X., H. Zou, C. Slaughter and X. Wang.** 1997. DFF, a heterodimeric protein that functions downstream of caspase 3 to trigger DNA fragmentation during apoptosis. *Cell* 89:175-184.
14. **Liu, X., P. Li, P. Widlak, H. Zou, X. Luo, W.T. Garrard and X. Wang.** 1998. The 40-kDa subunit of DNA fragmentation factor induces DNA fragmentation and chromatin condensation during apoptosis. *Proc. Natl. Acad. Sci. USA* 95:8461-8466.
15. **Liu, Z., L.M. Cashion and J.J. Twu.** 1997. A systematic comparison of relative promoter/enhancer activities in mammalian cell lines. *Anal. Biochem.* 246:152-155.
16. **Liu, Z., L.M. Cashion and H. Pu.** 1998. Protein expression both in mammalian cell lines and in yeast *Pichia pastoris* using a single expression plasmid. *BioTechniques* 24:266-271.
17. **Matsumoto, K., K. Montzka-Wassarman and A.P. Wolffe.** 1998. Nuclear history of a pre-mRNA determines the translational activity of cytoplasmic mRNA. *EMBO J.* 17:2107-2121.
18. **McCarty, J.S., S.Y. Toh and P. Li.** 1999a. Study of DFF45 in its role of chaperone and inhibitor: two independent inhibitory domains of DFF40 nuclease activity. *Biochem. Biophys. Res. Commun.* 264:176-180.
19. **McCarty, J.S., S.Y. Toh and P. Li.** 1999b. Multiple domains of DFF45 bind synergistically to DFF40: roles of caspase cleavage and sequestration of activator domain of DFF40. *Biochem. Biophys. Res. Commun.* 264:181-185.
20. **Mukae, N., M. Enari, H. Sakahira, Y. Fukuda, J. Inazawa, H. Toh and S. Nagata.** 1998. Molecular cloning and characterization of human caspase-activated DNase. *Proc. Natl. Acad. Sci. USA* 95:9123-9128.
21. **Novy, R., K. Yaeger, S. Monsma and M. Scott.** 1999. pTriEx-1 multisystem vector for protein expression in *E. coli*, mammalian, and insect cells. *inNovations* 10:1-5.
22. **Sakahira, H., M. Enari and S. Nagata.** 1999. Functional differences of two forms of the inhibitor of Caspase-activated DNase, ICAD-L, and ICAD-S. *J. Biol. Chem.* 274:15740-15744.
23. **Studier, F.W., A.H. Rosenberg, J.J. Dunn and J.W. Dubendorff.** 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* 185:60-89.
24. **Yang, T.T., L. Cheng and S.R. Kain.** 1996. Optimized codon usage and chromophore mutations provide enhanced sensitivity with the green fluorescent protein. *Nucleic Acids Res.* 24:4592-4593.

This work was supported by the Deutsche Forschungsgemeinschaft, INTAS and the Fonds der Chemischen Industrie. We would like to thank Ms. U. Steindorf and Ms. Helene Schäfer-Pfeiffer for technical assistance. Address correspondence to Dr. Gregor Meiss, Institut für Biochemie (FB 08), Heinrich-Buff-Ring 58, D-35392 Giessen, Germany. e-mail: gregor.meiss@chemie.bio.uni-giessen.de

Received 10 March 2000; accepted 14 June 2000.

G. Meiss, U. Böttcher, C. Korn, O. Gimadutdinov¹ and A. Pingoud
*Justus-Liebig-Universität
Giessen, Germany
¹Kazan State University
Kazan, Russian Federation*