Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7905–7909, September 1992
Genetics

Genomic targeting with a positive-selection *lox* integration vector allows highly reproducible gene expression in mammalian cells

(Cre recombinase/position effects/DNA targeting)

SHINICHI FUKUSHIGE AND BRIAN SAUER*

DuPont–Merck Pharmaceutical Co., Experimental Station E328, Wilmington, DE 19880-0328

Communicated by Sydney Breuner, May 7, 1992 (received for review February 15, 1992)

ABSTRACT **Stable transformants of mammalian cells from gene transfer often show extreme variability in expression of the introduced transgene. This occurs from the highly variable number of copies integrated into the genome and from position effects on gene expression due to random integration. We have eliminated both of these constraints on predictable gene expression by use of a *lox* recombination vector. The positive selection vector system is designed to directly select Cre-mediated DNA integration at a *lox* target previously placed into the genome of cultured mammalian cells. Proper targeting activates expression of a defective *lox*–neomycin phosphotransferase (*neo*) fusion gene target. With CHO cell lines containing this target, almost all of the selected transformants (54 of 56 independent G418-resistant colonies) were simple single-copy integrants of the targeting DNA. To monitor gene expression at a single chromosomal site, we used a β-actin promoter–*lacZ* reporter construct. Independent G418-resistant colonies from site-specific integration of the reporter gene all showed nearly identical levels of β-galactosidase activity when the reporter construct integrated at a particular chromosomal position. The same construct integrated at a second chromosomal position exhibited a slightly different level of activity, characteristic of that second position. These results show that Cre-mediated site-specific integration can facilitate the construction of isogenic cell lines and thereby permit reproducible gene expression in stably transformed cell lines.**

Targeted integration of DNA into the genome promises a simple solution to one of the major problems with gene transfer into mammalian cells. Cell lines made by standard DNA-transfer methods show variable gene expression from both chromosomal position effects and from copy-number variation between different clones (1). Hence, mutational analysis of transcriptional regulatory sequences using stable cell transformants is arduous at best. Site-specific integration of DNA at a preselected site in the genome should result in isogenic clonal lines having identical levels of reporter gene expression. We have confirmed this prediction by targeting DNA to a predefined genomic site with the Cre recombinase.

Cre is a 38-kDa recombinase protein from bacteriophage P1 that catalyzes reciprocal site-specific recombination between 34-base-pair (bp) *loxP* sites (2). In bacteria and *in vitro* Cre mediates both intramolecular (excisive or inversional) and intermolecular (integrative) recombination at *loxP* sites. Surprisingly, Cre also promotes both excisive (3–5) and integrative (6) recombination in eukaryotic cells.

To select targeted integration in a wide range of mammalian cells, we have designed an inactive *lox–neo* fusion gene to use as a *lox* chromosomal target. Cre-mediated integration of a promoter–ATG–*lox* targeting vector into the chromosomal target reconstructs a functional ATG–*lox–neo* fusion gene and renders cells resistant to the neomycin analogue G418. We constructed CHO cell derivatives with a single randomly placed genomic copy of this *lox* target and then characterized Cre-mediated targeting in these cells. To monitor gene expression, we placed a β-actin–*lacZ* reporter gene into the *lox* targeting vector, isolated a number of independent targeted lines, and measured the resulting level of β-galactosidase.

MATERIALS AND METHODS

Plasmids and DNA Constructions. Plasmids pBS185 (6), pBS74 (5), and pRH43 (7) have been described. The defective *lox–neo* gene fusion plasmid pSF1 (Fig. 1*A*) was constructed in a number of steps. First, the 2.4-kilobase (kb) *Eco*RI–*Bam*HI fragment of pRH43 containing *loxP* and the ampicillin-resistance (Ap) gene was ligated to the 2.8-kb *Eco*RI–*Bgl* II *neo* fragment from pBS74. The first five codons of the *neo* gene were removed and the *neo* coding region was fused in-frame to the *loxP* site by PCR overlap mutagenesis using synthetic primers (8), to give pSF1. The resulting product was sequenced and is shown in Fig. 1*A*. Because the N terminus of neomycin phosphotransferase is nonessential (9), we expected that provision of a translational start signal to the pSF1 construct (by Cre-mediated recombination; see below) would result in a functional *lox–neo* fusion protein. The 2.6-kb Chinese hamster DHFR minigene from pMG1 (10) was cloned into the *Bam*HI site of pSF1 to generate pSF14 (Fig. 1*A*).

The targeting vector pBS226 (Fig. 1*B*) was constructed by digesting pUC18 with *Asp*718, filling in the cohesive ends, and religating the DNA. Synthetic oligonucleotides were inserted into the polylinker to generate (*i*) a new AUG start having optimized eukaryotic and prokaryotic translational start signals, and (*ii*) a novel *loxG1C2* site with a *Sph* I site at one end. Finally, the 0.8-kb *Xba* I hCMV fragment from pBS185 was inserted to give pBS226. The human β-actin promoter–*lacZ* cassette (fusion is at position 52 of the β-actin sequence) with the simian virus 40 polyadenylylation site was obtained on a 3.95-kb *Bam*HI–*Bgl* II fragment (from J. Rossant, University of Toronto, through R. Scott, DuPont) and was cloned into the *Bam*HI site of pBS226 in both orientations to give pSF18 and pSF19 (Fig. 1*B*). The 600-bp promoter lacks both the upstream region and the enhancer-containing intervening sequence (11).

Cell Lines and Gene Transfer. The *lox–neo* target was placed into the DHFR$^-$ CHO cell line DG46 (12) by electroporating 10^7 cells in 0.8 ml with 1 μg of pSF14, using a single pulse of 450 V at 500 μF from a Bio-Rad Gene Pulser (13). One day later cells were selected for growth in α^- medium (which lacks deoxyribonucleosides and ribonucleosides) supplemented with 15% dialyzed fetal bovine serum. Southern

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DHFR, dihydrofolate reductase; hCMV, human cytomegalovirus.

*To whom reprint requests should be addressed.

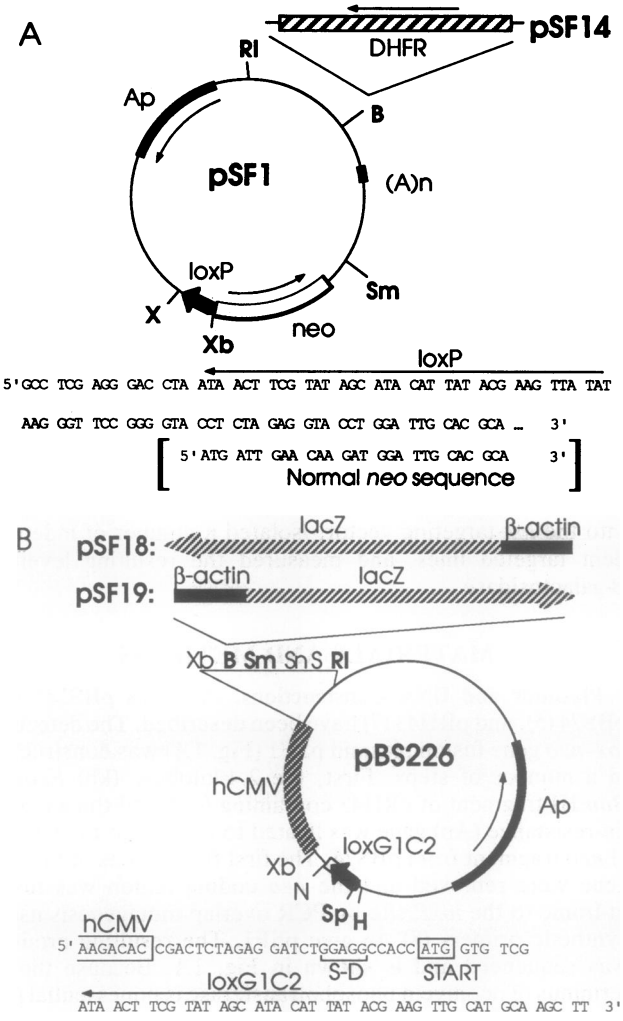

FIG. 1. Site-specific integration vectors. *lox* sites are indicated by short, stubby arrows. (*A*) Sequence of the *loxP* region in the *lox–neo* target vectors pSF1 and pSF14. The unmodified *neo* gene sequence is shown in brackets. DHFR, dihydrofolate reductase; Ap, ampicillin resistance; (A)n, simian virus 40 polyadenylylation signal. (*B*) Sequence of the *lox* region in the targeting vectors pBS226, pSF18, and pSF19. The human cytomegalovirus (hCMV) region and the ATG start used after integration are boxed. S–D indicates a prokaryotic ribosome binding site (Shine–Dalgarno site). Restriction sites: B, *Bam*HI; H, *Hin*dIII; N, *Nco* I; S, *Sal* I; Sm, *Sma* I; Sn, *Sna*BI; Sp, *Sph* I; X, *Xho* I; Xb, *Xba* I. Bold letters indicate unique restriction sites.

analysis of DNA (14) was performed after transfer to GeneScreen*Plus* (DuPont).

Cre-mediated integration of the *lox* targeting vectors used the same electroporation conditions with 10 μg of targeting vector and 20 μg of pBS185 to provide transient Cre expression. Each electroporated sample (≈10^7 cells in 0.8 ml) was plated into four 10-cm culture dishes. Two days later cells were selected for growth in medium with G418 (400 μg/ml). Colony formation was scored 12 days later and individual clones were selected for expansion.

Assay of β-Galactosidase. Cells (2×10^6) were plated in a 10-cm dish and cultured for 3 days. Confluent cells were harvested and kinetic β-galactosidase assays (15) were performed in a 96-well dish, using chlorophenol red β-D-galactoside (4 mg/ml). The OD_{575} was read at 25°C with a Molecular Devices Vmax kinetic plate reader. Protein was determined with the Bio-Rad protein assay.

Karyotype analysis was performed on metaphase spreads (16) stained with 4′,6-diamidino-2-phenylindole. The StatView Student computer program (Abacus Concepts, Berkeley, CA) was used for statistical analysis.

RESULTS

Construction of Cell Lines Having a Single *lox–neo* Target. Plasmid pSF1 contains a single *lox* integration target 5′ to a silent, but activatable, *neo* gene (Fig. 1*A*). The first five codons of the *neo* gene in pSF1 have been replaced with an in-frame *loxP* site but with no initiating AUG. Cre-mediated recombination of pSF1 with pBS226 (Fig. 1*B*) regenerates a functional *neo* gene: both an in-frame AUG and the promoter/enhancer of the major immediate early promoter of hCMV are provided from pBS226 (Fig. 2).

A DHFR minigene version of pSF1, plasmid pSF14, was electroporated into the $DHFR^-$ CHO cell line DG-46 to give 27 independent $DHFR^+$ colonies. By Southern analysis, 4 of these 27 isolates contained a single copy of an unrearranged *lox–neo* target. In all 4 isolates integration occurred in the 3.1-kb Ap region betwixt the *lox* site and the C terminus of the DHFR gene (data not shown). To confirm that each line is a distinct single-copy integrant, genomic DNA was digested with either *Eco*RI or *Xba* I and hybridized with *neo* (Fig. 3). As expected for independent single-copy integrants, each isolate exhibited a unique single band with each digest.

Site-Specific Integration of pBS226 into the Cell Line 14-1-18. Cell line 14-1-18 was used to verify site-specific integration of the targeting vector, pBS226, at the chromosomal *lox* target. Because recombination regenerates a functional *neo* gene, site-specific integrants can be selected directly by resistance to G418. Table 1 shows the results from transfection of pBS226 and of the Cre expression vector pBS185 into 14-1-18. Neither pBS226 nor pBS185 alone produced G418-resistant colonies. However, pBS226/pBS185 cotransfection stimulated production of G418-resistant colonies >100-fold. Southern analysis, similar to that shown below, of 24 independent isolates verified that all were single-copy site-specific integrants of pBS226 into the 14-1-18 chromosomal *lox* target (data not shown).

Site-Specific Integration of pSF18 and pSF19 into Recipient Cell Lines. To detect possible positional effects on gene expression, we cloned a β-actin promoter–*lacZ* reporter gene into the pBS226 targeting vector to give plasmids pSF18 and

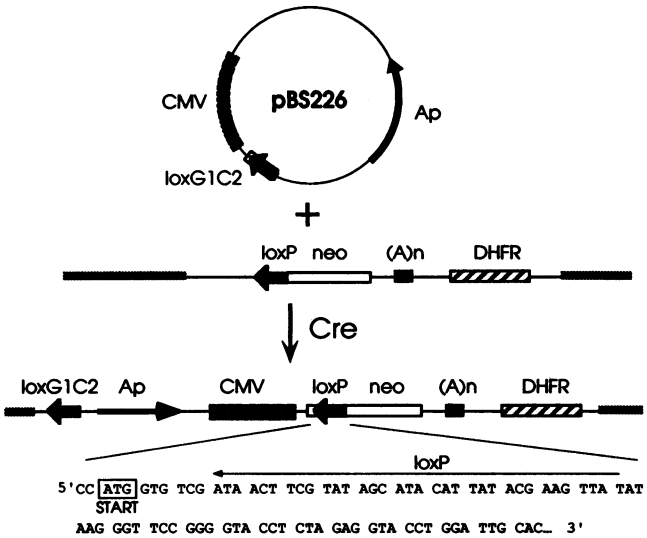

FIG. 2. Activation of a functional *lox–neo* gene by site-specific targeting. The circular targeting vector pBS226 and the chromosomal, nonfunctional *lox–neo* target of pSF14 are shown. Cre-catalyzed integration regenerates a functional *neo* gene. The resulting sequence is shown at the bottom.

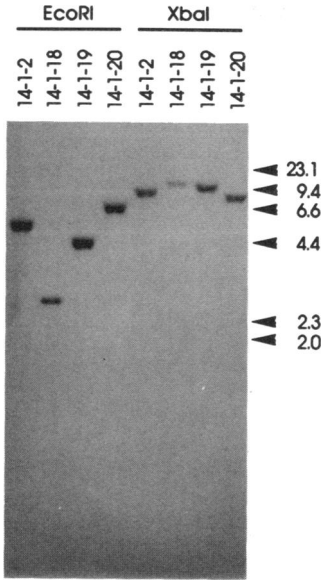

FIG. 3. Cell lines containing a single copy of the *lox–neo* target. DNA (5 μg) from four independent pSF14 transformants of DG46 was digested with either *Eco*RI or *Xba* I and analyzed by Southern blotting using a *neo* probe. Markers at right are in kilobases.

pSF19 (Fig. 1). These plasmids differ only in the orientation of the reporter gene: in pSF19 the β-actin and hCMV promoters are adjacent and direct divergent transcription; in pSF18 the β-actin promoter is 3.5 kb from the hCMV promoter and transcription of *neo* and *lacZ* occurs in the same direction.

The frequency of targeting (Table 2) with each of these two constructs was about the same in all four *lox* target cell lines, as indicated by the frequency of Cre-dependent G418 resistance. Only the 14-1-2 and 14-1-20 cell lines exhibited any spontaneous G418 resistance, and these non-Cre-mediated events were 40–100 times less frequent than G418 resistance due to targeting. There were slight differences: both 14-1-2 and 14-1-20 exhibited an ≈2-fold higher frequency of Cre-mediated G418 resistance than 14-1-19, and the frequency of targeting with pSF18 may have been higher than with pSF19. These differences are slight, however, and may be attributable to variation due to the electroporation procedure.

Site-specific integration in each G418-resistant colony was confirmed by Southern hybridization. Fig. 4*A* shows the *lox–neo* region in the parental cell line and the structures expected after single-copy targeting with either pSF18 or pSF19. DNAs digested with both *Eco*RI and *Xho* I were hybridized sequentially with a *neo*-specific probe and a hCMV-specific probe. The parental cell line 14-1-2 exhibited a 2.0-kb *neo*-specific fragment (Fig. 4*B*). Integration of pSF18 at the chromosomal *lox* site replaced this fragment with a

Table 1. Cre-mediated DNA targeting in cell line 14-1-18

DNA	$G418^R$ colonies	cfu plated	Frequency × 10^6 ($G418^R$/cfu)
Exp. 1			
pBS226	0	4.3×10^6	0
pBS185	0	4.3×10^6	0
pBS226 + pBS185	108	3.8×10^6	28
Exp. 2			
pBS226	0	3.7×10^6	0
pBS185	0	1.7×10^6	0
pBS226 + pBS185	77	1.7×10^6	45

Cells (10^7) were electroporated with 10 μg of the targeting vector pBS226, 20 μg of the Cre expression vector pBS185, or with both plasmids. $G418^R$, G418-resistant; cfu, colony-forming units.

Table 2. Targeting efficiency for various *lox* target CHO strains

Cell line	G418-resistant colonies, frequency × 10^6			
	pSF18		pSF19	
	− Cre	+ Cre	− Cre	+ Cre
14-1-2	0.5	22.5	0.1	10
14-1-18	0	16	0	7.6
14-1-19	0	11.4	0	4.9
14-1-20	0.5	22.7	0.5	14.8

Electroporation was performed as described in Table 1, using 10 μg of either pSF18 or pSF19 with (+ Cre) or without (− Cre) 20 μg of the Cre expression vector pBS185. Results shown are the average of two or more independent experiments.

3.2-kb band. Similarly, site-specific pSF19 integration replaced the 2.0-kb fragment with a 3.3-kb fragment. The eight pSF18 and the eight pSF19 G418-resistant transformants all exhibited the *neo* fragment diagnostic for site-specific integration at the chromosomal target *lox* site. As expected, the *neo* fragment of the pSF18 and pSF19 transformants also hybridized to the hCMV probe (Fig. 4*C*). The detection of but a single band with the hCMV probe argues both that there are no tandem multiple integration events at the chromosomal *lox* site and that illegitimate integration events have not occurred. Therefore in each G418-resistant transformant a single copy of the targeting vector has been integrated at the chromosomal *lox* site.

Southern analysis of eight pSF18 and eight pSF19 transformants of 14-1-20 indicated that these transformants also contained a single copy of the targeting vector integrated at the chromosomal *lox* target, although one of the pSF18 integrants, no. 7 (see below), contained a partial deletion of the *lacZ* reporter gene (data not shown). Interestingly, in addition to the 3.3-kb fragment characteristic of site-specific integration, two of the eight pSF19 transformants of 14-1-20 displayed hCMV-hybridizing bands diagnostic of illegitimate recombination. Only one of these illegitimate events retained an intact *lacZ* reporter gene (see below). Such nonselected cotransformation events are expected to occur randomly at low frequency. Of the 56 G418-resistant transformants analyzed, only 2 contained additional illegitimate integration events.

Gene Expression from the Targeted Single-Copy *lacZ* Gene. Each of the 14-1-2 and 14-1-20 derivatives described above has a single copy of a *lacZ* reporter gene integrated at the chromosomal *lox* target. To heighten responsiveness to chromosomal position effects, we used a β-actin promoter from which the enhancer had been removed (11). Transient expression experiments with CHO cells indicated that pSF19 produced 4- to 7-fold more β-galactosidase activity than pSF18 (data not shown), presumably because the β-actin promoter was adjacent to the hCMV enhancer in pSF19 and 3.5 kb away in pSF18. We therefore measured β-actin *lacZ* expression in the single-copy integrants derived from the cell lines 14-1-2 and 14-1-20 to see whether or not this orientation effect was maintained and to assess the reproducibility of expression in independent isolates.

With few exceptions, cell lines having the targeted plasmid showed very similar *lacZ* expression. All pSF18 integrants of 14-1-2 showed roughly the same *lacZ* activity, the mean value being 26.4 (Table 3). Integrants of 14-1-2 having *lacZ* in the opposite orientation (i.e., pSF19) were also remarkably uniform in expression and exhibited a 10-fold higher mean β-galactosidase activity, 256. Similarly, with cell line 14-1-20 the pSF18 integrants showed a mean β-galactosidase activity of 28.4 whereas the pSF19 integrants exhibited a 5-fold higher value, 152.

The exceptions expressing β-galactosidase at higher levels are surprising, however, because the integrated plasmid

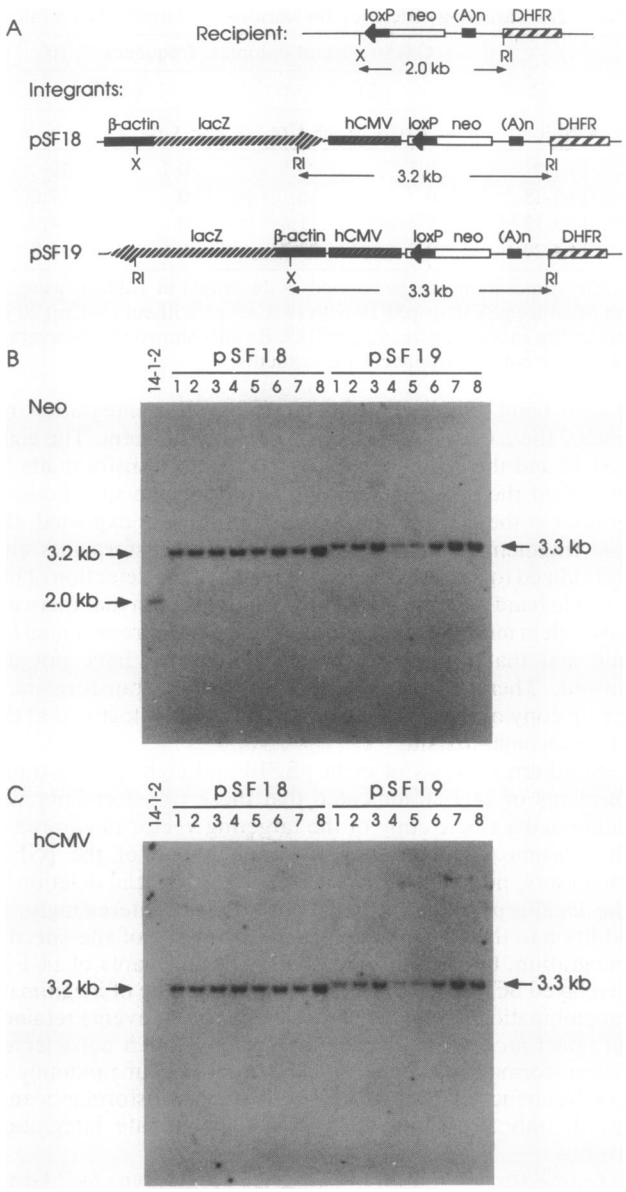

FIG. 4. Single-copy integration of the targeting vector. DNA (5 μg) from eight independent pSF18 and eight independent pSF19 Cre-directed integrants of 14-1-2 was digested with *Eco*RI and *Xho* I and analyzed by Southern blotting. (*A*) The chromosomal target before Cre-mediated integration (Recipient) and after targeting with either pSF18 or pSF19. See Figure 1. (*B*) Southern blot probed with *neo*. (*C*) The filter in *B* after removal of the *neo* probe and probing with the hCMV fragment.

construct and the chromosomal context are identical in all cell lines within a set. One anomolous transformant, pSF18 no. 6 of 14-1-2, was therefore examined in more detail. Although 70% of these cells showed a normal diploid karyotype ($2N = 22$), 30% of the cells were tetraploid ($4N = 44$). In contrast, the parental cell line 14-1-2 had the normal diploid karyotype. Chromosome duplication and/or tetraploidization therefore may contribute to the occurrence of these occasional, exceptional site-specific integrants.

Both 14-1-2 and 14-1-20 showed a slight position effect on expression of the β-actin–*lacZ* reporter gene. Application of the statistical *t* test to the data in Table 3 indicated that the higher level of expression from pSF19 in 14-1-2 compared with 14-1-20 was significant at the 99% confidence level but that the two lines showed little or no difference in expression when the β-actin promoter was located 3.5 kb from the hCMV enhancer (pSF18). We suspect that chromosomal

Table 3. Expression of β-galactosidase activity from stable transformants

Reporter plasmid	Transformant no.	β-Galactosidase activity* 14-1-2 parent	β-Galactosidase activity* 14-1-20 parent
pSF18	1	21.5	28
	2	20.5	31.5
	3	18.5	28.5
	4	27	34.5
	5	12.5	25
	6	56	25
	7	23	0†
	8	32.5	26
	(Mean ± SE)	26.4 ± 4.7	28.4 ± 1.3
pSF19	1	285	141
	2	210	120
	3	195	162
	4	235	390†
	5	157	139
	6	331	111
	7	267	217
	8	367	174
	(Mean ± SE)	256 ± 25.0	152 ± 13.6

*Results (pmol of chlorophenol red β-D-galactoside per min per mg of protein) are the average of assays from duplicate extracts from each transformant. Background β-galactosidase activity of 10 pmol per min per mg of protein in the 14-1-2 parent and 11 pmol per min per mg of protein in the 14-1-20 parent was subtracted from activities of the transformants.

†Omitted from the calculations of the mean and of the standard error because of deletion of the *lacZ* reporter (pSF18 integrant no. 7 of 14-1-20) or because of (illegitimate) integration of a second intact *lacZ* gene into the genome (pSF19 integrant no. 4 of 14-1-20). See text.

context may subtly influence the ability of the "enhancerless" β-actin promoter to recruit the adjacent hCMV enhancer for *lacZ* expression.

DISCUSSION

The advantages of phage λ site-specific DNA recombination for introducing DNA into the genome have long been appreciated by bacterial geneticists. Recombinant λ vectors carrying foreign DNA can integrate into the *Escherichia coli* chromosome at a specific location by site-specific recombination directed by the λ Int protein. This strategy can undoubtedly be extended to higher eukaryotes, since certain members of the Int family of site-specific recombinases (17) are active in higher eukaryotes. Functional heterologous expression of site-specific DNA recombinases in eukaryotic cells has been shown for the Cre protein of coliphage P1 (3, 4), the FLP protein of *Saccharomyces cerevisiae* (18, 19), and the pSR1 recombinase of *Zygosaccharomyces rouxii* (20).

Previously, a promoterless thymidine kinase (*tk*) *lox* vector (6) was used for Cre-mediated chromosomal targeting, but selection for *tk* limits such vectors to cells lacking thymidine kinase. We therefore developed a *lox–neo* fusion vector that can be used in any cultured cell sensitive to G418. Cre-directed targeting of the lox integration vector into the genomic *lox–neo* target is selected directly by resistance to G418. All 56 integrants analyzed have a copy of the targeting vector integrated at the chromosomal *lox* target. Only three "abnormal" transformants were observed: one showed a partial deletion of targeting vector sequences and two transformants carried an additional copy of the targeting vector randomly integrated elsewhere in the genome, presumably by illegitimate recombination. The remaining transformants had

a single copy of the targeting vector precisely integrated at the chromosomal *lox* target.

The ease and predictability of single-copy transgene targeting promises to be of quite general benefit. However, Cre-mediated targeting does not itself neutralize position effects; rather it ensures only that the same position effect (depending on the genomic location of the lox target) is imposed on the incoming reporter gene. Cre-mediated targeting thus allows the facile construction of isogenic cell lines. Routine targeting of a previously characterized chromosomal integration site in either embryonic stem cells or injected pronuclei also promises to speed the generation of transgenic animals that reliably express a desired transgene.

Control of positional and copy-number variation is important for analysis of gene expression in stable DNA transformants. We have shown that gene expression at a particular integration site is roughly the same in a number of independent isolates. Any observed variation must come from influences other than copy number and position. One source of such variation is fluctuation in chromosome number from aneuploidy or tetrapoidy in cultured cells. Differences in methylation patterns may also affect gene expression (21).

A small but reproducible position effect on β-actin–*lacZ* expression is seen with integrants at different sites, but only when the β-actin promoter and the hCMV promoter/enhancer are adjacent (pSF19). Cell line 14-1-2 shows 68% higher expression from pSF19 than 14-1-20, suggesting that chromosomal position may temper the ability of the hCMV enhancer to enhance transcription from the β-actin promoter. Results with pSF18, where the β-actin promoter is far from the hCMV enhancer, show little difference between the two cell lines, indicating that the effect on pSF19 is not a general one on β-actin expression. One possibility is that the hCMV enhancer is slightly less active at the 14-1-20 target site than at the 14-1-2 site. Dramatic differences in expression from the hCMV promoter/enhancer have been observed in transgenic mice (22, 23) indicating that this strong promoter is itself susceptible to chromosomal position effects.

Site-specific integration should help in understanding the basis of position effects. Specific DNA sequences probably organize the genome into independent transcriptional domains (24–26). Targeted insertion of these sequences with a reporter gene will facilitate their functional analysis. Strand-specific somatic hypermutation of immunoglobulin genes (27, 28) is a second type of position effect and may be linked to orientation of the immunoglobulin gene relative to the direction of DNA replication (29). Effects of orientation on hypermutation could be determined directly by targeted integration of DNA.

We thank Annie Colberg-Poley for advice on chlorophenol red β-D-galactoside/*lacZ* assays and for critical comments on the manuscript and Naomi Logsdon for assistance in karyotype analysis. S.F. was supported by a DuPont–Merck postdoctoral fellowship.

1. Lacy, E., Roberts, S., Evans, E. P., Burtenshaw, M. D. & Constantini, F. D. (1983) *Cell* **34**, 343–358.
2. Hoess, R. H. & Abremski, K. (1990) in *Nucleic Acids and Molecular Biology*, eds. Eckstein, F. & Lilley, D. M. J. (Springer, Berlin), pp. 99–109.
3. Sauer, B. (1987) *Mol. Cell. Biol.* **7**, 2087–2096.
4. Sauer, B. & Henderson, N. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5166–5170.
5. Sauer, B. & Henderson, N. (1989) *Nucleic Acids Res.* **17**, 147–161.
6. Sauer, B. & Henderson, N. (1990) *New Biol.* **2**, 441–449.
7. Abremski, K., Hoess, R. & Sternberg, N. (1983) *Cell* **32**, 1301–1311.
8. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K. & Pease, L. R. (1989) *Gene* **77**, 61–68.
9. Beck, E., Ludwig, G., Auserwald, E. A., Reiss, B. & Schaller, H. (1982) *Gene* **19**, 327–336.
10. Mitchell, P. J., Carothers, A. M., Han, J. H., Harding, J. D., Kas, E., Venolia, L. & Chasin, L. A. (1986) *Mol. Cell. Biol.* **6**, 425–440.
11. Ng, S.-Y., Gunning, P., Liu, S. H., Leavit, J. & Kedes, L. (1989) *Nucleic Acids Res.* **17**, 601–615.
12. Urlaub, G., Kas, E., Carothers, A. M. & Chasin, L. A. (1983) *Cell* **33**, 405–412.
13. Chu, G., Hayakawa, H. & Berg, P. (1987) *Nucleic Acids Res.* **15**, 1311–1326.
14. Nakamura, N., Sugino, H., Takahara, K., Fukushige, S. & Matsubara, K. (1991) *Cytogenet. Cell Genet.* **57**, 18–22.
15. Eustice, D. C., Feldman, P. A., Colberg-Poley, A. M., Buckery, R. M. & Neubauer, R. H. (1991) *BioTechniques* **11**, 739–743.
16. Biegel, J. A., Belasco, J. B. & Emanuel, B. S. (1988) *Cancer Genet. Cytogenet.* **36**, 211–215.
17. Argos, P., Landy, A., Abremski, K., Egan, J. B., Ljungquist, E. H., Hoess, R. H., Kahn, M. L., Kalionis, B., Narayana, S. V. L., Pierson, L. S., Sternberg, N. & Leong, J. M. (1986) *EMBO J.* **5**, 433–440.
18. Golic, K. G. & Lindquist, S. (1989) *Cell* **59**, 499–509.
19. O'Gorman, S., Fox, D. T. & Wahl, G. M. (1991) *Science* **251**, 1351–1355.
20. Matsuzaki, H., Nakajima, R., Nishiyama, J., Araki, H. & Oshima, Y. (1990) *J. Bacteriol.* **172**, 610–618.
21. Doerfler, W. (1983) *Annu. Rev. Biochem.* **52**, 93–124.
22. Furth, P. A., Hennighausen, L., Baker, C., Beatty, B. & Woychick, R. (1991) *Nucleic Acids Res.* **19**, 6205–6208.
23. Schmidt, E. V., Christoph, G., Zeller, R. & Leder, P. (1990) *Mol. Cell. Biol.* **10**, 4406–4411.
24. Grosveld, F., van Assendelft, G. B., Greaves, D. R. & Kollias, G. (1987) *Cell* **51**, 975–985.
25. Kellum, R. & Schedl, P. (1991) *Cell* **64**, 941–950.
26. Stief, A., Winter, D. M., Strätling, W. H. & Sippel, A. E. (1989) *Nature (London)* **341**, 343–345.
27. Golding, G. B., Gearhart, P. J. & Glickman, B. W. (1987) *Genetics* **115**, 169–176.
28. Lebecque, S. G. & Gearhart, P. J. (1990) *J. Exp. Med.* **172**, 1717–1727.
29. Rogerson, B., Hackett, J., Jr., Peters, A., Haasch, D. & Storb, U. (1991) *EMBO J.* **10**, 4331–4341.