# "Green light" for gene transfer

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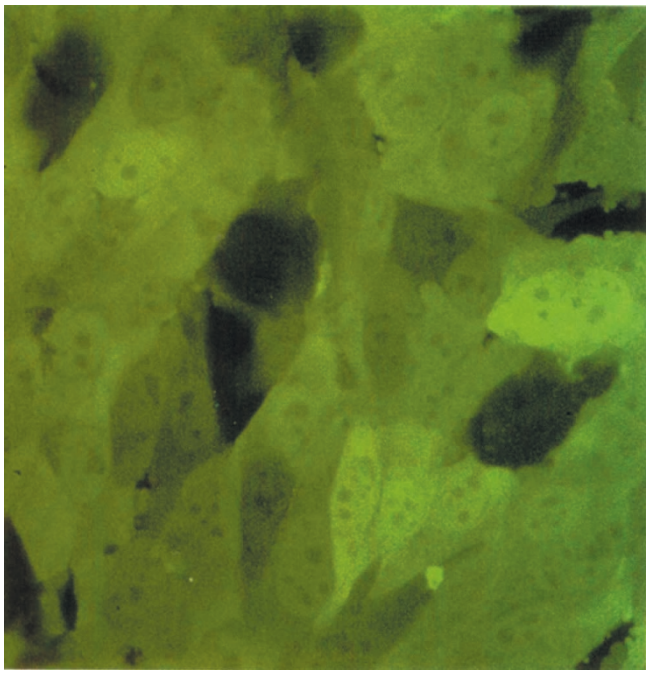

**Green fluorescent signal in transduced cells.**

One of the cherished goals of molecular biologists is to introduce genes into cells, tissues, and whole organisms and to study their expression. The problem, however, has been to identify the cell harboring the foreign gene. In a landmark paper in 1979, Mike Wigler and colleagues[1] demonstrated cotransfer of the herpes thymidine kinase gene (TK+transformants) with Φx174 DNA, pBR322, or the chromosomal rabbit β-globin gene. Since then, many selectable markers have been used, chief among them being neomycin resistance (Neo), mycophenolic acid resistance (GPT), hygromycin, histidinol, and so forth for transient transfection or to generate stable cell lines.

Other reporters used to select live cells from the background of untransfected cells include *E. coli* β-galactosidase, which requires the transport of fluoregenic substrate (FDPG) across cell membranes, and cell surface markers that require antibody staining. Neither of these methods is entirely satisfactory because it requires either a considerable time lag for positive selection in toxic media, or is prone to high endogenous background. Relief from the vagaries of drug or enzymatic selection methods is offered in two articles by Chang et al.[2] and Levy et al.[3] in the current issue of *Nature Biotechnology*, and another by Zolotukhin et al.[4] in the *Journal of Virology*. They have used green fluorescent protein (GFP) as a reporter, which results in a fluorescent signal in viable transduced cells. The signal is detectable by both fluorescence microscopy and fluorescence-activated cell sorter (FACS) analysis. The GFP is a 238 amino acid polypeptide (~27kD)[5] that acts as an energy-transfer acceptor under physiological conditions in the jellyfish *Aequorea victoria*. The chromophore is produced by autocyclization of three residues, serine-65, dehydrotyrosine-66, and glycine-67. GFP is inactive until cyclization and oxidation of the three residues generate a fluorescent chromophore. The resultant GFP is a very stable fluorescent chromophore. Furthermore, light-stimulated fluorescence is species independent and does not require cofactors, substrates, or additional gene products, thereby allowing detection in living cells. Attempts by several laboratories to use GFP as a marker have met with limited success; therefore, this technology[6] has not been widely used.

Two events have recently led to the improvement of this system, which is bound to revive interest in the use of GFP as a reporter marker. The first is the generation of a gain-of-function mutant of GFP, in which serine-65 is altered to threonine (S65T) resulting in a red-shifted excitation peak[7]. The S65T mutant protein has a vastly increased brightness (wavelengths of excitation and emission, 490 nm and 510 nm, respectively) and a greatly increased rate of fluorophore generation. It is also resistant to photobleaching. Another mutant in which three amino acids in the chromophore hexapeptide (amino acids 64–69) are mutated, has even greater fluorescence. A GFP, improved by molecular evolution using DNA shuffling, is generated that shows a 42-fold improvement over the wildtype GFP, but which requires ultraviolet light for detection[8]. The second event leading to the improvement of this system is the conversion of jellyfish GFP codons to human codon usage[4]. The "humanized" GFP is expressed at higher levels, presumably because of more efficient translation in mammalian cells.

Cheng et al.[2] and Levy et al.[3] have generated retroviral vectors containing either the mutant GFP or mutant "humanized" GFP, and show that GFP is efficiently transduced in recipient cells. Furthermore, the GFP retrovirus can be used to infect tumor cells, and a single copy of the integrated viral genome is sufficient to produce excellent fluorescence for detection either by fluorescence microscopy or by FACS[3]. Zolotukhin et al.[4] have generated a series of recombinant adeno-associated virus (AAV) and adenovirus vectors for the delivery and expression of GFP in mammalian cells. They have demonstrated the efficient transduction and expression of the humanized mutant GFP gene in the human 293 cell line, and also in vivo, within neurosensory cells of the guinea pig eye. Between one and ten copies of the AAV genome per cell are sufficient for fluorescence detection of GFP by fluorescence microscopy or FACS. No doubt, further improvements in the detection and sensitivity of GFP will be forthcoming. One obvious modification will be to introduce a nuclear localization signal so that all GFP-activated fluorescence is concentrated in the nucleus. The use of humanized mutant GFP will undoubtedly grow precipitously because of its extreme convenience and rapid detection. Selection of positively transduced cells can be done within 48 hours. GFP will also serve as a useful marker with which to study the lineage of stem cells. Molecular biology has thrived on rapidly emerging technologies, and the use of GFP as a marker is bound to be another potent tool in the growing arsenal.

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1. Wigler, M., Sweet, R., Sim, G.K., Wold, B., Pellicer, A., Lacy, E. Maniatis, T., Silverstein, S., and Axel, R. 1979. *Cell* **16**:777-785.
2. Cheng, L., Fu, J., Tsukamoto, A., and Hawley, R.G. 1996. *Nature Biotechnology* **14**:606-609.
3. Levy, J.P., Muldoon, R., Zolotukhin, S., and Link, C.J.,Jr. 1996. *Nature Biotechnology* **16**:610-614.
4. Zolotukhin, S., Potter, S., Hauswirth, W.W., Guy, J., and Muzyczka, N. 1996. *J. Virology* (In press).
5. Prasher, D.C., Eckenrode, V.K., Ward, W.W., Pendergast, F.G., and Cormier, M.J. 1992. *Gene* **111**:229-233.
6. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. 1994. *Science* **263**:802-805.
7. Heim, R., Chubitt, A.B., and Tsein, R.Y. 1995. *Nature* **373**:603-664.
8. Crameri, A., Whitehorn, E.A., Tate, E., and Stemmer, W.P.C. 1996. *Nature Biotechnology* **14**:315-319.