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Gorman C M, Moffat L F & Howard B H. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* 2:1044-51, 1982.
[Lab. Molecular Biology, Div. Cancer Biology and Diagnosis, Natl. Cancer Inst., Bethesda. MD]

This work describes the development of the first mammalian expression vectors producing the chloramphenicol acetyltransferase (CAT) protein. Because there is no endogenous CAT activity in eukaryotic cells and the assays for this bacterial enzyme are rapid and sensitive, these vectors provide a uniquely convenient system for monitoring foreign gene expression. A series of plasmids based on the SV40 early transcriptional control region is described. [The SC/® indicates that this paper has been cited in more than 4,410 publications, making it the most-cited paper published in this journal.]

CAT: An Easy Assay for Gene Expression

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The development of the chloramphenicol acetyltransferase (CAT) assay system for monitoring gene expression took place during 1980-1981 while I was a postdoctoral fellow at the National Cancer Institute (NCI). When I first spoke with Bruce Howard in the fall of 1980 he described work he had begun the year before, while a senior fellow in Paul Berg's lab at Stanford, leading to the development of one of the first widely used mammalian expression vectors, pSV2.

The idea to use the CAT protein, which was easily detectable in mammalian cells, to develop a rapid, sensitive assay system was a collaborative effort between Howard and Michael Gottesman. Gottesman had previously worked with prokaryotic systems where the CAT gene was widely used[1] and suggested it might prove useful as an easy system to monitor gene expression following transfection into cells in tissue culture. Work with the CAT gene began in Howard's group the months preceding my arrival by a summer student from Swarthmore, Leslie Moffat. Upon my arrival I took over the engineering- of the prototype CAT vector, pSV2CAT. I also began testing various potential assays for CAT activity in mammalian cells. The spectrophotometric assay, which was so useful in bacteria, did not seem sensitive for use in mammalian cells. The now well-known visual thin-layer chromatography (TLC) assay, which yields the characteristic three spots, was chosen because it was very sensitive and monitored the direct acetylation of chloramphenicol. With time many useful assays for CAT activity have been developed though many investigators seem to prefer the TLC assay which gives visual, if less quantitative, results.

The first transfection of any CAT plasmid was performed in February 1981 and was one of the few experiments in my scientific career which worked perfectly the first time. Excited about the development of a system which allowed gene expression to be monitored in any cell type, we submitted the initial work to *Nature* in the letter format. As has happened to many scientists, Nature's editorial staff judged that this work was *not of wide enough interest* for publication in *Nature.* This delayed the publication of the paper describing the CAT system for a year, when it was accepted by *Molecular and Cellular Biology,* then only in its second year of existence.

Despite the relative ease of monitoring transfection efficiency by the CAT assay, this system would not have become so well known or widely used if we had not entered into two collaborative efforts in 1982, just as the field of transcriptional control was blossoming. Being at the National Institutes of Health was important in establishing these collaborations. The development of this easy means to monitor gene expression coincided with one of the first descriptions of the transcriptional control regions called enhancers. George Khoury and Peter Gruss had just published work identifying species-specific control regions[2] and since Khoury and Howard were friends as well as colleagues, the team of L. Laimins, Khoury, and Gruss decided to use the CAT assay system to characterize the species-specific effect of enhancers.[3] Of equal importance in popularizing the CAT assay was a collaboration with Ira Pastan, chief of the Laboratory of Molecular Biology, NCI. Pastan and Yamamoto had previously characterized the first viral promoter present in a retroviral long terminal repeat (LTR), and the collaboration with Pastan allowed us to characterize in detail the transcriptional controls by the Rous sarcoma virus LTR.[4] These collaborations turned out to be two of the most rewarding of my career because of the personal benefit of collaborating with such fine scientists, and also because they expanded the vision of our original work.

1. **Shaw W** V. Chloramphenicol acetyltransferase from chloramphenicol-resistant bacteria. *Methods Enzymol.* 43:737-55, 1975. (Cited 390 times.)
2. **Levinson B, Khoury G, Vande Woude G & Grass P.** Activation of the genome by 72 base pair repeats of Moloney sarcoma virus. *Nature* 295:568-72, 1982. (Cited 235 times.)
3. **Laimins L, Khoury G, Gorman C, Howard B & Gruss P.** Host-specific activation of transcription by tandem repeats from SV40 and Moloney sarcoma virus. *Proc. Nat. Acad. Sci. USA* 79:6453-7. 1982. (Cited 510 times.)
4. **Gorman C M, Merlino G T, Willingham M C, Pastan I & Howard B H.** The Rous sarcoma virus LTR is a strong promoter in a variety of cell types when introduced by DNA-mediated transfection. *Proc. Nat. Acad. Sci. USA* 79:6777-81. 1982. (Cited 900 times.)

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