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Grunstein M & Hogness D S. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene.
Proc. Nat. Acad. Sci. US 72:3961-5, 1975.
[Department of Biochemistry, Stanford University School of Medicine, CA]

Bacterial colonies containing different recombinant DNA plasmids are replica-plated and then fixed to nitrocellulose filters *in situ* for radioactive RNA/DNA hybridization. The copy of the colony that anneals is then identified by autoradiography and picked from the replica plate. [The *SCI*® indicates that this paper has been cited in over 1,880 publications.]

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I came to Dave Hogness's laboratory at Stanford to do postdoctoral work in the fall of 1974. He had, by this time, initiated a comprehensive approach for the study of eukaryotic development that would take advantage of *Drosophila melanogaster*'s small genome, advanced genetics, and superb cytology. Genes involved in development would be cloned, and their products analyzed in mutant and wild-type flies. However, specific genes could not yet easily be cloned. While libraries containing random DNA fragments had been created, there was no simple means for identifying which bacterial colony contained the gene or DNA of interest.

Prior to my arrival in the lab, it was already known that bacteria could be fixed on cellophane and lysed enzymatically *in situ* for studies on DNA replication.[1] Dave suggested that a similar approach could potentially allow bacterial colonies carrying specific DNAs to be picked through their annealing to labeled cellular RNAs or their complementary DNAs. However, all my previous hybridizations to characterize ribosomal genes (in Max Birnstiel's lab) or histone genes (in Larry Kedes's lab) used purified components. Would RNA/DNA hybridization occur specifically in the presence of most of the cellular tailings? Could the DNA extruding from lysed colonies be fixed *in situ* without floating away?

I first grew bacteria to confluence on nitrocellulose filters lying on petri plates. But the cells, placed in liquid, easily sloughed off the filter paper. Furthermore, the NaOH solution used to denature DNA turned the filters brown and dissolved them. On my fourth attempt, four days later, I put together a method based on standard RNA/DNA hybridization and some guesswork. This involved transferring the filter carefully every few minutes from drop to drop of various solutions for cell lysis, DNA binding, and finally hybridization. The results of scintillation counting were encouraging. After several experiments, I finally repeated the hybridization with single bacterial colonies. This time, the result was monitored by autoradiography. It could not have worked better. Colonies carrying the plasmid could easily be distinguished after hybridization with ^{32}P-labeled cRNA made from the plasmid template. I then adapted colony hybridization for larger scale screening and showed that it was useful for isolating genes from a library of random *Drosophila* DNA fragments and for subcloning DNA between plasmids.

Why did everything work so rapidly? One reason was the unique cooperation and sharing of materials between labs in the Department of Biochemistry at Stanford. Another was the feeling of optimism and irreverence as cloning was being developed there. If the cell could do it, the scientist could do it. Everything was possible and established techniques were not sacred. Why is "Colony hybridization" widely quoted? It was the first annealing done to cell extracts *in situ* and, with RNA or DNA probes, is widely used in many phases of recombinant DNA work. This paper led directly to other important procedures for the identification of bacteriophage-carrying cloned DNA or for expressing eukaryotic proteins. It also led to the "colony hybridization" of yeast and mammalian cells. A review of some of these procedures is given in *Methods in Enzymology*.[2]

1. Schaller H, Otto B, Nusslein V, Hurl I, Hermann R & Beaucaillar F. Deoxyribonucleic acid replication *in vitro*. *J. Mol. Biol.* 63:183-200, 1972. (Cited 135 times.)
2. Wu R, ed. Screening and selection of cloned DNA. *Meth. Enzymol.* 68:379-442, 1979.