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Citation Classics

Gillespie D & Spiegelman S. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *Journal of Molecular Biology* **12**:829-42, 1965.

A method is described for performing RNA-DNA hybridization with DNA immobilized on a nitrocellulose membrane. The method is simple and convenient and eliminates the competing DNA reaction, allowing reliable quantitation of the RNA-DNA hybridization reaction. [The *SCI*® indicates that this paper was cited 1,227 times in the period 1961-1975.]

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"The technique of RNA-DNA hybridization using DNA immobilized on a nitrocellulose membrane was developed through insight, hard labor, and a stroke of luck. Most of the insight was provided by Sol Spiegelman, most of the labor by Sally Gillespie, and most of the luck by my errors. Sol immediately recognized the application of an article by Roy Britten describing the immobilization on glass of poly (U) networks formed by irradiation with ultraviolet light and pressured me to form similar networks of denatured DNA on nitrocellulose membranes. After several experiments, all outrageously successful, I inadvertantly eliminated the irradiated step and lo! the magic of DNA immobilization on nitrocellulose began. I use the word 'magic' advisedly for even today we do not understand the chemical basis for the DNA immobilization.

"I feel the reason our paper is so often cited is that the protocol we worked out has survived as the simplest, most convenient and most versatile form of the technique. For this, both Sol and I owe my wife, Sally, a large measure of gratitude. She did much of the detailed work that led to the success of the technique and she was never satisfied with an aspect of the method that simply 'worked.' To her the best form of technique was always apparent and striven for; to me this insight never came before repeated botching, and when we reached dead ends Sol was always there solving our problems with one or two words...

"It is one thing to recognize reasons for the success of a classic retrospectively, but it is quite another to envision them during the course of the project or even before it begins. I must admit that as a graduate student I didn't recognize the potential of what I was doing at the time and, in fact, I am still amazed and somewhat bewildered by the longevity of our paper. Sol saw it, however, right from the beginning. He must have said–about once a month in order that I remain sufficiently bouyed to continue–'Gillespie, I'm going to make you famous.' I looked upon the method primarily as a neat trick that would allow me to discover some 'really important' facts of biological interest. These facts, of course, remain to this day in the Library of Congress with my thesis....

"As I look back upon that period while attempting to decide why a 'classic' becomes one, especially in the area of methodology, I keep returning to the notion of developing an unimprovable method. But this notion is so obvious that it would seem to follow that every person developing a technique with the potential of reasonably wide use would end up with such a classic. This leads me to think that the distinction between a classic and a quickly outmoded method lies in the ability of the investigators to see the uses to which the method will be put and evaluate particular parameters accordingly and, as importantly, to take heed of the little irregularities that lead to significant improvements. I mentioned the (lack of) irradiation and magnesium as bits of luck and wisdom earlier, but there were many other smaller points that could have relegated us to the status of a 'good 1965 paper.' For example, we noticed once that DNA filters we had kept for a couple of days in a drawer gave more hybridization to RNA than those DNA filters that were freshly made. The difference was small enough to be ignored, but we didn't ignore it and it led to 'baking' the DNA filters, driving all the water off and causing the DNA to remain more stably bound to the filter during hybridization. Had we not picked up this and several other little irregularities surely someone in 1966 or 1967 would have, and their version would have been the one cited from then on."