

Soltis D E, Hauffler C H, Darrow D C & Gastony G J. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Amer. Fern J.* 73:9-27, 1983.

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Detailed recipes were published to assist investigators wishing to pursue studies in starch gel electrophoresis of enzymes. In addition, advice was provided as to which gel and electrode buffer systems were most likely to provide clear banding for individual enzymes. [The SC® indicates that this paper has been cited in more than 315 publications, making it the most-cited paper published in this journal.]

Starch Gel Electrophoresis of Enzymes—Betty Crocker Style

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During the late 1970s there was a great deal of interest in applying the technique of enzyme electrophoresis to a broad taxonomic spectrum of plants to address a diverse array of evolutionary and systematic questions. However, the gel and electrode buffer systems, grinding buffers, and staining schedules initially developed for *Drosophila* and widely applied to animals did not work well, sometimes not at all, for many flowering plants or ferns. I was at the time a graduate student at Indiana University working on the systematics of a genus of the flowering plant family Saxifragaceae. Repeated attempts using standard enzyme electrophoretic protocols failed with these plants. *Drosophila* was usually added as a control in my early studies, and it routinely worked excellently, leading us to refer to this famous beast as nothing more than a flying bag of enzymes.

I had a thesis at stake and the pace of experimentation quickened. We finally realized that plant groups containing abundant tannins and other secondary compounds were not easily amenable to the electrophoretic technique. Upon the grinding of leaf tissue, these compounds complex with the very enzymes we attempted to study. By modifying the grinding buffer and adding compounds that would preferentially bind to secondary metabolites, we started to overcome difficulties.¹ At this point the second author, Chris Hauffler, began making weekly visits to Bloomington, Indiana, from St. Louis, where he was a postdoc, and we attempted to

apply to ferns what we had so far learned in work with Saxifragaceae.

The result was a wealth of data on optimal electrophoretic conditions for plants with high concentrations of secondary chemical compounds. These conclusions had been obtained via numerous hours of trial and error research to develop new or modified electrophoretic recipes. As we began to present results of our isozyme studies of ferns and angiosperms, it was clear that many of our colleagues had encountered similar difficulties with secondary metabolites in many other groups of plants. We therefore began to receive numerous requests for our recipes. This made us feel a bit like Betty Crocker, as we suggested that colleagues preheat their incubators to 37° C and add a pinch of this and a tad of that to the staining recipe to improve results. Hence, the title of this contribution to *Current Contents*®.

The receipt of so many requests for our starch gel electrophoresis methods was the stimulus behind the preparation of the paper. Nonetheless, there was much discussion and debate regarding the merits of publishing what was essentially a list of recipes. It seemed too much like a cookbook to us, rather than a "real" scientific contribution. We nonetheless proceeded with the preparation of the manuscript, although with some trepidation, not entirely sure it was the right idea and also suspecting that the paper would be rejected for publication. This uncertainty regarding the merit of this paper also influenced our choice of a journal. We recognized that our methods might be of broad interest, but still wondered if the paper would be published by a major journal. We decided it probably would not be accepted, and we therefore added "of Ferns" to the original title, "Starch Gel Electrophoresis," and thought we would target mainly those pteridologists interested in applying enzyme electrophoresis.

We were surprised therefore by the large demand for reprints of this paper. Our Betty Crocker cookbook did turn out to be of broad interest to plant scientists, not just pteridologists. Researchers interested in enzyme electrophoresis of angiosperms, mosses, algae, and even fungi were all interested in these recipes. Even though more up to date and extensive "recipe" papers on enzyme electrophoresis have now been published,² we still receive requests for the original Betty Crocker recipe paper, and it continues to be frequently cited.

1. Soltis D E, Hauffler C H & Gastony G J. Detecting enzyme variation in the fern genus *Bommeria*: an analysis of methodology. *Syst. Bot.* 5:30-8. 1980.

2. Wendel W F & Weeden J F. Visualization and interpretation of plant isozymes. (Soltis D E & Soltis P S, eds.) *Isozymes in plant biology*. Portland, OR: Dioscorides. 1989. p. 5-45.

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