

Streamlined Strategies to Better Visualize Southern Blotting

DEREK M. DEAN

ABSTRACT

In this article, I describe an animated slideshow of Southern blotting that I have made freely available to other instructors. My hope is to provide a clear visualization of the logistics behind the technique so that instructors have a solid basis – as well as time freed up – to discuss its applications with students.

Key Words: *Southern blot; Northern blot; pedagogy.*

Since its development nearly 40 years ago by E. M. Southern, the Southern blot remains a standard molecular biology technique for determining the arrangement of DNA sequences within a vector or genome (Southern, 1975). Besides the utility of the technique, its use of labeled probes that are complementary to a target sequence serves as a useful reminder of DNA structure in the classroom. Therefore, college molecular biology instructors frequently cover Southern blotting, as well as the related, and humorously named, “Northern” blot, where RNA is detected in a similar fashion (Alwine et al., 1977).

Although Southern and Northern blots illustrate important principles about nucleic acid chemistry and hybridization, they are very difficult to describe in lecture: it is an elaborate process to separate nucleic acid molecules by size, move the nucleic acid from gel to blot, radioactively label a probe, hybridize the probe to a target sequence on the blot, and detect the hybridized probe by exposure of the blot to x-ray film. In my experience, the transitions between gel and filter and between filter and film present the most significant challenges to students. In all likelihood, this is because in the past, I was using static images to describe a very dynamic process. Students could benefit from demonstrations or a chance to perform a blot themselves, but either approach would be time-consuming (at least 2 days), technically difficult, and expensive, even though protocols have been highly standardized in more recent years (Sambrook & Russell, 2001).

In my experience, the transitions between gel and filter and between filter and film present the most significant challenges to students.

As a fast and inexpensive alternative to a live demonstration, I provide an animated slideshow on our school website that has worked well for me as I have walked students through Southern blotting (<http://biology.williams.edu/faculty-staff/ddean/publications/>). The slideshow, which should take no more than 12 minutes to present, first describes how DNA is restriction digested and separated by size using agarose gel electrophoresis. It then presents a labeled drawing of a blotting apparatus, showing, from bottom to top, a buffer reservoir, filter paper that acts as a wick, the agarose gel, nucleic acid binding filter, and a dry set of paper towels to draw the moisture upwards. Following this animated description, I would suggest interjecting a live demonstration: a standard agarose minigel – or, less expensively and more conveniently, a piece of paper labeled as a gel – is sprinkled with small droplets of food coloring or other liquids of varying colors, all up and down one lane of the gel (Figure 1A). Each droplet is a unique color and is explained by the instructor to represent a unique nucleic acid sequence that was separated from the others using gel electrophoresis.

A piece of paper is pressed upon the top of the gel or first paper sheet, allowing the colored droplets to soak into it (Figure 1B), and then the paper is inverted to show the class how capillary action caused the transfer of “DNA” in the same arrangement that it had in the gel (Figure 1C). Students are reminded that nucleic acid is not normally visible on the filter at this point, but upon adding a probe that is, for example, specific for the molecule represented by the red dye, that molecule will be detected on an autoradiogram. These final steps (i.e., probe hybridization through film exposure) are illustrated in the remaining slides of my PowerPoint presentation.

Interested instructors may download these PowerPoint slides from the Williams College Biology Department website for use in their classroom presentations (<http://biology.williams.edu/faculty-staff/ddean/publications/>). Any comments, requests, or suggestions are welcome.



Figure 1. A visualization of the Southern/Northern blotting process for use in the classroom. (A) Standard agarose minigel that has had droplets of different food colorings laid on top of it, within the confines of a single lane. Each color is said to represent a nucleic acid of a specific size that was hypothetically separated through gel electrophoresis. If an agarose gel is unavailable, a piece of paper or other surface may be used just as well, provided it is made clear that it represents a gel. (B) A piece of paper, which substitutes for the nitrocellulose or nylon membrane used in blotting, is laid on top of the gel, soaking up the food coloring. (C) The paper is inverted to show the “nucleic acids” that have been transferred by capillary action.

○ Acknowledgments

Thanks to Malcolm Campbell at Davidson College for DNA gel and Southern hybridization photographs that were used in the slideshow.

References

Alwine, J.C., Kemp, D.J. & Stark, G.R. (1977). Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. *Proceedings of the National Academy of Sciences, USA*, 74, 5350–5354.

Dean, D.M. (2011). Southern slideshow (PowerPoint presentation). Available online at <http://biology.williams.edu/faculty-staff/ddean/publications/>.

Sambrook, J. & Russell, S.W. (2001). *Molecular Cloning: A Laboratory Manual*, 3rd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Southern, E.M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology*, 98, 503–517.

DEREK M. DEAN is a Lecturer in the Department of Biology, Williams College, 59 Lab Campus Drive, Williamstown, MA 01267; e-mail: ddean@williams.edu.

Teachers in BIOLOGY

MASTER OF SCIENCE IN GENERAL BIOLOGY

TEACHERS! ONLINE DEGREE PROGRAM OFFERED!

Designed for K-12 teachers, this 33-hour program is taught online* and can be completed in two years.

Learn from faculty in the Department of Biological Sciences. Join students from around the world to earn a Master of Science degree through distance learning from Mississippi State University!

***The final capstone course is 8-10 days at MSU.**

For more information visit:
www.distance.msstate.edu/tib

or contact:
hstreet@aoce.msstate.edu

MSU is fully accredited by SACS.
MSU is an equal opportunity employer.

**MISSISSIPPI STATE
UNIVERSITY**