Ode to the humble Southern blot in the era of exomes

Alex R. Paciorkowski, MD

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Correspondence

Dr. Paciorkowski
Alex_Paciorkowski@urmc.
rochester.edu

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Methods to perform the Southern blot, the separation of pieces of DNA by size and then identification with a molecular probe, were first published in 1975,¹ before some of us in academic medicine had even entered preschool. It seems astonishing in this post-Human Genome Project era that the technique still has relevance in modern genetic diagnostics. Yet, even with the growing availability of exome sequencing, the Southern blot remains the method of choice to detect expansions in nucleotide repeats in the human genome. In this issue of *Neurology® Clinical Practice*, Wallace and Bird² remind us that neurologists forget this at our peril.

The coding portion of the human genome contains over 1,000 regions that comprise short tandem repeats. These monotonous regions can be many hundreds of nucleotides in length. Current exome sequencing technology does its work in read lengths of 100 base pairs at a time.³ For regions of the exome that are unique, it is no problem to then map each short read back to a single specific genomic location, and therefore to know exactly where a variation has occurred compared to the reference human genome. Exome technology excels at identifying single nucleotide variants—the point mutations in general parlance—above all else, and it does pretty well generally at identifying small insertions and deletions. But repeats are by definition not unique regions of sequence, and if the repeat region is longer than 100 base pairs mapping a run of CAGs (for example) back to a specific genomic address is not possible with current exome technology. In addition, several pathogenic repeat expansions occur in noncoding portions of the genome that are not evaluated by standard exome sequencing at all. It is for this reason that exome sequencing in the ataxia clinic is not the first line of testing, even in 2017.

Several solutions are on the horizon in the laboratories of researchers, but are not yet available to clinical practice. Longer read sequencing technologies, which can handle reads on the order of tens of thousands of base pairs in length, exist and allow expanded repeats flanked by unique sequence, and are therefore mappable.⁴ Short read whole genome sequencing (which includes the 3% of the genome that is coding, as well as the 97% that is noncoding) also allowed identification of repeat length with novel informatics techniques.^{5,6} However, these solutions are not yet ready for clinical use.

Wallace and Bird highlight an important message confronting neurologists in the genomic era. Amid the excitement of the newer massively parallel sequencing technologies, and the ability to assay the sequence of 20,000 genes simultaneously with exome, it is important to remember that not all genomic events are detectable by sequencing alone. Consultation with a medical geneticist familiar with genomic testing technologies is encouraged to assure valuable patient care time and resources are used most effectively, and the tests ordered are capable of identifying the disease suspected. In the case of detecting repeat expansions that cause many hereditary ataxias, this most capable test remains the unassuming Southern blot.

Departments of Neurology, Pediatrics, Biomedical Genetics, and Neuroscience, Neurogenetics Consultation Service, Hereditary Ataxia Program, Child Neurology, University of Rochester Medical Center, NY.

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References

1. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975;98:503–517.
2. Wallace S, Bird TD. Molecular genetic testing for hereditary ataxia: what every neurologist should know. *Neurol Clin Pract* 2018;8:27–32.
3. Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet* 2011;12:745–755.
4. Liu Q, Zhang P, Wang D, Gu W, Wang K. Interrogating the “unsequenceable” genomic trinucleotide repeat disorders by long-read sequencing. *Genome Med* 2018;9:65.
5. Dolzhenko E, van Vugt JJFA, Shaw RJ, et al. Detection of long repeat expansions from PCR-free whole-genome sequence data. *Genome Res* 2017;27:1895–1903.
6. Gymrek M, Golan D, Rosset S, Erlich Y. lobSTR: a short tandem repeat profiler for personal genomes. *Genome Res* 2012;22:1154–1162.