

TOOLBOX

Sequencing technique optimal for postmortem brains

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Good fit: A technique sequences degraded RNA (right) better than an older method does (left).

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A technique for measuring gene expression that detects short RNA messages is better suited than traditional methods for analyzing postmortem brain tissue, according to a study published 10 September in *BMC Genomics*¹.

Studies on gene expression typically look at **levels of messenger RNA**, or mRNA, the messages that code for protein.

Because gene expression is tissue-specific, however, autism studies often turn to postmortem brain samples to study gene expression in the brain. But RNA is easily damaged or degraded in

postmortem brains.

In the new study, researchers compared two methods of RNA sequencing: *in vitro* translation (IVT) and cDNA-mediated annealing, selection and ligation, or DASL. Researchers have used DASL to investigate gene expression in tissues from biopsies and autopsies, but this is its first use with postmortem brains.

Unlike IVT, DASL generates DNA copies of mRNA messages, called cDNA, and matches them with overlapping probes as small as 50 bases. The relatively small size of these probes makes this technique ideal for sequencing short, or damaged, RNA fragments.

In the new study, researchers first simulated the degraded RNA found in postmortem brains by exposing RNA to high temperatures for 10, 30 and 60 minutes. They then compared the gene expression results from these samples to a reference RNA sequence.

When RNA is degraded for 30 minutes, the IVT results vary significantly from the reference RNA, suggesting that IVT cannot reliably measure degraded RNA. However, results from the DASL technique are similar to the reference sequence, the study found. Results from both techniques vary from the reference sequence for RNA degraded for an hour, but those from DASL are more similar than the results from IVT.

The researchers then compared the accuracy and sensitivity of the two methods using 57 frozen postmortem brain samples and 4 chemically preserved brains. DASL sequencing is more sensitive and detects more genes than the IVT method does in frozen brain samples, the study found.

Both techniques have poor results with fixed brain tissue, however. A March 2011 study shows that researchers can **extract high-quality DNA** from frozen, but not fixed, postmortem brains².

References:

1: Chow M.L. *et al. BMC Genomics* **12**, 449 (2011) [PubMed](#)

2: Wintle R.F. *et al. Autism Res.* **4**, 89-97 (2011) [PubMed](#)