

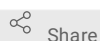
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Protocols for Northern Analysis of Exosome Substrates and Other Noncoding RNAs

Book Chapter

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ABSTRACT

Over the past decade a plethora of noncoding RNAs (ncRNAs) have been identified, initiating an explosion in RNA research. Although RNA sequencing methods provide unsurpassed insights into ncRNA distribution and expression, detailed information on structure and processing are harder to extract from sequence data. In contrast, northern blotting methods provide uniquely detailed insights into complex RNA populations but are rarely employed outside specialist RNA research groups. Such techniques are generally considered difficult for nonspecialists, which is unfortunate as substantial technical advances in the past few decades have solved the major challenges. Here we present simple, reproducible and highly robust protocols for separating glyoxylated RNA on agarose gels and heat denatured RNA on polyacrylamide–urea gels using standard laboratory electrophoresis equipment. We also provide reliable transfer and hybridization protocols that do not require optimization for most applications. Together, these should allow any molecular biology lab to elucidate the structure and processing of ncRNAs of interest.

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KEYWORDS

lncRNA, ncRNA, northern blot, hybridization, probes

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Over the past decade a plethora of noncoding RNAs (ncRNAs) have been identified, initiating an explosion in RNA research. Although RNA sequencing methods provide unsurpassed insights into ncRNA distribution and expression, detailed information on structure and processing are harder to extract from sequence data. In contrast, northern blotting methods provide uniquely detailed insights into complex RNA populations but are rarely employed outside specialist RNA research groups. Such techniques are generally considered difficult for nonspecialists, which is unfortunate as substantial technical advances in the past few decades have solved the major challenges. Here we present simple, reproducible and highly robust protocols for separating glyoxylated RNA on agarose gels and heat denatured RNA on polyacrylamide–urea gels using standard laboratory electrophoresis equipment. We also provide reliable transfer and hybridization protocols that do not require optimization for most applications. Together, these should allow any molecular biology lab to elucidate the structure and processing of ncRNAs of interest.

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RNA Electrophoresis in Agarose Gels
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