Rapid and accurate mRNA detection in plant tissues

April 17 2014

Gene expression is the process whereby the genetic information of DNA is used to manufacture functional products, such as proteins, which have numerous different functions in living organisms. Messenger RNA (mRNA) serves as an important intermediary during gene expression, by relating the genetic information of DNA to the molecular mechanisms involved in manufacturing proteins.

By examining the different types and amounts of mRNA molecules present in an organism at a given time, researchers can determine which specific genes are being expressed. This, in turn, offers tremendous insight into the genes responsible for producing different morphological forms (or phenotypes).

Scientists generally rely on in situ hybridization (ISH) techniques (using mRNA-specific probes) to determine the presence or absence of particular mRNA molecules in plant tissues. Most traditional ISH methods, however, are time- and labor-intensive and lack the sensitivity necessary to precisely quantify the amount of expression of each gene.

In a new study in the April issue of *Applications in Plant Sciences*, researchers from Dow AgroSciences demonstrate the effectiveness of a new ISH method, called RNAscope ISH, for studies of gene expression in plants. In contrast to traditional approaches, RNAScope ISH is significantly faster and highly sensitive, permitting researchers to not only detect but also quantify, with confidence, the expression levels shown by genes of interest.

RNAScope ISH was developed by Advanced Cell Diagnostics (ACD) Inc., initially for studies of gene expression in animal (and especially human) tissues. It is a type of branched DNA ISH that uses pairs of 'Z-probes,' which are highly specific to target genes, but also small enough to easily diffuse into the tissues under study. Once the Z-probes bind to the mRNA molecules of interest, this allows label molecules to also bind, which then makes the mRNA molecules detectable, either via fluorescence or chromogenic detection.

According to Dr. Andrew Bowling, lead author of the paper and a research scientist at Dow Agrosciences, detected mRNA molecules appear as individual spots within the cell, making the expression results very easy to interpret.

"This method has a very low background level, which allows for higher confidence in the results obtained. With the conventional method, there seemed to always be a point where you were trying to determine if one tissue or cell was 'bluer' than another, or 'bluer' than the control. With the RNAscope method, the experimental sections have spots and the control sections don't. The results are very clear."

Bowling adds that RNAscope ISH is also a very accessible method compared to more traditional ISH approaches.

"The vendor provides probe design and synthesis services, greatly reducing the suite of skills that are required to carry out a successful ISH experiment. You don't have to be (or collaborate with) an RNA in vitro transcription expert to make your labeled probes. You don't have to synthesize plasmids to transcribe. You don't have to run northern blots to confirm efficacy and specificity. That is all part of the probe design and synthesis service. Also, it only takes about two weeks from emailing them a sequence to obtaining the probe in the mail from them."

"Furthermore, the [RNAScope ISH] method is faster. It takes about 8–10 hours to do an experiment. The classic ISH methods could sometimes require one or even two overnight incubations."

More information: Andrew J. Bowling, Heather E. Pence, and Jeffrey B. Church. 2014. Application of a novel and automated branched DNA in situ hybridization method for the rapid and sensitive localization of mRNA molecules in plant tissues. *Applications in Plant Sciences* 2(4): 1400011. DOI: 10.3732/apps.1400011.

Provided by American Journal of Botany