CELLULAR LOCALIZATION OF TRANSCRIPTS VIA *In situ* RNA HYBRIDIZATION

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In situ hybridization is a widely used method that permits the localization of target mRNAs in a preserved tissue section. Most *in situ* hybridization methods use either non-isotopic or isotopically labelled riboprobes. The use of digoxigenin (DIG) compound is popular in the former type of labelling. For this, hybridized DIG-labelled riboprobes are normally detected with high affinity anti-digoxigenin (anti-DIG) antibodies that are conjugated to alkaline phosphatase that consequently allows for colorimetric visualization of the anti-DIG antibody conjugate using NBT (Nitro blue tetrazolium chloride)-BCIP (5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt) substrate solution. There is also the alternative method of using fluorescence-labelled probes. In addition, various other *in situ* hybridization protocols have been developed and reported over the years.

In situ hybridization, however, can be problematic when it comes to detecting low abundance mRNA or diffusely localized mRNA (McFadden, 1995). For oil palm samples, the previously used protocols based on Jackson (1992) and Coen *et al.* (1990) worked well only on certain transcripts. On some occasions, the difference between the sense and antisense hybridized sections was not distinct enough. Even after several optimization attempts on hybridization temperature, stringency washes, *etc.*, the signals were still indistinguishable.

We have thus modified and optimized the conventional standard protocol (Coen *et al.*, 1990; Jackson, 1992) for *in situ* hybridization of oil palm tissues (*Figures 1* and *2*). This modified protocol offered as a service by MPOB is faster, simpler and uses fewer reagents.

SERVICE OFFERED

In situ RNA hybridization is a highly technical methodology that requires experienced and skilled expertise. The main equipment required for *in situ* hybridization include the microtome, embedding station and shaker incubators. With the modified method, a time-saving of approximately 6 hr as well as cost savings are achieved as compared to the previously used standard protocol.

For the service, samples can be provided in the following forms:

- plant tissues in fixative solution, OR
- tissues embedded in paraffin moulds AND
- DIG-labelled RNA probes (sense and antisense probes).

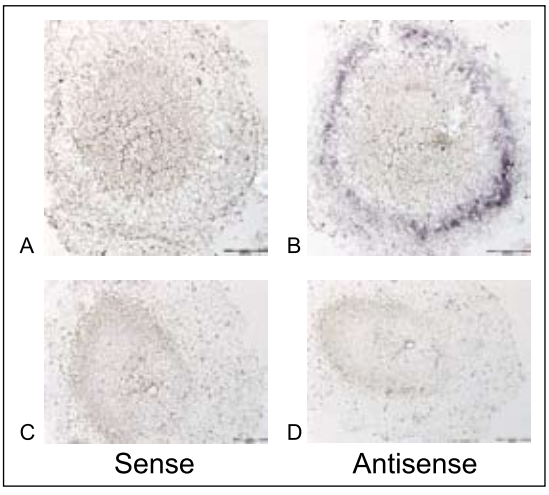

Figure 1. Comparison of in situ *hybridization results on oil palm suspension calli using the different protocols: modified protocol (A, B) and standard protocol (C, D).*

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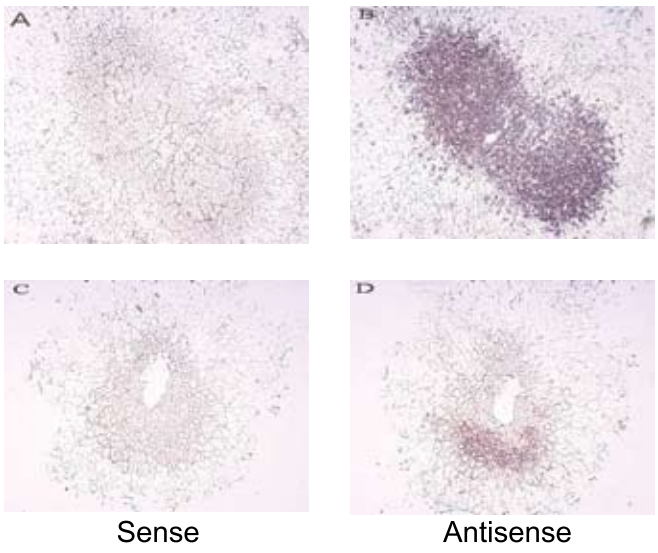

Figure 2. Oil palm suspension callus hybridized with two different probes: elongation factor 1α (A, B) and ACC oxidase (C, D).

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