

SUPPLEMENTARY MATERIAL FOR:

## Rolling circle amplification-RACE: a method for simultaneous isolation of 5' and 3' cDNA ends from amplified cDNA templates

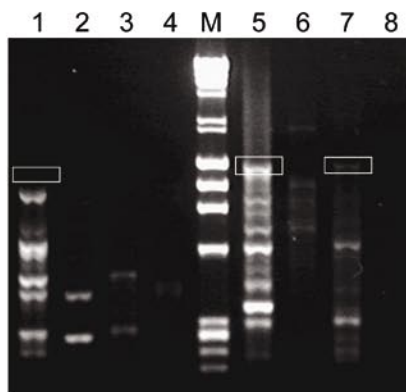
Alexios N. Polidoros<sup>1</sup>, Konstantinos Pasentsis<sup>1</sup>, and Athanasios S. Tsiftaris<sup>1,2</sup><sup>1</sup>Institute of Agrobiotechnology and <sup>2</sup>Aristotle University of Thessaloniki, Thessaloniki, Greece*BioTechniques* 41:35-42 (July 2006)

To test the efficiency of circularization and the possibility of directly isolating a transcript from the circular cDNA pool, a diagnostic inverse PCR was performed just after circularization in 50  $\mu$ L volume containing 0.2 mM dNTPs, 0.4  $\mu$ M primers, and 1 U Vent (exo<sup>-</sup>) DNA polymerase (New England Biolabs, Ipswich, MA, USA) or DyNAzyme™ II DNA polymerase (Finnzymes, Espoo, Finland) in the 1 $\times$  reaction buffer supplied by the manufacturers. For each reaction, a 2.5- $\mu$ L aliquot of the purified circularized cDNA was used as template in PCRs with the maize InvF (5'-CGAGTGGGAAGTGGACTTGCT-3') and InvR (5'-TGGACATAAGC CGTAGCATG-3') primers, or the crocus Inv1 (5'-GCGCTTCGAGAA GGTGACCTG-3') and Inv2 (5'-GGCGGCCTCTCAAGAAAGC-3') primers. A control reaction containing only the forward primers Inv2 or InvF was also performed. The cycling parameters were 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 54°C for 45 s, 72°C for 1.5 min, and a final extension step of 72°C for 10 min. The PCR products were separated on an agarose gel and stained with ethidium bromide. Several PCR products were observed. Therefore, the gel was blotted onto nylon membrane and hybridized with digoxigenin-labeled *CsAPIa* and *Aox1a* probes as described (13,14). Hybridizing bands of the expected size were cut out from the gel, purified, cloned, and sequenced.

Using the outward pointing primers InvF/InvR for maize and Inv1/Inv2 for crocus, specific PCR products could result from circular templates or from concatemers of each transcript,

although the latter was not expected by the particular ligase. For PCR, we used a standard DyNAzyme II DNA polymerase, as well as Vent (exo<sup>-</sup>) DNA polymerase, which lacks 5' to 3' exonuclease activity, so that possible 5' to 3' hydrolysis of the newly synthesized strand after completion of the cycle on the circular template can be avoided. A control reaction with no reverse primer was also included to test whether PCR products could be produced from noncircularized molecules with traces of remaining oligo(dT)-adaptor.

As shown in Figure S1, PCR products could be observed in all but one reaction and appeared different



**Figure S1. Inverse PCR on circular cDNA templates.** Lanes show amplification products of crocus *CsAPIa* (lanes 1–4) and maize *Aox1a* (lanes 5–8) genes using Vent (exo<sup>-</sup>) (lanes 1–2, 5–6) and DyNAzyme II (lanes 3–4, 7–8) DNA polymerases on circular cDNA. Lanes 2, 4, 6, and 8 are the control reactions with only the forward primers used. Boxed bands of the expected size were excised, cloned, and sequenced. M indicates the  $\lambda$ HindIII/ $\phi$ X174HaeIII molecular weight marker (Finnzymes).

for the two DNA polymerases used. Multiple bands were observed with Vent (exo<sup>-</sup>) for amplification of both crocus *CsAPIa* and maize *Aox1a* genes (Figure S1, lanes 1 and 5, respectively), while in the control PCR without the reverse primers, two to three weak bands were observed in crocus and two to three very faint bands could be discerned in maize (Figure S1, lanes 2 and 6, respectively). Using DyNAzyme II, two to three weak bands were observed for both genes (Figure S1, lanes 3 and 7, respectively), while in the control PCR without the reverse primers, a very faint band could be observed in crocus, and no band was evident in maize (Figure S1, lanes 4 and 8, respectively). The Vent (exo<sup>-</sup>) PCR products could hybridize with a *CsAPIa* and an *Aox1a* probe, while with the DyNAzyme II polymerase, hybridization signals were observed only for the maize *Aox1a* gene (data not shown). The bands expected from the full-length template were of higher molecular weight than all the other hybridizing bands and, in the case of *Aox1a*, were of higher molecular weight from all clearly evident bands on the gel. The expected approximate 1200-bp band of *CsAPIa* [only from the Vent (exo<sup>-</sup>), not evident on the gel] and expected approximately 1300-bp bands of *Aox1a* (resulted from both polymerases and evident on the gel) that gave strong hybridization signals were excised from the gel (boxed bands in Figure S1), cloned, and sequenced. Results confirmed that sequences of both genes corresponded to those expected from a circular full-length cDNA template. These data indicated that inverse PCR directly after circularization was confronted by high background problems and might be not very useful for isolation of unknown transcripts. For direct cloning of an unknown transcript, optimization of PCR conditions might improve performance, and nested PCR with internal primers could be the most appropriate solution to avoid high background problems. However, our results suggested that if a gene-specific probe is available, then selection of the longest hybridizing band for cloning could lead to isolation of the full-length transcript of the gene.