Application Note

Comparison:
Agarose vs. Elchrom's Spreadex® Precast Gels

Analysis of pre-mRNA splice variants in wild type and NMD mutant
Arabidopsis thaliana

Introduction

Most eukaryotic genes are transcribed into precursor messenger RNAs (pre-mRNAs) containing exon and intron sequences followed by intron removal in the process of pre-mRNA splicing. Interestingly, many pre-mRNAs can be alternatively spliced thereby increasing the transcriptome diversity. Alternative splicing can lead to mRNA variants encoding different proteins or affect transcript features such as mRNA stability and hence contribute to quantitative gene control. Thus, the elucidation of splicing patterns provides important insight into fundamental aspects of gene expression and regulation. As steady state transcript levels are defined by transcription and splicing on one side and mRNA turnover on the other side, the usage of mRNA turnover mutants allows detection of splice variants, which are usually rapidly degraded. For example, the *lba1* mutant is impaired in the nonsense-mediated decay (NMD) pathway, which is responsible for degradation of aberrant mRNAs. Besides serving as an mRNA surveillance mechanism, NMD coupled to alternative splicing is also employed to mediate gene expression control. Relative accumulation of certain splice variants in the *lba1* mutant compared to wild type plants indicates that the respective mRNAs are NMD substrates.

Steady state transcript patterns can be analyzed by reverse transcription of mRNA followed by PCR amplification of cDNA regions and separation and visualization of DNA products. One major limitation of this approach is the separation of PCR products with only small size differences, which is difficult to achieve by classical electrophoresis systems.

Results

Separation of PCR products with small size differences is limited by classical electrophoresis. With high resolution Spreadex® gels, the number of visible splice variants increases in one of the displayed examples from 2 up to 5 (indicated with arrows). In addition, the increased sensitivity of Spreadex® gels (only half of the amount of DNA was loaded per lane) facilitates the detection of low abundant splice variants.

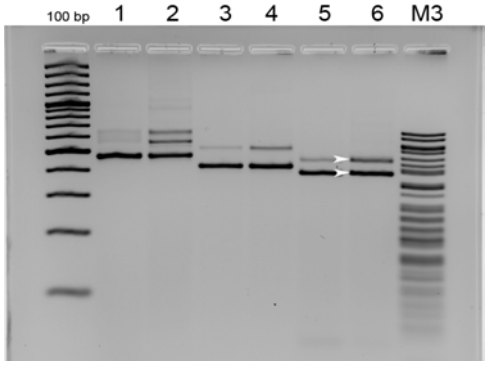

Fig. 1 3% agarose gel
7 µl of PCR amplified cDNA separated on a 3% agarose gel. Uneven numbers (1,3,5) represent the WT splice variants of the mRNAs . Even numbers (2,4,6) represents the splice variant of the *lba1* mutant. In addition to the M3 marker (Elchrom Scientific) the 100bp ladder (Fermentas) was used.

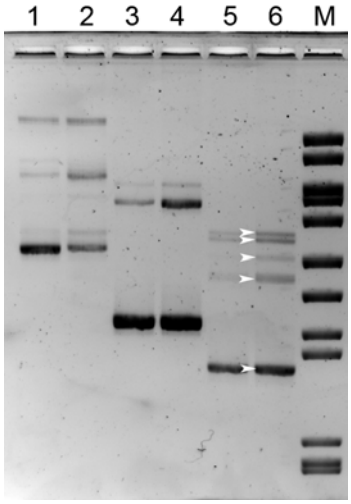

Fig. 2 Spreadex® EL1200 precast gel
3 µl of PCR amplified cDNA separated on precast Spreadex® EL1200 gel. Uneven numbers (1,3,5) represent the WT splice variants of the mRNAs. Even numbers (2,4,6) represent the splice variants of the *lba1* mutant. The marker indicated with M corresponds to the M3 marker from Elchrom Scientific.

Both gels where run at conditions for optimal band separation (e.g. Spreadex® EL 1200 run for 240 min., 120 V and 55 °C).

Advantages of the method include:

• Separation of PCR products with small size differences

• Spreadex® precast gels are mechanically stable and easy to handle

• Simple downstream analysis; fragments of interest can be analysed further by direct sequencing (no gel extraction)

• Results are reliable and highly reproducible

Experimental Procedures

Equipment

Electrophoresis was performed using the ORIGINS by Elchrom™ Scientific submarine electrophoresis system. PCR Products were separated on a 3% agarose gel as well as on a precast Spreadex® 1200 gel.

Sample Preparation and Running Conditions

Total RNA was isolated from 14 days old wild type or *lba1* mutant seedlings and subjected to reverse transcription using oligo dT. Subsequently, partial cDNA sequences were amplified by PCR with three different primer combination revealing steady state levels of various splicing variants for the corresponding genes X, Y and Z. 3 µl of each PCR product were separated on a Spreadex® EL1200 gel at 120 V, 55°C for 240 min. The marker corresponds to the M3 marker from Elchrom Scientific.
For comparison, the same samples were analyzed on a 3% agarose gel by loading 7 µl of each sample and gel run for 120 min at 80 V at RT. In addition to the M3 marker, the 100bp ladder (Fermentas) was used, which contains DNA fragments of 100bp increments starting with 100bp.

Detection

Upon completion of the gel run, gels were stained in Ethidium Bromide solution and destained in water followed by UV illumination for visualization of DNA bands.

References

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2. Reddy, A.S. (2007).
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   Annu Rev Plant Biol 58, 267-294.
3. Soergel, D., Lareau, L., Brenner, S (2006)
   Regulation of gene expression by coupling of alternative splicing and NMD.
   In Nonsense-mediated mRNA decay, L. Maquat, ed (Georgetown, Texas: Landes bioscience), pp. 175-190.
4. Yoine, M., Ohto, M.A., Onai, K., Mita, S., and Nakamura, K. (2006).
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   Plant J 47, 49-62.

Acknowledgement

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Ordering Information

Product	P/N
ORIGINS by Elchrom™ Scientific, 230 V	2100E
ORIGINS by Elchrom™ Scientific, 115 V	2100U
Spreadex® EL 1200 Wide Mini S-2x13 *	3431
Spreadex® EL 1200 Wide Mini S-2x25*	3447
Spreadex® EL 1200 Wide Mini S-4x13**	3457
Spreadex® EL 1200 Wide Mini S-2x104*	3457
Spreadex® EL 1200 Wide Mini S-2x104L*	3477
40 x TAE Running buffer (20 tubes a 50 ml)	3031
Easy Stain Tray	2344
Power Supply, 200 V/2000 mA, incl. timer	2029E
PeelIT™	2355

* 1 box of 2x6 gels
** 1 box of 6x4 gels

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