Fast screening of Tobacco leaves for recombinant protein expression.

Tobacco Leaves

CASE STUDY

Dr. Loïc Faye, ANGANY Genetics, France.

Overview

- **Keywords:** Protein expression, plant screening, recombinant protein
- **Aim of the study:** Selection of the best producers of a recombinant protein used for allergic patients desensitization
- **Application:** Western Blot analysis
- **Sample name:** Leaves
- **Material:** FastPrep-24™ homogenizer, 2 mL Lysing Matrix D tubes containing 1.4 mm ceramic spheres
- **Buffer:** Laemmli denaturing sample buffer containing 60 mM Tris-Cl pH 6.8, 1% SDS, 10% glycerol, 2% beta-mercaptoethanol

Protocol and Parameters

1. Snap one tobacco leaf disc (10 mg) in a Lysing Matrix D tube
2. Add 200 µL of denaturing buffer to the Lysing Matrix D tube
3. Homogenize the plant tissue with the FastPrep-24™ instrument for 60 seconds at a speed setting of 4.0 m/s
4. Transfer supernatant from the Lysing Matrix D tube into an Eppendorf tube
5. Boil each sample for 5 min and then centrifuge 1 min at 12,000 rpm to pellet the cellular debris
6. Load 15 µL of each supernatant in a 15% polyacrylamide gel for protein separation by electrophoresis

After SDS-PAGE proteins are either stained in the gel or transferred on a membrane for Western Blot analysis.

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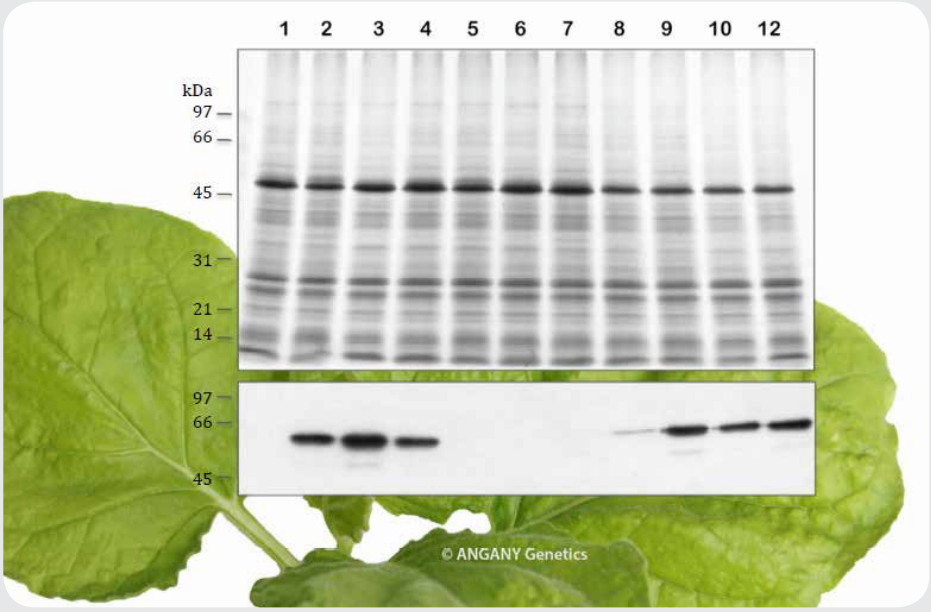
Results

Screening for expression of a recombinant allergen from house dust mite in tobacco plants using the FastPrep® instrument for sample preparation.



Each lane in the upper panel shows the protein pattern (SDS-PAGE) obtained for one leaf disc cut out from 12 different tobacco plants used for transient expression of the recombinant allergen. The lower panel is a Western Blot used for identification of the best allergen producers.

The same samples were used in the upper and lower panels. This procedure is part of the Allergo- Pur™ production platform developed in ANGANY Genetics, France.

Conclusion

- The FastPrep® system is a powerful tool to rapidly obtain protein extracts with high reproducibility, ready for electrophoresis (SDS-PAGE) and Western Blot analysis.
- The FastPrep-24™ has also been successfully used for protein extraction from other plants (Arabidopsis, lettuce etc...) in the same conditions using less than 15 mg of plant material for sample preparation.
- Similar experiments have been performed for protein extraction in non-denaturing conditions using ice cold buffer (100 mM Tris buffer, pH 7.4 containing 10% sucrose, 5 mM EDTA, 0.28% b-mercaptoethanol, 5 mL PMSF and 0.3 mL aprotinin) and plant samples stored at -70°C in Lysing Matrix D tubes before extraction.
- The process is then scaled-up by using the BigPrep™ adapter for homogenization in 2 x 50 mL tubes. Two large protein samples are prepared simultaneously from 1-2 g of plant tissues in 50 mL Lysing Matrix D tubes.

MP Biomedicals
Americas: 800.854.0530 | custserv@mpbio.com
Europe: 00800.7777.9999 | custserv.eur@mpbio.com
APAC: 65.6775.0008 | enquiry_ap@mpbio.com

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