

# Natural Method for Allergen Identification using FastPrep-24™ 5G technology

## CASE STUDY

Andrea Brazdova, Oumsaad Naas, Nicolas Visez, Jean-Pierre Sutra, Hélène Sénéchal et Pascal Poncet Hôpital d'Enfants Armand Trousseau, Laboratoire de Biochimie, Equipe "Allergie & Environnement", 26 avenue du Dr Arnold Netter, 75012 - Paris. Université de Lille, Laboratoire de Physico-chimie des Processus de Combustion et de l'Atmosphère, Lille.

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### Overview

- **Keyword:** Allergen, IgE immunoreactivity, pollen homogenization, hypersensitivity community, DNA extraction
- **Aim of the study:** Identification of fast method for protein extraction from pollen grains
- **Application:** Western blot analysis
- **Sample name:** Birch, Nettle, Wall Pellitory pollens
- **Sample type:** Pollen
- **Material:** FastPrep-24™ 5G instrument, CoolPrep adapter, 2 mL Lysing Matrix C & E tubes
- **Buffer:** PBS

### Protocol and Parameters

#### 1 - Incubation method

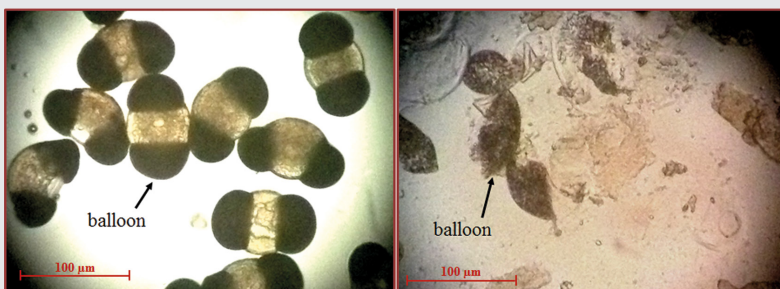
- Add 50 mg of pollen and 500 µL of PBS in a tube
- Place the tube in a shaker for 18 hours, in cold room
- Centrifuge the suspension 20 mins at 18 000 x g, 4°C
- Keep the supernatant at -20°C prior to analyses

#### 2 - Grinding method

- Add 50 mg of pollen and 500 µL of PBS in 2 mL Lysing Matrix C or E tube.
- Load Lysing Matrix tubes in a CoolPrep Adapter, containing dry ice.
- Process with the FastPrep-24™ 5G: 40 sec at a speed setting of 6.0 m/s.
- Centrifuge the Lysing Matrix tubes 20 mins at 18 000 x g, 4°C to pellet debris.
- Keep the supernatant at -20°C prior to analyses

### Results

#### Total destruction of the pollen grain structure with FastPrep-24™ 5G instrument and Lysing Matrix C



Optical microscope observation of pine pollen (X 200) before and after grinding with the FastPrep-24™ 5G System.

Left: pollen grain before grinding.

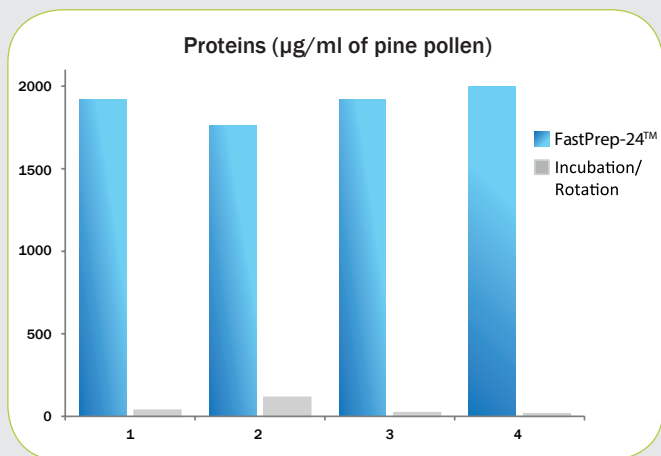
Right: homogenized pollen grain with FastPrep-24™ 5G, 40 s at speed 6 m/s with Lysing Matrix C.

(FastPrep-24™ 5G, MP Bio, CoolPrep, 40 sec, 6 m/s)

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## Results

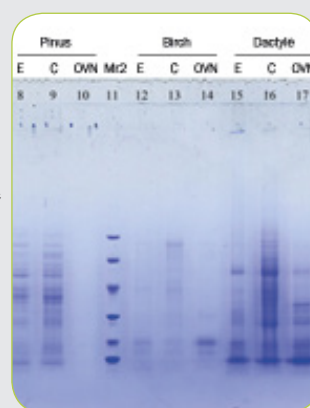
### Up to 2 mg/ml of protein extracted with FastPrep-24™ 5G System



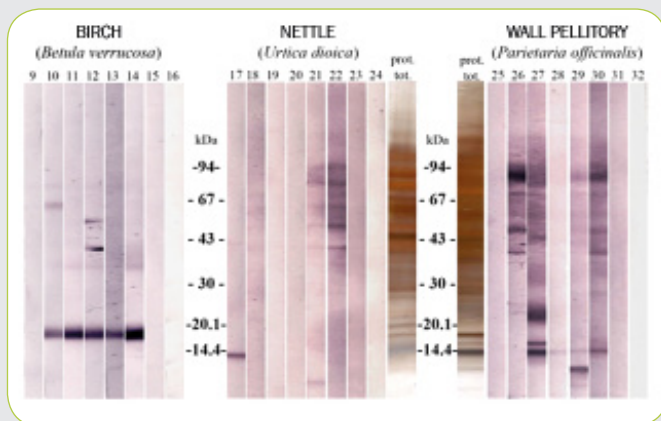
Comparison of 8 pine pollen protein extracts obtained by standard or FastPrep® method. Experience is repeated 4 times using 4 different pollen batches. Protein concentration is determined using Bradford assay.

### Effective protein extraction, with the FastPrep-24™ 5G System, for all the tested pollen

Comparison of protein extraction with standard (OVN) or FastPrep® method, using Lysing Matrix C or E. Coomassie blue gel staining.

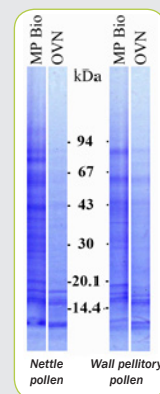


### IgE immunoreactivity conservation for pollen extracts



Conserved IgE immunoreactivity in pollen extracts obtained with FastPrep-24™ 5G instrument. Left, IgE immunoreactivity of 6 birch pollen allergic patients tested against birch pollen protein (9-14 strips). Middle and right, IgE immunoreactivity of 12 patients, allergic to herbaceous pollen, tested against nettle and wall pellitory pollen protein (17-22 & 25-30). Relative masses expressed in kDa. 15, 16, 23, 24, 31 & 32 strips correspond to negative controls.

### Wide variety composition of protein extracted with FastPrep® method



Protein profiles comparison of nettle and wall pellitory pollen obtained by FastPrep-24™ 5G (MP Bio) homogenization or by overnight incubation (OVN). The 4 extracts are used without dilution or concentration. SDS-PAGE 8-18 %. Coomassie blue gel staining.

## Conclusion

- Protein extraction from pollen samples with the FastPrep-24™ 5G showed to be highly effective compared to the standard method based on overnight incubation. The effectiveness of the FastPrep® method is quantitative, higher protein yield, and qualitative, wide variety composition of protein extracts.
- The FastPrep® system is a powerful tool to get rapidly and with a very high reproducibility protein extracts ready for electrophoresis (SDS-PAGE) analysis.
- IgE immunoreactivity is conserved in protein extracted with the FastPrep-24™ 5G instrument.