

Protein extraction from yeast *Pichia pastoris* with Minilys

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CONTEXT

This laboratory develops biomolecular solutions for plant biomass production. The proteins extraction from methylotrophic yeast is a part of the laboratory's daily work.

MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-004 (0.5 mm glass beads).
- Samples: $5 \cdot 10^8$ to $1 \cdot 10^9$ of *Pichia pastoris* (pellets).
- Buffer: 500 μ L of *Pichia* breaking buffer (pH7.4, 50mM Na-Phosphate, 1mM EDTA, 5%(v/v) glycerol).

PROTOCOL

- Minilys: Two different speeds and different durations of cycle were tested to lyse yeast cells. Duplicate (5000 rpm) or triplicate (4000 rpm) by condition.
 - 5000 rpm 20 sec x 2, 4, or 6 with 60 sec break on ice between each cycle.
 - 4000 rpm 20 sec x 2 with 60 sec break on ice between each cycle.
- Analysis: SDS-PAGE.

RESULTS

The following treatment 4000 rpm 20 sec x 2 already gave good extraction of protein. There were no sign of the excess disintegration of the proteins even after 5000 rpm 20 sec x 6 treatment. Even the 5000 rpm 20 sec x 6 treatment gave no excess heat and the tube was less than 30°C after each treatment.

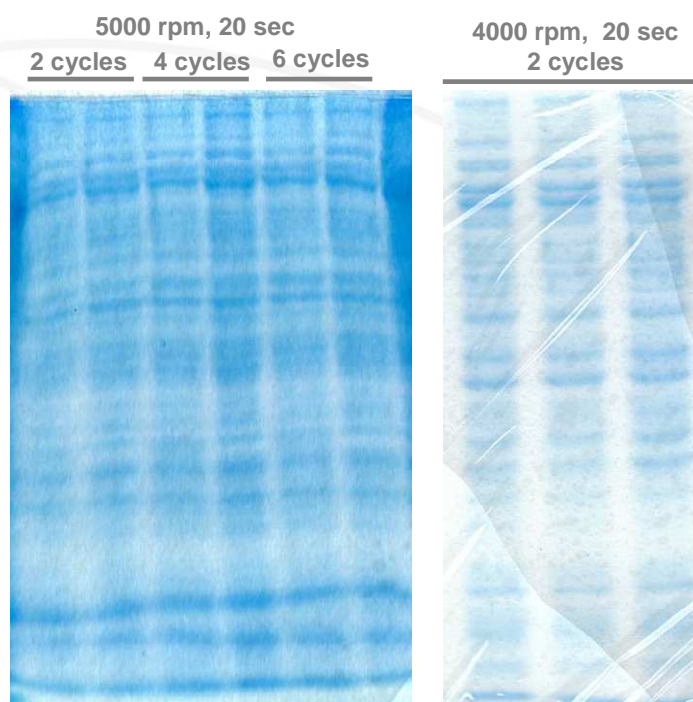
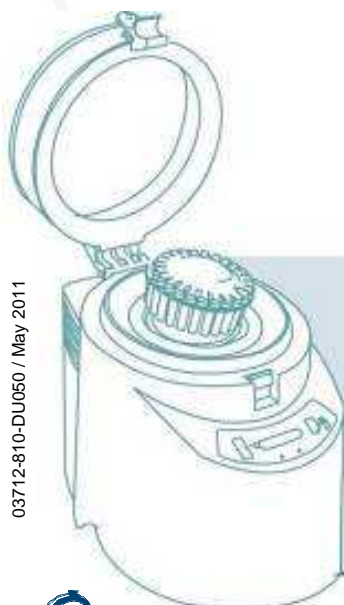


Figure 1: SDS-PAGE of the protein extracted from *Pichia pastoris*



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CONCLUSION

Buffer extraction with 4000 rpm, 20 sec x 2 treatment allows to have enough protein yield for the experiment while further treatment up to 5000 rpm 20 sec x 6 gave no sign of excess disintegration of the extracted proteins.

Minilys's ease of handling and good extraction power provide efficient and cross-contamination free throughput when the number of samples are small.

For more details, please contact precellys@bertin.fr

