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Polysomes immunoprecipitation and isolation of translating RNA from specific cell types of mouse cerebellum

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CONTEXT

Precellys®24 homogenizer has been tested to perform **polysomes immunoprecipitation (IP) and translating RNA extraction from IP**. In our system, polysomes of some specific cell types of the cerebellum are immunoprecipitated, by expressing a GFP tagged ribosomal protein in these specific cell types with CRE mediated recombination. As these cell types represent a few percent of the whole cerebellum, we should observe an enrichment of specific genes in the IP fraction compared to the input fraction (whole cerebellum lysate before IP).

MATERIAL

- **Precellys®24.**
- Precellys lysing kit: CK14_2mL (KT03961-1-003.2).
- Sample: new born or adult mouse cerebellum (10 mg to 50 mg).
- Buffer: 300µl to 1mL polysome extraction buffer described by Heiman et al., Cell 135, 738-748, 2008.

PROTOCOL

- Precellys®24: 6500 rpm, 2x10 sec, 10 sec break (new born cerebellum) or 2x20 sec, 20 sec break (adult cerebellum).
- 5 min incubation on ice, lysate transfer to Eppendorf tube, 10 min 13000g centrifugation 4 ° C.
- 1/20th of the lysate is saved for RNA extraction from the input (whole cerebellum RNA).
- IP: the remaining lysate is incubated with anti-GFP coupled magnetic beads.
- High salt rinses of magnetic beads / Ab / polysomes.
- RNA extraction from input and IP samples with Qiagen RNeasy microkit.
- RT and qPCR to determine gene enrichment or depletion in the IP fraction compared to input.

RESULTS

In the IP fraction, we could obtain an enrichment of RNA from our specific cell types (i.e. Purkinje cells and GABAergic interneurons), compared to RNA obtained from whole cerebellum (RNA from input fraction). Genes only expressed by Purkinje cells (Pcp2, Calb1, Fgf7) or genes expressed by both Purkinje cells and GABAergic interneurons (Parv, Gad65) are enriched in the IP fraction, whereas genes that are not expressed in these cell types (Gfap, Mbp, Glast, NeuroD1) are depleted in the IP fraction (Figure 1).

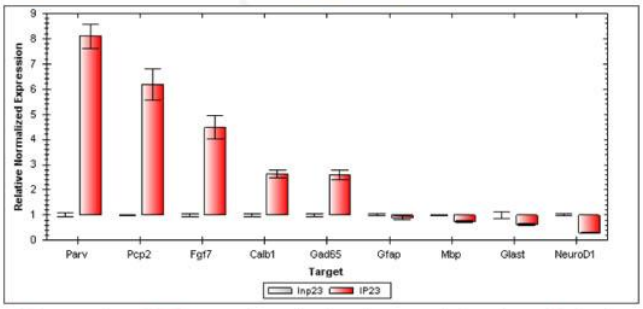

Figure 1: qPCR results obtained on adult mice. Relative normalized expression of different genes in IP fraction (IP23) compared to input fraction (Inp23).

CONCLUSION

Precellys®24 homogenizer is suitable and convenient for **polysomes immunoprecipitation and subsequent translating RNA purification** from mouse cerebellum.

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