MicroRNA expression profiling of individual rat hypothalamic nuclei

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**CONTEXT**

MicroRNAs (miRNAs) finely tune messenger RNA (mRNA) expression. As the brain is a highly heterogeneous tissue, physiologically relevant miRNA expression profiling greatly benefits from sampling brain regions or nuclei. miRNA expression profiling from individual samples is also important for investigating potential differences between animals according to their physiological and pathophysiological status[1].

**RESULTS**

cDNA library sequencing using a GAIIx machine provided individual miRNA expression profiles from single hypothalamic nuclei with a read depth >10^6. Individual miRNA expression profiles are shown for five paraventricular nuclei (PVN). All harbored products from more than 200 miRNA genes, the twenty most abundant of which accounted for 78-85% of the whole profiles (figure 1).

Overall, our results showed that cDNA libraries can be constructed with RNAs of 16-30 bases that have been purified from individual rat hypothalamic nuclei.

**MATERIAL**

- Precellys 24 & Cryolys cooling option.
- Precellys lysing kit: 03961-1-003 (CK14).
- Sample: ~1-3 mm^3 of frozen hypothalamic tissue.
- Extraction solvent: 700 µl QIAzol lysis reagent (Qiagen).

**PROTOCOL**

- Precellys 24: 5500 rpm, 1x20 sec.
- Small RNAs were purified using the miRNeasy Mini Kit (Qiagen) with the two-column system.
- Small RNAs were recovered in a volume of 30 µL of RNase-free H2O and size-fractionated on a denaturing urea (8M) polyacrylamide (17%) gel.
- RNAs of 16-30 bases were eluted in 0.4M NaCl by overnight incubation under gentle shaking at +4°C, then precipitated with the addition of 3 volumes of ethanol in the presence of 0.04 µg/µL glycogen. cDNA libraries were constructed using an Illumina-like protocol.

[1] Lamar et al., MicroRNA expression profiling of hypothalamic arcuate and paraventricular nuclei from single rats using Illumina sequencing technology. Journal of Neuroscience Methods; http://dx.doi.org/10.1016/j.jneumeth.2012.05.003

**CONCLUSION**

The quality of the cDNA libraries proved the quality and efficiency of the Precellys 24 when working with hypothalamic tissues.

Precellys 24 homogenizer alone can be used at least for hypothalamic tissues. For other tough tissues requiring a longer time of homogenization, Cryolys cooling option is strongly recommended.

For more details, please contact precellys@bertin.fr

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