

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].

MATERIAL

- Precellys 24 & Cryolys cooling option.
- Precellys lysing kit: 03961-1-003 (CK14).
- Sample: ~1-3 mm³ of frozen hypothalamic tissue.
- Extraction solvent: 700µl 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 30µL 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 0.04µg/µL glycogen. cDNA libraries were constructed using an Illumina-like protocol.



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[1] L.Amar 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.2012.05.033>

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.

RESULTS

cDNA library sequencing using a GAIIIX machine provided individual **miRNA expression profiles** from single hypothalamic nuclei with a read depth >10⁵. 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.

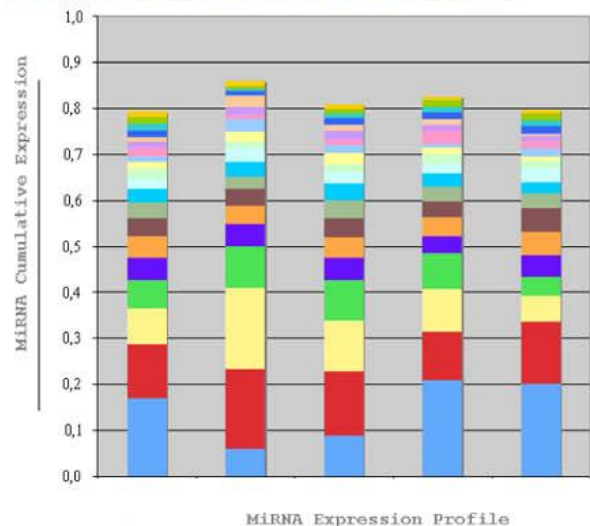


Figure 1: **MiRNA expression in PVN profiles.** Expression of the 20 most highly expressed miRNAs are shown for the five PVN profiles. Different miRNAs are shown in different colors.

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