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Extraction of RNA from various *Caenorhabditis* species (Nematodes)

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CONTEXT

Ageing, immunity and stress tolerance are inherent characteristics of all organisms. These traits in *Caenorhabditis elegans* have been shown to be regulated at least in part by the *daf-16* gene. Through our research we aimed to establish a correlation between the expression of *daf-16* and observed phenotypes (lifespan, immunity and stress tolerance) among four closely related *Caenorhabditis* species (*C. elegans, C. briggsae, C. remanei and C. brenerri*).

For such studies we required optimal concentration of high quality extracted RNA with minimal genomic DNA contamination. The added complication of using entire organisms for RNA extraction also meant that a very effective method be used to produce homogenized samples for RNA Isolation.

MATERIAL

-Precellys®24 Homogenizer

-Precellys® lysing kit: 03961-1-004 (0.5mm glass beads)

-Sample & buffer: Suspension of M9 buffer with *C.elegans* from a full 9cm petri dish.

[1] AMRIT, F. R., BOEHNISCH, C. M. & MAY, R. C. (2010) Phenotypic covariance of longevity, immunity and stress resistance in the *Caenorhabditis* nematodes. PLoS One, 5, e9978.

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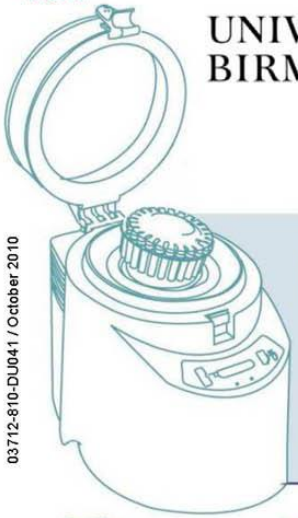

PROTOCOL

- Animals (either mixed stage, or staged, depending on requirements) were washed off petri plates using M9 buffer, gently pelleted by centrifugation (1500rpm for 1min) and washed three times with M9 buffer before re-suspending in 500µl of M9 buffer.
- Precellys®24: 6400rpm, 2x10secs twice for a total time of 40secs.
- Analysis: RNA was isolated directly from the extract using Qiagen RNeasy kit before being checked for quality and quantity using NanoDrop analysis.

RESULTS

Employing this methodology we were able to successfully isolate required amounts and quality of RNA from four species of the *Ceanorhabditis* genus at various stages of development. On average a yield of 1.5 – 2 µg/µl of RNA was obtained per 9cm petri plate. This RNA was then used for RT-PCR to quantify gene expression studies as shown in Figure 1.

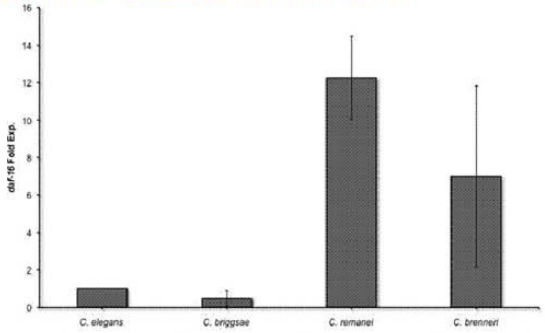

Figure 1: Expression levels of daf-16 (normalized to the reference gene gpd-3) among mixed populations - nematodes at various stages of development adapted from Amrit et al, 2010 [1].

CONCLUSION

The use of the **Precellys®24** to produce homogenized extracts for RNA isolation significantly improved the concentration and quality of extracted RNA as well as improving the speed of the isolation protocol.

For more details, please contact precellys@bertin.fr

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