Robust and reproducible automated tissue homogenization

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Introduction

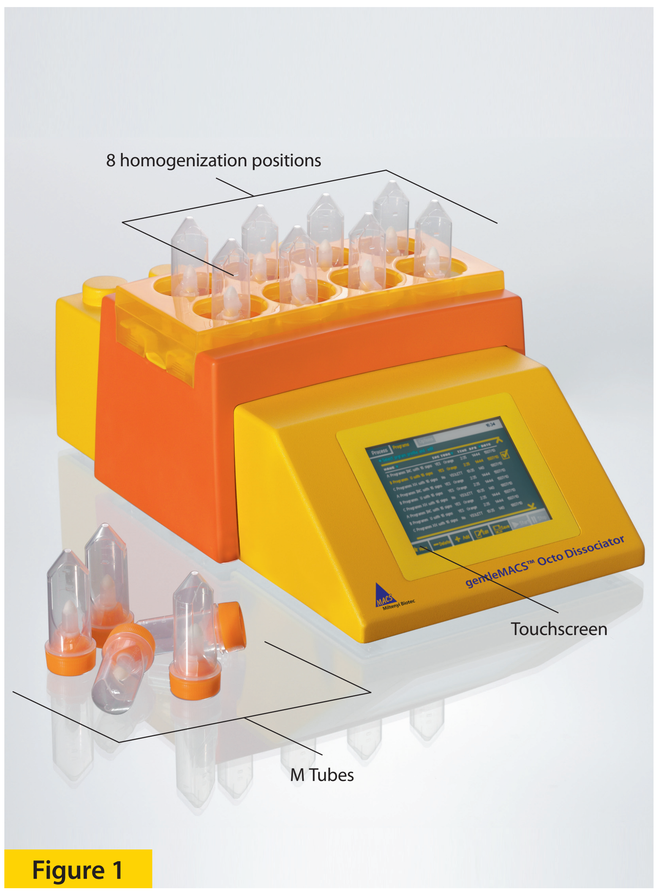

Figure 1

High-quality total RNA is a prerequisite for successful gene expression analysis. Samples are derived either from cells or tissues, which are generally stored frozen until lysis. However, homogenization of frozen tissue is still a challenging task as traditional methods have major drawbacks.

- Samples damage re-usable blades of rotor-stator homogenizers, which also require laborious cleaning.
- Crushing in liquid nitrogen is a tedious, time-consuming procedure.
- When using a bead mill the sample size is not flexible and the risk of thawing and RNA degradation is high, as all samples have to be processed simultaneously.

To overcome these disadvantages, we have developed the gentleMACS™ Octo Dissociator (fig. 1), an easy-to-use instrument for automated tissue homogenization, which uses disposable tubes with an integrated rotor-stator built into the lid.

Results

1 Comparison of standard homogenization procedures

gentleMACS Octo Dissociator

Load M Tube with lysis buffer and sample (e.g. whole organ).

Start automated homogenization of the first sample.
Note: Alternatively, up to eight samples can be homogenized simultaneously.

Homogenize the next samples.
Note: Homogenization of samples can be started at any time, even during homogenization of the previous samples.

Processing time (8 samples) < 3 min

Rotor-stator homogenizer

Load tube with lysis buffer and sample.
Note: Depending on stator size, whole organ can be used.

Homogenize the sample by manual agitation.
Note: Processing frozen whole organs implies risk of damaging the blade.

Clean rotor-stator probe.

Homogenize other samples consecutively.

Clean rotor-stator probe after each homogenization.

Processing time (8 samples) ~12 min

Bead mill

Optionally: Place tubes containing one stainless steel bead on dry ice for 15 min.

Fill samples into the tubes containing a stainless steel bead.
Note: As tube capacity often is not large enough to process whole organs, an appropriate piece must be cut out. This implies the risk that the particular piece does not show representative gene expression.

Optionally: Incubate on dry ice for 15 min.

Optionally: Incubate samples at room temperature for 2 min.
Note: Samples must not thaw; lysis buffer would freeze on dry ice.

Immediately add lysis buffer to all tubes.

Quickly start automated homogenization (2–5 min).

Processing time (12 samples) 5–35 min

2 Total RNA extraction

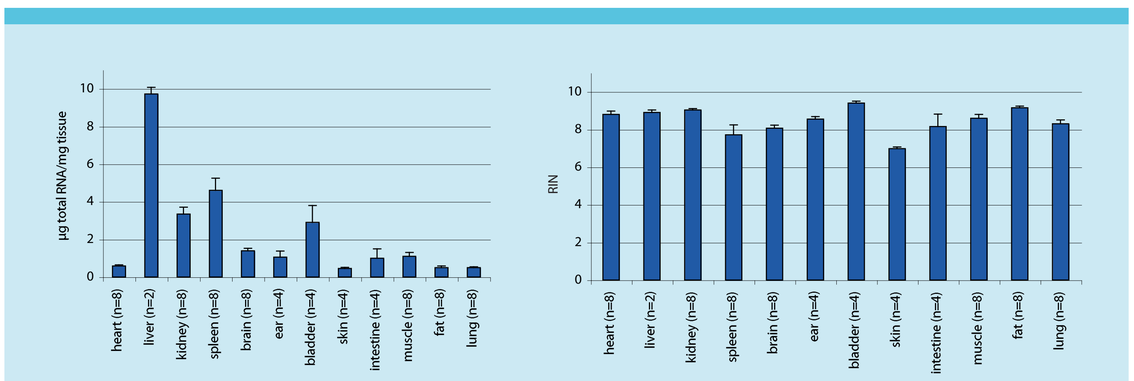

Figure 2A

Figure 2B

Whole mouse organs or 100–150 mg of skin, gut, muscle, or fat tissue were homogenized with the gentleMACS Octo Dissociator in 1–10 mL TriReagent (Sigma) depending on tissue weight. All tissues were successfully homogenized. Average total RNA yields (µg per mg of tissue, fig. 2A) as well as RNA integrity numbers (RIN, fig. 2B) show consistent results.

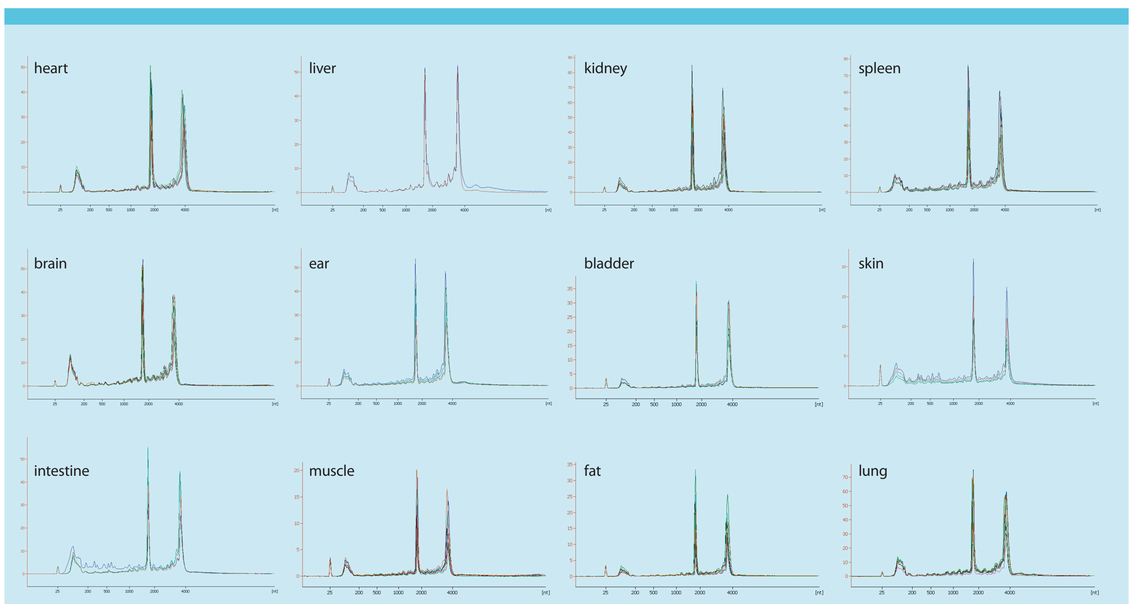

Figure 2C

One µL of total RNA samples was loaded on an Agilent Bioanalyzer 2100. Figure 2C shows overlaid electropherograms of various tissues.

3 mRNA preparation

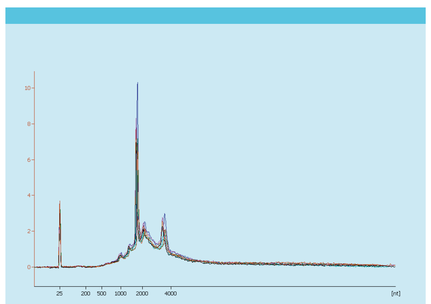

Figure 3

150 mg of frozen mouse liver were homogenized in 5 mL of lysis buffer, and mRNA was purified from 1 mL of lysate with the µMACS™ mRNA Isolation Kit. One µL of the sample was loaded on an Agilent Bioanalyzer.
Figure 3 shows the overlaid electropherograms of eight preparations with a typical mRNA distribution from 1–6 kb and residual rRNA peaks showing the integrity of the mRNA samples. The average yield was 8.3 ng mRNA/mg liver (CV: 15%).

Conclusion

The gentleMACS Octo Dissociator shows robust and reproducible homogenization results and outperforms other homogenizer types by offering the following benefits:

- The buffer volume used for homogenization is highly flexible (0.3–10 mL), allowing the homogenization of up to one gram of tissue—crucial for the analysis of whole rodent organs where gene expression might vary locally.
- The instrument processes samples without the need for prior crushing in liquid nitrogen. In case of rotor blockage, the process is repeated automatically after a few seconds.
- The device is user-friendly with minimal hands-on time. Sample processing can be started simultaneously or separately on each of the eight positions.
- By using single-use tubes, the system avoids cross-contamination of samples and cumbersome cleaning of re-usable blades.
- Homogenization takes place in a closed system. The instrument is highly recommended for the use of toxic lysis buffers or infectious samples. The tubes confine foam, which might occur during the homogenization process.