RNA extraction: PureLink kit vs. Qiagen kit

Introduction

According to the Foundation for Biomedical Research (FBR), 95% of all lab animals are mice and rats. For regular experiments, scientists typically use mice because they are small in size, generally mild-tempered, and adaptable; they also reproduce quickly and have a short lifespan. Many research studies require harvesting various organs and tissues from mice, isolating nucleic acids from the tissues, and performing downstream analysis by RT-qPCR, next-generation sequencing (NGS), or other techniques. For both ethical and practical reasons, it is desirable to extract the largest quantity of high-purity nucleic acids (DNA or RNA) possible.

Here we compare the performance of two commercially available kits—the Invitrogen™ PureLink RNA Mini Kit and Qiagen™ RNeasy™ Mini Kit—in the extraction of RNA from heart, liver, and brain tissue of mice. As shown in Table 1, a larger percentage of organ tissue can be purified with a single PureLink column than with a Qiagen column.

Materials and methods

Kits tested:

- PureLink RNA Mini Kit (Cat. No. 12183018A)
- Qiagen RNeasy Mini Kit (Cat. No. 74104)

Flash-frozen mouse tissues (5 mg or 30 mg) were homogenized using each kit's respective lysis buffer scaled for each input, following the manufacturer's protocol. For PureLink kits, an input of 150 mg was also tested. One volume of 70% ethanol was mixed with each lysate, and samples were processed using the spin cartridges and buffers provided in each kit. All samples were analyzed in triplicate. Treatment with Invitrogen™ DNase was not performed for either kit. RNA yields were determined using the Invitrogen™ Qubit™ 3 Fluorometer and Qubit™ RNA BR Assay Kit (Cat. No. Q10210). RNA samples were analyzed using the Thermo Scientific™ NanoDrop™ 8000 Spectrophotometer.

Table 1. Comparison of relative mouse organ weight and column input capacity.

Organ	Average weight (mg)*	PureLink kit—max input capacity (mg)	Qiagen kit—max input capacity (mg)
Brain	427	200	30
Heart	150	200	30
Liver	1,350	200	30

* Organ weights are for 26-week-old male mice of the C57BL/6J strain.

To assess purity visually, 1 µg of RNA was run on a 1% agarose TAE gel. Samples were allowed to migrate for 45 min at a constant 84 V. The RNA integrity number (RIN) was measured on the Agilent™ 2100 Bioanalyzer instrument with the Agilent™ RNA 6000 Nano Kit.

DNA contamination in the RNA samples was assessed by RT-qPCR using an Applied Biosystems™ TaqMan® Gene Expression Assay (Cat. No. 433182) for the mRNA target *RPLP0*. A control that omitted the reverse transcription (RT) step was included. The TaqMan assay was run on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System.

Results

RNA yield

RNA yields from mouse heart, liver, and brain tissue samples were analyzed using the Qubit 3 Fluorometer, and the results were compared for the PureLink and Qiagen kits used to extract the RNA (Figures 1–3).

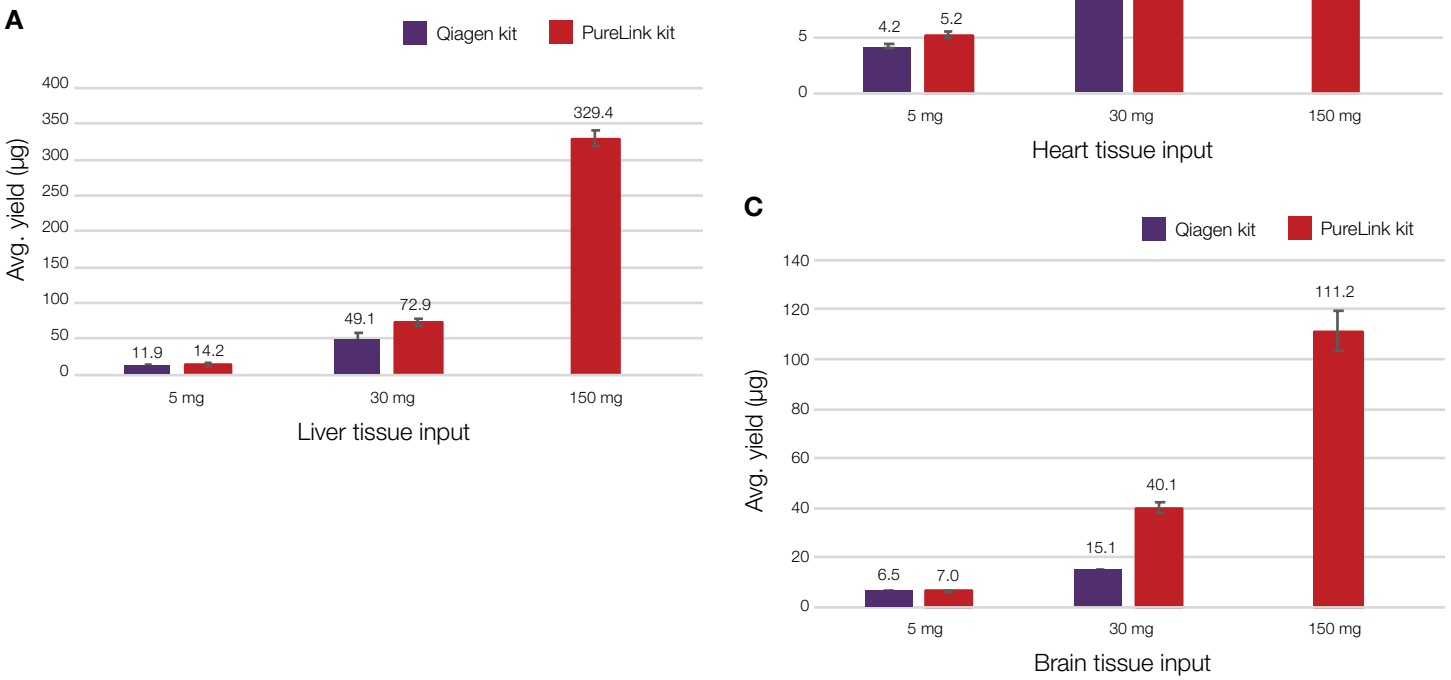


Figure 1. RNA yields, measured using the Qubit 3 Fluorometer. RNA yield from **(A)** liver, **(B)** heart, and **(C)** brain tissue of mice was determined by analysis on the Qubit 3 Fluorometer, which specifically measures intact RNA but not other nucleic acids. The Qiagen kit has a recommended sample input of up to 30 mg, while the PureLink kit enables processing of more than 150 mg of tissue. For 5 mg and 30 mg inputs, the PureLink kit delivered higher RNA yield than the Qiagen kit, for all three tissue types analyzed.

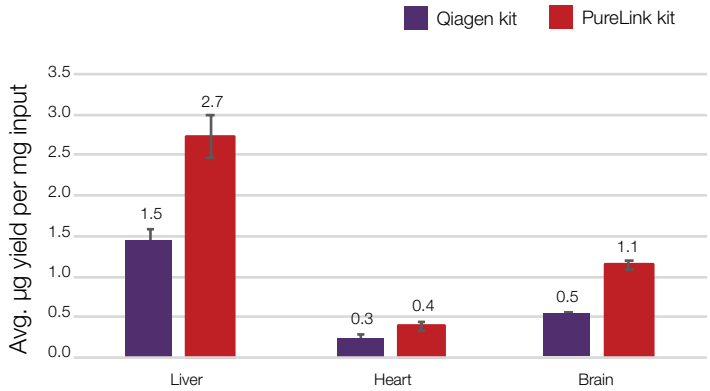


Figure 2. Comparison of RNA yields from 30 mg of mouse liver, heart, and brain tissue. For all tissue types, the PureLink kit yielded more RNA than the Qiagen kit. For liver and brain tissue, the PureLink kit recovered approximately twice as much RNA as the Qiagen kit. RNA yield was measured on the Qubit 3 Fluorometer.

Figure 3. Comparison of PureLink and Qiagen columns. The PureLink column (right) has a larger bed than the Qiagen column (left), supporting higher input capacity.

RNA purity

The purity of RNA from three different tissue types was assessed using the NanoDrop spectrophotometer (Figure 4), evaluated for integrity by both gel electrophoresis (Figure 5), and analyzed on the 2100 Bioanalyzer instrument using the RNA 6000 Nano Kit (Figure 6).

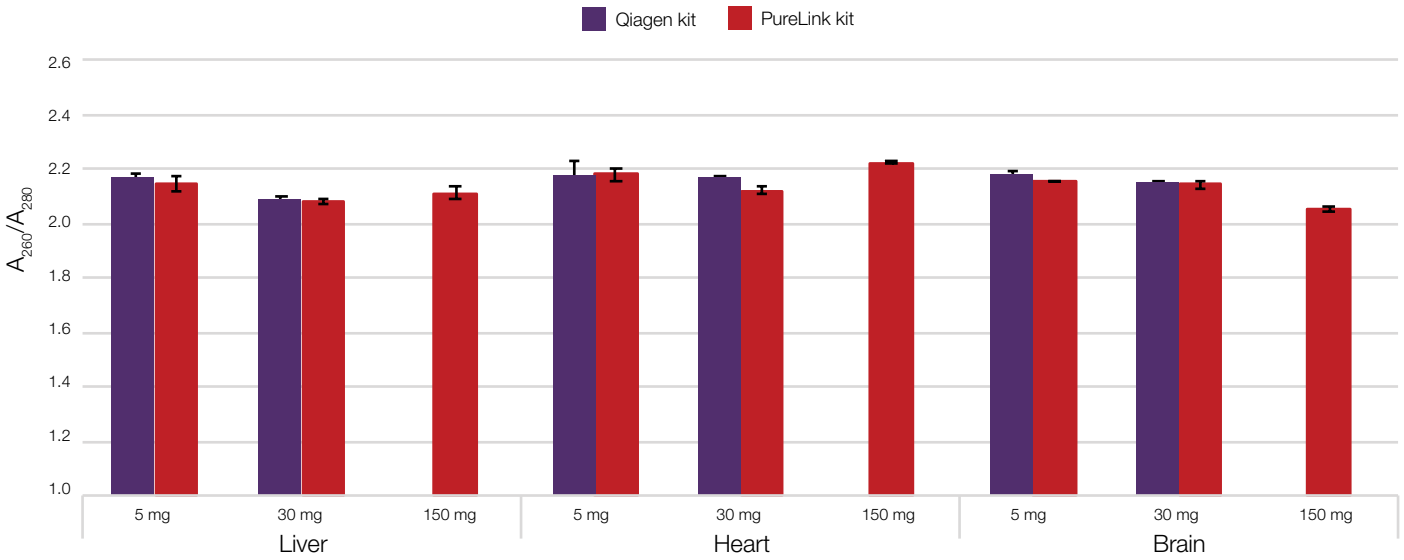


Figure 4. Purity analysis of RNA. When RNA samples were analyzed on the NanoDrop spectrophotometer, all had A_{260}/A_{280} between 2.1 and 2.2, indicating high RNA purity.

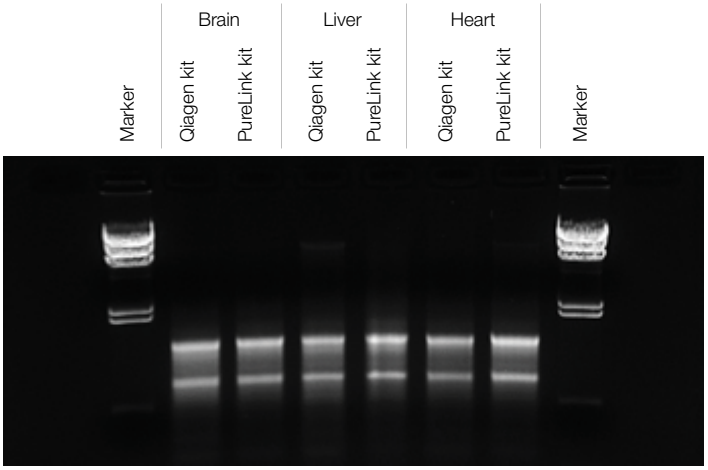
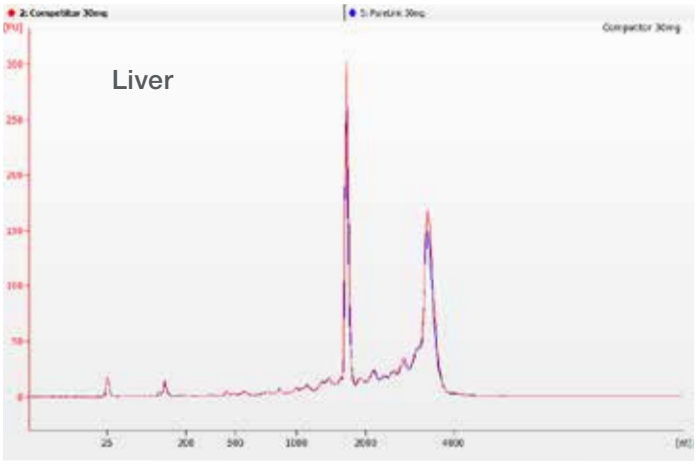


Figure 5. Visual inspection of RNA integrity by agarose gel electrophoresis. All samples were from 30 mg tissue input and showed a high level of RNA integrity.

A



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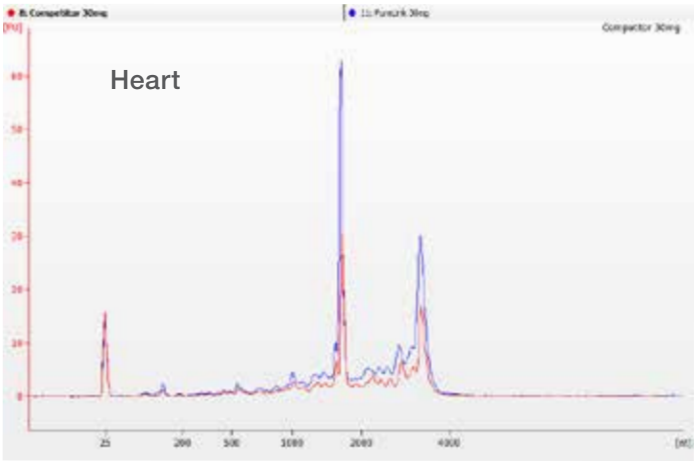

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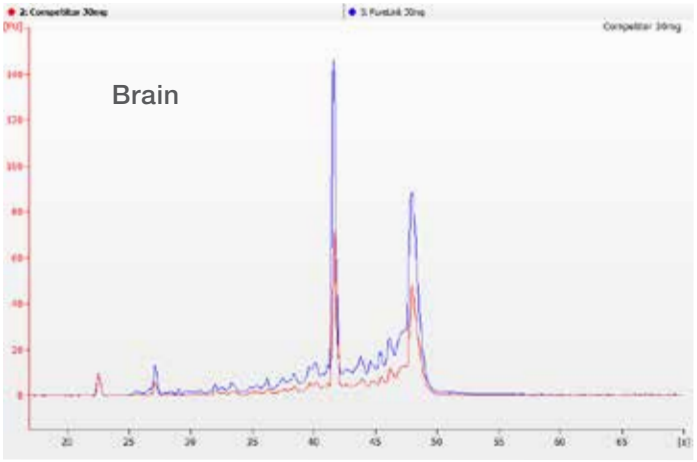

D

Tissue	Input (mg)	Qiagen kit (RIN)	PureLink kit (RIN)
Liver	5	9.1	9.0
	30	8.8	8.6
Heart	5	7.5	7.9
	30	7.6	7.9
Brain	5	8.5	8.3
	30	8.4	8.0

Figure 6. RIN values of RNA from different tissues. RNA profiles are shown for **(A)** liver, **(B)** heart, and **(C)** brain tissue at 30 mg input. **(D)** RIN values at two different input amounts of mouse tissue. All samples had high RIN values and produced similar profiles using the Qiagen and PureLink kits.

Measurement of DNA contamination

To determine the amount of residual DNA present in RNA samples after purification, RT-qPCR analysis was carried out for the mRNA target *RPLP0* (Figure 7). DNase treatment was not performed.

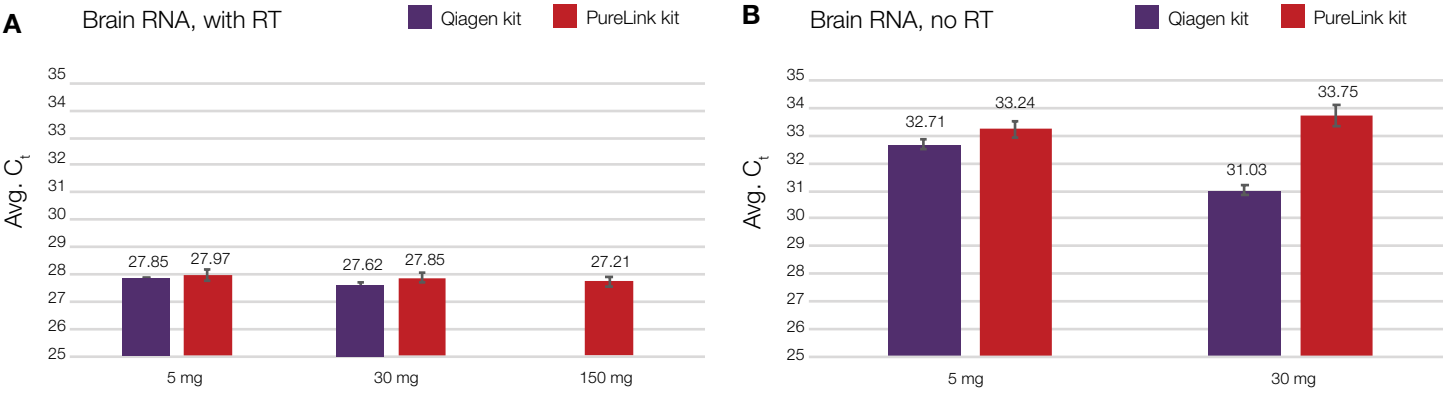

Figure 7. RT-qPCR analysis to evaluate DNA contamination. RT-qPCR was performed for **(A)** mRNA target *RPLP0*, along with **(B)** a control omitting the RT step. RNA was amplified as expected, and for the no-RT control, C_t values were higher by 5–6 cycles, indicating efficient depletion of DNA from the RNA samples during precipitation steps. In RNA from brain tissue, there was less residual DNA with the PureLink kit than with the Qiagen kit. However, similar amounts of residual DNA were present in RNA extracted from both liver and heart tissue, with both kits (data not shown).

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Conclusions

Column purification is a simple and efficient way to obtain purified RNA from different tissue types. In this study, PureLink and Qiagen kits were successfully utilized to purify high-quality RNA from liver, heart, and brain tissue samples at various input amounts.

- The PureLink kit consistently yielded more RNA across the three tissue types. The higher capacity of the PureLink columns enables high RNA recoveries.
- Both the PureLink and Qiagen kits delivered high-quality RNA as determined by spectrophotometry, agarose gel, and 2100 Bioanalyzer instrument measurements.
- RNA samples obtained using the PureLink and Qiagen kits had similar quantities of residual DNA, except in the case of brain tissue where using the PureLink kit resulted in less residual DNA.

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