Head-to-head of Commercial Total RNA Extraction Kits Reveals 'Practical Differences'

Dec 04, 2014 | Madeleine Johnson

Premium

NEW YORK(GenomeWeb) — Extraction of total RNA from complex tissue types or very small samples can be challenging. Commercial kits, either manual or automated, purport to recover high-quality RNA, but there are few studies directly comparing them.

In an attempt to address this, a group led by scientists at Miami University in Ohio recently tested five kits from three commercial sources — the RNeasy Plus Mini and RNeasy Plus Universal from Qiagen, the Maxwell 16 LEV SimplyRNA and SimplyRNA HT from Promega, and MagMAX-96 from Thermo Fisher brand Ambion — to determine the quality and quantity of RNA from a number of different tissue types.

The study, published last month in BMC Biotechnology, revealed "practical differences" between the RNA extraction kits that should be taken into account when selecting methods for isolating RNA designated for gene expression analysis, according to its authors.

"The purpose was to give people a sense of what's useful, what worked in our hands, and how reproducible things were," said Andor Kiss, a coauthor on the article, in an interview with GenomeWeb last week. Kiss is the supervisor for the Center for Bioinformatics and Functional Genomics at Miami University in Ohio and also runs a training lab in the biology department there. He noted that he has no commercial interest in any of the companies whose products were evaluated.

"We really focus on training undergraduates, graduate students, and postdocs to use the equipment," he said. "In many cases we have the question: 'Which is the best kit to use?' That is a really difficult question for us to address because we have so many different users using so many different organisms and tissues for so many different downstream applications."

Generally speaking, the Promega kits fared the best in the study, while Kiss said he'd be cautious about recommending the MagMAX to other researchers.

The study compared performance of the kits in extracting total RNA from less than 15 milligrams of eukaryotic tissue. Sample types included blood, spleen, kidney, larvae, and embryos of Pimephales promelas, otherwise known as the fathead minnow.

The group chose minnows partly because it hopes to do other downstream work with them, Kiss said. The popularity of zebrafish as a model organism is increasing, and with the advent of technologies like CRISPR, genetic experiments in "non-model organisms" are now possible, he said.

The study calculated cost per sample for each extraction, and found the Qiagen kits were the most expensive while the Promega Maxwell "works out to be about the same, or even a little bit cheaper, than traditional Trizol methods, which is pretty astounding when you think about it," Kiss said.

The Promega Maxwell 16 is also "the most reliable for the vast majority of people," he noted.

However, one disadvantage of the Maxwell kits is the price of the extraction instrument they run on — around $24,000, Kiss said.

"I hesitate to say it is the best one because you actually have to buy the instrument … This is probably a great unit for a group purchase, or a departmental purchase that can be a shared resource," he said.

The Qiagen kits yielded more RNA as a rule, Kiss said, but the Maxwell also extracted "very good quality RNA" as assessed by RNA integrity number (RIN).

Qiagen kits also removed the bulk of genomic DNA, Kiss said. "There is a miniscule amount [of gDNA], so if you were doing something like single-molecule detection, then I would say do a DNAse treatment. But for the majority of people, even ones doing RNA-seq, we say … you probably don't need [that step]."

In terms of consistency, Kiss said that in his lab graduate students use the Maxwell to teach undergraduates how to perform RNA extraction. The method is "very nice because you can set 16 samples up, it takes about 10 minutes of prep to get the samples ready, and the machine takes an hour to do the extraction."

There are people who prefer the Qiagen kits because of the quantity of RNA they yield, Kiss noted. Also, some tissue types are more problematic in the Maxwell. "Some users work on muscle tissue, and that has so much protein … that it interferes with extraction. So, we can't just recommend one approach for everybody, it's not one size fits all."

Anecdotally, he said the lab has found that researchers "who are very gung-ho about Qiagen [kits], when they did the comparison themselves on their particular organism and tissue, they found … that the Maxwell 16 gave them a better result, and they were really surprised."

For labs that don't have a Maxwell machine or aren't doing next-gen sequencing, it would be "overkill" to aim for RNA with a high RIN value simply to run qPCR, Kiss noted. "One has to think about the downstream purpose."

The lab was also impressed with the large-scale Promega kit that runs on a robotic platform, called Simply RNA HT. The study used a beta version, and "put it through the toughest test," but for future use, Kiss said he would likely modify the program because "each instrument is configured slightly differently."

The group was surprised that the Ambion kit performed "less than what we'd hoped for," he said. His impression was that the kit was designed quite a few years ago, and it hasn't been updated very

much since then.

Importantly, Kiss also noted that technical support differed among the companies, and that this might be an important consideration for other researchers.

"Because of the nature of the work — RNA work is sometimes very peculiar, very particular, very sensitive — when you call [tech support], it is really great to be able to talk to a technician or somebody who worked on that particular product's development," said Kiss. Products from smaller companies may cost more, but built into the price is "the fact that you can pick the phone up and you can talk to them," Kiss observed.

Other researchers can now run their samples on one of the kits his group evaluated in the study, Kiss suggested, and similar results might suggest they'll also have equivalent experiences with the other kits.

"I would put the caveat out there that you really do have to ask yourself: 'What am I going to use [the RNA] for?'" For a non-model organism, or difficult-to-obtain wild-caught animal, he also suggested obtaining tissue from a similar animal first to get a feel for a particular kit.

Filed Under Sample Prep PCR Sequencing Technology Promega Qiagen Thermo Fisher

Related Articles

Apr 03, 2014
Product Watch: Bio-Rad Precision Blue qPCR Dye; Promega Maxwell RNA FFPE Kit

Jun 10, 2014
Danish Team Scrutinizes Strategies for Sequencing RNA, DNA from FFPE Samples

Nov 17, 2014
Study Tracks Kissing Consequences on Mouth Microbiome

Apr 10, 2014
Study Compares Bisulfite Conversion Kits from Analytik Jena, Qiagen, Zymo for Methylation Analyses

Jan 23, 2014

Analytik Jena Launches Polymer-mediated Extraction Kit for cfDNA

Mar 13, 2014

Exosome's $27M Series B to Drive Internal, Qiagen-Partnered MDx Development