The Importance of RNase Inactivation in RNA Sequencing Labs

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Working with RNA can be intimidating. Environmental RNase contamination sources include microbial contamination from room air as well as RNAses from human skin, hair, or saliva. RNase inactivation methods range from DEPC treatment of water followed by autoclaving to more involved methods such as: chemical decontamination of surfaces, baking glassware, rinsing equipment in RNase-free water after chemical treatment, and frequent glove changes - all while continually using freshly opened disposables. Such cleaning methods can be costly in terms of money but more importantly they are time consuming, slowing experimental progress. Unfortunately, much of the available information regarding specific methods for RNase inactivation have been passed from scientist to scientist and form an informal institutional knowledge base rather than codified standards.

The single most important aspect of RNA protocols is isolating and maintaining full length, un-degraded RNA for analysis or use as a reaction substrate. Hindering this process is the presence of RNase. Whether preparing total RNA libraries for NGS or looking at individual RNAs (iCLIP), degradation by RNases is a recurring laboratory handling issue requiring diverse cleaning methods.

Once a package of disposables is opened, the contents can become contaminated and no longer suitable for RNA work and need to be cleaned or replaced. Pipettes left out on the bench can accumulate dust and microbial contamination from the room air and need frequent re-cleaning for use with RNA protocols. Cleaning surfaces and equipment with sprays and rinses can leave chemical residues, an additional type of contamination, which may interfere with downstream biochemical reactions. Furthermore repeated exposure to cleaning solutions or soaking may corrode metal or degrade plastic surfaces. How clean is clean enough? Clean enough occurs when you don't need to repeat lengthy protocols because of degraded RNA. Even trace amounts of RNase have a big impact on RNA sequencing, due to its catalytic action.

An alternative to chemical or thermal decontamination is inactivation of RNase with ultraviolet light (UV). High-intensity UV light has been shown to effectively, irreversibly inactivate RNase[1]. Rapid inactivation has also been demonstrated, with complete inactivation feasible in less than 1 minute for very high irradiance[2]. There are three advantages to UV inactivation of RNase:

1. Fast
2. Inexpensive
3. No residue

1. Irreversible inactivation of RNase A on a surface by UV LED; Thompson T, Eliason G, Pasquantonio J; American Society of Human Genetics Scientific Sessions 2017 (poster)
2. Rapid inactivation of RNase A by high irradiance UV LEDs; Thompson T, Eliason G, Pasquantonio J; American Society of Cell Biology/European Molecular Biology Organization Scientific Sessions 2017 (poster)

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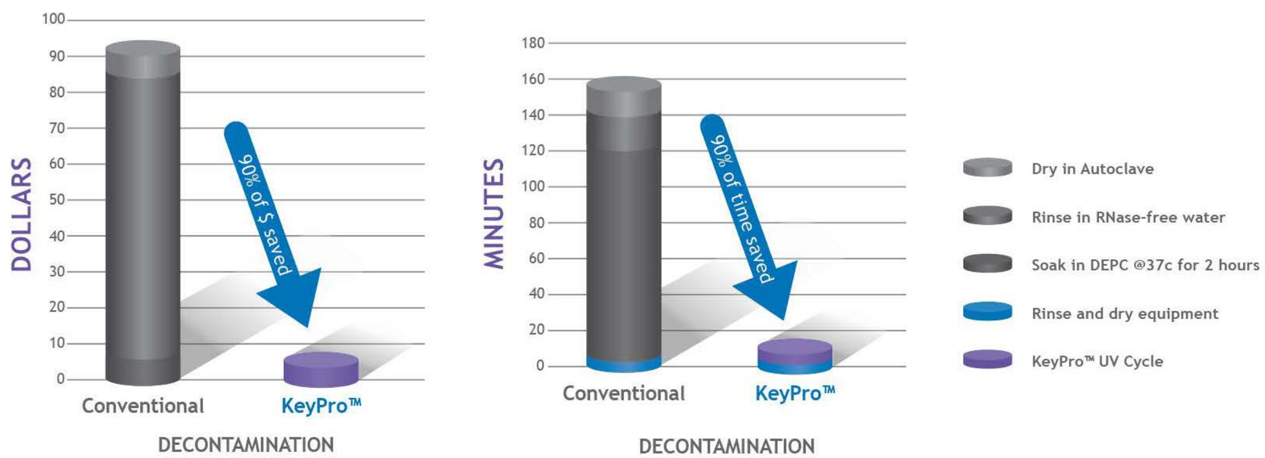

In addition, UV can be used on all common laboratory surfaces (plastic, metal, glass, or ceramic).

RNA Protocols that benefit from UV inactivation of RNase

1) Ultra-low input and Single-cell RNA sequencing
2) Ribosome profiling
3) RNA Exome Capture sequencing
4) Targeted RNA sequencing
5) Small RNA sequencing
6) Total RNA sequencing
7) mRNA sequencing
8) CRAC
9) iCLIP
10) NGS of RNAs

Accuracy and consistency are important goals when working with RNA. When one can save time and money while at the same time improving RNA protocol results, it helps the individual researcher and the lab as a whole. High-intensity, UV decontamination of RNase enables researchers to save time and money while ensuring consistent, accurate results.