Democratizing Molecular Biology: RNAGEM

Total RNA extraction from cells, tissues, insects, bacteria, and viruses

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Introduction

*RNA*GEM is a rapid RNA extraction solution:

- temperature-driven, single-tube approach preserves all RNA material
- uses a broad-specificity, thermophilic proteinase to obtain gene expression from a small number of cells
- lyses cells, eliminates RNases, and stabilizes RNA in less than 15 minutes
- ideal for preparing RNA, including miRNA, from cells, tissues, insects, bacteria, and viruses.
- significantly reduces the risk of pathogen exposure, cross-contamination and human error
- no harsh chemicals or additional purification needed
- hands-off protocol uses a thermocycler or even a water bath
- suitable for extractions from slides, tubes, or plates in 24, 96, 384, or 1536 well formats
- compatible with most downstream applications (qPCR, RT-PCR, cDNA synthesis, LAMP, Nanostring, and RNAseq).

Most methods for extracting RNA rely on solid phase purification or solvent-based extraction and precipitation. In some cases, lysis is achieved using inhibitory agents, such as SDS, that must be neutralised or removed from the solution before the nucleic acids are usable for analysis. This requirement increases the number of manipulations and, if solid phase or solvent methods are used, reduces yields and introduces bias into the population of mRNAs. *RNA*GEM's single-tube approach solves these challenges, producing high-quality extracts in minutes, not hours.

Technology and Workflow

*RNA*GEM uses a rapid, single-tube protocol that releases RNA and DNA with excellent recovery across a wide range of cell numbers. The method is automatable, closed-tube, and does not require further purification of the RNA for accurate down-stream analysis. The *RNA*GEM reagents efficiently lyse the cells, hydrolyse nucleases, and clear protein from strands of nucleic acids, preparing them for analysis. Greater sensitivity is achieved, especially with low abundance transcripts and small sample volumes as all nucleic acids are preserved in the process. Reduced handling and efficient template preparation mean *RNA*GEM generates mRNA profiles as close to the biological reality of the sample as possible. In addition, multiple wash steps are not needed since no ionic detergents are added. Removing these purification steps minimizes loss, limits contamination and human error, and drastically reduces environmental impacts from hazardous waste and excess plastic.

The workflow is significantly faster than conventional methods and is much simpler (**Figure 1a**):

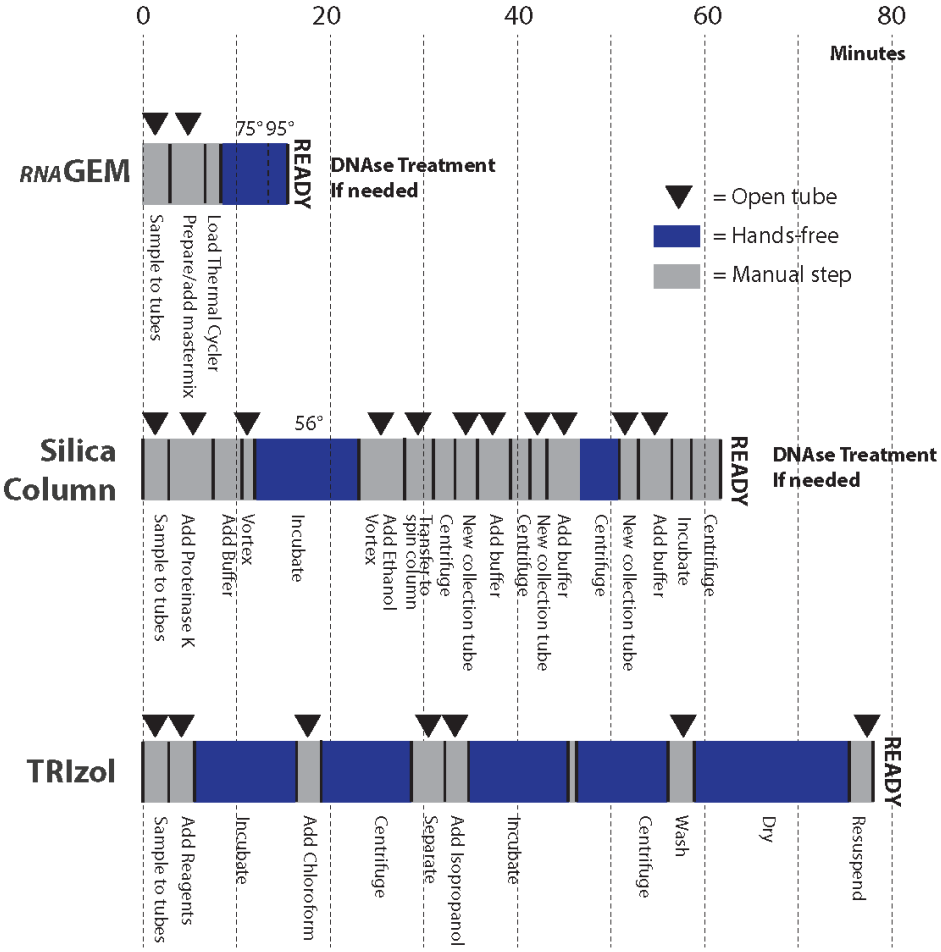

Figure 1a : *RNA*GEM Workflow Contrasted with Conventional Methods

- Prepare sample (centrifugation step or homogenization might be required for some samples)
- Mix sample and reagents
- Place in a thermocycler, thermoblock, or water bath for 5-10 minutes at 75°C
- Denature *RNA*GEM enyzmes at 95°C for 5 minutes
- Optional DNA digestion step at 37°C for 10 minutes

The extraction process consists of only a few steps (**Figures 1b and 1c**):

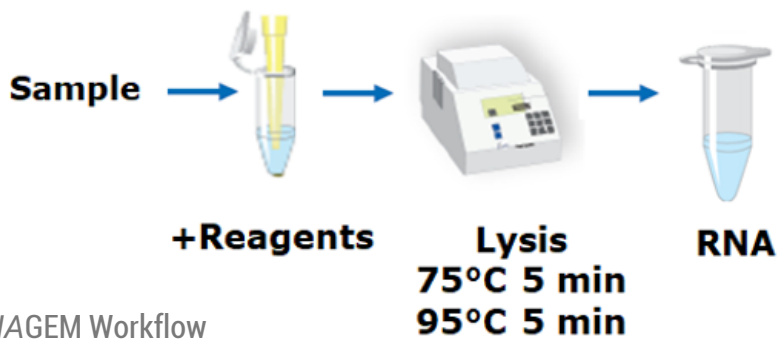

Figure 1b: Simple *RNA*GEM Workflow

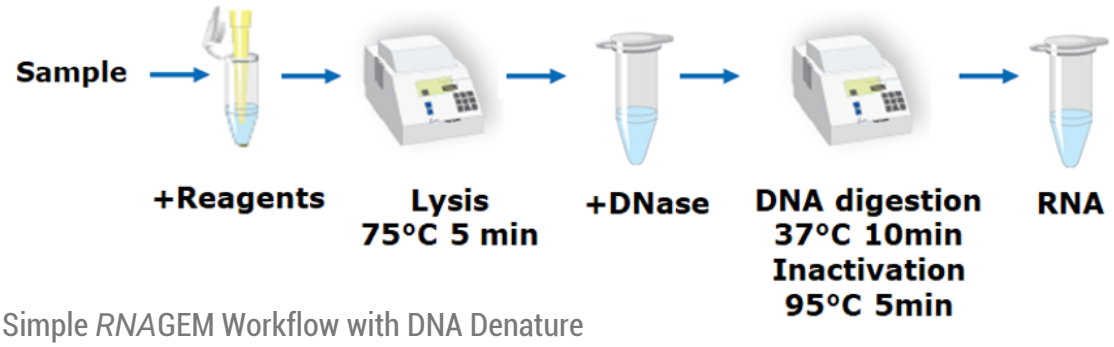

Figure 1c: Simple *RNA*GEM Workflow with DNA Denature

The BLUE buffer used by *RNA*GEM is compatible with polymerases, reverse-transcriptases, ligases, and many other nucleic acid modifying enzymes, so the extracts can be used without purification in PCR, RT-PCR, qPCR, and RT-qPCR.

The *RNA*GEM kit is highly adaptable across a range of extraction volumes and can be scaled from a few cells (where a small volume is desired) to~5x10^5 cells (**Table 1**). These reagents can be added directly to the RNA sample for a simple, streamlined 96-well plate protocol. For cell numbers greater than 100,000, extractions can be scaled upwards from the 50,000-100,000 while keeping the incubation time at 10 minutes. The lowest practical reaction volume that can be used for larger cell densities is generally limited by the viscosity of the extract. This is caused by the presence of very high molecular weight DNA and can be reduced by DNase treatment, vortexing or repeated pipetting to shear the DNA. The lowest achievable volume for extraction when working with low cell numbers is limited only by evaporation. With specialised equipment, sub-µl extractions are possible.

Cells	Volume	*RNA*GEM	Incubation Time
50,000 – 100,000	50 – 100 µl	1 µl	10 minutes
5,000 – 50,000	20 – 50 µl	1 µl	5 minutes
100 – 5,000	5 – 20 µl	0.5 µl	5 minutes
<100	1 – 15 µl	0.2 µl	5 minutes

Table 1: Reagent Scalability

*RNA*GEM Effectively Degrades RNase A from a Solution

RNA extraction is a challenge due to the instability of RNA and rapid breakdown in the presence of RNase contamination. Even tiny amounts of contamination are sufficient to destroy the RNA. RNase A contamination can be present in lab reagents, glassware, and even in lab water. Conventional RNA extraction methods require careful cleaning of consumables to limit RNase contamination.

Figure 2 shows that *RNA*GEM eliminates RNase A from a solution. *RNA*GEM extraction reagents were spiked with RNase A ranging from 0.00001 - 10 Kunitz units and incubated at 70°C for five minutes. On completion, the residual activity was assayed using a dual labelled RNA oligonucleotide where the presence of RNase is detected by an increase in fluorescence over time. The plot demonstrates that *RNA*GEM, at working conditions, completely inactivates up to 0.1Kunitz units of RNase A, an amount that greatly exceeds expected levels of RNase in a biological sample and shows the superb dynamic range of this chemistry.

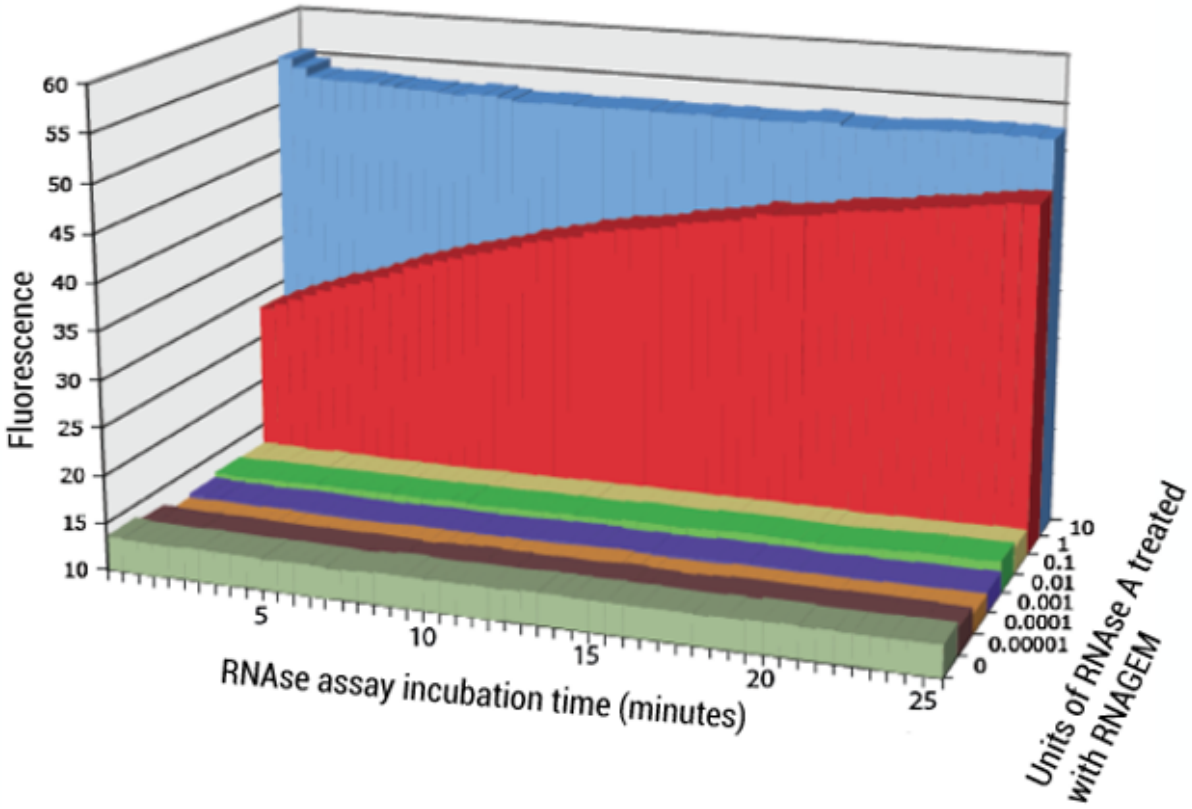

Figure 2: Residual Activity of Dilutions of RNase A. Residual activity of dilutions of RNase A treated with RNAGEM for 5 minutes. Eight different amounts of RNase A were used in separate experiments ranging from 0Kunitz units to10Kunitz units (shown on the Z axis). In the presence of residual activity, fluorescence (Y axis) increases with time (X axis). Only the experiments with 1 full unit or more showed increasing fluorescence over time. Hence up to 0.1units of RNase A are effectively cleared by RNAGEM during extraction.

RNAGEM Comparison with TRIzol and RNAeasy Mini QIAGEN

The simplicity of the *RNA*GEM method results in high yields that are linear over a wide range of cell numbers. The plots shown in **Figure 3** were obtained when 5µl of *RNA*GEM extracts from HeLa cells were added directly to an RT-qPCR reaction. Cell numbers ranging from 10–50,000 were extracted with *RNA*GEM and plots generated from a high abundance mRNA (ACTB;ß-actin) and a low abundance mRNA (BRCA1;breastcancerearlyonset). The clean traces with gradients similar to the standards demonstrate the lack of inhibition, and the plots obtained from as few as 10 cells demonstrate the sensitivity of the method with low cell numbers.

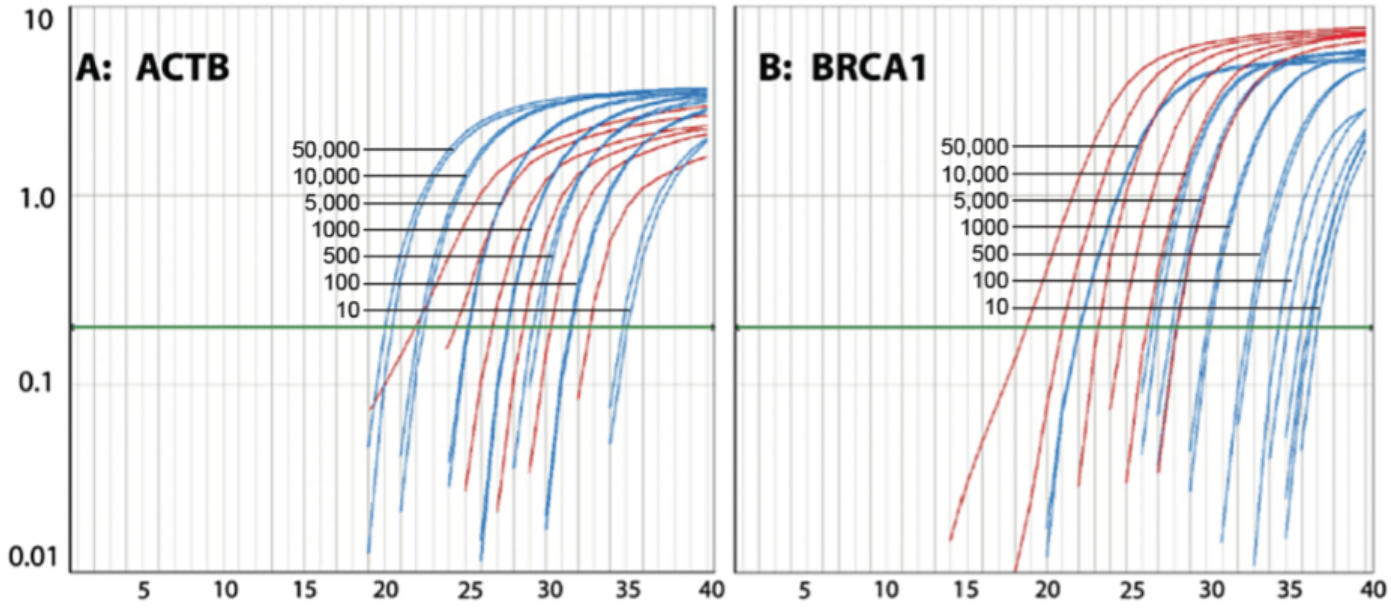

Figure 3: RT-qPCR plots of RNA extracted from a dilution series of HeLa cells from 10-50,000 cells. A: high copy number mRNA (ACTB) B: low copy number mRNA (BRCA1). Red=standards; Blue=duplicate mRNA curves.

The efficiency of *RNA*GEM extraction is constant over a broad range cell numbers from single cells to ~5x10⁴. **Figure 4** compares *RNA*GEM's yields to TRIzol®. *RNA*GEM maintains a linear extraction efficiency from low to high cell numbers unlike TRIzol which suffers RNA loss with low abundance samples. *RNA*GEM releases both DNA and RNA into the extract. Use of intron primers and intron-spanning primers can normalise expression measurements in a single extraction. If it is necessary to remove DNA, DNase is provided in the kit.

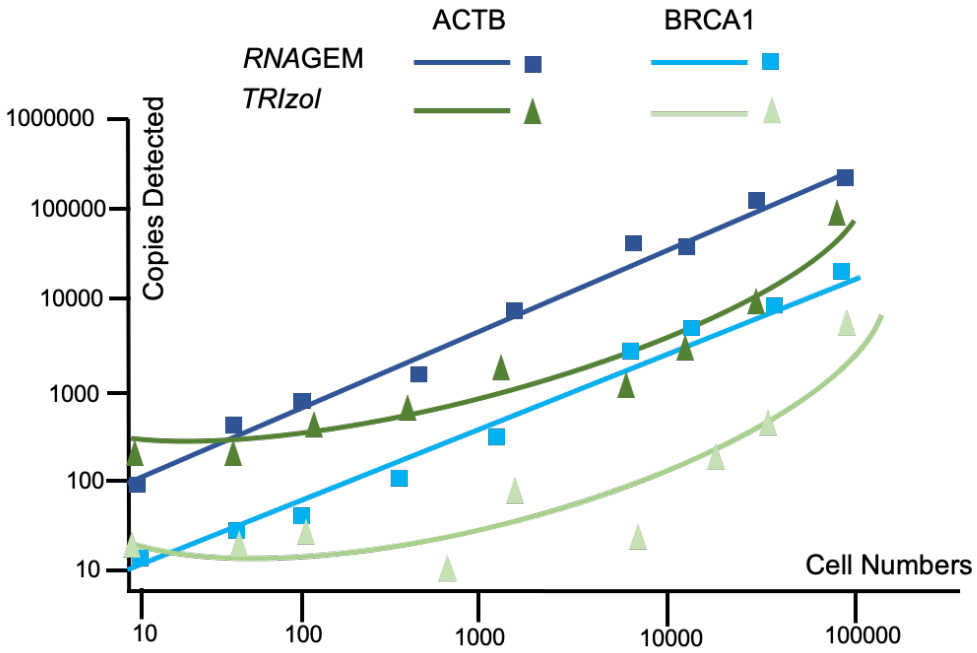

Figure 4: Log/Log plots of HeLa cell numbers versus mRNA copies detected. Both high copy number mRNAs (ACTB) and low copy number (BRCA1) are shown. Two extraction methods are used: *RNA*GEM and TRIzol

A recent publication compared *RNA*GEM and RNeasy Mini QIAGEN to determine whether the qRT-PCR results were comparable.[1] The data showed that RNA extracted from transfected or non-transfected by siRNA WiDr colorectal cell line with the *RNA*GEM extraction method led to TNFRSF11A gene expression levels two-times higher than the QIAGEN method.

Rapid RNA Extraction from Different Sample Types

As shown in **Table 2**, *RNA*GEM can extract total RNA, including microRNA (miRNA) and non-long coding RNA (lnc-RNA)[2–5] , from a variety of samples and is suitable for downstream applications such as PCR-based methods[6,7], Loop-mediated Isothermal Amplification (LAMP)[8], Nanostring[9] and RNAseq[10]. It has been successfully used for the extraction of total RNA from a variety of samples such as mammalian cell culture[11], laser capture microdissection[7] and FACS-prepared cell populations, exosomes[2,3], tumour tissues[12] , insects[13], bacteria[10] and viruses.[8]

Sample type	RNA type	Analysis	Reference
SH-SY5Y cells	mRNA	RNA extraction; cDNA synthesis; qPCR	Ayazgök et al., 2018. J Biochem Mol Toxicol.[14]
LMD frozen brain tissue (rat)	mRNA	RNA extraction; cDNA synthesis; TaqMan gene expression FAM-labeled assays	Michaelides et al., 2018. Mol Psychiatry[15] Michaelides et al., 2017. Neuropsycholpharmacology[7] Michaelides et al., 2013. Brain Res.[16]
DLD1, PC3, TC1 and B16 cells	mRNA	RNA extraction; cDNA synthesis; qPCR	Batista et al. 2020. Preprint BioRxiv[17]
J558L cells; Extra-cellular vesicles (EV)	mRNA; miRNA	RNA extraction; cDNA synthesis; Taqman MicroRNA Reverse Transcription; qPCR	Almanza et al., 2018. Sci. Rep.[2] Almanza et al., 2015. Mol. Ther. - Nucleic Acids[3]
Rat mammary gland tissue	lnc-RNA	RNA extraction; cDNA synthesis; qPCR	Bhan et al., 2014. Journal of Steroid Biochemistry & Molecular Biology[5]
Canine Distemper Virus (CDV) from culture, animal fecal and sera samples	Viral RNA	RNA extraction, RT-PCR, RT-LAMP	Segawa et al., 2014. Journal of Virological Methods.[8]
Fresh mammalian cells infected with intracellular bacteria (e.g., Salmonella typhimurium strain 14028	Bacterial RNA	RNA extraction; RNA clean up with RNAClean XP beads; Bioanalyzer analysis; cDNA synthesis; RNAseq	Avraham et al., 2016. Nature Protocols[10]
Tumour xenograft tissue	mRNA	RNA extraction; cDNA synthesis; qPCR	Ansari et al., 2013. British Jounral of Cancer[12]
Equine H3N8 and H7N7 viruses from nasal swab	Viral RNA	cDNA synthesis; EI H3 TaqMan assay	Foord et al., 2009. Veterinary Microbiology[18]
CD8+ T cells	mRNA miRNA	RNA extraction; qRT-PCR	Sheppard et al., 2014. Journal of Translational Medicine[4]
Single blood circulating tumour cell	mRNA	RNA extraction; cDNA synthesis; qPCR	Hoshino et al., 2015. J. Circ. Biomarkers[6]
Mosquitoes	mRNA	RT-PCR, Amplicon Sequencing	Müllerová et al., 2018. Polar Biol.[13]
Blastocytes	mRNA	cDNA synthesis; qPCR; nCounter analysis (Nanostring)	McLean et al., 2014. Biology of Reproduction[9]

Table 2: Select references for RNA extraction from a variety of samples using *RNA*GEM

Examples of Extraction from Single Cell, Laser-capture Micro-dissected Brain and Tumour Tissues

Most methods for extracting RNA rely on solid phase purification or solvent-based extraction and precipitation. These methods are laborious and require several manipulations, such as washes and centrifugation steps, to remove toxic agents used in the lysis. Each of these steps increases the risk of contamination and reduces yield. Therefore, these methods fail to extract RNA from small samples or single cells.

In contrast, *RNA*GEM has been proven to extract RNA and DNA from very small samples sizes. Hoshino et al (2015) combined immunofluorescence and laser microdissection to isolate RNA from blood circulating tumour cells. Following the immunomagnetic assay, the RNA from a single isolated cell was placed on the cap of a PCR tube to reduce the volume of the reagents. For the extraction, 0.2µl of *RNA*GEM were incubated at 75ºC for 5 minutes, and, for long storage of the RNA, 0.28 µl of TE buffer was added. Subsequently, the extracted RNA was converted in cDNA using qScript cDNA Super Mix (Quanta Biosciences) and preamplified by 20-23 cycles before being successfully analysed in the microfluidic qPCR system (BioMark HD System, Fluidigm Corporation and StepOne Plus, Applied Biosystems).[6]

In their study showing that NAc D2R regulates both peripheral glucose levels and glucose-dependent reinforcement learning behaviors, Michaelides et al (2017) used *RNA*GEM to extract RNA from laser microdissected frozen brains sectioned (20µm) at the striatum using a cryostat (Microm HM560, Thermo Scientific, Rockford, IL) and a dissecting microscope. The synthetized cDNA was analysed using TaqMan gene expression assay (Applied Biosystems, Carlsbad, CA) to analyse Drd2 mRNA expression.[7]

*RNA*GEM can also be used to extract RNA from larger samples, such as tumour tissues. Ansari et al (2013) demonstrated the key role of MLL1 in hypoxia signaling, vasculogenesis and tumour growth. To study gene expression, the excised tumours from euthanized mice were flash frozen in liquid nitrogen and homogenized prior to RNA extraction with *RNA*GEM and analysis by qPCR.[12]

Examples of Non-coding RNA Extraction from Cell Lines, Tissues, and Exosomes

Due to the small size and instability of microRNA (miRNA), a specific extraction kit needs to be used. In contrast, *RNA*GEM can extract messenger RNA (mRNA) and miRNA from cell lines and exosomes.[2,3]

Almanza et al (2015) demonstrated that B-cells can be easily programmed to synthetize and release multiple short non-coding RNAs (sncRNA) and extracellular vesicles (EVs) enriched with sncRNA. The authors utilized *RNA*GEM to extract the total RNA, including miRNAs, from 5×10^5 transfected or untransfected J558L cells, and 1 ml of culture supernatant and EVs produced by J558L cells. The mir-150 and anti-miR-155 were analysed by Taqman MicroRNA Reverse Transcription Kit.[3]

The same research group (2018) demonstrated that EVs laden with a tumour suppressor miRNA (miR-335) produced in B cells by plasmid DNA induction (iEVs) can markedly inhibit tumour growth in vivo. Total RNA, including miRNAs, was extracted using *RNA*GEM[2]. Bhan et al (2014) used *RNA*GEM to extract the lnc-RNA HOTAIR from flash frozen rat mammary gland tissue to study its expression by bisphenol-A (BPA) and diethylstilbestrol (DES)[5].

Examples of RNA Extraction from Insect, Bacteria and Virus

*RNA*GEM is the ideal solution to extract RNA from non-mammalian samples such as insect, bacteria and virus.

Avraham et al (2016) published an RNAseq protocol to allow the simultaneous analysis of host and bacterial pathogen transcriptome. *RNA*GEM was employed to extract RNA from a limited number of host cells (~10^5 cells). Following the extraction, the RNA was cleared by using RNAClean XP beads, the quality evaluated by Bioanlyzer and quantified by RNA Pico Kit. Up to 100 ng of RNA was placed in a tube and spiked with 1:2000 dilution of ERCC spike-in mix 1. The mixed sample was subjected to reverse transcription, cDNA amplification and sequencing[10]. This study confirmed the ability of *RNA*GEM to extract RNA from both eukaryote and prokaryote cells and its compatibility with RNA-seq.

In another study, Müllerová et al (2018) screened over 11,000 specimens of mosquitoes collected between 2012 and 2016 on Greenland (Kangerlussuaq) and Svalbard (Petuniabukta) for the presence of viruses transmitted to vertebrates via arthropod vectors (arboviruses) which had previously been reported in latitudes up to 70°N. Whole mosquito bodies were removed from RNA stabilizing solution, transferred to 1 ml of cooled PBS solution, and homogenized using a TissueLyser II (QIAGEN, Germany). RNA was isolated from 280 µl of homogenate by *RNA*GEM and subsequently analysed by RT-PCR. PCR amplicons from positive control and potentially positive samples were purified by the QIAquick PCR Purification Kit (QIAGEN, Germany) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA) by Biogen PRAHA (Czech Republic).[13]

In a study by Foord et al (2009), *RNA*GEM performed at least as well as other commercially available kits, including RNAeasy, MagNA Pure, and MagMAx, to extract viral RNA from a nasal swab to detect by TaqMan assay equine influenza (EI) H3N8 and H7N7 viruses in horses. To evaluate the four kits, extractions were performed from serial dilution of five different nasal swab samples. The authors modified the *RNA*GEM extraction protocol by mixing 50µl of nasal swab transport medium with 80µl of buffer and 1µl of enzyme. The reaction mix was incubated for 15 minutes at 75°C and 5 minutes at 95°C. The extracts from the four kits were analysed in triplicates by EI H3 TaqMan assay. The results showed that all extraction kits were able to extract quantifiable amounts from most samples, indicating that in general the four kits produce similar results.[18]

GEM RT-LAMP: an Example of RNAGEM's Versatility and Reliability

*RNA*GEM is an extraction solution that can be combined with several downstream applications.

Segawa et al (2014) used *RNA*GEM to establish GEM-LAMP, a method that combines reverse transcription loop-mediated isothermal amplification (RT-LAMP) and *RNA*GEM. This method detects viral RNA from different samples, such as sera and faeces, from cattle, pigs, horses, dolphins, penguins, and sea lions with SYBR green within 70 minutes in a single-tube using only a water bath. Moreover, the authors showed that GEM RT-LAMP is 100-fold and 100,000-fold more sensitive than standard RT-LAMP and modified RT-LAMP, which utilized QiAmp Viral RNA Mini kit and thermal extraction respectively. GEM-LAMP, combined with *RNA*GEM, can be used to detect pathogens in clinical, veterinary, and environmental samples as well as for point-of-care testing.[8]

Conclusions

*RNA*GEM is a versatile reagent that efficiently extracts DNA and RNA from many sample types. Easily scaled, this single-tube extraction is fast, simple, and can be conducted in a thermocycler, thermoblock or even a simple water bath. RNA can be extracted from slides, tubes, or plates in 24, 96, 384, or 1,536 well formats in 5-15 minutes. In contrast to conventional methods, total RNA, including miRNA, can be extracted using the same methodology. The single-tube approach does not require harsh chemicals and drastically reduces the use of plastic consumables. The extracted RNA is compatible with downstream applications such as qRT-PCR, RT-PCR, cDNA synthesis, LAMP, Nanostring and RNAseq.

*RNA*GEM quickly and efficiently lyses cells, eliminates RNases, and stabilizes and preserves the integrity of the RNA, making it the ideal solution for gene expression using very small sample amounts.

For additional information about RNAGEM, visit www.microgembio.com/products/rna/

References

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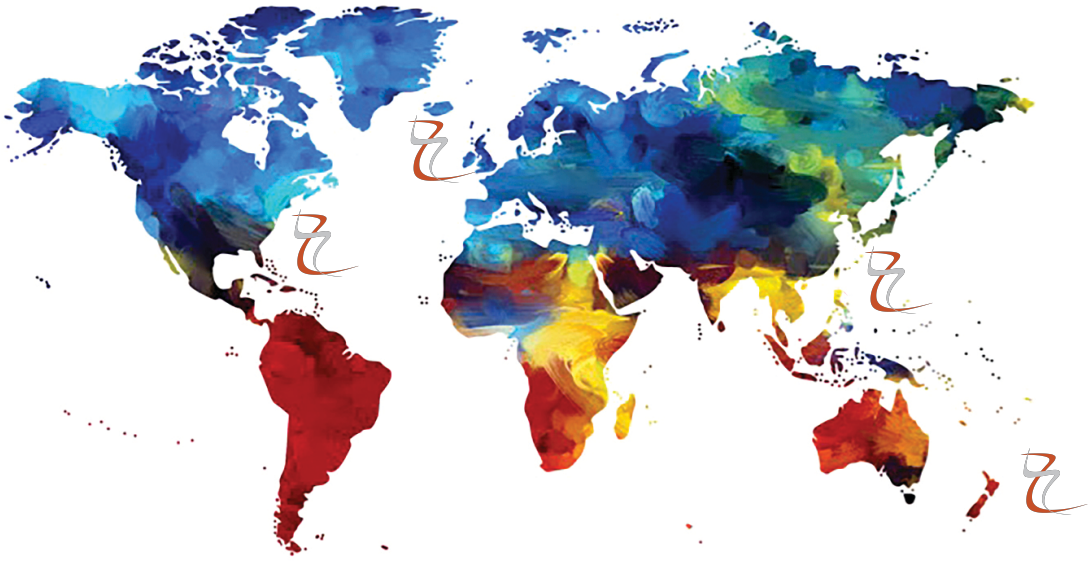

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At MicroGEM, our goal is to democratize molecular biology, enabling a broader spectrum of users to both employ and benefit from molecular techniques. The first step is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

MicroGEM

Committed to minimal packaging, reduced plastic use, and a vibrant, sustainable world.