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RNASwift: a rapid, versatile RNA extraction method free from phenol and chloroform.

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Abstract

RNASwift is an inexpensive, versatile method for the rapid extraction of RNA. Existing RNA extraction methods typically use hazardous chemicals including phenol, chloroform and formamide which are often difficult to completely remove from the extracted RNA. RNASwift uses sodium chloride and sodium dodecyl sulphate to lyse the cells and isolate the RNA from the abundant cellular components in conjunction with solid phase extraction or isopropanol precipitation to rapidly purify the RNA. Moreover, the purified RNA is directly compatible with downstream analysis. Using spectrophotometry in conjunction with ion pair reverse phase chromatography to analyse the extracted RNA, we show that RNASwift extracts and purifies RNA of higher quality and purity in comparison to alternative RNA extraction methods. The RNASwift method yields approximately 25 µg of RNA from only 10^8 *Escherichia coli* cells. Furthermore, RNASwift is versatile; the same simple reagents can be used to rapidly extract RNA from a variety of different cells including bacterial, yeast and mammalian cells. In addition to the extraction of total RNA, the RNASwift method can also be used to extract double stranded RNA from genetically modified *E. coli* in higher yields compared to alternative methods.

Introduction

Ribonucleic acid (RNA) is chemically-labile and susceptible to endo- and exo-nuclease mediated degradation. Therefore the extraction, purification and downstream storage of RNA are challenging. A variety of methods have been employed for the extraction of RNA from bacterial cells, however these methods can often result in low yields or low quality total RNA [1]. One of the earliest RNA extraction methods used guanidinium isothiocyanate to lyse cells and denature proteins in conjunction with ultracentrifugation with a caesium chloride cushion to separate RNA from cellular components [2]. Alternative methods utilizing hot phenol replaced caesium chloride gradients [3] but yielded RNA that was not consistently of high quality [4]. Methods that combined guanidinium isothiocyanate and phenol to extract RNA improved the RNA quality [5].

For many RNA downstream processing applications, the preservation of RNA integrity during RNA extraction is paramount. The accurate quantification of mRNA used for gene expression profiling depends on the integrity of extracted RNA. The use of RNA of poor quality during quantification of mRNA levels may compromise the accuracy of gene expression results [6, 7]. Therefore, concerted efforts have been made over the years to develop RNA extraction methods that will yield quality RNA for various applications. RNA extraction methods are often limited by either the toxicity of the reagents used, the complexity of the procedure, poor quality of RNA generated or by the isolation of only a subset of the total RNA present. For instance, most existing methods which extract RNA of sufficient quality use phenol and chloroform in their procedures. In addition, extracting both low molecular weight RNAs (such as tRNAs and short RNAs) as well as the abundant larger RNA remains a significant challenge. Furthermore, a large number of these methods are complex,

either involving multiple transfer steps or requiring cumbersome precautions to avoid RNA degradation.

The lysis of *E. coli* cells with the aid of SDS-based buffers is not entirely new, but is often combined with phenol or phenol/chloroform extractions in order to separate the RNA from other cellular components [8]. Further developments have included the use of a hot-SDS/hot phenol RNA method in conjunction with DNase treatment to remove DNA [9]. RNA extractions using this approach have been shown to generate high quality RNA from *E. coli* [9]. The increasing concern over the toxicity of phenol or phenol/chloroform means that the method suffers the same disadvantage as all the phenol-chloroform based RNA extraction methods [3, 9]. Moreover, the hot-SDS/hot phenol method is time-consuming, requiring overnight incubation at -80 °C [9].

A variety of alternative RNA extraction methods have been developed and optimised for extracting specific RNA species or extracting RNA from specific cell types or tissues. Formamide-based RNA extractions were developed to efficiently extract total RNA from bacterial cells [10]. This single-step method termed RNAsnap™, generated similar quality and yield compared to the commercial guanidium isothiocyanate - phenol/chloroform based methods [10]. RNA extractions centred on guanidium thiocyanate in conjunction with histidine and arginine affinity chromatography have been developed for the purification of RNA from prokaryotic and eukaryotic cells [11, 12]. Additional methods include, LogSpin, an RNA extraction method based on guanidium hydrochloride and spin column purification [13], modified TRIzol-based methods for extraction of RNA from polyethylene glycol-

based hydrogels [14], methods that utilise RNase inhibitors combined with different protocols using guanidium thiocyanate–phenol extraction [15] and modified Lithium-based protocols for extraction of viral dsRNA from plants [16, 17].

In this study the aim was to develop a simple, versatile method for the rapid extraction of high quality RNA from bacterial cells without the use of toxic reagents such as phenol/chloroform. In addition, the developed method should be amenable for large scale extractions and directly compatible with downstream analysis such as ion pair reverse phase chromatography and RT-PCR. The developed method termed RNASwift results in higher purity RNA compared to alternative methods and is suitable for the extraction of high quality total RNA from a wide range of organisms including *E. coli*, yeast and mammalian cells. In addition, the method is effective for extraction of long-chain dsRNA and does not require modification in the procedure or reagents for different cell types. The method is simple, time-effective and efficient in isolating RNA that is consistently of high quality. RNASwift is also an inexpensive method, using reagents consisting of small quantities of cheap and less-hazardous chemicals, such as, sodium chloride, SDS, isopropanol and ethanol.

Materials and Methods

Chemicals and reagents

Synthetic genes were synthesised by GeneArt® Gene Synthesis (Invitrogen Life Technologies). Ampicillin sodium salt, tetracycline hydrochloride, Isopropyl β-D-1-thiogalactopyranoside (IPTG) ≥99%, sodium dodecyl sulphate (SDS), 99% , sodium chloride (NaCl), 99% were all obtained from (Sigma-Aldrich, Poole, UK), TRIzol® Max™ Bacterial RNA Isolation Kit with TRIzol®, Max Bacterial Enhancement Reagent (Life Technologies) and the Ribopure™ bacterial RNA extraction kit (Life Technologies) were used for RNA extractions.

Expression of dsRNA gene using *E. coli* HT115 (DE3).

The *E. coli* strain, HT115 (DE3) [18] was obtained from Cold Spring Harbor Laboratory, NY, USA. A plasmid pCOIV that contains an in-house designed 765 bp sequence flanked on both sides with T7 promoter was ordered from Gene Art® Gene synthesis (Invitrogen). The *E. coli* HT115 (DE3) cells were transformed with pCOIV. A colony from the transformed cells was inoculated into 5 mL LB media containing 10 ng/mL tetracycline and 100 µg/mL ampicillin and incubated overnight at 37°C. This was followed by seeding 2 mL of the overnight culture into 50 mL LB media containing the same concentration of antibiotics, incubated at 37°C and allowed to reach an $OD_{600\ nm}$ of 1. Then IPTG was added to the culture to 1 mM final concentration followed by further incubation at 37 °C for 3 hours.

Cell growth

For *E. coli* growth a single colony from a fresh plate was inoculated into 5 mL LB media and was grown with shaking at 37°C to $OD_{600\ nm}$ 0.4 (3.2×10^8 mL^{-1}) and

aliquoted into an Eppendorf tube. A colony of *Saccharomyces cerevisiae* strain S288C from a fresh plate was inoculated into 5 mL YPD media and incubated overnight at 30 °C. The culture was diluted and aliquoted 10^7 cells per Eppendorf tube. Chinese hamster ovary cell line (CHO-S derived Herceptin like IgG producer, Cobra Biologics) was grown in CD-CHO media supplemented with 8 mM L-glutamine, 2 mM HT and 12.5 µg/ml puromycin. Cells were grown in 30 ml cultures in a dry shaking incubator at 140 rpm, 5% CO_2 at 37 °C and counted daily using a Vi-Cell.

Development of RNASwift

E. coli cells were harvested by centrifugation at 4500 rpm at 4°C for 10 minutes. Approximately 10^8 *E. coli* cells, 10^7 yeast cells or 10^7 Chinese hamster ovary cells were suspended in pre-warmed 100 µl LB1 lysis reagent (4% SDS pH 7.5, 0.5 M NaCl) or LB2 lysis reagent (4% SDS pH 7.5, 0.5 M NaCl, 2% DMSO). For *E. coli* and mammalian cells, lysis was achieved by pipetting and incubating for 3 minutes. However, for optimisation of RNA yield from yeast and *E. coli* cells expressing dsRNA, the suspended cells were heated for 4 minutes at 90°C and homogenised by pipetting. The lysate was then centrifuged for 4 minutes at 13,000 rpm and the supernatant transferred to a new 2 mL Eppendorf tube. 250 µL of 1.0 M guanidine hydrochloride (Gu-HCl) (Thermo Scientific), 40 µL 5 M NaCl and 250 µL Isopropanol were added prior to purification using solid phase extraction (SPE). These extractions are termed either RNASwift+Gu-HCl or RNASwift+Gu-HCl+DMSO for clarity. For the SPE, the sample mix was applied to a silica-membrane column (Qiagen/Invitrogen) and centrifuged at 13000 rpm for 1 minute. The flow through was

discarded and 700 µL wash buffer, (15 mM TRIS-HCl, 85% ethanol, pH 7.4) added followed by centrifugation at 13000 rpm for 1 minute. The flow through was discarded and the dry column was re-centrifuged. The RNA was eluted with 100 µL RNase-free water (Ambion).

RNASwift extraction

For RNASwift RNA purification, 100 µL of pre-warmed LB1 lysis reagent (4% SDS pH 7.5, 0.5 M NaCl) was used to lyse cells as described previously. After lysis, 50 µL of 5 M NaCl was added. The lysate was then centrifuged for 4 minutes at 13,000 rpm and the supernatant transferred to a new 2 mL Eppendorf tube. 500 µL 60% Isopropanol was added prior to purification using a silica-membrane column (Qiagen or Invitrogen). After loading the column was centrifuged at 13000 rpm for 1 minute. The flow through was discarded and 700 µL wash buffer (15 mM TRIS-HCl, 85% ethanol, HCl-final pH 7.4) was added followed by centrifugation at 13000 rpm for 1 minute. The flow through was discarded and the dry column was re-centrifuged. The RNA was eluted with 100 µL RNase-free water (Ambion).

Extraction with TRIzol® Max™ Bacterial RNA Isolation and Ribopure™ bacterial RNA

The Ribopure bacterial RNA extraction kit (Ambion) and TRIzol Max Bacterial RNA Isolation kit (Invitrogen) were used to extract total RNA following the manufacturer's instructions. Steps described as optional but that may improve quality or yield of RNA were followed and every effort made to ensure that the extracted RNA using

each method met the manufacturer's guidelines, including the number of *E. coli* cells used for the extractions. However, no DNAse I treatment was performed for any RNA sample used in this study. For TRIzol® Max Bacterial RNA Isolation, RNA pellet was dissolved in 100 µL RNase-free water (Ambion). For Ribopure™ bacterial RNA extraction, the RNA was eluted in 100 µL RNase-free water (Ambion).

Analysis of RNA quality and quantity

The quality and quantity of RNA was determined using a NanoDrop™ 2000c spectrophotometer (Thermo Scientific). RNA concentrations were determined by absorbance at 260 nm. In order to determine the dsRNA yield, total RNA amount was first determined by multiplying the total RNA concentration from Nanodrop^{Tm} measurement (40 ng cm/µl) with the total volume of eluted RNA. This value was then multiplied by the ratio of dsRNA peak area: total RNA peak area derived from IP RP HPLC trace. The A260/280 nm and A260/230 nm ratios were obtained using the NanoDrop™ instrument. RNA quality was determined by performing ion-pair reverse phase chromatography using a 10 µl injection from the 100 µl of eluted/re-suspended RNA. Subsequently, ribosomal RNA (rRNA) percentage and 23S:16S rRNA ratios were determined using the chromatographic RNA peak areas. The percentage (%) degradation index was calculated from the IP RP HPLC chromatogram by dividing the sum of the peak areas within the region where the 16S (or 18S as the case may be) and the 5S rRNAs elute by the total RNA peak area and then multiplying by 100.

Ion pair-reverse phase high performance liquid chromatography (IP-RP HPLC)

All samples were analysed by IP-RP-HPLC (WAVE HPLC system, Transgenomic, San Jose, USA) using a Proswift RP-1S Monolith column (4.6 X 50 mm I.D., ThermoFisher). Chromatograms were acquired using UV detection at 260 nm with a column temperature of either 50 °C or 75 °C. The chromatographic analysis was performed using the following conditions: solvent A 0.1 M triethylammonium acetate (TEAA) pH 7.0 (Fluka, UK); solvent B 0.1 M TEAA, pH 7.0 containing 25% acetonitrile (ThermoFisher). RNA was analysed using the following gradients. Gradient (1) starting at 25% B the linear gradient was extended to 27% B in 2 minutes, followed by a linear increase to 57% B over 15 minutes, followed by a linear increase to 70% B over 2.5 minutes at a flow rate of 1.0 ml/min. Gradient (2) starting at 25% B the linear gradients were extended to 30% B in 2 minutes, then to 65% buffer B over 15 minutes, and to 80% B over 2.5 minutes at a flow rate of 1.0 ml/min.

Results and Discussion

Development of RNAswift for the extraction of RNA from bacterial cells

A wide range of RNA isolation methods are suitable for the extraction of total RNA but are limited by the presence of hazardous and or toxic chemicals in the RNA extraction reagents. In addition, residual chemicals from these extraction reagents often contaminate the RNA and may interfere with the downstream processing or analysis. RNA precipitation steps utilised as part of RNA extraction methods are often ineffective at removing these potential contaminants and are associated with low yields of certain species of RNA. In order to address these problems, we developed a less-hazardous, rapid and versatile RNA purification method that separates RNA from the bulk of the DNA and proteins without a phenol-chloroform extraction step and in conjunction with a solid phase extraction (SPE) step to purify the RNA.

In developing RNASwift we took advantage of the chemical properties of sodium dodecyl sulphate (SDS), an anionic surfactant known for its ability to aid in the lysis of cells and denature proteins. Additionally, sodium chloride (NaCl) facilitates cell lysis by exerting osmotic pressure which ruptures cell membrane and is also known to promote binding of SDS to proteins. Initial work focused on optimising cell lysis using SDS and NaCl, in conjunction with ensuring minimal RNA degradation through denaturation of cellular ribonucleases.

Following lysis of the bacterial cells using the NaCl-SDS reagent and centrifugation to remove the majority of cell debris containing proteins, genomic DNA and other insoluble cellular material, the RNA was subsequently purified using SPE. However, it was observed that excess SDS precipitates in the presence of the organic solvents

necessary for binding the RNA to the silica columns used in the SPE, resulting in lower yield of RNA. By adding 4 M guanidinium-HCl we were able to solubilize the SDS prior to purification of the RNA using SPE. Following purification of the total RNA from bacterial cells using the above method, the RNA was analysed using IP RP HPLC (see Fig. 1a). The results show the purification of high quality total RNA using this approach. No significant degradation of the rRNA was observed. Further optimisation was performed using additional reagents/denaturants in an attempt to further improve the yield and quality of total RNA extracted. Fig. 1b shows the total RNA purified with the addition of 2% dimethyl sulfoxide (DMSO) to the lysis buffer. The results show that the yield and purity of the total RNA extracted with the addition of DMSO was not significantly affected by the addition of DMSO.

Further analysis of IP RP HPLC chromatograms of the extracted RNA revealed only low amounts of the small RNAs (5S/tRNAs) in the total RNA fraction were recovered using this approach (see Fig. 1c). In addition, although the extraction methods were effective in isolating high quality total RNA, the addition of guanidinium-HCl (4 M) was necessary during the procedure. As our objective was to minimize the use of potentially hazardous chemicals and expensive reagents, without compromising RNA yield and quality, we therefore further modified the extraction method. Further optimisation of the RNA extraction method was performed in an attempt to both retain the small RNAs and remove the requirement for guanidinium-HCl prior to SPE purification of the RNA.

We observed that precipitation of the SDS/NaCl solution increased as a function of increasing total SDS and NaCl concentration indicating the formation of SDS

micelles and their aggregation. This effect of inorganic salts on ionic surfactants has been extensively studied and is explained by the increased stability of hydrophobic interactions involving the aliphatic C_{12} groups in a solution at high ionic strength together with inter-micellar binding via ion-dipole interactions between neutralised sulfate groups and sodium ions [19]. It has been observed that NaCl enhances cooperative binding of SDS to proteins [20] which would also explain the partitioning, via hydrophobic interactions, of proteins into the SDS micelles. Therefore during cell lysis in the presence of sodium chloride, SDS and heating, the hydrophobic/neutralised genomic DNA is denatured and subsequently binds with denatured proteins coated with SDS molecules. The high concentration of sodium ions in the neutralization buffer induces precipitation and therefore in addition to the cell debris the genomic DNA/proteins can be removed by centrifugation [21]. The RNA remains in the supernatant and is further purified.

Taking into consideration the dissociation of NaCl and SDS ions in solution, the total molality of NaCl and SDS ($\underline{m}$) and the mole fraction of SDS ($\underline{x}$) can be defined by the equations, $\underline{m} = 2m_1 + 2m_2$ and $\underline{x} = 2m_2/ m = m_2/(m_1 + m_2)$ where m_1 and m_2 is the molality of NaCl and SDS, respectively [11]. Following lysis of the cells using the SDS-NaCl buffer, the lysate with initial 0.5 M NaCl, was adjusted to 2.25 M NaCl, centrifuged at 14000 rpm for 4 minutes and the supernatant transferred to a new tube. Subsequently, 500 µL of 60% isopropanol was added to the supernatant and RNA purified using SPE. No precipitation was observed with addition of isopropanol, which suggests significantly lower SDS content in the supernatant and that SDS is salted and therefore precipitates along with insoluble cell material. The eluted RNA was subsequently analysed by IP RP HPLC (see Fig. 2a/b). The results show the purification of high quality total RNA using this approach. No significant degradation

of the rRNA was observed, similar to previous analysis (see Fig. 1). In contrast to previous analysis using guanidinium-HCl (4 M) prior to SPE where poor recovery of small RNAs was observed, the IP RP HPLC analysis shows the representative recovery of high quality total RNA including the small RNAs. By adding adequate amounts of isopropanol to the recovered supernatant we are able to increase the binding of smaller RNA to silica columns thereby achieving a more representative recovery of all RNA species (compare Fig. 1C and 2B).

Analysis of RNA quality, purity and yield extracted using RNASwift

Following optimisation of RNA extractions using RNASwift, further assessment of the purity, quality and yield of RNA extracted from bacterial cells was compared to a number of alternative RNA extraction methods. RNA extracted using RNAswift was compared against RNA extracted using Ribopure^{Tm} and TRIzol® max. Analysis was performed using UV spectrophotometry in conjunction with IP RP HPLC. A number of metrics were used, including A260/280 nm and A260/230 nm measurements. In addition, IP RP HPLC was used to measure the integrity of the total RNA using a combination of the rRNA percentage, 23S/16S rRNA ratio and degradation index.

A summary of the comparative UV spectrophotometry analysis is shown in Table I and Table II. The results show that for all extraction methods the A260/280 nm ratios of the extracted total RNA was approximately 2. An A260/280 nm ratio of 1.8 - 2.0 is indicative of minimal protein contamination. In contrast, the A260/230 nm ratio measurements demonstrate differences between the different extraction methods. An A260/230 nm ratio of < 1.5 was obtained for Ribopure and TRIzol max. However, an A260/230 ratio of > 2 was obtained for the RNASwift. An A260/230 ratio of >2.0 is

indicative of minimal contamination from chemicals that absorb at 230, including EDTA, phenol, carbohydrates. In summary, these results demonstrate that each of the extraction methods generate RNA of high purity with minimal protein contamination. However the RNASwift extracts RNA with the lowest levels of contaminants that absorb at 230 nm. It is likely residual phenol from the extractions was present in the RNA extracted using the Ribopure^{Tm} and TRIzol® extraction methods.

The integrity of the total RNA extracted in each of the different methods was assessed using IP RP HPLC analysis. In each case 100 µL of RNase free water was used to elute and resuspend the RNA following extraction and 10 µL analysed using IP RP HPLC (see Fig. 3). A combination of the rRNA percentage, 23S/16S rRNA ratio and degradation index was used. Based on the ratio of 23S/16S rRNA both the RNASwift and Ribopure^{Tm} extraction methods isolate good quality RNA with ratios >1.2. In contrast, using TRIzol max the 23S/16S rRNA ratio was less than 0.5 suggesting lower quality total RNA. The percentage of 23S and 16S in the total RNA was above 80% for the RNASwift and 92.13% and 29.17% for Ribopure and TRIzol® max extraction methods respectively (see Table I). The fact that ribosomal RNA constitutes more than 80% of the cellular RNA suggests that there was minimal degradation of rRNA in each of the methods with the exception of TRIzol max. Also the degradation index shows a value of 2% (minimal degradation) for all methods except for TRIzol max whose average degradation index was more than 60%.

For *E. coli*, the analysis showed an increase in yield of total RNA isolated using RNASwift compared with the Ribopure bacteria extraction. The data showed the highest yield was from the TRIzol® max however a significant amount of degraded rRNA was observed (see Table I).

Extraction of total RNA from *Saccharomyces cerevisiae* and Chinese Hamster Ovary (CHO) cells

Following optimisation of the RNASwift extraction methods for bacterial cells (*E. coli*), the versatility of the method was examined by isolating RNA from both *S. cerevisiae* and Chinese Hamster Ovary (CHO) cells. Following extraction from the different cells, the RNA was analysed using IP RP HPLC in conjunction with UV spectrophotometry (see Fig. 4 and Table II). The results show that the RNASwift method effectively isolated total RNA from each of the different cells. Furthermore, consistent with previous extractions from bacterial cells, the result is that RNASwift recovered all the expected RNA species, including the low M_W RNAs (see Fig. 4). Minimal degradation (<2%) of the rRNA was observed from both *Saccharomyces cerevisiae* and CHO cells (see Fig. 5b/c). Furthermore, the 25S rRNA/18S and 28S rRNA/18S rRNA ratios from RNA isolated from yeast and mammalian cells using RNASwift was >1.3 (see Table II). Consistent with previous extractions from bacterial cells an A260/280 nm ratio of approximately 2 was obtained and an A260/230 nm ratio of > 2 demonstrating the extraction and purification of RNA of high purity and minimal contamination.

Extraction of dsRNA from bacterial cells

The potential to synthesize large quantities of dsRNA in both bacterial systems and via *in vitro* transcription [22, 23] for RNA interference applications has generated significant demand for the development and application of high throughput analytical tools for the rapid extraction, purification and analysis of dsRNA. A number of alternative approaches have been used for the extraction of dsRNA from bacterial

cells including methods using phenol/chloroform [18] and a number of non-phenol chloroform extraction methods have been developed for extraction of dsRNA from plants and fungi [17, 24].

Therefore, the RNASwift method was used to extract dsRNA from bacterial cells engineered to express dsRNA. *E. coli* HT115 (DE3) cells were transformed with plasmid pCOIV to express a dsRNA (~756 bps). Following induction and transcription of the dsRNA we evaluated a number of alternative commercially available extraction methods including TRIzol Max™ Bacterial RNA Isolation and the Ribopure bacterial RNA extraction kit to extract the dsRNA. Following extraction of the dsRNA, analysis was performed using IP-RP HPLC in conjunction with UV spectrophotometry (see Fig. 5 and Table II). The results show that the dsRNA was not extracted using the Ribopure bacterial RNA extraction kit (see Fig. 5a). However, the dsRNA was successfully extracted using both the RNASwift and TRIzol® Max™ methods. Approximately 20 µg of dsRNA per 10^8 cells was extracted using these methods, demonstrating no significant difference in the yield of dsRNA obtained. Consistent with previous extractions from bacterial cells the RNA extracted using RNASwift resulted in an A260/280 nm ratio of approximately 2 and an A260/230 ratio of > 2, demonstrating the high purity of the RNA extracted (see Table II). In contrast the dsRNA extractions using TRIzol® Max an A260/230 nm ratio of 1.67 was obtained.

RNASwift is a single-step RNA isolation method

During the development of the RNASwift method for the extraction of RNA from bacterial, yeast and mammalian cells we used an SPE step to purify the RNA following cell lysis. Further development of the RNASwift method employed

isopropanol precipitation as an alternative to SPE. Following RNASwift extraction from *E. coli*, yeast and mammalian cells RNA from supernatant was directly precipitated using isopropanol prior to IP RP HPLC (see Fig. 6 and Table I and 2). Apart from the A260/230 nm ratio, which reduced to approximately 1.8, the RNA quality, purity and size distribution obtained using direct RNA precipitation from the supernatant was not significantly different from the result obtained using SPE column purification. A260/280 nm ratios of >2 were obtained for all RNA extractions in conjunction with isopropanol precipitation (see Fig. 4/6, Table I and 2). This demonstrates that RNASwift can be used to isolate RNA in conjunction with isopropanol precipitation without the need for SPE since RNA recovery with the two methods is similar.

Conclusions

RNA extraction methods are often limited by either the toxicity of the reagents used, the complexity of the procedure, isolation of poor quality RNA and the enrichment of only a subset of the total cellular RNA present. We have developed a new method termed RNASwift which is a simple, rapid, effective and reproducible method for RNA isolation. RNASwift does not require the use of phenol/chloroform and therefore utilises less hazardous and inexpensive reagents to isolate RNA from a variety of cell types. RNASwift uses sodium chloride and sodium dodecyl sulphate to lyse the cells and isolate the RNA from the abundant cellular components in conjunction with solid phase extraction or isopropanol precipitation to rapidly purify the RNA. RNA extractions using RNASwift routinely can be completed in less than 20 minutes. Moreover, the purified RNA is directly compatible with downstream analysis including IP RP HPLC. RNASwift extracts high quality intact RNA with minimal degradation. We have shown that the purity of the RNA isolated is superior to a number of alternative RNA extraction methods based upon a number of metrics including A260/230 nm measurements. We have also shown that RNASwift efficiently recovers a wide range of cellular RNAs including both small RNAs and more abundant larger rRNAs that represent the cellular complement of RNA. Furthermore, the method is versatile and can efficiently extract total RNA from a wide range of different cells including bacteria, yeast and mammalian cells. The method is also suitable for the extraction of dsRNA from bacterial cells and is cost-effective for the large scale extraction of RNA.

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Legends to Fig:

Fig. 1 IP RP HPLC chromatograms of total RNA extracted from *E. coli* using RNASwift. **a)** IP RP HPLC chromatogram of total RNA from *E. coli* cells extracted using RNASwift + Gu-HCl. The 23S/16S rRNA ratio = 1.76±0.09 with <2% degradation index. **b)** IP RP HPLC chromatogram of total RNA from *E. coli* cells extracted using RNASwift +Gu-HCl+DMSO. The 23S/16S rRNA ratio = 1.82±0.07 with <2% degradation index. **c**) IP RP HPLC chromatogram highlighting the low molecular weight RNAs extracted using RNASwift + Gu-HCl.

Fig. 2 IP RP HPLC chromatograms of total RNA extracted from bacterial cells using RNASwift. **a)** IP RP HPLC chromatogram of total RNA from *E. coli* cells extracted using RNASwift. The corresponding rRNA is highlighted. The 23S/16S rRNA ratio = 1.82±0.05 with <2% degradation index. **b**) Enhanced view of the IP RP HPLC chromatogram highlighting the small RNAs present. 10 µl of extracted total RNA was analysed using gradient condition 1 at 75°C and 260 nm UV detection.

Fig. 3 IP RP HPLC chromatograms of total RNA extracted from *E. coli*. **a)** IP RP HPLC chromatogram of total RNA from *E. coli* cells extracted using Ribopure™ bacterial extraction kit. The 23S/16S rRNA ratio = 1.55±0.03 with <2% degradation index. **b)** IP RP HPLC chromatogram of total RNA from *E. coli* cells extracted using TRIzol® Max™ Bacterial RNA Isolation Kit. The 23S/16S rRNA ratio = 0.47±0.21 with >60% degradation index. **c**) IP RP HPLC chromatogram of total RNA from *E. coli* cells extracted using RNASwift. The corresponding rRNA and dsRNA are

497 highlighted. The 23S/16S rRNA ratio = 1.82±0.05 with <2% degradation index. 10 µl
498 of extracted total RNA was analysed using gradient condition 1 at 75 °C and 260 nm
499 UV detection.

500

501 **Fig. 4** IP RP HPLC chromatograms of total RNA isolated from bacterial, yeast and
502 mammalian cells using RNASwift. **a)** IP RP HPLC chromatogram of total RNA
503 isolated from *E. coli* cells using RNASwift. The 23S/16S rRNA ratio = 1.82±0.05 with
504 <2% degradation index. **b)** IP RP HPLC chromatogram of total RNA isolated from *S.*
505 *cerevisiae* cells using RNASwift. The 25S rRNA/18S rRNA ratio = 1.40±0.01 and 2%
506 degradation index **c)** IP RP HPLC chromatogram of total RNA isolated from CHO
507 cells using RNASwift. The 28S/18S rRNA ratio = 1.50±0.01 with <2% degradation
508 index. 10 µl of total RNA was analysed using gradient condition 1 at 50 °C for *E. coli*
509 and CHO cells and gradient 2 for yeast at 50 °C and 260 nm UV detection.

510

511 **Fig. 5** IP RP HPLC chromatograms of total RNA extracted from *E. coli* cells
512 engineered to express dsRNA. **a)** IP RP HPLC chromatogram of total RNA isolated
513 using Ribopure™ bacteria extraction kit from *E. coli* HT115 (DE3) cells transformed
514 with plasmid pCOIV following induction with IPTG. **b)** IP RP HPLC chromatogram of
515 total RNA isolated using TRIzol® Max™ Bacterial RNA Isolation Kit from *E. coli*
516 HT115 (DE3) cells transformed with plasmid pCOIV following induction with IPTG. **c)**
517 IP RP HPLC chromatogram of total RNA isolated using RNASwift + Gu-HCl + DMSO
518 from *E. coli* HT115 (DE3) cells transformed with plasmid pCOIV following induction
519 with IPTG. **d)** IP RP HPLC chromatogram of total RNA isolated using RNASwift from
520 *E. coli* HT115 (DE3) cells transformed with plasmid pCOIV following induction with

IPTG. . 10 µl of total RNA was analysed using gradient condition 2 at 50 °C and 260 nm UV detection.

Fig. 6 IP RP HPLC chromatograms of total RNA isolated from different cells using RNASwift in conjunction with isopropanol precipitation. **a)** IP RP HPLC chromatogram of total RNA isolated from *E. coli* cells using RNASwift in conjunction with isopropanol precipitation. The 23S/16S rRNA ratio = 1.62±0.14 with <2% degradation index. **b)** IP RP HPLC chromatogram of total RNA isolated using RNASwift procedure in conjunction with isopropanol precipitation from *E. coli* HT115 (DE3) cells transformed with plasmid pCOIV following induction with IPTG. **c)** IP RP HPLC chromatogram of total RNA isolated from CHO cells using RNASwift in conjunction with isopropanol precipitation. The 28S/18S rRNA ratio = 1.30±0.02 with <2% degradation index. 10 µl of total RNA was analysed using gradient condition 1 at 260 nm UV detection at the indicated temperatures. The corresponding rRNA is highlighted

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