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1

2 # Filter paper-based spin column for low throughput
3 # nucleic acid purification

4

5

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Abstract

We describe a method of recharging used spin column or assembling homemade spin column using filter paper as binding material for low throughput nucleic acid purification. We evaluated the efficiency of filter paper based spin columns in the purification of different type of nucleic acids. For instance, by following protocols of respective commercial kits, we found that filter paper to be a useful binding material for purification of many types of nucleic acids, including plant genomic DNA, plant total RNA, PCR product, and DNA from agarose gels. We also found that filter paper has a weak binding affinity to plasmid DNA in tested miniprep protocols. Also, we present the protocols of using filter paper recharged spin column or homemade spin column for low throughput purification of plant genomic DNA and plant total RNA with commercial kit buffer leftover and less expensive homemade buffer.

Introduction

As a basic molecular biology technique, nucleic acid purification is the starting point for many molecular biology applications [1, 2]. Classic nucleic acid purification methods are based on organic extraction followed by ethanol-based precipitation. However, classic methods are time-consuming and require the use of toxic solvents, such as chloroform and phenol which can be harmful to the user and the environment [1, 2].

Commercial kits usually follow solid-phase purification approaches whereby nucleic acids in an extraction solution are absorbed by a solid-phase binding material under appropriate chaotropic conditions, followed by washing of non-nucleic acids remaining on the binding material using appropriate buffer solutions, and elution of purified nucleic acids from the binding

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40 material using low salt solutions [1, 3, 4]. Commercial kits using this approach permit for fast
41 purification of high-quality nucleic acids, although they are expensive to use.
42 The success of commercial kits largely relies on the spin columns or spin plates assembled
43 with solid-phase nucleic acid binding material which allow easy binding, washing, and elution of
44 nucleic acids in the purification process. The most widely adopted nucleic acid binding material
45 in the past has been mineral based silica material in the form of a matrix (glass fiber filter or
46 silica membrane) or powder (glass milk or silica slurry). Guanidine based buffer is usually
47 adopted to provide a chaotropic condition for nucleic acid binding [1]. Plant-based cellulose
48 material has been applied in DNA and RNA purification. For example, Su and Comeau [5]
49 adopted cellulose fiber to purify nucleic acids by using sodium chloride and polyethylene glycol
50 as binding reagents. MeganCel paramagnetic cellulose particles are an example of a
51 commercialized product from Promega [6]. Also, modified cellulose such as DEAE-modified
52 cellulose membranes can be applied as anion exchange materials for nucleic acid purification [7].
53 In a previous report, we found that filter paper made from cellulose fiber to be a viable
54 replacement for silicon-based material for purification of plant genomic DNA from CTAB/NaCl
55 extractions [8]. Another recent report also indicated that the filter paper tip could be used to
56 purify nucleic acids from crude extract with NaCl in a concise time for subsequent PCR based
57 analyses [9]. Since cellulose-based filter paper is an inexpensive and readily available material in
58 laboratories, we hypothesized that filter paper might have more widespread usefulness in solid-
59 phase based nucleic acid purification. We, therefore, described our attempts to use filter paper in
60 assembling recharged or homemade spin column suitable for low throughput nucleic acids
61 purification.
62

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63 # Material and methods

64 ## Plant materials, plasmid DNA and PCR product

65 Tomato (*Solanum lycopersicum L.*) plant samples were collected from the field or greenhouse of
66 Mountain Horticultural Crops Research & Extension Center of North Carolina State University
67 at Mills River, NC. USA. Tobacco (*Nicotiana tabacum L.*) plant samples were collected from
68 laboratory or growth chamber experiments being carried out by the Department of Crop and Soil
69 Science at North Carolina State University, Raleigh. For DNA purification, collected fresh
70 samples can be used immediately, or stored at -20ºC before use. Usually, about 50 to 100 mg leaf
71 samples were collected and put into 2 ml screw cap tubes for grinding using mechanic
72 homogenizer, or 1.5 ml Eppendorf tube for grinding using plastic pellet pestles. The sample for
73 RNA purification should be frozen in liquid nitrogen quickly after collection and grind into fine
74 powder in liquid nitrogen, then transfer 50 to 100 mg ground sample into the tube and to use
75 immediately or store at -80ºC before use.

76 pUC19 and pBI121 plasmids were purchased from Invitrogen (Carlsbad, CA). The GUS gene
77 fragment was amplified from the pBI121 plasmid or transgenic tobacco by PCR using the
78 following primer:

79 The forward primer, 5'- TGACCTCGAGGTCGACGATATCGTCGTCATGAAGATGCGGAC-
80 3'

81 Reverse primer, 5'- CTAGACTAGTCCCGGGGGTACCATCCACGCCGTATTCGGTG-3'.

82

83 ## Filter paper-based spin column preparation

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84 Recharging of used commercial spin columns is initiated by separating plastic parts and treating
85 them with 10% bleach for at least 10 mins, followed by thorough rinsing with sterilized water
86 several times, and air-drying. Filter paper discs were punched from sheet of Whatman™
87 qualitative filter paper, Grade 3 (GE Healthcare Life Science, UK) or equivalent filter paper
88 using 3/16-inch (~8mm) paper puncher, and then one or two layers of filter paper disc is added to
89 the column by pushing down them to bottom of column tightly using the end of a 200 µl pipette
90 tip (Fig 1A). The assembled spin column could be autoclaved and air dried.

91

92 **Fig 1. Illustration of recharging spin column and homemade spin column using filter**
93 **paper.** (A) Recharged used spin column with a flat bottom and net structure to support filter
94 paper discs. (B) Homemade filter paper-based spin column prepared by using 0.5 ml tube with
95 the bottom part cut, and adding the top part of 10 µl pipette tip as supporting tube to support two
96 layers of filter paper discs loaded on it within the tube. The homemade spin column is based on
97 0.5 ml PCR tubes. In brief, the bottom of 0.5 ml tubes is initially cut off, followed by the
98 insertion of the upper part of 10 µl pipette tips to serve as supporting tubing, then load one or
99 two layers of filter paper discs on the supporting ring in tube, and push tightly using upper end of
100 10 µl tip (Fig 1B). For homemade spin column based on 0.5 ml PCR tube, filter paper discs were
101 punched using a standard 1/4-inch (6.35 mm) paper puncher from filter paper sheet.

102

103 Purify plant genomic DNA using filter paper-based spin 104 column with Qiagen plant DNeasy kit buffer or homemade 105 buffer

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106 This protocol is an example of using filter paper-based spin columns to purify plant genomic
107 DNA with Qiagen DNeasy Plant mini kit buffers referred to Qiagen DNeasy® plant handbook
108 (March 2018) or less expensive homemade buffer as described by Lemke et al [10].

109 To lysis plant material, add 400 µl AP1 buffer of Qiagen kit or homemade lysis buffer (0.5%
110 SDS, 8% PVP-10, 250 mM NaCl, 25 mM Na-DETA, 200 mM Tris-HCl pH7.5) along with 4 µl
111 RNase A stock solution (100 mg/ml, Qiagen) into 2 ml screw cap tube with 50 to 100 mg frozen
112 or fresh plant material, then add two tungsten carbide beads (Qiagen) or similar beads into tube
113 and homogenize samples using homogenization equipment like FastPrep FP120 cell
114 homogenizer (Savant Instruments Inc, Holbrook, NY) followed the manual. Alternatively, grind
115 50 to 100 mg frozen or fresh sample in 1.5 ml Eppendorf tube with 400 µl lysis buffer and 4 ul
116 RNase A stock solution using disposable plastic pellet pestles (USA Scientific, Ocala, FL).

117 Tubes with lysis mixture are incubated at 65ºC for 10 min (mix by inverting the tube 2 to 3
118 times), then add 130 µl P3 buffer of Qiagen kit or 130 µl homemade precipitation buffer (5M
119 KAc, pH 6.5) and maintenance on ice for at least 5 mins. Centrifuge sample tube using a
120 microcentrifuge at its top speed (≥16,000 g) for 10 mins to clear lysate.

121 In case lysate is not well cleared, an option step followed is to place a spin column with one
122 layer of filter paper into a new 2 ml tube which is used as collection tube, then transfer lysate to
123 the spin column and centrifuge at 6,000 × g for 1 min followed another round of centrifuge at top
124 speed for 1 min to get cleared lysate in collection tube.

125 To bind DNA onto filter paper discs in spin column, transfer cleared lysate from collection
126 tube into a new tube and mix with 1.5 volume of AW1 buffer of Qiagen kit or homemade
127 binding buffer (2M Guanidine Hydrochloride, 75% ethanol) by pipetting or inversion, transfer
128 mixture to new spin column assembled with two layers filter paper disc, which placed in a new

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129 2-ml collection tube, centrifuge at 6000 × g for 1 min, discard flow through and repeat to load
130 remaining solution for another round of centrifuge step to allow all cleared lysate flow through
131 filter paper discs in spin column.
132 To wash filter paper discs in the spin column, add 500 µl AW2 buffer of Qiagen kit or
133 homemade washing buffer I (10 mM NaCl, 10 mM Tris-HCl pH6.5, 80% ethanol) to the column,
134 spin at 6000 × g 1 min, discard flow through and reuse collection tube. Add 500 µl AW2 buffer
135 of Qiagen kit or homemade washing buffer II (95% ethanol), spin at 6000 g for 1 min and then
136 transfer spin column to the new collection tube. Centrifuge columns at the top speed for at least 2
137 mins.
138 For DNA elution from filter paper discs, insert a spin column to new 1.5 ml collection tube, air
139 dry for a while and add 100 µl AE buffer of Qiagen kit or 10 mM Tris-HCl (pH8.5) on filter
140 paper in spin column, and maintain at room temperature for 5 mins, then centrifuge at 6000 g for
141 1 min to elute DNA into collection tube.
142

143 Purification of plant RNA using filter paper-based spin 144 column with commercial kit buffer or homemade buffer

145 This protocol is an example of using filter paper-based spin columns to purify plant total RNA
146 with Qiagen RNeasy Plant mini kit buffers according to RNeasy® Mini Handbook (Fourth
147 edition, June 2012) or less expensive homemade buffer presented by Yaffe et al [11].
148 In brief, add up to 100 mg grinded frozen sample into 1.5 or 2 ml tube, add 450 µl buffer RTL
149 or RLC of Qiagen kit with 1% (v/v) b-mercaptoethanol or homemade lysis buffer (8 M

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150 guanidine hydrochloride, 20 mM MES hydrate and 20 mM EDTA) along with 1% (v/v) b-
151 mercaptoethanol.
152 Tube with lysis mixture is incubated at 55ºC for 1 to 3 min (mix by inverting the tube 2 to 3
153 times), and then centrifuge tube using a microcentrifuge at top speed (≥16,000 × g) for 5 to 10
154 mins to clear lysate. If lysate is not clear enough, then insert a spin column assembled with one
155 layer of filter paper in a 2 ml new collection tube, and transfer all cleared lysate to spin column
156 and centrifuge at 8,000 × g for 1 min followed another round of spin at top speed for 1 mins to
157 get cleared lysate.
158 Transfer all cleared lysate to a new 1.5 ml tube and mix with half amount of ethanol by
159 pipetting or inversion. Place a new spin column assembled with two layers of filter paper discs
160 into a new 2 ml collection tube, then transfer lysate/ethanol mixture to spin column and
161 centrifuge at 8000 × g for 1 min, discard flow through, and reuse collection tube and repeat to
162 load remaining solution and centrifuge again.
163 To wash column, add 700 μl RW1 buffer of Qiagen kit or homemade washing buffer I (3 M
164 Na-acetate pH 5.2) to the spin column, then spin at 6000 × g 1 min and discard flow through
165 from collection tube. Add 500 μl RPE buffer of Qiagen kit or homemade washing buffer II (70%
166 ethanol), and centrifuge at 8000 × g for 1 min, transfer spin column to new collection tube and
167 centrifuge spin column at the top speed for at least 2 mins to eliminate remaining ethanol. Insert
168 a spin column to new 1.5 ml collection tube, air dry for a while, then add 50 μl RNase free water
169 and maintain at room temperature for 1 mins, then centrifuge at 8000 × g for 1 min to elute
170 RNA.
171 For RNA purification, all reagent and buffer should be treated by diethyl pyrocarbonate
172 (DEPC) to eliminate RNase and also keep working area clean.

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173

174 Evaluation of purified nucleic acids

175 DNA yield and quality were checked by 1 to 1.5 % agarose electrophoresis stained using
176 Ethidium Bromide (EB), RNA integrity and quality were evaluated by 1 % MOPs-formaldehyde
177 denaturing agarose electrophoresis with EB in RNA loading buffer [12]. Ethidium bromide
178 stained DNA, or RNA fluorescent bands were visualized and recorded by Bio-Rad Gel Doc™
179 XR+ gel image system (Bio-Rad Laboratories, Hercules, CA), or FOTODYNE system
180 (FOTODYNE incorporated, Hartland, WI).

181 DNA and RNA quality and quantity were also estimated by Nanodrop 2000 UV spectrometer
182 (Fisher Scientific, Waltham, MA). Also, DNA concentration was measured using Hoefer
183 fluorometer DQ300 (Hoefer, Holiston, MA) with double DNA specific H33258 dye (Sigma-
184 Aldrich).

185 Quantitative PCR (qPCR) and quantitative reverse transcription PCR (qRT-PCR) approaches
186 were used to further precisely check whether the purified DNAs and RNAs contain
187 contamination which could interfere with the efficiency of PCR reaction. In brief, 10 µl SYBR
188 green-based qPCR reaction was prepared using Luna® Universal Probe qPCR Master Mix (New
189 England Biolabs, Ipswich, MA, USA) with 0.5 µM primer set NtTublin_1 for tobacco [13]. For
190 DNA evaluation, we added 50 ng genomic DNA purified by different approaches in reaction. For
191 RNA evaluation, cDNAs were reverse transcribed from 200 ng purified RNA in 10 µl reverse
192 transcription reaction prepared using iScript™ cDNA Synthesis Kit (Bio-Rad), and cDNA
193 amount to 2.5 ng RNA was added to the qRT-PCR reaction. Both qPCR and qRT-PCR were run
194 on CFX96™ Real-Time System followed standard two step PCR program as suggested by

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195 Luna® Universal Probe qPCR Master Mix manual. Amplification efficiencies of different input
196 templates were evaluated using CFX Maestro™ Software version 1.1 (Bio-Rad) based on
197 quantification cycle (Cq) value [14], which is calculated fractional cycle at which the target
198 DNA amplicon associated fluorescent accumulated up to an arbitrary threshold.

199

200 Results

201 Recharged used spin column and homemade spin column
202 using filter paper

203 We previously described a method to prepare filter paper-based 96 well spin plates suitable for
204 high throughput nucleic acid purification [8], and another recent publication also reported filter
205 paper dipstick designed for fast purification of DNA and RNA used in PCR based applications
206 that do not require large quantities of nucleic acids [9]. However, compared to the multiple well
207 spin plate and dipstick, spin column assembled with filter paper should be the more appropriate
208 format for low throughput nucleic purification conducted in most biological labs. Therefore, we
209 have made attempts to prepare a filter paper based spin column.

210 Our efforts start from recharging of used commercial spin columns with filter paper disc(s). As
211 described in the Methods section, such recharged spin column can be prepared based one a
212 commercial spin column which has a flat bottom but equipped with net structure to support
213 binding material, such as those Wizard® SV minicolumns of Promega (Madison, WI) (Fig 1A).
214 Alternatively, a spin column with a conical bottom (V shape bottom) equipped drip opening,
215 such as a miniprep column of Qiagen can be adopted. Both formats are convenient for reloading

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216 of filter paper discs with a diameter of 5/16 inch (~8 mm). A recent version of a spin column
217 named as a microspin column, such as those used in NEB kit can be recharged by using filter
218 paper discs with a diameter of 5/16 inch or using filter paper disc(s) with a diameter of 3/16 inch
219 (6.35mmm).

220 Since filter paper is relatively physically stronger to hold its shape compare to the soft silica
221 based membrane, such as glass fiber filter, therefore, we prefer to exclude fixing ring (O-ring)
222 and support plastic frit adopted in many types of the commercial spin column in the recharging
223 of these used spin column. The advantage of excluding these items is to simplify the recharging
224 process, and also help to avoid the problem of solution leftover on the fixing ring during
225 purification experiment.

226 In addition to the recharged used spin column, we also try to prepare homemade spin column
227 assembled with filter paper. In the previous report, Borodina et al [15] have described a way to
228 prepare glass fiber filter based homemade spin column using a 0.5 ml PCR tube. They added
229 silica based glass fiber material to the bottom of the tube with several small holes punched,
230 however, it is harder to push filter paper discs down to the bottom and seal the bottom of the tube
231 properly like soft glass fiber filter adopted in the homemade silica spin column. For example, if
232 we push filter paper too hard, then filter paper did fill the bottom of the tube but might be too
233 tight to block flow through of solution. Otherwise, it might leave space and result in leaking of
234 the sample solution.

235 After many attempts, we found that adding upper part of 10 ul tip as supporting ring in the
236 tube, and then can load filter paper disc(s) on the supporting ring to form a simple homemade
237 spin column. For better performance, we chose the upper part of the tip with additional edges
238 (Fig 1B), which can provide enough supporting area to support filter paper disc and also leave

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239 space between supporting ring and wall of the tube for allowing solution flow through filter discs
240 without leftover.
241

242 Evaluation of filter paper in the purification of different 243 nucleic acids

244 Though filter paper has been successfully adopted in method using NaCl as a binding reagent [5,
245 8, 9], we also found that filter paper work well in DNA purification followed protocol using
246 guanidine-based reagent, which is commonly adopted in commercial kit for nucleic acid binding,
247 and therefore, it is possible to use filter paper-based spin column as substitute for nucleic acid
248 purification using commercial kit.

249 To investigate whether filter paper-based spin column can be used in commercial kit,, we
250 reassembled spin columns of Qiagen kit by just replacing of original silicon membrane with filter
251 paper discs, then apply such reassembled spin column in purification of different types of nucleic
252 acids using respective Qiagen kits, including DNeasy® plant mini kit for plant genomic DNA,
253 RNeasy® plant mini kit for plant total RNA, QIAquick PCR purification kit for PCR product,
254 QIAquick gel extraction kit for DNA in agarose gel, and QIAprep spin miniprep kit for plasmid
255 DNA. For comparison of effectiveness, we included original spin columns and reassembled spin
256 column using glass fiber filters (Whatman™ glass microfiber filters, Grade GF/F, GE), a silica
257 based material usually adopted for recharging or preparing homemade spin column [10, 15].

258 In these experiments, the filter paper based-spin columns shown better performance for
259 purification of tomato genomic DNA and yield higher amount final DNA than two silica-based
260 spin column, including original Qiagen kit spin column and glass fiber-reassembled spin column,

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261 (Fig 2A). Filter paper-based spin column was also functioned in purify plant total RNA (Fig 2B),
262 DNA from PCR (Fig 2C) and recover DNA from agarose gel (Fig 2D), although the yields of
263 final nucleic acid are relatively lower (half) compare to yield from experiments using silica-
264 based spin columns.

265

266 **Fig 2. The efficiency of filter paper for purification of different types of nucleic acid using**
267 **respective Qiagen kits.** (A) Tomato genomic DNAs purified using Qiagen DNeasy® plant mini
268 kit. (B) Tomato total RNAs purified using Qiagen RNeasy® plant mini kit. (C) PCR products of
269 GUS fragment purified Qiagen QIAquick PCR purification kit. (D) PCR products of GUS
270 fragment recovered from agarose gel using a Qiagen QIAquick gel extraction kit. (E) pUC-19
271 plasmid DNAs purified Qiagen QIAprep spin miniprep kit.

272

273 For each panel, lane from left to right labeled with original column, glass fiber and filter
274 paper is the same volume of nucleic acid elution from purification experiments using
275 original Qiagen spin column, reassembled spin column using two layers of Whatman™
276 glass microfiber filters, (Grade GF/F), and reassembled spin column using two layers of
277 Whatman™ qualitative filter paper, (Grade 3) respectively.

278

279 Apart from these finding, we also noticed that filter paper reassembled spin column does not
280 work well for purification of plasmid DNA using QIAprep spin miniprep kit protocol since only
281 a weak plasmid DNA band was detected by agarose gel electrophoresis (Fig 2E).
282 According to these results, it seems that filter paper works well for purifying long linear double
283 strand nucleic acids, such as plant genomic DNA followed protocol for silica-based binding

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284 material. On another hand, the filter paper was substantially less effective for supercoiled
285 plasmid DNAs in the present experiments. Nevertheless, these experiments still indicated that
286 filter paper could serve as an alternative binding material to replace silicon material for
287 purification of many types of nucleic acids followed the protocols for silica-based material,
288 except plasmid DNA using protocols developed for silica based nucleic binding material.
289

290 Purification of nucleic acids using filter paper-based spin 291 column with commercial kit buffer or homemade buffer

292 Since it was confirmed that filter paper-based spin column could apply in the purification of
293 many types of nucleic acids followed the protocol of commercial kit originally optimized for the
294 silica-based spin column, we expected that filter paper based spin column can use as a substitute
295 of the commercial spin column in an experiment using the commercial kit buffer leftover to save
296 resources. Also, filter paper-based spin column might also work for a homemade buffer which
297 mimics commercial kit's for further reduce the expanse in the laboratory. For instance, as to our
298 interested plant nucleic acids, there are already several in-house protocols developed for
299 purification using the silica based material for purification of plant DNA [10, 16-18] or plant
300 RNA [11, 19]. Therefore, we have tried this idea using tobacco plant material.

301 Followed the protocol described in material and method, we have successfully purified tobacco
302 genomic DNA using filter paper-based recharged or homemade spin column following protocol
303 using Qiagen kit buffers and an in-house protocol modified from Lemke et al [10] using
304 homemade buffers. As shown in Fig 3A, agarose gel electrophoresis can clearly show the similar
305 performance of purification of tobacco genomic DNA using Qiagen original spin column; filter
306 paper recharged spin column and filter paper based homemade spin column with a buffer of

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307 Qiagen. The EB stain bands of genomic DNA band purified from the tobacco leaf by using either
308 Qiagen original spin column, filter paper recharged spin column, and homemade spin column
309 with Qiagen kit buffers are similar in size (integrity) and density (yield), i.e., the filter paper
310 based spin column can be substitute of commercial silica based spin column in plant DNA
311 purification using commercial kit buffer. On another hand, our results also indicated the higher
312 yield of genomic DNAs purified from tobacco sample using homemade buffer compared to
313 experiments using Qiagen kit buffer, especially for filter paper recharged spin column.
314
315 **Fig 3. Evaluation of purification of tobacco genomic DNA and total RNA using filter paper-**
316 **based spin column with respective Qiagen kit buffer and homemade buffer.** (A) Agarose gel
317 electrophoresis for 2.5 µl tobacco genomic DNAs elution from purification experiments using
318 Qiagen DNeasy® plant mini kit buffers with Qiagen original spin column (Lane Q/Q), filter
319 paper recharged used spin column (Lane Q/R) and filter paper-based homemade spin column
320 (Lane Q/H), followed by tobacco genomic DNAs purified using homemade buffer with Qiagen
321 original spin column (Lane H/Q), filter paper recharged used spin column (Lane H/R) and filter
322 paper-based homemade spin column (Lane H/H). The start material amount is 100 mg tobacco
323 young leave tissue for experiments using a Qiagen spin column or filter paper recharged spin
324 column, and 50 mg plant sample for homemade spin column purification. Final DNAs were
325 eluted using 100 µl elution buffer in these experiments. (B) Typical UV spectrum curve of
326 tobacco DNAs purified using filter paper recharged spin column with Qiagen kit buffer (Q/R,
327 blue curve) or homemade buffers (H/R, red curve) from the same amount leaf tissue. Y-axis is
328 UV absorbance, and X-axis is wavelength (nM). (C) Amplification plots for three duplicated
329 qPCR reactions contain 50 ng DNA purified using Qiagen kit (Q/Q, Blue curves) and three

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330 qPCR reaction duplicates for 50 ng DNA purified from filter paper recharged spin column with
331 homemade buffer (H/R, Red curves). The x-axis is PCR cycle numbers, Y-axis is level of SYBR
332 fluorescence, and the green line is an arbitrary threshold to determine the Cq value (the fractional
333 cycle number at which amplification curve meet threshold level). (D) MOPS-formaldehyde
334 denaturing agarose gel electrophoresis separated 5 μl RNA elution from purification experiment
335 using Qiagen RNeasy® plant mini kit buffers with Qiagen original spin column (Lane Q/Q), filter
336 paper recharged used spin column (Lane Q/R) and homemade buffer filter paper-based
337 homemade spin column (Lane Q/H), followed tobacco total RNAs purified by using homemade
338 buffer with Qiagen original spin column (Lane H/Q), filter paper recharged used spin column
339 (Lane H/R) and filter paper-based homemade spin column (Lane H/H). The start material
340 amount is 100 mg tobacco young leave in purification experiments using Qiagen spin column,
341 and filter paper recharged used spin column, and 50 mg for homemade spin column purification.
342 All these purifications used 50 μl elution buffer. (E) UV spectrum of tobacco total RNA
343 purified using filter paper recharged spin column with Qiagen RNeasy® plant mini kit buffers
344 (Q/R, blue curve) or homemade buffers (H/R, red curve). Y-axis is UV absorbance, and the X-
345 axis is wavelength. (F) Amplification plots of three duplicated qRT-PCR reactions contain
346 cDNA amounts to 2.5 ng RNA purified using Qiagen kit (Q/Q, Blue curves) and three duplicated
347 qRT-PCR reactions with cDNA amounts to 2.5 ng RNA purified using filter paper recharged
348 spin column with homemade buffer (H/R, Red curves).
349
350 UV spectrum meter analysis shown tobacco leaf DNA purified using filter paper-based
351 recharged spin column with either Qiagen kit buffer or homemade buffer all shown standard
352 DNA absorbance curve with the highest peak at 260 nm (Fig 3B).

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353 To further check the quality of purified DNA, we adopted qPCR to see whether DNA purified
354 by commercial kit and filter paper-based spin column with the homemade buffer can be
355 amplified in the same efficiency. As shown in Fig 3C, amplifications of the same amount of
356 DNAs purified by Qiagen kit and filter paper recharged spin column with homemade buffer
357 shown the same amplification plots with nearly identical Cq value across the threshold. Based
358 on assumption that PCR amplicon double their amount after each PCR cycle, there are no
359 difference in PCR analysis for these DNAs (Fig 3C), and confirmed that DNA purified using the
360 filter paper-based with homemade buffer approach is the same quality and has no additional
361 inhibitor of PCR reaction as DNA purified using commercial kit.

362 DNA purified by using recharged and homemade spin column is more suitable for low
363 throughput experiments, such as confirmation of transgenic plants via PCR. We also successfully
364 use tomato and tobacco DNAs purified by homemade spin column with homemade buffer for
365 molecular marker development experiments, including PCR based Cleaved Amplified
366 Polymorphic Sequences (CAPS) or Kompetitive Allele Specific PCR (KASP) (unpublished
367 data).

368 In addition to genomic DNA purification, we tested an in-house protocol and buffered
369 described by Yaffe et al [11] for plant RNA purification. Our experiments confirmed that this
370 protocol could be adopted for purification of tobacco plant RNA from leaf tissue using filter
371 paper-based spin column as well. As shown in Fig 3B, the performance in the purification of
372 tobacco total RNA using filter paper-based spin column with homemade buffers is similar to that
373 of Qiagen Plant RNeasy mini kit. RNAs purified by filter paper recharged spin column with
374 homemade buffer show the same pattern and density of EB stain rRNA bands as those purified
375 by Qiagen kit, which indicated the purified RNAs have similar integrity and abundance (Fig 3D).

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376 Analysis using UV spectrum meter shown standard RNA absorbance curve for tobacco leaf
377 RNA purified using filter paper recharged spin column with Qiagen kit buffer or homemade
378 buffer (Fig 3E). qRT-PCR analysis of RNAs purified by these different method also shown no
379 noticeable difference as to the amplifications efficiencies between RNAs purified by Qiagen kit
380 and RNA purified using filter paper bases spin column with homemade buffer, since Cq of these
381 reactions are similar as indicated the cross point of their amplification curves with threshold (Fig
382 3F). Such results suggested the same efficiency in both of reverse transcription or real time PCR
383 reaction using RNAs purified by these different approaches. Therefore, filter paper based spin
384 column can be adopted for plant RNA followed commercial kit protocol using kit buffer leftover
385 to save resources, or using an in-house protocol with homemade buffer to further reduce the cost
386 in the lab.
387 RNA purified by filter paper based spin column using a homemade buffer is ready to use for
388 many downstream experiments, such as RT-PCR analysis of transgene expression in a transgenic
389 plant, RACE for cDNA cloning, and even construction of mRNA deep sequencing libraries
390 (unpublished data).
391

392 Discussion

393 Development of filter paper-based spin column would be useful for the utility of filter paper in
394 nucleic acid purification since the spin column is a more adopted format which can operate using
395 a conventional desktop centrifuge for low throughput bench-scale nucleic acid experiments
396 required in daily practices of the biology lab. Here we found either recharged or homemade filter
397 paper-based spin columns are suitable for such an application, and they can incorporate with
398 commercial kits or in-house protocol by replacing expensive commercial spin column. The

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399 apparent advantage of such practice is to save on laboratory monetary resources. For instance,
400 the price per commercial spin column usually ranges from \$0.3 to over \$1 on the market. In
401 preparing recharged or homemade spin column with filter paper, the filter paper discs cost less
402 than 1 cent per column, though plastic ware of homemade spin column is about \$0.1, all plastic
403 wares can be reused by washing using bleach solution which costs very little. Of course, labor in
404 spin column preparation should be considered, but the extra effort should be acceptable for low
405 throughput applications, mainly followed the simplified recharging processes without adding
406 plastic fixing ring and support frit which commonly adopted in many types of commercial spin
407 column.

408 Another way to save lab resources is to take advantage of the extra buffer of commercial kit or
409 using a less expensive homemade buffer. To use the extra buffer, some labs regenerated spin
410 column after regeneration treatment, such as using MaxXBBond regeneration kit [11]. Here we
411 used filter paper to recharge spin column which is easy to perform. Also, using homemade spin
412 column based on 0.5 ml tube can start from reduced amount sample and buffer, and therefore,
413 more efficient in using the commercial kit buffer leftover. Purified nucleic acid by this way still
414 yield final nucleic acid at μg scale and is enough for many downstream applications. The less
415 expensive homemade buffer would be another attractive choice for reducing the cost. The
416 homemade buffer used in in-house protocol might result in higher yield than commercial kit, and
417 also save more than 75% in total expense compared to commercial kit [10, 11, 16].

418 We found that filter paper has the weak binding ability to nucleic acids in lysate without
419 adding a binding buffer in the protocol of Qiagen DNeasy or RNeasy plant kit. Therefore, we
420 used filter paper spin column to filter cell debris instead of using QIAshredder spin column from
421 Qiagen kit. This filtering step might reduce final yield about 10% based on experiments using

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422 cleared lysate (data not shown), but it could help to increase the amount of cleared lysate for
423 samples which is hard to get clean lysate by centrifugation only. The similar strategy can apply
424 to filter cell debris of *E. coli* for quick plasmid DNA purification (data not shown) as well.

425 As a solid phase nucleic acid purification approach, the protocols adopted for filter paper-based
426 spin column eliminate the usage of toxic solvent, such as chloroform or phenol, and is safe to
427 conduct. Punching filter paper disc process is safe while punching and cutting silica-based glass
428 fiber filter might release airborne glass fiber which is a potential hazard to operator [20]. Also,
429 recycle used spin column by recharging help to reduce the releasing of plastic waste from the lab
430 to the environment and therefore is environment-friendly.

431 Apart from these advantages, some issues should be cautious in the application of the filter
432 paper recharged or homemade spin column. One is the centrifuge speed. For the recharged spin
433 column with V shape bottom or homemade spin column based on 0.5 ml tube, they should be
434 centrifuged at relatively lower speed when filling with a solution. We usually centrifuge them no
435 more than 8000 × g. Otherwise, it might result in leaking of the solution which does not pass
436 through the filter and in turn reduce the binding of nucleic acids. However, when centrifuge
437 these spin column without a solution, such as at the step of drying filter, it would be OK to
438 centrifuge at full speed of microcentrifuge (>16000 × g). As to spin using a spin column with the
439 net structure at the bottom to support filter discs (Fig 1A), those spin columns can be centrifuged
440 as the full speed of micro centrifuge all the time. However, in steps of binding plant genomic
441 DNA or RNA, we still prefer relative lower speed, such as no more than 8000 × g during binding
442 and washing step, except the last drying filter paper step, which needs full speed to eliminate
443 residue ethanol.

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444 In the purification of plant DNA, we found that the final DNA elution might contain RNA if
445 samples contain high abundant RNA or use less RNase in lysis buffer. DNA with RNA can
446 result in additional low molecular smear visualized in agarose gel electrophoresis, and an also
447 higher ratio of absorbance at 280/260 in UV spectrometer measurement due to higher 280 value.
448 Although DNA with RNA might not affect PCR, it might lead to overestimated DNA
449 concentration in calculation based on UV absorbance value at 280 nm. In case of this, we
450 recommend reducing the amount of sample, such as using 50 mg per purification experiment or
451 extend the time of lysis. We do not recommend increase RNase amount since the cost of RNase
452 is the major portion of total expense in purification using a homemade buffer. For instance, 4ul
453 RNase from Qiagen will cost more than 30 cents based on marker price. We sometimes use a
454 reduced amount of RNase in plant genomic DNA purification with longer time of incubation or
455 spin longer time at centrifuge. Purified DNA can also be quantified by approaches other than UV
456 spectrometer for accurate concentration for experiments sensitive to DNA input amount. For
457 tobacco, we usually use fluorometer and an H33258 dye-based assay to quantify tobacco
458 genomic DNA for PCR based molecular marker analysis [21]. DNA quantification based on UV
459 absorbance at 280 nm might not be consistent for DNA purified from different plant species
460 using different commercial kits [22].

461 As for RNA purification, DNA contamination needs to be considered [23]. Though we found
462 that filter paper-based spin column did accommodate with in-column DNA digestion protocol as
463 suggested by Qiagen kit manual, we still suggest treating RNA elution using DNase-free^{TM} kit
464 (Invitrogen) or similar kits to eliminate remaining DNA. This step is important for DNA
465 contamination sensitive experiments, such as qRT-PCR for quantifying of low expressed genes.
466 On another hand, if the abundance of detected transcript sequence is much higher than that of the

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467 background of DNA, such as in detection of pathogen transcript [9, 19], the DNA treatment
468 might be ignored for reducing the cost if no reverse transcription control could verify the
469 situation. In experiments like a Rapid amplification of cDNA ends (RACE), which is a PCR
470 using one gene-specific primer combined with one arbitrary primer anneal to sequence of polyT
471 adaptor or 5' adaptor added to cDNA, contaminated DNA in RNA might not be a big issue
472 because DNA cannot be amplified in the PCR with only one gene specific primer, and no
473 additional DNase treatment step also helps to eliminate the worry of reducing RNA integrity
474 which is important for RACE like experiments.

475 At last, the successfully nucleic acid purification using filter paper mainly relies on cellulose,
476 the major component of filter paper. Cellulose seems has the similar feature as silica-based
477 material to bind nucleic acids in chaotropic condition, but only secondary fibril-associated
478 cellulose found in filter paper was found able to isolate a wide range of nucleic acids, another
479 type of cellulose with smooth surfaces was found is less efficient in recover DNA from solution
480 [5]. On another hand, we found that the cellulose-based filter paper is more efficient in the
481 purification of high molecular weight genomic DNA, which is in consistent with similar
482 phenomenon reported for Promage's Paramagnetic cellulose. DNA purified using this
483 commercial format of cellulose product shown much better performance in plant genomic DNA
484 purification [6]. In addition, we found that filter paper is less efficient for plasmid DNA
485 purification followed miniprep protocol. These phenomena suggested that cellulose based
486 material does not exactlly followed the same mechanism as silica-based material in binding and
487 elution process for nucleic acids. Unlike silica-based nucleic acids purification process which has
488 been extensively studied [24], less report associated with interaction between cellulose and

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489 nucleic acids [9], and therefore, more studies are needed to facilitate application of the filter
490 paper-based nucleic acid purification.
491

492 Conclusions

493 We found that filter paper can replace silicon material for purification of many types of nucleic
494 acids. Filter paper can be easily adopted in the form of recharged used spin column or homemade
495 spin column for low throughput application using commercial kit buffer leftover or homemade
496 buffers to reduce the cost, therefore, can be an important component for nucleic acid purification
497 in molecular biology laboratories.
498

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501

502 Reference

503 1. Tan SC, Yiap BC DNA, RNA, and protein extraction: The past and the present. J Biomed
504 Biotechnol. 2009; 574398. doi: 10.1155/2009/574398. doi: 10.1155/2009/574398 PMID:
505 20011662
506 2. Thatcher SA. (2015) DNA/RNA preparation for molecular detection. Clinical chemistry.
507 2015; 61:89-99. doi: 10.1373/clinchem.2014.221374 PMID: 25451869
508 3. McCormick RM. A solid-phase extraction procedure for DNA purification. Anal Biochem.
509 1989; 181(1):66–74. PMID: 2554759

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510 4. Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheimvandillen PME, Vandernoordaa J.
511 Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 1990;
512 28(3):495-503. PMID: 1691208
513 5. Su X, Comeau AM. Cellulose as a matrix for nucleic acid purification. Anal Biochem. 1999;
514 267:415–8. doi: 10.1006/abio.1998.2987 PMID: 10036150
515 6. Moeller JR, Moehn NR, Waller DM, Givnish TJ. Paramagnetic cellulose DNA isolation
516 improves DNA yield and quality among diverse plant taxa. Applications in Plant Sciences.
517 2014; 2(10): 1400048 doi: 10.3732/apps.1400048 PMID: 25309836
518 7. Vanhuynh N, Motte JC, Pilette JF, Decleire M, Colson C. Sequential elution of denatured
519 proteins, hydrolyzed RNA, and plasmid DNA of bacterial lysates adsorbed onto stacked
520 DEAE-cellulose membranes. Anal Biochem. 1993; 211(1): 61-5. PMID: 7686721
521 8. Shi R, Panthee DR. A novel plant DNA extraction method using filter paper-based 96-well
522 spin plate. Planta. 2017; 246(3):579-584. doi: 10.1007/s00425-017-2743-3 PMID: 28707084
523 9. Zou Y, Michael MG, Wang Y, Wee E, Turni C, Blackall PJ, Trau M, Botella JR. Nucleic
524 acid purification from plants, animals and microbes in under 30 seconds. PLoS Biology.
525 2017; 15(11):e2003916. doi: 10.1371/journal.pbio.2003916 PMID: 29161268
526 10. Lemke L, Rex M, Zyprian E, Töpfer R. A simple, inexpensive and environmentally friendly
527 method for high throughput DNA extraction from grapevine (Vitis spp.). VITIS-Journal of
528 Grapevine Research. 2011; 50(1):7–10.
529 11. Yaffe H, Buxdorf K, Shapira I, Ein-Gedi S, Zvi MM, Fridman E, Moshelion M, Levy M.
530 LogSpin: a simple, economical and fast method for RNA isolation from infected or healthy
531 plants and other eukaryotic tissues. BMC research notes. 2012; 5(1): 45. doi: 10.1186/1756-
532 0500-5-45 PMID: 22260178

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533 12. Shi R, Sun YH, Zhang XH, Chiang VL. Poly (T) Adaptor RT-PCR. In Next-Generation
534 MicroRNA Expression Profiling Technology. Humana Press; 2012; 53-66. doi: 10.1007/978-
535 1-61779-427-8_4 PMID: 22144191
536 13. Głowacka K, Kromdijk J, Leonelli L, Niyogi KK, Clemente TE, Long SP. An evaluation of
537 new and established methods to determine T-DNA copy number and homozygosity in
538 transgenic plants. Plant, cell & environment. 2016; 39(4):908-17. doi: 10.1111/pce.12693
539 PMID: 26670088
540 14. Lefever S, Hellemans J, Pattyn F, Przybylski DR, Taylor C, Geurts R, Untergasser A,
541 Vandesompele J, RDML consortium. RDML: structured language and reporting guidelines
542 for real-time quantitative PCR data. Nucleic acids research. 2009; 37(7):2065-9. doi:
543 10.1093/nar/gkp056 PMID: 19223324
544 15. Borodina TA, Lehrach H, Soldatov AV. DNA purification on homemade silica spin-
545 columns. Analytical biochemistry. 2003; 321(1):135-7. PMID: 12963065
546 16. Ivanova NV, Fazekas AJ, Hebert PD. Semi-automated, membrane-based protocol for DNA
547 isolation from plants. Plant Molecular Biology Reporter. 2008; 26(3):186. doi
548 10.1007/s11105-008-0029-4
549 17. Li JF, Li L, Sheen J. Protocol: a rapid and economical procedure for purification of plasmid
550 or plant DNA with diverse applications in plant biology. Plant Methods. 2010; 6(1):1. doi:
551 10.1186/1746-4811-6-1 PMID: 20180960
552 18. Takakura, K. I., & Nishio, T. (2012). Safer DNA extraction from plant tissues using sucrose
553 buffer and glass fiber filter. Journal of plant research, 125(6), 805-7. doi: 10.1007/s10265-
554 012-0502-x PMID: 22695723

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555 19. Yang F, Wang G, Xu W, Hong N. A rapid silica spin column-based method of RNA
556 extraction from fruit trees for RT-PCR detection of viruses. Journal of virological methods.
557 2017; 247:61-7. doi: 10.1016/j.jviromet.2017.05.020 PMID: 28583858
558 20. National Institute for Occupational Safety and Health: Fibrous Glass Dust.
559 https://www.cdc.gov/niosh/npg/npgd0288.html
560 21. Drake K, Lewis RS. An introgressed Nicotiana rustica genomic region confers resistance to
561 Phytophthora nicotianae in cultivated tobacco. Crop Science. 2013; 53(4):1366-74. doi:
562 10.2135/cropsci2012.10.0605
563 22. Li X, Wu Y, Zhang L, Cao Y, Li Y, Li J, Zhu L, Wu G. Comparison of three common DNA
564 concentration measurement methods. Analytical biochemistry. 2014; 451:18-24. doi:
565 10.1016/j.ab.2014.01.016 PMID: 24495734
566 23. Bustin SA. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR):
567 trends and problems. Journal of molecular endocrinology. 2002; 29(1):23-39. PMID:
568 12200227
569 24. Rimola A, Costa D, Sodupe M, Lambert JF, Ugliengo P. Silica surface features and their role
570 in the adsorption of biomolecules: computational modeling and experiments. Chemical
571 reviews. 2013; 113(6):4216-313. doi: 10.1021/cr3003054 PMID: 23289428

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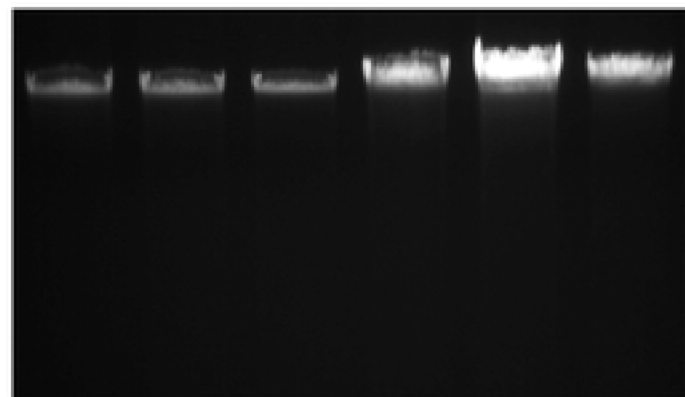

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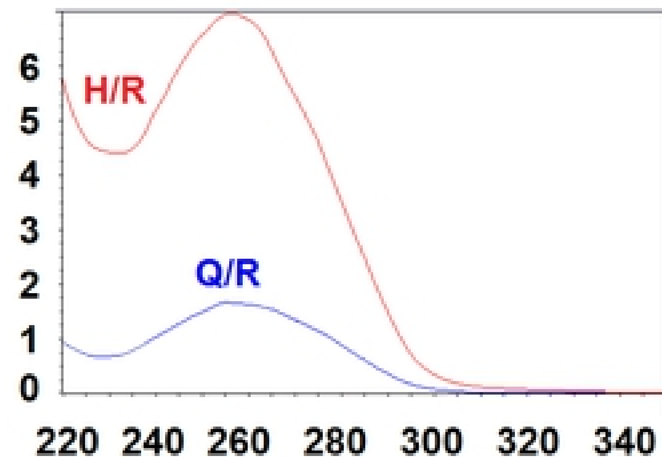

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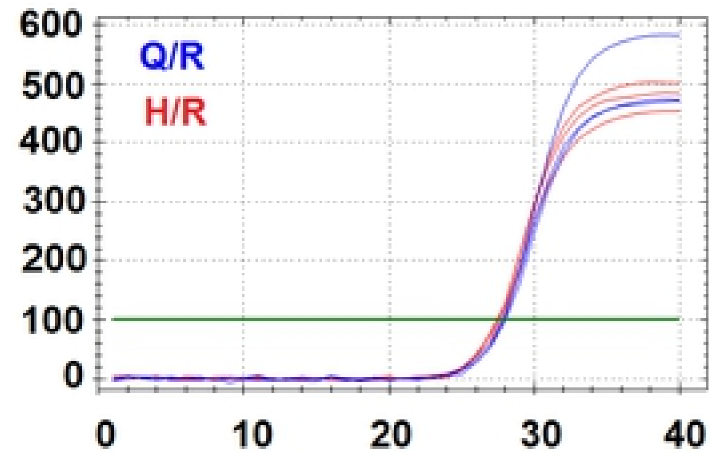

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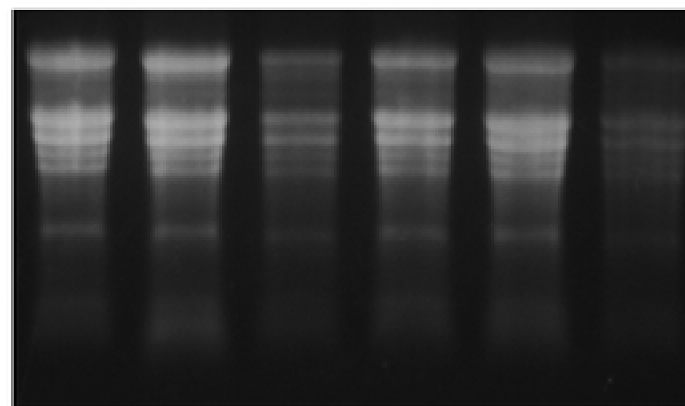

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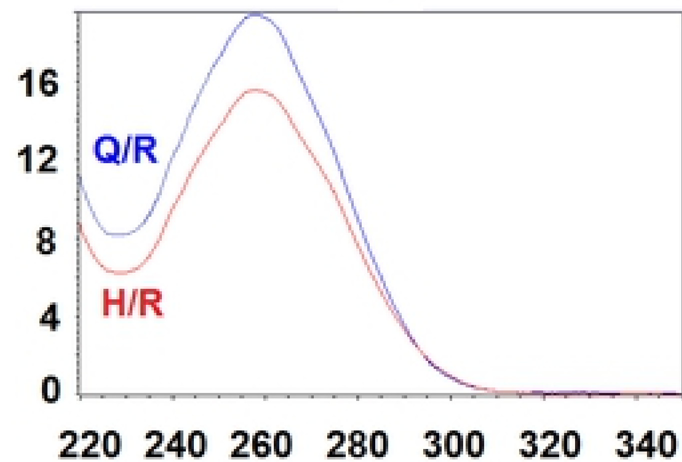

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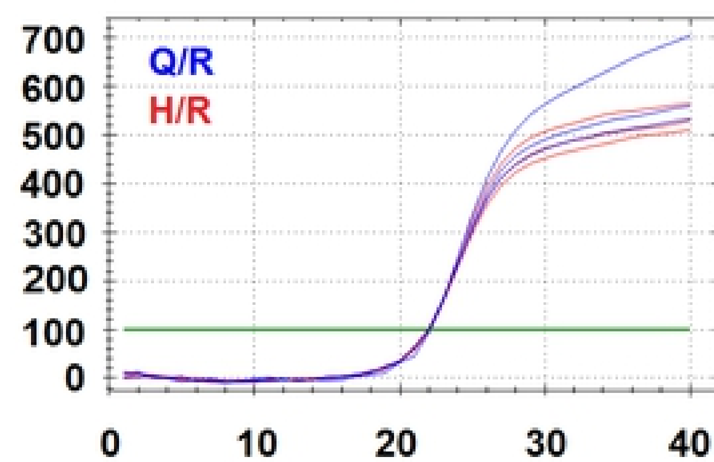

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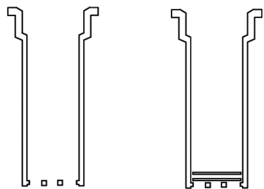

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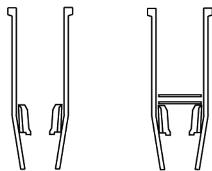

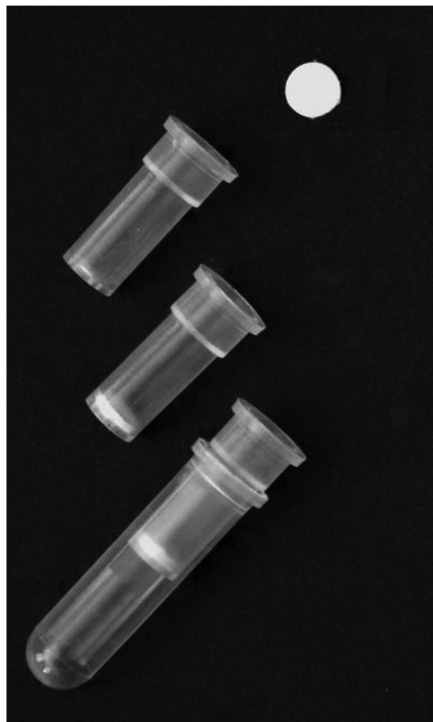

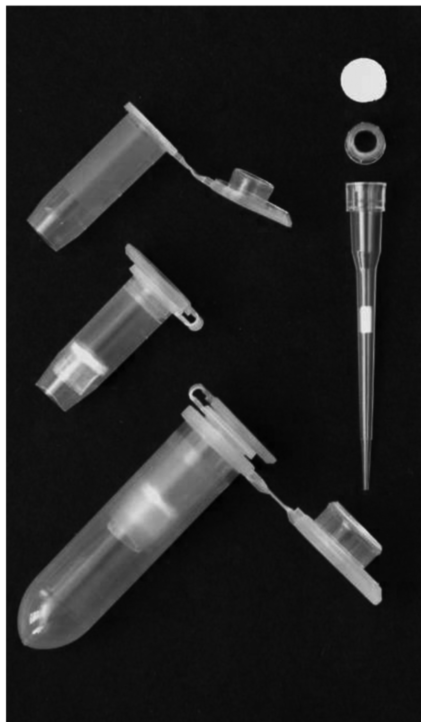

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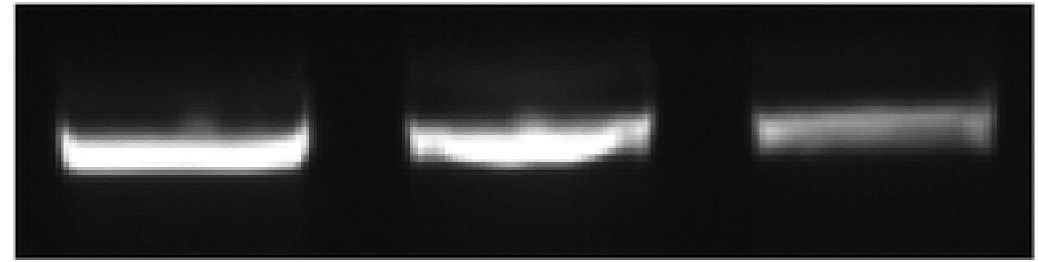

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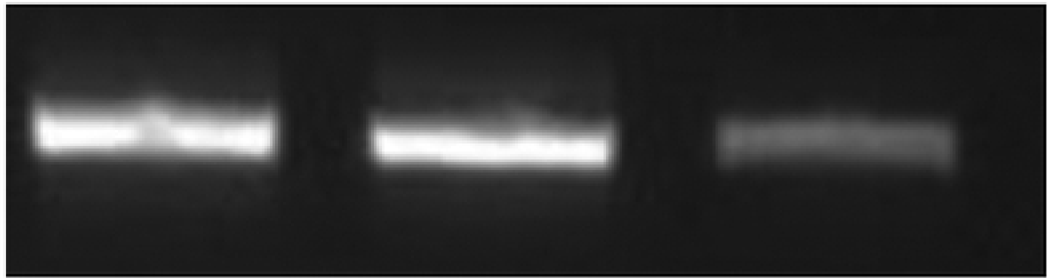

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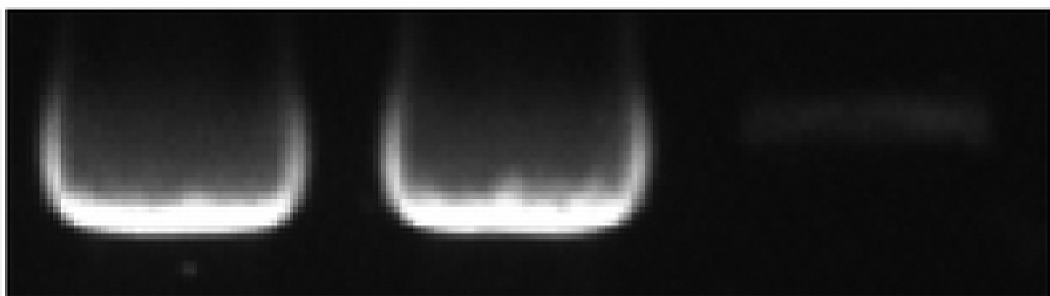

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