# RapidExtract, a novel biodegradable filter cartridge for capture and containment of biomolecules.

**Siun Chee Tan[1], Chin Eng Ong[3], Yuen Kah Hay[4], and Beow Chin Yiap[2]***

1. School of Postgraduate Studies & Research, International Medical University, No.126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia
2. Division of Pharmacy,School of Pharmacy and Health Science, International Medical University, No.126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia
3.School of Medicine and Health Sciences, Monash University Sunway Campus, Jalan Lagoon Selatan,46150 Bandar Sunway, Selangor, Malaysia
4. School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

**** Corresponding author email:*** beowchin_yiap@imu.edu.my

## INTRODUCTION

In the field of molecular biology, isolation and purification of nucleic acids (DNA and RNA) is the fundamental step before any manipulating process such as Polymerase Chain Reaction (PCR) and DNA sequencing for DNA sample, microarray for RNA. The quality and integrity of the isolated nucleic acid influence the results of all subsequent scientific research directly(1).In this case, the purification of DNA and RNA become one of the crucial steps for analytical and preparative purposes in molecular biology (2).

Many specialized techniques for the purification of DNA or RNA has been developed nowadays. In general, the extraction process can be grouped into two main methodologies -- the conventional method and the commercially available solid-phase extraction method. Most of these methods, especially the solid-phase extraction, have been developed into commercial kit in order to simplify the extraction process into a rapid and efficient method (3). A number of materials, such as cellulose, silica, glass powder and diatomaceous earth, have been employed in solid-phase extraction method as column matrices. These materials capture and separate the desired DNA or RNA from impurities such as carbohydrates and protein. (4-8). Nucleic acid may bind to the solid phase depending on the pH and the salt content of the buffer. The binding of nucleic acid to the solid phase involved the presence of chaotropic salts, for example sodium iodide, sodium perchlorate or guanidinium thiocyanate ((8-10). However, guanidine salt is a hazardous chemical which can be harmful when in contact with skin (11). Therefore, it is important to design an environmental-friendly extraction method, without the usage of hazardous chemical, to replace the current available method.

Solid phase nucleic acid extraction is normally performed by using a spin column and required the application of large instrument such as centrifuge machine as it operates under centrifugal forces (12) . Most of the kits available in the market are only able to extract single type of nucleic acid at a time with various protocols. Some may involve protocols which are tedious and complicated to users. Therefore, a new, simple and user-friendly spin column-based extraction method has been designed. The designed spin column utilized biodegradable cellulose as DNA and RNA capturing matrix. This method is capable to extract DNA and RNA from various organisms by using single protocol with 4 easy and basic steps. Moreover, it is environmental-friendly as it does not require the usage of organic-solvent.

## METHODS AND MATERIALS

### *Preparation of spin-column*

0.03g of cellulose was weighed and compacted into a disc-shaped filter with 7mm diameter by using TDP-1.5 single punch tabletting machine. The compacted filter disc was viewed under Scanning Electron Microscope (TM3000 Tabletop Microscope, Hitachi) at 300 x magnification, for physical characterization. The filter was treated with 80% palladium and 20% gold before viewing under SEM.

In order to prepare a spin column, the compacted filter disc was sandwiched with two-disc-shaped holding filter paper (brand?) ~~membrane~~, at the upper side and lower side of the filter's surface. Then, it was inserted into the spin column and tighten with an O-ring to avoid the movement of the filter disc during purification process.

For RNA extraction, the column was treated with 0.1% of diethylpyrocarbonate (DEPC) (Sigma Aldrich) water overnight. After the treatment, the column was autoclaved at 121°C for 45 minutes to remove the DEPC residual and was dried in incubator after the autoclaving process.

***Preparation of model organism***

*Escherichia coli* (*E.coli*) strain TOP 10 carrying plasmid pcDNA3.1 His A *DSN1* has been chosen as the model organism in customising the new nucleic acid extraction method. This plasmid was grown in Luria-Bertani (LB) broth (Pronadisa, Conda, Spain) containing 1% of 50 µg/mL ampicilin (USB, USA) and incubated with shaking overnight at 37°C.

Plasmid sample in 2 mL as staring material was spinned down at 10,000rpm for 2 minutes in room temperature. The supernatant was discarded after the sample has been pelleting down and vortexed the tube to loosen the sample pellet that attached to the bottom of the tube.

Besides the model organism, various organisms have been extracted by using the same protocol which will be described below. These organisms included:

Bacteria (DH5-alpha), which was grown in LB broth and incubated with shaking overnight at 37°C
Recombinant yeast, which was grown in YPD broth and incubated with shaking overnight at 37°C
0.2 g plant leaf
0.2 g shrimp (dried sample)

Samples (c) and (d) were grinded to break down the cell wall after freezing with liquid nitrogen.

***Plasmid DNA, genomic DNA and RNA extraction process***

A set of basic extraction solutions, consisting of solution DRoP1 and DRoP3 (DeRiPro™ DNA, RNA and Protein extraction kit; TerraJu Life Sciences Sdn Bhd) was provided by manufacturer. Solution DRoP1 is a cellular disruption solution, containing detergent salt and metal chelating agent. Meanwhile, solution DRoP3 is an extraction solution containing polyalkylene alcohol and salts. These solutions are for cell lysis, nucleic acid binding onto the filter disc and contaminant clean up by washing step.

For DNA extraction, 200 µL of DRoP1 solution was added and mixed with the plasmid sample by inverting 6 to 10 times. Next, 2 µL of 10mg/mL RNase (Bio Basic, Canada) was added into the lysate and inverted to mix. The lysate was incubated at 37°C for 5 minutes in order to denature RNA contaminant. For sample such as yeast, plant leaf, shrimp and anchovy, incubation at 65°C for 5 minutes was recommended. After the incubation, 200 µL of DRoP3 was added and inverted to mix. The cell lysate mixture was transferred into spin column and was centrifuged at 10,000 rpm for 2 minutes. The supernatant was discarded. 500 µL of diluted DRoP3 solution (1 volume of DRoP3 with 1 volume of ultrapure water) was added into the column and was centrifuged at 10,000 rpm at 3 minutes to wash out the remained impurities from the column. After the washing step, 100 µL of ultrapure water was added in order to elute DNA from the column.

The same protocol was applied for RNA extraction except the RNase treatment that was performed after the addition of D1. DNase treatment was carried out by using RNAse-free DNase I (Fermentas). RNA template in 10 µL was digested with 1 U of DNase and 10x $MgCl_2$ reaction buffer and marked up to 20 µL by DEPC-treated water. The sample was incubated at 37°C for 30 minutes to denature DNA contamination.

***Visualization and quality control of extracted DNA/RNA product***

1% of agarose (Vivantis, Malaysia) in 1X TAE, which prepared from 50X TAE ( 242 g Tris base, 57.1 mL glacial acetic acid and 100 mL of 0.5M pH 8 EDTA), was prepared and routinely used for separation of genomic and plasmid DNA, PCR product, restriction enzyme digestion product and RNA.

The agarose gel was pre-stained with ethidium bromide (Vicantis, Malaysia) during the gel preparation. DNA samples were mixed with 5x loading buffer (Bioline, London) before loaded into the gel well. On the other hand, RNA samples were mixed with formamide (Calbiochem, Canada) in the amount giving a final concentration of 60% (v/v) formamide, with 5uL of 2x RNA loading dye. The samples were then denatured by heating at 65°C for 5 minutes and chilled on iced immediately for another 5 minutes after the incubation. The gel was run at 100 volts for 40 minutes and visualization of the DNA was carried out by UV transillumination using GelDoc™ 2000.The Hyperladder I DNA ladder mix (Bioline, London) and RiboRuler™ High Range RNA ladder (Fermentas) were used as the standard DNA and RNA molecular weight marker.

Besides using visualization method, the purity and concentration of the extracted product was tested by using NanoQuant-plate™ (Tecan, Austria) with absorbance at wavelength 260 and 280.

***PCR amplification and RE digestion***

Amplification of *DSN1* gene at 1731 bp was carried out by using Live Express Thermal Cycler (Bioer, China) in 20 µl reaction volumes. PCR mix was prepared with 10 µL of BioMix™ 2x PCR master mix (Bioline, London), 0.5 µl of 10x dilution of 29719 pmol forward primer ( F_010W_forward 5'-ATG AGT CTG GAA CCC ACA CAA ACG-3' ) and 26887pmol reverse primer (R_010W_Reverse 5'-TCA TAA ATC CAG TTT TTT ACT GAA AGA TAA ACT TAA TCC-3'). 0.5 µL of template DNA was added and marked up with ultrapure water until 20 µl of total volume. The PCR was performed with an initial denaturation at 95°C for 5 minutes and continued with 28 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 2 minute. The amplification reaction was completed by the final extension at 72°C for 5 minute.

pcDNA *DSN1* DNA template was also subjected to restriction endonuclease digestion with restriction enzyme Hind III (New England BioLabs, USA), in 50 µL reaction volumes. The reaction mix was prepared with 2 µl of enzyme Hind III with 10X NEbuffer 2 provided, 5 µL of template DNA and marked up ultrapure water to 50 µL. The whole process was carried out by incubating at 37°C for 2 hours.

## RESULTS AND DISCUSSION

In this study, a new purification method using cellulose powder as spin column capturing matrix has been designed. (please highlight that the cellulose powder is non-treated) This purification method provided a single protocol which is applicable to isolate and purify biomolecules from a number of organisms. It is a fast and easy method for either DNA or RNA extraction. Furthermore, the solutions that employed in this extraction method is environmental-friendly as it does not required the usage of organic solvent such as phenol and chloroform and chaotropic salt. Figure 1 showing the assembly of the novel designed spin column.

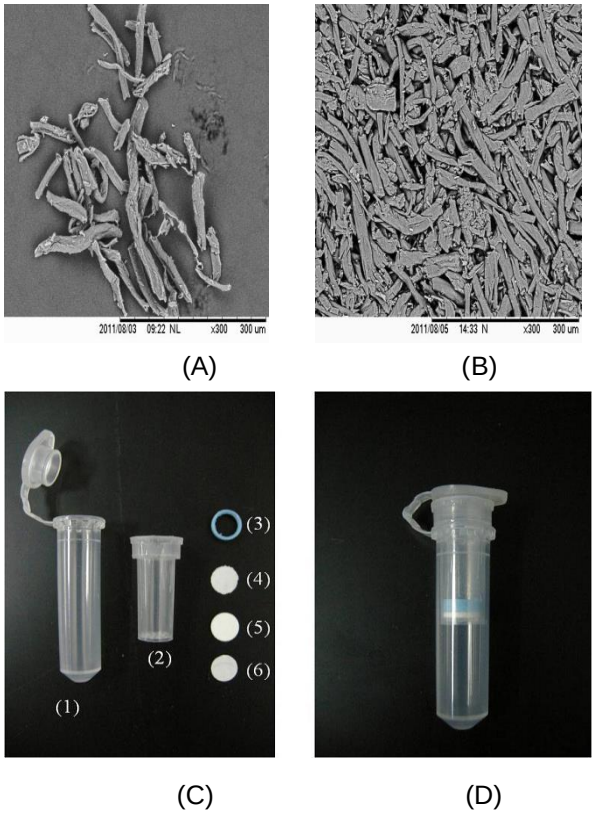


Figure 1: Assembly of spin column. (A) SEM image for cellulose powder before compaction, under 300 x magnification. (B) SEM image for an intact filter disc after compaction, under 300 x magnification. (C) Spin column before assembly: (1) collection tube; (2) minicolumn; (3) O-ring; (4) membrane holder; (5) disc-shaped filter; (6) membrane holder. (D) A complete spin column.

In Figure 2, the purified DNA sample of pcDNA *DSN1* as a model organism in optimizing the final purification protocol has been visualized by UV transilluminator after gel electrophoresis. It obtained a high purity at around 1.8 (as shown in Table 1), showing that the DNA sample is free from impurities such as protein. Usually, DNA or RNA sample which has a purity value ($A_{260}/A_{280}$) at around 1.7 to 2.0 considered to be a good estimation of purity. The purified plasmid DNA was then amplified at 1.7kb with designed-primer in PCR (Figure 2) and digested with *Hind III* ~~Hind III~~ enzyme to manifest the usability of the purified product in downstream process. Besides, the concentration and purity of plasmid DNA extracted with novel developed extraction method has been compared with different commercial kit methods (Table 2). It was found that the concentration and purity of plasmid DNA extracted with new method was comparable with DNA extracted using commercial kits.

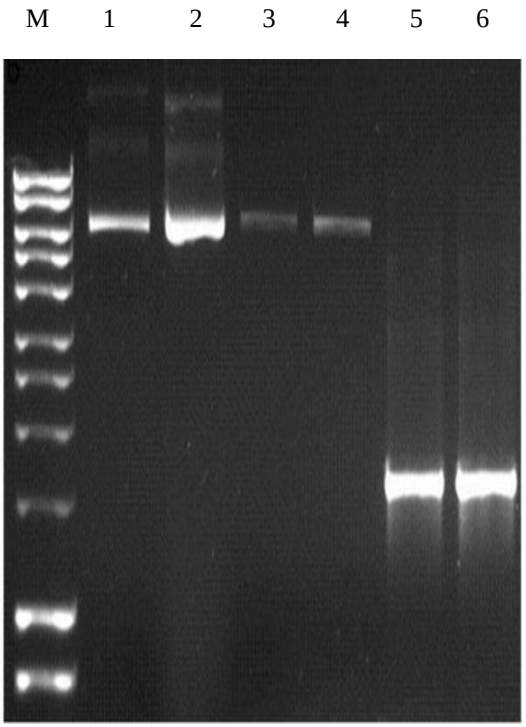


Figure 2: Plasmid DNA sample and downstream process product. M: DNA marker in bp (from top: 10,000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000, 800); Lane 1 and 2: extracted DNA product for pcDNA *DSN1*; Lane 3 and 4: RE digested product of pcDNA *DSN* at ~ 7.2 kb; Lane 6 and 7: pcDNA *DSN1* PCR product at ~1.7 kb

Table 1: Purified plasmid DNA purity and concentration.

| Lane | Sample | Concentration (ng/ µL) | Purity (260/280) |
|---|---|---|---|
| 1 | Plasmid DNA | 120.45 | 1.83 |
| 2 | Plasmid DNA | 123.05 | 1.79 |

Table 2: Comparison of plasmid DNA concentration and purity extracted using different extraction method.

| No. | Method | Concentration (ng/ µL) | Purity (260/280) |
|---|---|---|---|
| 1 | Novel developed method | 146.33 | 1.86 |
| 2 | Commercial kit method A | 172.70 | 1.91 |
| 3 | Commercial kit method B | 168.76 | 1.96 |
| 4 | Commercial kit method C | 63.37 | 1.90 |

Besides plasmid DNA purification, the newly designed-purification method is applicable to isolate genomic DNA from different organisms, by using single protocol. Results for genomic DNA from bacteria, yeast, plant, shrimp and anchovy have been shown in Figure 2. Meanwhile, Figure 3 showing a gel electrophoresis results for RNA purification of pcDNA *DSN1* and DH5-alpha. Total nucleic acid has been purified from the sample organisms. After the purification process, the total nucleic acid sample was subjected to DNase treatment in order to obtain a pure RNA sample.

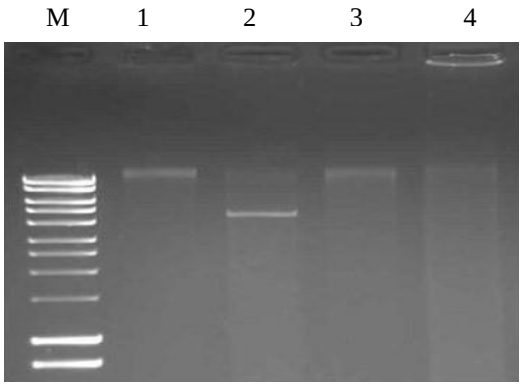


Figure 3: Genomic DNA sample for various organisms. M: DNA marker in bp (from top:10,000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000, 800); Lane 1: Bacteria; Lane 2: Recombinant yeast; Lane 3: Plant leaf ; Lane 4: Shrimp (Dried sample)

Table 3: Purified genomic DNA purity and concentration.

| Lane | Sample | Concentration (ng/ µL) | Purity (260/280) |
|---|---|---|---|
| 1 | Bacteria | 207.85 | 1.75 |
| 2 | Recombinant yeast | 280.15 | 1.90 |
| 3 | Plant leaf | 68.70 | 1.90 |
| 4 | Shrimp | 374.70 | 1.81 |

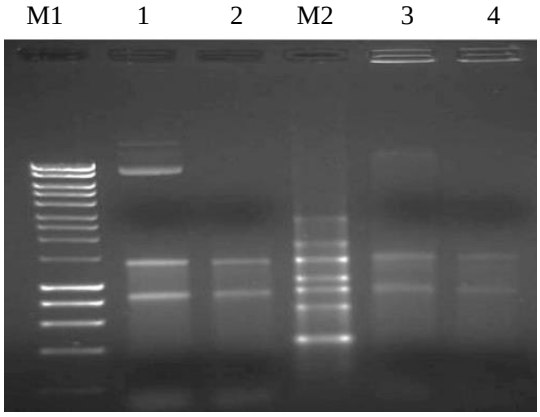


Figure 4: RNA sample pcDNA *DSN1* and DH5-alpha. M1: DNA marker in bp (from top: 10,000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000, 800, 600, 400, 200); Lane 1: pcDNA *DSN1* nucleic acid product (before DNase treatment); Lane 2: pcDNA *DSN1* RNA product (after DNase treatment); M2: RNA marker in bp (from top: 6000, 4000, 3000, 2000, 1500, 1000, 500); Lane 3: DH5-alpha nucleic acid product (before DNase treatment ); Lane 4: DH5-alpha RNA product (after DNase treatment).

Table 4: Purified RNA purity and concentration.

| Lane | RNA Sample | Concentration (ng/ µL) | Purity (260/280) |
|---|---|---|---|
| 1 | pcDNA *DSN1* | 55.72 | 2.02 |
| 2 | DNase-treated pcDNA *DSN1* | 33.64 | 1.96 |
| 3 | DH5-alpha | 44.28 | 1.84 |
| 4 | DNase-treated DH5-alpha | 21.32 | 1.95 |

A few differences were achieved using this novel purification method compared with commercial kit methods. In this newly developed nucleic acid purification method, good quality of DNA and RNA could be extracted within 15 minutes, with four simple steps compared to conventional or commercialized kit method. The binding of DNA and RNA onto the column does not require the employment of guanidinium thiocyanate, a

hazardous chaotropic salt. For RNA extraction, the application of beta-mercaptoethanol, which normally used to avoid the release of RNase during cell lysis, is not required.

Cellulose powder has been selected as the finalized filtering material from various fibrous materials that have been tested for the containment and capture of nucleic acid in this study. It is a natural biopolymer that is renewable and biodegradable. It is negatively charged because of its carboxyl and hydroxyl group and hard to be dissolved in a variety of solvents but very reactive with water(13, 14). The wide application of cellulose used in chromatography and also nucleic acid purification basically attributed to their special properties, for example sub-micron or nano-dimentional particle size, large specific area and inertness or many organic or inorganic substances (15). Nucleic acid purification by using cellulose and its derivatives as filtering material has been described a few decades ago(6, 16-18). However, the purification process is long, tedious and required the application of hazardous solution such as carbodimide, which aided in the binding of DNA onto the filtering matrix. Therefore, a novel purification method utilizing cellulose powder as filter has been designed. It is an environmental–friendly and simple purification process.

Cellulose powder that used in the study is negatively charged as it was not treated with any anion exchanger such as DEAE. DEAE-cellulose column has been widely utilized in nucleic acid extraction, together with the presence of chaotropic salt. The positively charge DEAE capture the negatively charged nucleic acid in the presence of chaotropic salt. However, it was found that the binding of nucleic acids to the untreated negatively charged cellulose surface can be achieved using certain concentration of salt and polyalkylene alcohol solution (16). Nucleic acid, which possesses a phosphodiester backbone that is negatively charged, is neutralized and nucleic acid aggregates are formed in the presence of monovalent salt, such as NaCl, KCl and so on. that can be found in solution DRoP3 (19). The nucleic acid aggregate suspension is allowed to bind to the cellulose in the column reversibly via van der waals force (18) and impurities were washed away from the column with diluted solution DRoP3, where 1 volume of DRoP3 was diluted with 1 volume of ultrapure water. The desired nucleic acid which bind to the cellulose column could be eluted out by using a low salt concentration buffer, such as TE buffer or water.

## CONCLUSION

Simple and efficient nucleic acid purification by using biodegradable cellulose-based spin column has been designed. This method is able to purified DNA or RNA of different organisms using a single protocol. It is an extraction method with low costing as only two solutions would be used in the whole process. No harmful solvent such as phenol is required and the filtering matrix that used in spin column is biodegradable and renewable.

## REFERENCES

Cseke LJ, Wu W, Tsai CJ., 2004. Chapter 1: Isolation and purification of DNA. In: Cseke LJ, Kaufman PB, Podila GK, Tsai CJ, editors. Handbook of Molecular and Cellular Methods in Biology and Medicine. Second ed. Florida: CRC Press LLC 1 - 19.

Wink M., 2006. Chapter 9:Isolation of DNA and RNA. An Introduction to Molecular Biotechnology Molecular Fundamentals, Methods and Applications in Modern Biotechnology. Weinheim, Germany: WILEY-VCH 165-9.

maxXbond, 2006. First Regeneration System for DNA Binding Silica Matrices [database on the Internet]. Nature Publishing Group. 2006 [cited 15 Oct 2009]. Available from: http://www.nature.com/naturemethods.

Makro MA, Chipperfield R, Birnboim HC., 1982. A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder. Anal Biochem 121:382-7.

Li JF, Li L, Sheen J., 2010. Protocol: a rapid and economical procedure for purification of plasmid or plant DNA with diverse applications in plant biology. Plant Methods. 6(1):1.

Shih TY, Martin MA., 1974. Chemical linkage of nucleic acids to neutral and phosphoreylated cellulose powders and isolaion of specific sequences by affinity chromatography. Biochemistry.13(16):3411-8.

McCormick RM., 1989. A solid-phase extraction procedure for DNA purification. Anal Biochem. 15;181(1):66-74.

Tanaka J, Ikeda S., 2002. Rapid and efficient DNA extraction method from various plant species using diatomaceous earth and a spin filter. Breeding Science. 52:151-5.

Vogelstein B, Gillespie D., 1979. Preparative and analytical purification of DNA from agarose. Proc Natl Acad Sci U S A. 76(2):615-9.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J., 1990. Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 28(3):495-503.

Guthner T, Mertschenk B, Schulz B., 2006. Guanidine and derivatives. Ullmann's Encyclopedia of Industrial Chemistry. : Wiley-VCH Verlag GmbH & Co. KGaA.

Gjerde DT, Lee H, Hornby D., 2009. RNA extraction, separation, and analysis. RNA Purification and Analysis: Sample Preparation, Extraction, Chromatography. Weinheim, Germany: WILEY-VCH; 1-16.

Klemm D, Schmauder HP, Heinze T., 2005. Cellulose. Biopolymers Online: Wiley-VCH Berlag GmbH & Co. KGaA; 275-87.

Gardner DJ, Oporto GS, Mills R, Samir MASA., 2008. Adhesion and surfaces issues in cellulose and nanocellulose. J Adhes Sci Technol. 22:547-67.

Li XF, Ding EY, Li GK., 2001. A method of preparing spherical nano-crystal cellulose with mixed crystalline forms of cellulose I and II. Chin J Polym Sci. 19(3):291-6.
Nargessi RD, Pourfarzaneh M, inventors; Cortex Biochem Inc. , assignee., 2007. Isolation and purificaiton of nucleic acids. US patent US 7264927 B2.
Eilat D, Ziv E, Lapidot Y., 1975. Purification of peptidyl-tRNA on benzoylated DEAE-cellulose columns. Nucleic Acid Research. 2(12):2237-50.
Caffin NA, Mackunlay AG., 1975. Fractionation of DNA on benzoylated DEAE-cellulose. Anal Biochem. 63:442-51.
Su X, inventor The Theobald Smith Research Institute Inc., assignee, 2006. Method for isolating nucleic acids patent US 5804684.