A short overview about technologies for isolation of nucleic acids - a journey through time

Life Science Conference
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Timo Hillebrand
The molecule of life

Cambridge, in 1953. Watson and Crick proposed a double helix structure of the DNA. In 1962 the Nobel Prize of Physiology or Medicine was awarded to Watson, Crick and Wilkins.
The molecule of life

In 1869 Johann Friedrich Miescher detects a substance in an extract of pus. He termed the substance „nuclein“ – derived from the word nucleus.
Friedrich Mischer developed the first method for isolation of DNA.
Friedrich Mischer's DNA extraction method (1869)

**Steps:**

- **a:** Washing with sodium sulfate
- **b:** Diluted sulfuric acid
- **c:** 24 hours at 4°C
- **d:** Jod solution, water, ether
- **e:** Washing with alcohol, diluted sodium carbonate, dilute sulfuric acid
... 100 years later
The extraction of DNA -
the phenol/chloroform method

Isolation of Deoxyribonucleic Acid from Mammalian Tissues

BY K. S. KIRBY AND E. A. COOK
Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

(Received 29 November 1966)

1. DNA has been isolated from different mammalian tissues. The DNA preparations were free from RNA, protein and polysaccharides and have a similar range of sedimentation coefficients (approx. 24s). 2. Protein was removed by a two-stage extraction with a phenol–cresol mixture by using a detergent with 4-amino salicylate in the first stage and sodium chloride in the second. 3. Polysaccharides remained in solution when DNA was precipitated with 2-butoxyethanol in the presence of 0.5M-sodium chloride and 1.5M-sodium benzoate. 4. Ribosomal RNA was removed by precipitation in the presence of 3M-sodium chloride at 0°, when DNA remained soluble.

The isolation and characterization of DNA from different tissues is important in deciding whether there are variations in the nature of DNA–protein binding in the tissues (Kirby, 1964). The means by which DNA may be released and the yield of DNA ribosomal RNA remaining insoluble, but DNA of higher molecular weight does not dissolve in this solution so that the separation of DNA from RNA is made more difficult.

The criteria of the quality of the product have
Phenol-chloroform extraction

Phenol-chloroform extraction is a liquid extraction technique. Phenol and chloroform and the sample are mixed or the sample is lysed with SDS and after lysis mixed with phenol-chloroform, forming a biphasic mixture. DNA is precipitated from the upper phase and finally diluted in water.

PCE – extracted DNA is from excellent quality and yield!

BUT

Very time consuming procedure and the used chemicals are hazardous!
The extraction of DNA
The revolution...

Preparative and analytical purification of DNA from agarose
(NaI/acetone precipitation/DNA-glass complexes/molecular hybridization)

BERT VOGELSTEIN* AND DAVID GILLESPIE†

Section on Molecular Hybridization, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20014

Communicated by T. O. Doiener, November 1, 1978

ABSTRACT Two procedures were developed for removing DNA from agarose after electrophoretic separation of DNA fragments according to size. Both involve dissolving the DNA-containing agarose in NaI. The preparative technique uses binding of DNA to glass in the presence of NaI. The method is rapid and convenient, and DNA of all molecular weight ranges can be recovered in high yield and without degradation. The DNA is free of agarose and remains susceptible to digestion by restriction enzymes. The analytical technique uses selective precipitation of DNA with acetone and has been adapted to molecular hybridization scans of sequences in agarose gels. The sequence-monitoring system is quantitative, directly measuring the proportion of the probe complementary to a given DNA fragment and vice versa. It is especially suitable for analyzing restriction enzyme digests of DNA in mapping experiments.

The best studied glass powder was flint glass prepared from ground scintillation vials (American Flint Glass Co.). Glass particles were separated according to size by sedimentation through water at unit gravity. "Large" particles sedimented faster than 6 cm/min, "medium" particles sedimented between 1 and 6 cm/min, and "powder" sedimented more slowly than 0.25 cm/min. Silica gel and porous glass beads were unsuitable for DNA purification (see Results).

Methods I: Analytical Electrophoresis and Removal of DNA from Agarose. Vertical slab gels (5 mm depth) of 0.3–3.0% agarose were cast in a Bio-Rad model 220 support. For small numbers of samples and agarose gels of 0.6% or greater, combs were used to form the sample wells, usually 20 mm wide. For low-percentage agarose gels, posts of agarose were fixed to the top of the slab with melted 0.3% agarose. To eliminate "trailing" of the DNA at the edge of the slot, we applied DNA samples to the slab gels in the following manner. The sample wells were nearly filled with 5 mM NaOAc/2 mM EDTA/40 mM Tris acetate, pH 7.8. The DNA sample was held at 45°C for 10 min, then mixed with glycerol and melted low-temperature agarose to 2–10% and 0.2% final concentrations (wt/vol), respectively. When the sample grinded, the buffer was with-
Silica-based DNA extraction

The DNA is adsorbed by silica in the presence of chaotropic salt solutions with high ionic strength. A chaotrope denatures biomolecules by disrupting the shell of hydration around them. This allows positively charged ions to form a salt bridge between the negatively charged silica and the negatively charged DNA backbone in chaotropic high salt concentration. The DNA can then be washed with high salt and ethanol and is ultimately eluted with a low salt buffer.
Silica-based DNA extraction

and ... everybody knows the technology!

binding material (glass fibre paper) or silica powder, diatom earth, magnetic particle, etc.
Silica-based DNA extraction
Interesting application

DNA extraction from Pleistocene bones by a silica-based purification method
Matthias Höss and Sivante Pääbo
Institute of Zoology, University of Munich, Postfach 2123, D-80021 Munich, Germany

Received May 12, 1992; Revised and Accepted July 4, 1993

The polymerase chain reaction has made it possible to include sensitive species and even populations in molecular studies of phylogeny and evolution (1). This emerging field, however, is hampered by technical obstacles because of which high-quality pure DNA preparations cannot be achieved. The extraction of DNA from ancient materials is a labor-intensive process and in several cases allows the study of late Pleistocene animals and humans to be made available to other researchers. A layer of approximately 1 mm is removed from the surface of the bone samples by grinding with a drill in order to reduce contamination from the previous handling. The sample is ground in a polyethylene tube containing 1 M NaOH, 2 M KCl, 1 M EDTA, pH 12.0, and 1.36 M ICI. This is then incubated at 37°C for 30 min. After centrifugation for 5 min at 13,000 × g, the supernatant is removed and added to a mixture of 500 μl of extraction buffer and 40 μl of silica suspension prepared as in ref. 6. The mixture is incubated for 5 min at room temperature. Subsequently, the silica pellet is washed twice with a buffer consisting of 0.3 M Tris-Cl and 0.2 M EDTA, pH 8.0, and 1.3 M ICI, and 1.0 M Tris-Cl, pH 8.0, and 1.3 M ICI. This is then added to 80 μl of sample, which is subjected to a second extraction. After centrifugation for 3 min at 13,000 × g, the supernatant is removed, and the pellet resuspended in 20 μl of 1 M NaOH. This solution is passed through a glass disk, and the eluate is neutralized with 1 M NaOH. Finally, the DNA is isolated by ethanol precipitation and dissolved in 10 μl of distilled water.

In the present paper we describe a rapid and sensitive method for the simultaneous isolation of total DNA and genomic plus low-molecular-weight DNA from apoplastic cells. Using this method, we were able to detect a DNA ladder from apoplastic cells in less than 48 h. In addition, the DNA preparation obtained by this method is not hampered by the presence of RNA in the sample.

One of the most characteristic phenomena of apoptosis (programmed cell death) is the activation of endonucleases leading to the fragmentation of genomic DNA (1). Fragmentation of DNA from apoptotic cells is a consequence of the programmed death of cells, and the subsequent separation by gel electrophoresis yields a pattern of DNA fragments characteristic for apoptosis. The fragmented DNA is isolated with high efficiency using silica spin columns that are easily loaded and cleaned.

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Silica-based DNA extraction

Interesting application

Detection of Adenoviruses and Entroviruses in Polluted Waters by Nested PCR Amplification

MONTSE GRUÈS,* JORDI SOLER, FRANCISCO LUCENA, ANGELA ALLADO,* GONZALO WAJLLE, and ROSINA GRIÈVES*†

Department of Microbiology, University of Barcelona, 08028 Barcelona, Spain. *Present address: Spanish National Institute for Aerospace Research, 28049 Madrid, Spain.

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A procedure has been developed for the rapid detection of adenoviruses and entroviruses in environmental samples. Several strains for DNA extraction and concentration of nucleic acid were tested by adding adenovirus type 3 and poliovirus type 1 to different water samples. The most promising method for these samples involved the lysis of the virus in the sample and the purification of viral DNA using silica-based filters. The efficiency of the method was assessed in water samples with different concentrations of adenovirus and entrovirus DNA. Finally, the method was applied to water samples from different river water systems and showed good results. The method was successfully used for the detection of adenovirus and entrovirus DNA in environmental samples.

Interesting application

We describe a rapid, simple, and reliable procedure for detection of hepatitis B virus (HBV) DNA in serum. HBV DNA can be amplified from 0.5 ml serum samples in less than 3 h, and HBV DNA can be amplified from 0.5 ml serum samples in less than 3 h. The method is based on the principle that HBV DNA is amplified by a nested polymerase chain reaction (PCR) with two specific primer sets. The first primer set targets the S gene, and the second primer set targets the P gene. The sensitivity of the method is 10 copies of HBV DNA per milliliter of serum.

* Corresponding author. Mailing address: Department of Microbiology, University of Barcelona, Hospital Sant Joan de Déu, 08034 Barcelona, Spain. Phone: 34-93-507-11-61. Fax: 34-93-507-11-61. E-mail address: rosina@structuresib.edu.

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AJINUSCRE

Rapid Purification of Hepatitis B Virus DNA from Serum

B. ROCHER,* A. J. SOLÉ,* J. BERTHELIN,† P. M. E. WERTH-VAN DILLIEN,†* and J. F. VAN DER BORDRA

Department of Virology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, and Department of Virology, Eindhoven University, 5600 DB Eindhoven, The Netherlands

Received 5 July 1999/Accepted 11 June 1999

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* Corresponding author. Mailing address: Department of Virology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Phone: 31-20-463-9493. Fax: 31-20-463-9883. E-mail address: rocher@mcu.medamc.nl.

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* Corresponding author. Mailing address: Department of Virology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Phone: 31-20-463-9493. Fax: 31-20-463-9883. E-mail address: rocher@mcu.medamc.nl.
Another silica-based DNA extraction

The DNA is adsorbed by silica in the presence of nonchaotropic salt solutions with high ionic strength...

The DNA can then be washed with high salt and ethanol, and ultimately eluted with low salt.
Another silica-based DNA extraction:
Hofmeister serie

**Antichaotropic salt**

**Chaotrophic salt**

**Protein stability**

**Hydrophobic interaction**
...and the new silica based extraction procedure is very easy and don’t need hazardous components...
Another silica-based DNA extraction

Lysis + Isolation of DNA from E.coli.

Subsequent amplification of an E.coli specific target sequence...

it works!

Isolation of DNA from E.coli.
Subsequent amplification of an E.coli specific target sequence

Bind

Wash

Elute

Everybody can do it!
Another silica-based DNA extraction

The DNA is adsorbed by silica and other surfaces in the presence of a mixture of chaotrophic and nonchaotrophic salts with low ionic strength...

The DNA can then be washed with ethanol, and ultimately eluted with low salt.
Another silica-based DNA extraction

**Isolation of DNA from orcideen species**

**Isolation of DNA from cigarettes butts and subsequent STR-analysis**

**Isolation of DNA from whole blood**

**Isolation of microbial DNA from different types of soil**

...and it works, too!
Silica-based DNA extraction - conclusion

<table>
<thead>
<tr>
<th>Adsorption on silica</th>
<th>1979</th>
<th>1998</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>salt solution</td>
<td>chaotrop</td>
<td>nonchaotrop</td>
<td>Chaotrop + nonchaotrop</td>
</tr>
<tr>
<td>ionic strength</td>
<td>strong</td>
<td>strong</td>
<td>low</td>
</tr>
</tbody>
</table>

The same procedure for isolation of DNA from complex starting material, but permanently changed binding chemistry!
2012 –
all problems of sample preparation dissolved?
PMI – Technology
Polymer Mediated Isolation

a new technology for enrichment of virusses or circulating DNA
Sample preparation is the essential step for virus diagnostic or tumor diagnostic

- More sensitivity by higher sample volume
- Reduced amount of extraction reagents for higher sample volumes
- Reduced hands on time
- High efficiency of enrichment is an important demand
PMI – Technology
enrichment and isolation of viruses
Current approaches

Magnetic beads coated with antibodies (Sato et al. 2003)

Lab on chip devices (Lien et al. 2007)

Use of funnel column system
(www.roche-applied-science.com)

Reloading normal column more times
www.clontech.com

Plasma serum
Binding
Washing
Elution
PMI – Technology enrichment and isolation of viruses
general procedure

Sample mixed with two components and incubation for 1 minute

Centrifugation for 1 minute

Dissolving of pellet
PMI – Technology
enrichment and isolation of virusses
application: electron micrograph of polymer and Camelpox virus

Electron micrograph of polymer and Camelpox virus
A-B: Electron micrograph of polymer complex;
Bars: A = 500 nm, B = 200 nm

(data kindly provided by P. Patel; RKI Berlin)
PMI – Technology
enrichment and isolation of virusses
application: electron micrograph of polymer and Camelpox virus

Electron micrograph of polymer and Camelpox virus
C: Electron micrograph of Camelpox virus
D: Electron micrograph of polymer-virus complex after enrichment
Bars: C = 200 nm, D = 200 nm

(data kindly provided by P. Patel; RKI Berlin)
PMI – Technology enrichment and isolation of viruses
application: electron micrograph of polymer and Camelpox virus

Electron micrograph of polymer and Camelpox virus
E-F: Electron micrograph of polymer-virus complex after enrichment
Bars: E = 1 µm
F = 1 µm

(data kindly provided by P. Patel; RKI Berlin)
PMI – Technology
enrichment and isolation of virusses
application: yellow fever virus recovery from sample matrix by virus enrichment

Yellow fever virus recovery from sample matrix by virus enrichment and analysis by virus titration and real-time PCR

A) Virus titration of YFV stock solution by plaque assay

B) Virus titration of YFV recovered from plasma sample by plaque assay

C) Virus titration of YFV recovered from urine sample by plaque assay

D) results by virus titration and real-time PCR analysis

data kindly provided by P. Patel; RKI Berlin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plaque assay (PFU/ml)</th>
<th>RT and real-time PCR (Geq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YFV stock</td>
<td>1.0 * 10^6</td>
<td>3.1 * 10^7</td>
</tr>
<tr>
<td>YFV spiked in Plasma (Enrichment)</td>
<td>8.6 * 10^4</td>
<td>3.9 * 10^6</td>
</tr>
<tr>
<td>YFV spiked in Urine (Enrichment)</td>
<td>3.4 * 10^2</td>
<td>2.5 * 10^7</td>
</tr>
</tbody>
</table>
PMI – Technology enrichment and isolation of virusses
application: direct real time PCR

complexing reaktion; 1 min

centrifugation; 1 min / washing 1 min

dissolving and heating; 1 min

real time PCR for detection of a DNA virus spiked in 200 µl serum sample; recovery from sample matrix by virus enrichment and subsequent direct real time PCR vs. standard virus DNA extraction

blue) standard prep (30 min)

brown) PMI and direct real time PCR (4 min)
PMI – Technology enrichment and isolation of viruses
application: combination PMI and Virus RNA prep (1ml sample)

1 ml sample + 200 µl + 200 µl Lysis Buffer
+ 400 µl Binding Buffer

1 ml sample + 1 ml Lysis Buffer
+ 2 ml Binding Buffer

6 x
real time PCR for detection of FMD virus spiked in 1 ml serum sample; recovery from serum sample matrix by virus enrichment and subsequent viral RNA prep vs. standard viral RNA prep by multiple loading steps

black) standard prep (multiple loading steps)
brown) PMI and standard prep

real time PCR for detection of FMD virus spiked in 1 ml serum sample; recovery from urine sample matrix by virus enrichment and subsequent viral RNA prep vs. standard viral RNA prep by multiple loading steps

magenta) standard prep (multiple loading steps)
brown) PMI and standard prep
PMI – Technology
enrichment and isolation of circulating free DNA

State of the art (isolation of cf DNA from 1 ml serum sample)

1. Incubation of 1 ml sample + 100 µl PK + 1.8 ml lysis buffer + carrier RNA in a 50 ml tube at 60 °C for 30 minutes
2. Addition of 1.8 ml binding buffer and mixing
3. Incubation on ice for 5 minutes
4. Transfer the sample mix over a spin column using vacuum
5. Washing step 1 using vacuum
6. Washing step 2 using vacuum
7. Washing step 3 using vacuum
8. Drying the spin filter column by centrifugation and subsequent incubation for 10 min at 56°C
9. Addition of elution buffer, incubation for 3 min and centrifugation for 1 minute
PMI – Technology enrichment and isolation of circulating free DNA
application: combination PMI and cf DNA prep (1ml sample) vs state of the art method

real time PCR assay for GAPDH; recovery of gDNA (10 ng/ml) spiked in a serum sample; PMI prep vs. standard state of the art method

green) state of the art prep
blue) PMI and standard prep
Thank you for attention!