METHODS BETA

DNA Extraction

Deoxyribonucleic acid (DNA) extraction is the process by which DNA is separated from proteins, membranes, and other cellular material contained in the cell from which it is recovered. This extraction can be one of the most labor-intensive parts of DNA analysis. Extraction methods may require an overnight incubation, may be a protocol that can be completed in minutes or a couple of hours, or may be a recent procedure that employs reagents for which this step can be skipped completely.

The DNA extraction process requires careful handling of biological material to prevent sample contamination and crossover. Tubes should be carefully labeled, especially when transfers are required. Robots may be employed to extract reference samples and some evidence samples, but other evidentiary samples may require the direct attention of a DNA analyst.

From: Forensic DNA Biology, 2013

Related techniques:

Plasmids, Genomic DNA, Lysis, DNA Template

Protocols

From: Chapter 2 - DNA and RNA Extraction Protocols

John T. Corthell Ph.D.

Basic Molecular Protocols in Neuroscience: Tips, Tricks, and Pitfalls • 2014

DNA Extraction

17. Remove any remaining aqueous phase from the DNA/protein extract.
18. Add 300 µl of 100% ethanol per 1 ml guanidium solution to the sample. Vortex to mix and let sit for 3 min at room temperature.
19. Centrifuge samples at 2000g for 5 min at 4°C.
20. Transfer the colored supernatant (protein extract) to a new tube and keep on ice.
21. Wash the pellet with 1 ml of a sodium citrate/ethanol solution (0.1 M sodium citrate, 10% ethanol, pH 8.5), mix by inverting the tube, and let sit for 30 min at room temperature.
22. Centrifuge samples at 2000g for 5 min at 4°C.
23. Remove supernatant and wash pellet with 1 ml of the sodium citrate/ethanol solution, mix by inverting the tube, and let sit for 30 min at room temperature.
24. Centrifuge samples at 2000g for 5 min at 4°C.
25. Remove supernatant. Add 1.5 ml of 75% ethanol to tube, mix by inverting the tube, and let sit for 10 min at room temperature.
26. Centrifuge samples at 2000g for 5 min at 4°C.

27. Remove supernatant. Invert tube and allow pellet to air dry. Air drying typically takes 10–20 min.

28. Resuspend DNA pellet in 8 M NaOH and store at –20°C. Evaluate samples using a spectrophotometer and gel electrophoresis.

    As with RNA extraction, the volume of NaOH used to resuspend the DNA is up to you. Increased volumes result in more dilute DNA. Do not use a vacuum microcentrifuge for this sample, as decreasing the volume increases the concentration of both your DNA and NaOH, possibly harming your DNA. I highly recommend using a cleanup protocol from a kit so that you can get your DNA out of NaOH solution and into either water or TE.

Key researchers

Didier A. Raoult

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140	100,490	2,976
h-index	Citations	Documents

James P. Landers

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56	10,066	325
h-index	Citations	Documents

Relevant methods

Human T-Cell Lymphotropic Virus Type I Sequences Detected by Nested Polymerase Chain Reactions Are Not Associated With Multiple Sclerosis

Naraporn Prayoonwiwat M.D., Larry R. Pease Ph.D, Moses Rodriguez M.D

Mayo Clinic Proceedings • Volume 66, Issue 7 • July 1991 • Pages 665-680

> #### DNA Isolations
>
> Peripheral blood mononuclear cells were separated from heparinized blood samples of 22 patients with MS and all the control donors.30 DNA was extracted from each peripheral blood mononuclear cell sample, three pieces of white matter from two patients with MS, and three pieces of normal white matter from three autopsy specimens.31

Guidance Cues at the Drosophila CNS Midline: Identification and Characterization of Two Drosophila Netrin/UNC-6 Homologs

Robin Harris, Laura Moore Sabatelli, Mark A Seeger

Neuron • Volume 17, Issue 2 • August 1996 • Pages 217-228

> #### cDNA Isolation, Sequence Analysis, and Molecular Characterization
>
> Standard molecular techniques were carried out following Sambrook et al. 1989. cDNAs were isolated from a λgt11 phage library made from 9–12 hr embryonic poly(A)+ RNA (Zinn et al. 1988). Netrin cDNA clones were sequenced initially from a set of Exonuclease III nested deletions using the fmol DNA cycle sequencing system (Promega, Madison, WI). Genomic sequence and confirmation of the cDNA sequence was determined using a set of specific oligonucleotide primers. Sequences were analyzed using programs of the Wisconsin GCG sequence analysis package, and database searches were performed using the BLAST program (Altschul et al. 1990).

Anatomical studies of DNA fragmentation in rat brain after systemic kainate administration

S. Weiss, O. Cataltepe, A.J Cole

Neuroscience • Volume 74, Issue 2 • 19 July 1996 • Pages 541-551

cDNA Isolation, Sequence Analysis, and Molecular Characterization

Seventy-two hours after kainate administration, animals were killed by decapitation and brains were rapidly dissected on ice. One hemisphere was taken for in situ DNA fragmentation analysis, while the other was microdissected on a cold plate for regional DNA isolation. Blocks of tissue of 2mm3 were dissected from the hippocampus, centromedial thalamus, amygdala and frontal suprarhinal cortex. Samples were homogenized using a hand-held dounce in 500μl of buffer A (10mM Tris–HCl, pH 8.0, 1mM EDTA, pH 7.4). Two volumes of buffer B [10mM Tris–HCl, pH 7.4, 10mM EDTA, 0.5% (w/v) sodium dodecyl sulfate, 10μg/ml RNase A] were added and the mixture was incubated at 37°C for 15min. Proteinase K (20μg/ml) was added and samples were incubated overnight at 50°C. Following two phenol extractions and a chloroform–isoamyl alcohol (24:1, v/v) extraction, DNA was precipitated with 0.1volumes of 3M sodium acetate, pH 5.2, and 2volumes of absolute ethanol. Pellets were resuspended in Tris–EDTA and optical densities were measured to estimate yield. On average, 10–15μg of DNA were isolated from each sample.

Related content

Chapter 7: DNA extraction: finding the most suitable method

Cristina Barbosa, Sofia Nogueira, Mário Gadanho, Sandra Chaves

Molecular Microbial Diagnostic Methods • 2016 • Pages 24-36

On DNA transfer: The lack and difficulty of systematic research and how to do it better

Annica Gosch, Cornelius Courts

Forensic Science International: Genetics • Volume 40 • May 2019 • Pages 95-125

Large scale DNA identification: The ICMP experience

Thomas J. Parsons, Rene M. L. Huel, Zlatan Bajunović, Adnan Rizvić

Forensic Science International: Genetics • Volume 30 • January 2019 • Pages 236-244

DNA-based techniques for authentication of processed food and food supplements

Yat-Tung Lo, Pang-Chui Shaw

Food Chemistry • Volume 240 • 1 February 2018 • Pages 767-774

Forensic Sciences | DNA Profiling

Adrian M. T. Linacre

Encyclopedia of Analytical Science (Third Edition) • 2019 • Pages 17-22