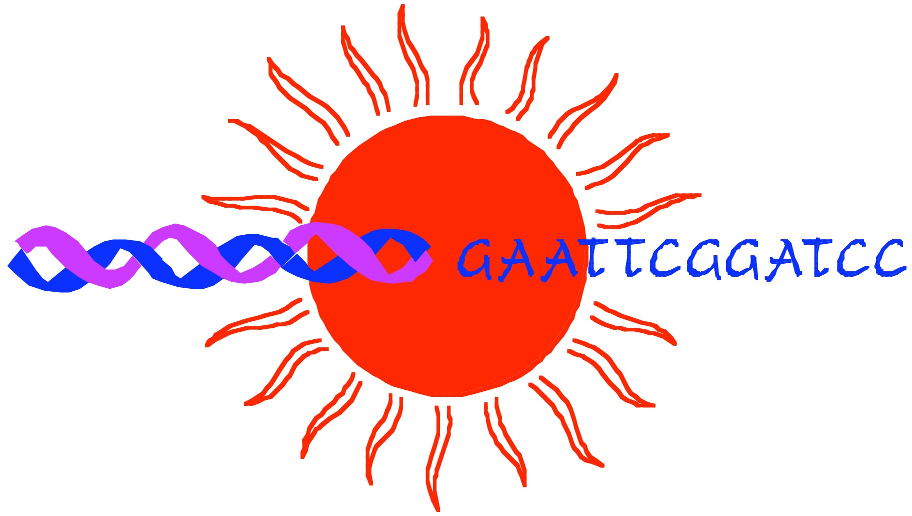
Email: DNA.Core@UCHSC.edu
Web Site: http://loki.uchsc.edu



CU-CANCER CENTER

DNA SEQUENCING & ANALYSIS CORE

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CU-Cancer Center DNA Sequencing & Analysis Core
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The CU CANCER CENTER DNA SEQUENCING CORE was established in the fall of 1995. It offers rapid sequencing of DNA to Cancer Center members as well as to academic and industrial researchers in Colorado. The Core started with two automated single-lane, four-color fluorescent slab-gel automated sequencers (ABI 373A-XL and ABI 377-XL). These have been replaced with two automated DNA sequencers; a 16-capillary (ABI 3100) and a 48-capillary (ABI 3730, the only in Colorado), which can provide over 650 and 900 bp of usable DNA sequence, respectively. The Core also offers DNA profiling for identification and authentication of cell lines. In collaboration with the UCHSC DNA Diagnostic Laboratory, the DNA sequencing Core offers CAP-approved screening of clinical samples for mutations in the exons of the VHL, p53, pTEN, FLT3 genes, which are implicated in cancer.

Customers can supply the DNA as one of the following: plasmid DNA, an *E. coli* transformant bearing plasmid DNA, DNA fragments (restriction or PCR products), lambda phage DNA, cosmid DNA, PAC DNA, or BAC DNA. A Beckman Biomek 2000 robot allows the Core to offer automated, high-throughput template (plasmid) preparation for large-scale sequencing projects and preparation of high density arrays on membrane filters of different gene libraries.

The Core offers expert technical advice on the best strategy for individual sequencing projects, troubleshooting of PCR and DNA sequencing projects, and analysis of sequences with a suite of computer programs. The results are provided, in confidence, as chromatograms and computer disk files, with backup copies kept in-house. These services are priced very competitively for both academic and industrial researchers.

These services are provided on the condition that the Core's customers acknowledge use of the CU-CANCER CENTER DNA SEQUENCING CORE's services in their publications and provide the Core with information of the grants that support the use of the Core. Please contact the Core for specific details.

DNA Sequencing Core

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DNA SEQUENCING CORE PRICES FOR SERVICES

(Effective July 1, 2005)

Price List for Cancer Center Members Only

DNA Sequencing

- Premium Full Service Single-Pass Sequencing Reaction — $12.50
  Core quantifies DNA and performs sequencing reaction. Use of standard sequencing primers included: M13 Forward, M13 Reverse, SP6, T7, T7 neo, T7 terminator, and T3. Repeats included, if fault due to Core
- Normal Mid-Level Single-Pass Sequencing Reaction — $9.00
  Customer mixes DNA and desired primers at specified DNA and primer concentrations. Core does not provide any sequencing primers. Core performs sequencing reaction. No repeats.
- High Through-put Mid-Level Single-Pass Sequencing Reaction
  See brochure or separate description for details on sample preparation for this procedure.
  Batches of 48 or greater of Samples (price per sample) — $ 8.00
- Budget Single-Shot Sequencing — $4.00
  Core runs customer's processed sequencing reaction on its automated sequencers. No repeats.

DNA Profiling

- DNA Profiling with the ABI Profiler Plus kit (per sample DNA extracted from a cell line by the customer) — $40.00

Template Preparation

- Plasmid Minipreps (from colonies or liquid cultures)
  High Copy Number Plasmids — $15.00
  Low or Medium Copy Number Plasmids — $20.00
- Purification of PCR Products from Reaction Mixtures — $10.00
- Semiautomatic, High-Throughput Plasmid Minipreps
  (Price per 96 cultures) — $500.00
- Quantification of Templates of Unknown Concentration — $5.00
- Miscellaneous Services (e.g., dilution of primer) — $\geq$$5.00

Hybridization Filters

- High Density Hybridization Filters for Screening DNA Libraries
  2 copies — $180.00
  3-7 copies — $200.00

DNA Analysis and Additional Services

Available on request. Please call for quotation for Primer Walking, Large-scale Preps of Sequence Quality Plasmid DNA, Preparation of Lambda or Cosmid DNA Templates, Construction and Screening of DNA Libraries (In vitro Transposon Libraries in Plasmids and Cosmids), Preparation of Sequence Submissions.

Price List For Academic and Non-Profit Institutions

DNA Sequencing

- Premium Full Service Single-Pass Sequencing Reaction $15.00
  Core quantifies DNA and performs sequencing reaction. Use of standard sequencing primers included: M13 Forward, M13 Reverse, SP6, T7, T7 neo, T7 terminator, and T3. Repeats included, if fault due to Core

- Normal Mid-Level Single-Pass Sequencing Reaction $11.00
  Customer mixes DNA and desired primers at specified DNA and primer concentrations. Core does not provide any sequencing primers. Core performs sequencing reaction. No repeats.

- High Through-put Mid-Level Single-Pass Sequencing Reaction
  See brochure or separate description for details on sample preparation for this procedure.
  Batches of 48 or greater of Samples (price per sample) $ 9.00

- Budget Single-shot Sequencing $4.50
  Core runs customer's processed sequencing reaction on its automated sequencers. No repeats.

DNA Profiling

- DNA Profiling with the ABI Profiler Plus kit (per sample DNA extracted from a cell line by the customer) $50.00

Template Preparation

- Plasmid Minipreps (from colonies or liquid cultures)
  High Copy Number Plasmids $15.00
  Low or Medium Copy Number Plasmids $20.00
- Purification of PCR Products from Reaction Mixtures $10.00
- Semiautomatic, High-Throughput Plasmid Minipreps
  (Price per 96 cultures) $500.00
- Quantification of Templates of Unknown Concentration $5.00
- Miscellaneous Services (e.g., dilution of primer) ≥$5.00

Hybridization Filters

- High Density Hybridization Filters for Screening DNA Libraries
  2 copies $180.00
  3-7 copies $200.00

DNA Analysis and Additional Services

Available on request. Please call for quotation for Primer Walking, Large-scale Preps of Sequence Quality Plasmid DNA, Preparation of Lambda or Cosmid DNA Templates, Construction and Screening of DNA Libraries (In vitro Transposon Libraries in Plasmids and Cosmids), Preparation of Sequence Submissions.

Price List For Commerical/Non-Academic Researchers and For-Profit Institutions

DNA Sequencing

- Premium Full Service Single-Pass Sequencing Reaction $16.00
  Core quantifies DNA and performs sequencing reaction. Use of standard sequencing primers included: M13 Forward, M13 Reverse, SP6, T7, T7 neo, T7 terminator, and T3. Repeats included if fault due to Core

- Normal Mid-Level Single-Pass Sequencing Reaction $12.00
  Customer mixes DNA and desired primers at specified DNA and primer concentrations. Core does not provide any sequencing primers. Core performs sequencing reaction. No repeats

High Through-put Mid-Level Single-Pass Sequencing Reaction
See brochure or separate description for details on sample preparation for this procedure.
Batches of 48 or greater of Samples (price per sample) $ 10.00

- Budget Single-shot Sequencing $ 6.00
  Core runs customer's processed sequencing reaction on its automated sequencers. No repeats.

DNA Profiling

- DNA Profiling with the ABI Profiler Plus kit (per sample DNA extracted from a cell line by the customer) $60.00

Template Preparation

- Plasmid Minipreps (from colonies or liquid cultures)
  High Copy Number Plasmids $22.00
  Low-Medium Copy Number Plasmids $27.00
- Purification of PCR Products from Reaction Mixtures $15.00
- Automated, High-Throughput Plasmid Minipreps
  (Price per 96 cultures) $600.00
- Quantification of Template of Unknown Concentration $5.00
- Miscellaneous Services (e.g., dilution of primer) ≥$5.00

Hybridization Filters

- High Density Hybridization Filters for Screening
  2 copies $270.00
  3-7 copies $300.00

DNA Analysis and Additional Services

Available on request. Please call for quotation for Primer Walking, Large-scale Preps of Sequence Quality Plasmid DNA, Preparation of Lambda or Cosmid DNA Templates, Construction and Screening of DNA Libraries (In vitro Transposon Libraries in Plasmids and Cosmids), Preparation of Sequence Submissions.

DNA SEQUENCING SERVICES

The primary function of the CU-Cancer Center DNA Sequencing Core is to sequence DNA samples brought to us as purified DNA. We can also purify DNA from bacterial colonies for sequencing. However, we highly recommend that the customer confirm that the *E. coli* transformant does in fact bear the correct plasmid before submitting it for DNA purification and sequencing.

We offer several levels of sequencing service - from our Premium Full-Service Single-Pass Sequencing to budget "Single-Shot" sequencing, where the customer performs the sequencing reaction themselves and submits the reaction products ready for electrophoresis on one of our automated sequencers. The Core's two capillary ABI fluorescent sequencing instruments can provide between 650 and 950 bp of reliable DNA sequence per AmpliTaq FS cycle sequencing reaction with dRhodamine and BigDye labelled dye-terminators.

Sequencing results will only be delivered electronically as follows: customers will receive an email informing them that their results are ready. In the message, there will be a link to our website from where the results can be downloaded, using the customer's name and password. The data will be available on the server for one month from date of posting; afterwards customers will need to contact the Core directly for the data. We will no longer print electropherograms of sequencing results.

On this website customers will have access to the text files (.seq), electropherogram files (.ab1), and .pdf files of the electropherograms. The electropherogram (.ab1) files will be editable, but the .pdf files will not. Viewing the .ab1 files you will need to download either Editview (for MACs) or Chromas (for PCs). Both are free of charge and available at the following websites:

Editview: http://www.appliedbiosystems.com/support/software/dnaseq/installs.cfm

Chromas: http://www.technelysium.com.au/chromas.html

Xplorer Lite: http://www.dnatools.com/download.html

Premium Full-Service Single-Pass Sequencing

This is the highest level of sequencing service, which includes performance and processing of the sequencing reaction, loading the products on a sequencing instrument, and preparing and troubleshooting the results for the customer. The DNA samples should be quantified by the customer using agarose gel electrophoresis and be submitted to the Core at the following concentrations:

Premium Service DNA and Primer Concentrations

Type of DNA	**DNA Concentration** (nanograms/ microliter)	**Primer Concentration** (micromolar)
Plasmid DNA	150.	5.
Purified Uncloned PCR Product	15.	5.
Purified DNA Restriction Fragments	15.	5.
Lambda, PAC, BAC, Cosmid DNA	500.	100.

The DNA and primers should be submitted in **1.5-1.7 mL microfuge tubes** and the labels on the tubes must be exactly as that submitted through the on-line ordering system.

To estimate the concentration and purity (i.e., presence of RNA and genomic DNA) of your DNA samples, we recommend that you electrophorese your samples on an agarose gel and compare the intensity of the ethidium bromide bands of your DNA sample against that of the bands present in a ladder of mass standards. Three commercially available mass standards that can be used are: PGC Scientifics Gene Choice DNA Ladder I (Cat. No. 62-6108-00), which we sell and you thereby avoid paying shipping costs. New England Biolabs (NEB) also have a 1KB Ladder (Cat. No. N32325), which is available in the NEB freezer in the UCHSC School of Medicine Room 5626. Inter-Mountain Scientific Company (ISC Bioexpress) sells the Gene Mate Quanti-Maker 1 kb Ladder (Cat. No. C-5087-200), which is very similar to the mass ladder from PGC Scientifics. When it comes to DNA concentration, more is not always better. Too much DNA or too concentrated primer solutions can give results with high background or appear to be a mixture of sequences. We will assume the concentration of your sample is the one appropriate for your samples (see above table) and will run the reactions accordingly. If the reaction fails because of a possible error on the part of Core personnel, the reaction will be repeated at no extra charge.

We can provide several standard sequencing primers (M13 Forward, M13 Reverse, SP6 promoter, T3 promoter, and T7 promoter primers, see section below for primer sequences) at no additional cost to the customer. We also have the primer T7neo, which is specific for the mammalian expression vectors derived from the pCIneo and pSI vectors. The customer may also bring their own primer for sequencing their DNA samples. We can sequence different types of DNAs: plasmids, PCR fragments, restriction fragments, cosmid, PAC, and lambda phage. The Core will provide the

customer with the sequencing results as three electronic files per sample as described above.

Normal Mid-Level Single-Pass Sequencing

For **each** reaction, the customer should mix the below specified amounts of DNA and the desired primer made up in a total volume of **17.0 microliters** using either water or 10 mM Tris-HCl (pH 8) in a labelled 1.5-1.7 mL microcentrifuge tube (**Please note tube size!**).

Mid-Level Service DNA and Primer Quantities

Type of DNA	**DNA Quantity** (nanograms)	**Primer Quantity** (picomoles)
PLASMID DNA	1250.	10.
Purified Uncloned PCR Product	50.	10.
Purified DNA Restriction Fragments	50.	10.
Lambda, PAC, BAC, Cosmid DNA	3000.	100.

Do not use TE, because the EDTA will inhibit the reaction. The Core does not supply any primers for this level of service. Instructions for labelling the tubes are found below and on the order form specific for this service. The Core will add an aliquot of this mixture to a tube containing the sequencing reagents, perform and process the cycle-sequencing reactions, load the samples on a gel, and provide the customer with the sequencing results as described above. Only failures clearly due to an error of the sequencers will be repeated.

High Throughput Mid-Level Single-Pass DNA Sequencing

These options include running and analysis of sequencing reactions and emailing of electronic copies of the data. The customer mixes specific amounts of DNA and primer (see below) and we do the rest. No repeats unless due to Core error. Turn-around time approximately 2 working days.

In order to offer these inexpensive sequencing options, we must specify how the samples are brought to the Core. The samples must be in multiples of either **47** or **95** samples and pre-loaded in an Applied Biosystems MicroAmp Optical 96-well Reaction plate (Part Number N801-0560 which can be purchased through the ABI Freezer Program in the DNA Core; currently $40.70 per box of 10 plates). The wells must be covered with either strips of 8 caps (ABI part # N801-0535 currently $55.50 for 300 strips of 8 caps) or with optical adhesive cover sheets (AB part # 4311971; currently $122.00 for package of 100 cover sheets). Each of the wells must contain one sample of DNA mixed with a single primer in the amounts below, depending on the type of DNA. The last well in the batch (#48 = H6 or #96 = H12) should be left empty for the addition of a control template provided by the Sequencing Core. The DNAs and primers must be dissolved in water. We suggest quantifying the DNAs using a mass ladder as described above.

High-Through-put Mid-Level Service DNA and Primer Quantities

Type of DNA	**DNA Quantity** (nanograms)	**Primer Quantity** (picomoles)	**Total Volume** (microliters)
Plasmid (3 - 20 kb)	150	5	10
PCR Fragment (Purified, i.e., not cloned	15	5	10
DNA Restriction Fragment (Purified, i.e., not cloned	15	5	10
BAC (bacterial artificial chromosome)	600	100	10
Lambda phage DNA, Cosmid DNA (> 30 kb)	600	100	10

We suggest that DNAs with unusual features that can make them difficult to sequence (e.g., very GC rich regions, known hairpins structures) be submitted for Premium Full Service Sequencing instead of attempting to sequence them by any of the Mid-Level DNA sequencing services and that the customer inform the Core personnel of these potential problems.

Budget Single-shot Sequencing

The customer performs the sequencing reactions with either of the ABI dye-terminator cycle sequencing ready reaction kits: the dRhodamine (ABI part number 403044 or 403045) or BigDye dye-terminator kit (ABI part number 4303149 or 4303150). The customer must perform and process the cycle-sequencing reactions per instructions from ABI and from the Core. Instructions for labelling the tubes are on the order form specific for this service. Core personnel will electrophorese the reaction products on on the appropriate automated DNA sequencing instrument and provide the customer with the sequencing results as described above. Results will only be repeated if due to a failure of the automated sequencers.
Please specify on the order form which ABI sequencing chemistry kit was used!!

TEMPLATE PREPARATION SERVICES

It is necessary to use DNA of high purity to obtain quality sequencing results. The most critical step is the preparation of the DNA template for sequencing - from

choosing the bacterial strain, to its culturing, to template purification. Included in this brochure and a separate handbook are some recommendations of how our customers may best prepare the DNA themselves. Alternatively, we offer the following template preparation services.

Plasmid Mini-Preps

Individual cultures of *E. coli* containing high-copy number plasmids are inoculated from individual colonies on plates supplied by the customer. The plasmid DNAs are isolated by alkaline lysis of the bacterial cells and purified with Qiagen or other resins. The DNAs are quality controlled on agarose gels prior to sequencing. *E. coli* containing low to medium copy number plasmids require additional labor and therefore there is a slightly higher charge for their preparation.

Purification of PCR Products from Reaction Mixtures

PCR products can be sequenced if they are of sufficient quantity and purity. However, it is essential that, prior to purification, the PCR reaction produced a single band of amplified DNA. Otherwise, one must purify the DNA band of interest and, in our hands, these products are less likely to yield unique sequences. It is best to optimize the PCR conditions so that a uniques product is obtained. “Touch-down” PCR conditions are extremely helpful in obtaining a high yield of unique PCR products. The Qiagen QIAquick PCR purification kit and the Qiagen QIAquick DNA fragment purification kit yield DNA of sufficient quality for sequencing, if combined with specific changes recommended by the Core. Alternatively, the Microcon PCR centrifugal filter devices (Cat. # UFC7PCR50 or UFC7PC250) are faster, less expensive, and produce equally satisfactory results. ExoSAP-IT from USB (Cat. # 78202) is currently being used by the core. Contact the core for recommendation on an alternative protocol.It is critical that the concentration of the primers are not excessive (i.e., < 200 nanomolar) in the original PCR reaction, because they can be carried over through these purification procedures and will then produce mixed sequences. Similarly, the concentration of the dNTPs in the original PCR reactions should not exceed 200 micromolar each. See the section below on the preparation of PCR products for additional information.

Semi-Automated High-Throughput Plasmid Mini-Preps

From cultures provided by customers, plasmid-bearing *E. coli* are grown in deep-well plates to a high cell density before processing. This procedure does not work well with low and medium copy number plasmids. A Beckman Biomek 2000 Robot extracts the sequence quality plasmid DNA by alkaline lysis and resin purification. Large numbers of cultures can be processed to yield high quality templates for sequencing.

Quantification of Customer Templates

Cycle sequencing works best with an optimal concentration of DNA. Therefore, the quantity/concentration of the DNA to be sequenced must be accurately estimated by the customer. The easiest quantification method is by electrophoresing a 1 microliter sample of plasmid DNA or 3-5 microliters of a purified restriction fragment or a PCR product in parallel with a DNA standard of known concentration, for example, either known amount of a maxi- or midi-preparation of plasmid DNA or an aliquot of one of the DNA mass ladders described above from PGC Scientifics, New England BioLabs, or ISC BioExpress as described above. We offer this service for $5.00 per sample to our customers to help ensure satisfactory results.

PREPARATION OF PLASMID DNA FOR SEQUENCING

Sample Names

We require that the DNA samples be named only with English alphanumeric characters and underscores. **Please do not use Greek letters, dashes, dots, colons, slashes, nor signs such as #.** This simplifies the naming of the DNA sequence files so that they are acceptable to both IBM/DOS/Windows and Mac formats. Please limit the clone name to a maximum of 8 characters and the primer name to 5 characters.

Escherichia coli Strains

Numerous *E. coli* strains are used for cloning of DNA; however, two factors that interfere with obtaining optimal DNA sequencing results are cell wall polysaccharides and intrinsic DNA nucleases. The ideal strain from which to prepare plasmid DNA for sequencing is DH5α, because it has low amounts of polysaccharides and is mutant in the gene for endonuclease I (i.e., it bears the *endA1*-minus allele). Non-suitable strains are: NM544, C600, BL21, TG1, JM101. The strains MV1190, XL1 Blue, and JM109 give variable results due to slow growth and higher amounts of polysaccharides, and do not increase plasmid yields in response to Terrific Broth medium.

Choice of plasmids

The *E. coli* vectors of the pUC, pGEM, or pBluescript series yield high amounts of plasmid DNA per cell (>300 copies per cell). Generally, vectors derived from pBR322 or pACYC/p15A series of plasmids have much lower copy numbers (≈ 1-30 copies per cell). Very large DNA inserts (≥20 kb) can reduce copy number for both types of vectors and certain inserts can be toxic to the cell. Consider these factors when choosing the plasmid for cloning the DNA to be sequenced and the size of culture needed to isolate sufficient amounts of plasmid DNA. If you submit a culture for plasmid isolation, it is important to indicate the type of plasmid (high copy or medium-low copy). **We highly recommend that the customer confirm that the *E.***

***coli* transformant brought to the Core does in fact bear the correct plasmid before submitting it for DNA purification and sequencing.**

Growth of Bacterial Culture

The concentration and care of the antibiotic used to maintain plasmids in bacterial strains is a critical step in obtaining optimal amounts of plasmid DNA. We recommend growing the bacterial culture in either Luria Broth (LB with the usual amount of NaCl, 1%) or Terrific Broth, and if the plasmid selection is ampicillin resistance, use either 100 micrograms/mL ampicillin on plates or 50 micrograms/mL for liquid cultures or 50 micrograms/mL of the more stable carbenicillin in both plates or liquid.

Adequate aeration of bacterial cultures is ensured with a liquid to container volume ratio of 1:5 and rotary shaking at 250 rpm. Most bacterial strains grow optimally at 37°C with optimal yields at 18 + hours, depending on the aeration rate. Often, 12-16 hours can be too little time for satisfactory yields of plasmid DNA. Prolonging growth to 18-20 hours can increase plasmid yields, but again this will depend on the aeration rate. Long-term storage of cultures on ampicillin plates is to be avoided; colonies become quite clumpy and lose viability due to cell lysis.

If the Core is to purify the DNA for you from your clone, please use fresh cultures on fresh plates of selective medium and ALWAYS streak out one (1) SINGLE colony sufficiently to produce single well-separated colonies. This will help ensure we obtain a unique sequence. Again, since we do not know the structure of your plasmid, be certain of what you give us to sequence.

DNA Preparation and Purity

We use ABI's FS Recombinant AmpliTaq polymerase and dRhodamine and BigDye version 1.1 dye-terminator-based cycle sequencing kits to sequence DNA. High DNA purity is essential for optimal results. We recommend using alkaline lysis and the Qiagen products and protocols for purifying plasmid DNA, as **MODIFIED** by the Core; contact the Core for details. Boiling preps, unless subsequently cleaned up, usually do not produce DNA suitable for sequencing, because of the high amount of detergent left in the sample. It is also critical that the samples be **free** of RNA. A good alternative, especially for large amounts of plasmid DNA, is the method of Feliciello and Chinali (Anal. Biochem. **212**: 394-401, 1993), when followed by precipitation of DNA with polyethylene glycol, a 70% ethanol rinse, and a re-precipitation and rinse with ethanol. Carefully remove the supernatants so as not to lose the sample. We have our detailed version of this protocol available for Core customers.

Other protocols for preparing DNA using glass-based or diatomaceous-earth- or glass-based resins (e.g., Promega's Wizard or Wizard-Plus, or Clontech's S.N.A.P. miniprep kit) can produce suitable results, if one ensures that all EDTA, ethanol, and NaCl have been removed from the DNA prep before resuspending the dried DNA pellet in **sterile and pure water** or 10 mM Tris-HCl, pH 8. The solution originally used to elute DNA from the Wizard™ columns of Promega contained EDTA and sodium chloride, both shown to interfere with AmpliTaq-based cycle sequencing. Therefore, if you use one of these older protocols, precipitate the eluted DNA with sodium or potassium acetate and either ethanol or isopropanol at ROOM TEMPERATURE, rinse the DNA pellet with 70-80% ethanol at ROOM TEMPERATURE, and dry the pellet. Carefully remove the supernatants so as not to lose the pelleted sample.

Remember: DNA pellets can be accidentally lost after ethanol precipitation and especially after isopropanol precipitation. If you need to concentrate a DNA sample resuspended in water, you may evaporate the DNA solution and resuspend it in the desired volume of water. However, remember you will also concentrate any contaminants present.

With all plasmid isolation methods involving alkaline lysis, it is critical to keep the incubation time in the alkaline solution short (< 5 minutes at room temperature) before neutralization of the solution and precipitation of the chromosomal DNA and cell debris with the acetate solution on ice. Longer times produce a DNA conformation that migrates ahead of the main covalently closed circular (CCC) band and it is probably denatured and may not be digestible by certain restriction enzymes. After centrifugation to remove the cellular debris and genomic DNA, precipitate the DNA with isopropanol at **ROOM TEMPERATURE** and rinse with 70% ethanol at **ROOM TEMPERATURE** to remove excess salts. Dry the pellet. DNA template submitted for sequencing should be resuspend in water or 10 mM Tris-HCl (pH 8). TE (10 mM Tris-HCl, 1 mM EDTA, pH 8) should not be used since the EDTA can inhibit the AmpliTaq polymerase by chelating the Mg^{2+} in the cycle-sequencing reaction.

DNA Concentration

DNA concentration is often determined by measuring the absorbance of a DNA solution at 260 nm (A_{260nm}) with subtraction of the absorbance at 320 nm (A_{320nm}) to correct for light scattering due to debris. The actual measured A_{260nm} value should be between 0.2 and 1.0, using a 1 cm cuvette and the sample should not contain RNA or nucleotides. **NOTE:** Mini-preps of DNA are too dilute to accurately measure the absorbance in this manner and still have enough of the sample left for sequencing.

Therefore, we recommend that a sample of the DNA (ideally, 1.0 microliter for plasmid DNA and 3-5 microliters for PCR products and restriction fragments) be electrophoresed on a 0.75% agarose gel along with a known amount of similar sized DNA. One can use for example an aliquot of either a maxi- or midi-prepared plasmid DNA that has been accurately quantified. Alternatively, a DNA mass ladder such as

those described above can be used. Agarose gel electrophoresis will ensure that DNA is present in the expected/desired amounts and it is free of RNA and genomic DNA. Photograph the gel without over exposing so that the band intensity is proportional to the amount of DNA. Also, it is critical that the amount of RNA in the DNA sample be minimal. Even a slightly visible RNA band on a gel can indicate that 50% or more of the nucleic acid in the sample is RNA. This is due to RNA not binding ethidium bromide as efficiently as double-stranded DNA.

Plasmids

For the Premium Full-Service sequencing, we require each plasmid template to be dissolved at a concentration of 150 nanograms/microliter in water or Tris-HCl (pH8) per sequencing reaction/primer combination. Kindly provide us with minimum of 5 microliters of DNA solution **per reaction** at this concentration in a **1.5** mL microcentrifuge tube. The extra amount DNA is so that we have enough for repeats or pipetting errors. We will gladly return whatever we do not use; however, we will discard DNA samples and primers after one calendar month. Please indicate the concentration of the DNA on the order form.

For uncloned PCR products or linear restriction fragments, we need that the DNA samples have a concentration of 15 nanograms/microliter.

For Lambda, PAC, and BAC DNA, we need the DNA concentration be 500 nanograms per microliter and the primer concentration be 100 micromolar. Please do not dilute and we will gladly provide advice on protocols for the isolation of these DNAs.

Unusual Templates

DNA templates with high G+C content, palindromes (e.g., tRNA genes, transcription terminators, iRNA), or homopolymeric regions can be difficult to sequence. AmpliTaq FS can have difficulty in polymerizing through such structures, because the sequencing reaction mixtures contain dITP instead of dGTP; dITP is used to minimize such electrophoretic artifacts as sequencing compressions. However, if we know of potential problems beforehand, we have developed some unpublished methods that can increase the likelihood of successfully sequencing through these regions. Please indicate at the time of ordering the sequencing whether a DNA sample is known to contain such features and whether you wish us to modify our normal protocol for your samples.

Although we can use a Big Dye sequencing kit containing dGTP to sequence through difficult regions, it has the drawback that compressions will occur in the sequencing results and therefore this kit is only used for very difficult templates. PLEASE NOTE, we do not guarantee that the modified reactions will produce satisfactory sequencing results.

PREPARATION OF PCR PRODUCTS FOR SEQUENCING

Synthesis of PCR Products

PCR products are **linear** DNA fragments and **not** ones that have been cloned into a vector after amplification. Once the DNA is cloned, it is considered to be plasmid DNA. To successfully sequence PCR DNA products they must electrophorese as clear, distinct, and unique bands after one round of PCR amplification. If more than one product is produced, the desired product should be gel purified and tested for homogeneity (e.g., complete digestion with a specific restriction enzyme). Be aware that two rounds of PCR amplification (e.g., nested PCR or re-amplification with the original primers) can introduce heterogeneities in to the amplified DNA fragment and therefore produce mixed sequences.

To sequence a mixture of DNA fragments, use of a nested primer for sequencing may improve the success rate. If a pair of primers produces a mixture of similar size fragments, a second primer known to hybridize only to and within the desired fragment can be used to sequence the PCR product with some degree of success.

Purification of PCR Products

It is absolutely critical to remove or destroy the primers and the dNTPs after PCR amplification; otherwise, sequencing will either not produce a signal due to excess dNTPs in the reaction or the different primers will give multiple (i.e., mixed) sequences. The Microcon™ PCR filter units from Millipore (catalogue number UFC7PCR50 or UFC7PC250) are simple to use and work well for purifying PCR products from a reaction. Avoid the use of oil in the PCR reactions.

Alternatively, Qiagen's QIAquick PCR Purification spin columns and similar products work well to purify DNA fragments away from nucleotides, primers, and oil used to prevent evaporation during PCR. By either method, if the primers are long or can form dimers, they may not be totally removed and can thus produce spurious results. Qiagen's QIAquick Gel Purification spin columns work well to extract DNA fragments from agarose, if used according to modifications available from the Core. With both of Qiagen's products, the DNA should be eluted with water, not TE. If eluted with TE, precipitate the DNA with ethanol and a salt (e.g., Na-acetate), rinse the pellet with 70% ethanol, dry, redissolve in water and quantify on an agarose gel. Millipore offers an alternative method for purifying DNA fragments from agarose (Amicon Ultrafree-DA spin columns, Millipore catalog no. 42600).

Primers

The primers used to produce the PCR product can be used successfully to sequence a PCR DNA fragment. However, even gel-purified PCR fragments may contain more than one product as well as inhibitory compounds from the agarose or polyacrylamide. Therefore, it may be preferable to use internal "nested" primers (i.e., ones which anneal to the desired sequence between the original PCR primers) to increase the specificity of the sequencing reactions.

PREPARATION OF LAMBDA AND COSMID CLONES FOR SEQUENCING

Lambda Clones

We recommend using a modification of the protocol by Lee and Clark (Biotechniques 23: 598-600, 1997) with the Qiagen Midi kit. Supply the sequencing primers at 100 micromolar. For details, contact the DNA Sequencing Core.

Cosmid Clones

Cosmid DNA can be isolated by alkaline lysis followed by either a PEG precipitation step or by using the Qiagen Plasmid Kit. The simple plasmid preparation method of Feliciello and Chinali (Analytical Biochemistry **212**: 394-401, 1993) combined with a PEG precipitation step (a copy of our modification of this method is available upon request) can also produce excellent template DNA. Remember to increase the culture volume to compensate for the lower copy number of cosmid vectors as compared to the pUC / pGEM / pBluescript series of vectors. Supply the sequencing primers at 50 micromolar.

BAC and PAC Clones

These large cloned DNAs are more difficult to sequence. We recommend using Qiagen kits specific for isolating these DNAs. Supply the sequencing primers at 100 micromolar. Because of the size and complexity of the DNA, it may be easier to sequence the DNA that has been digested with restriction enzymes, so long as a restriction site is not located between the primer site and the region to be sequenced.

DNA Quality and Concentration

For each reaction, we need 5 microliters of 500 nanograms per microliter of DNA in water. Please confirm the DNA concentration on an agarose. **Do not freeze these large DNAs as this may make them more difficult to dissolve**. Please note on the order form if your DNA is one of these types.

SEQUENCING PRIMERS - CONCENTRATION, DESIGN, AND CHOICES

Primer Concentration

For most sequencing experiments, we require primers dissolved in water or Tris-HCl (pH 8) at 5 micromolar. For sequencing of large DNA templates (phage, PACs, BACs, cosmids, the primer should be at 100 micromolar . Please note the concentration units (i.e., PLEASE do not use micrograms per milliliter!). Please supply enough primer for three reactions of 1 microliter each with a **minimum** of 10 microliter of each primer.

Primer Design

Many commercially available computer programs can help circumvent several problems encountered in primer design. We have in-house programs for primer design. Primers should have a 40-65% GC content, and 16-30 bases in length (depending on Tm). A C or G at its 3' end provides a stronger bond to the target, but an A or T at the 3' end may provide higher specificity of annealing. Those that can form hairpins or dimerize with themselves should be avoided. To prevent primer mispairing and slippage during annealing, avoid stretches of 5 or more identical bases (especially G or C), in particular, at the 3' end of the primer. Detailed considerations and recommendations for both sequencing and PCR primers are available on request.

Universal Primers

We can supply the following primers for Premium Service sequencing your DNA sample.

Primer	Length	Sequence (5'-->3')	Tm /Td
M13/pUC Forward	18	TGTAAAACGACGGCCAGT	57° 54°
M13/pUC Reverse	22	TCACACAGGAAACAGCTATGAC	58° 64°
T7 (Promega)	20	TAATACGACTCACTATAGGG	46° 56°
T7neo	19	TAATACGACTCACTATAGG	41° 52°
T7 Terminator	19	GCTAGTTATTGCTCAGCGG	53° 58°
T3 (Promega)	20	ATTAACCCTCACTAAAGGGA	53° 56°
SP6 (Core version)	23	GCTATTTAGGTGACACTATAGAA	50° 62°

Important Notes

The sequences of these primers have been optimized for cycle sequencing and will match most vectors; however, always check your vector sequence and clearly indicate which vector and primer combination you desire. Please note that the primer annealing during cycle sequencing is done at 50 °C.

There are two different T7 primers commercially available and they are **not** cross compatible. Check to see whether the sequence of our primer will work with your template.

Tm was determined by calculating the stacking energy method of Breslauer et al., Proc. Nat. Acad. Sci. 83, 3746-50, 1986

Td was determined by the Wallace et al. (1979) equation: Td (in °C) = 2° x (number A + T) + 4° x (number G + C).

HIGH DENSITY HYBRIDIZATION FILTERS FOR SCREENING OF DNA LIBRARIES

The Beckman Biomek 2000 Robot allows production of high-density hybridization filters of clones from a library that can be screened for the presence of specific genes or their cognates/homologues. The customer need only bring the selected colonies in 96-well microtiter dishes and specify the number of membrane copies needed. The robot can transfer samples from one plate (96 colonies) up to twenty-five plates (2400 colonies) onto one hybridization filter. We can make up to seven copies of the filters. We can also make hybridization filters from 384-well plates, transfer libraries from both 96 and 384 well plates to fresh dishes, and condense libraries from 96-well dishes to 384-well dishes.

DNA ANALYSIS SERVICES

The ABI sequencing instruments contain several algorithms for calling the bases from the electropherograms of the sequencing reactions. Because of some idiosyncrasies of the AmpliTaq FS polymerase and the stereochemical interactions of the dye labelled dideoxynucleotides, these routines can sometimes have difficulty calling the bases correctly. The sequences should be examined and edited manually in the electropherogram files. To minimize the number of miscalled bases, we recommend that prior to performing further analyses of the DNA sequences, the electropherograms be examined **CAREFULLY** and **edited as needed**.

Manual Editing of ABI Base-Calling

The chromatogram of each sequencing reaction is analyzed visually on a computer monitor and corrections are made to regions of ambiguity in the sequence, based on the expertise of the Core personnel. This provides the highest quality sequencing results, but can be labor-intensive.

The customer can also edit their own electropherograms using the free-ware Mac program Editview 1.0.1.1 from Applied Biosystems (can be downloaded at the following address:
http://www.appliedbiosystems.com/support/software/dnaseq/installs.cfm

For PCs, the program Chromas is available at the following web site:
http://www.technelysium.com.au/chromas.html

Sequence Assembly

Customer sequences are stored on-site and can be assembled into contiguous sequences of any size using the Sequencher assembly programs. It is best to align edited electropherogram files instead of the unedited sequence text files.

Basic Sequence Analyses

Assembled DNA sequences can be analyzed for base composition and restriction enzyme sites. The sequence can then be examined in a variety of ways. New primers can be designed for extending the sequence, or amplifying the sequenced DNA by the PCR, or similarity searching using BLAST against the non-redundant databases including dbEST (expressed sequence tagged sites) provide a powerful approach to gene identification.

Upon completing the sequencing of the gene of interest, comparison of its deduced amino acid sequence with that of potentially homologous proteins can provide valuable insight into its function and cellular role. We recommend that as a first step in understanding the biological role of your favorite gene, you analyze it by: Prosite Analyses, Prediction of Protein Physical and Chemical Properties, BLITZ Gapped Alignment Searches, and Multiple Sequence Alignments. Below are listed the different analyses that we can perform on your sequences. They are available individually or as packages.

Prosite Analyses

Comparison of the deduced protein sequence with several databases of specific conserved functional motifs in proteins can provide clues to the protein's biological function.

BLITZ Searches and Alignments

Comparison of your deduced protein sequence by the BLITZ gapped alignment algorithm to protein databases can identify homologues of your protein.

Prediction of Protein Physical and Chemical Properties

Calculation of the molecular size, isoelectric point, hydropathicity profile, and net charge of the deduced protein sequence may confirm known biochemical properties of the purified protein.

Primer Design

Primers are designed and checked for self-complementarity, possibility for primer-dimer formation, avoidance of common repeats, primer-melting temperature, and sequence annealing temperature, and the results manually compared to the original chromatogram to ensure that they are picked from a high-quality portion of the sequence. The most important segment of a primer is the 5-6 nucleotides at the 3' end; verify that they do not anneal elsewhere in the template.

Multiple Sequence Alignments

Using the results from the above analyses, customer nucleic acid and protein sequences can be aligned against each other or against database sequences, to identify possible mutations and functionally conserved regions.

NEW CORE SERVICE: ASSAY FOR IDENTITY & AUTHENTICITY OF HUMAN CELL LINES

The contamination and overgrowth of cell lines is a well documented, but often ignored phenomenon. Contamination by HeLa cells is probably the most well known, notorious example. However, it illustrates that any rapidly growing cell line can overtake both tumor cell cultures as well as normal cell lines. In the culturing of mammalian cells, laboratory personnel usually are more concerned about microbial (yeast, mold, mycoplasma) contamination, while cross-contamination between different cell lines (both intra- and inter-species) is often overlooked.

Nelson-Rees et al. (1981) were among the first to document the extent of this problem, and still researchers, journal editors, and review committees do not routinely require evidence for the authenticity of reported cell lines. Recognition of this problem could jeopardize substantial numbers of published research results. MacLeod et al. (1999) recently surveyed the extent of this problem in 252 human tumor cell lines that were submitted by the originators to the DSMZ - German Collection of Microorganisms and Cell Cultures in Germany and five other cell repositories. They found 18% of the cultures to be intraspecies cross-contaminants. The most prolific cross-contaminants were tumor cell lines that included eleven instances of HeLa cells. The most cited endothelial cell line, ECV304, was found not to be derived by spontaneous immortalization of normal cells, but rather due to intraspecies cross-contamination. van Bokhoven et al. (2001a, 2001b, 2003) showed that cross-contamination frequently occurred among human prostate cancer cell lines. Among nemerous supposedly independent prostate tumor cell lines, they found only 17 genuinely unique lines; the others were intraspecies cross-contaminants or even interspecies contaminants. The UCCC DNA Sequencing & Analysis Core assisted Drs. Adrie van Bokhoven and Gary Miller in identifying this problem. The Miller-Nordeen laboratory now routinely quarantines new cell lines until they are confirmed genotypically. In addition to intraspecies cross-contamination, there is the problem of interspecies contamination, which is discussed and tackled in a report by Parodi et al. (2002).

The standard approach for determining the authenticity of a cell line has been cytogenetics. This of course requires that a characteristic alteration be present. Two common methods of karyotype analysis are (1) G-banding patterns and (2) multi-FISH labeling patterns using chromosome-specific probes tagged with different fluorophores (e.g., SpectraVysion probes of Vysion Inc.). These procedures are currently available from the CU Cancer Center Cytogenetics Core (Director: Dr. Marileila Varella-Garcia). While very useful, the analysis requires careful examination by trained personnel and is costly, especially when repeated confirmations of the same cell line might be required.

Alternatively, one can use DNA profiling to compare cell lines, The UCCC DNA Sequencing & Analysis Core employed this technique, in collaboration with van Bokhoven et al. (2001a, 2001b, 2003), to demonstrate whether cell lines were of common or independent origin. Furthermore, this technique can be used to verify that a new derivative cell line is, in fact, derived from the original cell line or tissue and has not arisen due to a contamination of the culture.

Table 1 - Comparison of Loci Amplified by Three DNA Profiling Kits

ABI Profiler Plus (9 Loci + Gender Marker)		**Promega Powerplex 16** (16 Loci + Gender Marker)	**Promega Powerplex 1.2** (8 Loci + Gender Marker)
Shared Loci	**Dye color**	**Shared Loci**	**Shared Loci**
Amelogenin	Green	Amelogenin	Amelogenin
D13S317	Yellow	D13S317	D13S317
D18S51	Green	D18S51	
D21S11	Green	D21S11	
D3S1358	Blue	D3S1358	
D5S818	Yellow	D5S818	D5S818
D7S820	Yellow	D7S820	D7S820
D8S1179	Green	D8S1179	
FGA	Blue	FGA	
vWA	Blue	vWA	vWA
		Unique Loci	**Unique Loci**
		CSF1PO	CSF1PO
		D16S539	D16S539
		Penta D	
		Penta E	
		THO1	THO1
		TPOX	TPOX

DNA profiling entails analyzing DNA samples for polymorphic short tandem repeat (STR) markers. Masters et al. (2001) compared two different kits for short tandem repeat DNA profiling to characterize over 250 cell lines and recommended this technique as an international standard for characterizing cell lines. There are several

kits available commercially from Promega Corporation and Applied Biosystems Inc. Table 1 lists the loci tested for tetranucleotide repeats by three commercial kits. The American Type Culture Collection (ATCC) uses the Promega Powerplex 1.2 kit. We are using AmpF*l*STR Profiler Plus PCR Amplification kit from Applied Biosystems. With this ABI kit, the repeat regions of the nine tetranucleotide short tandem repeat loci and the Amelogenin gender marker listed in Table 2 are co-amplified and their sizes determined on an ABI 3100 capillary automated DNA sequencer. The results are easy to tabulate for cell line identification and comparison. This kit is commonly used in forensic investigations for human identification and yields results that provide, on average, a probability of identity of 1 in 8.2 x 10^{10}. Because the kit is used for forensics and subjected to extensive quality controls by Applied Biosystems, it is expensive for an individual researcher with relatively few samples.

Table 2 - Tetranucleotide STR Amplified by ABI's Profiler Plus Kit

Locus	Chromosomal Location	Common Sequence Motif	Size Range (bp)	Dye Label	Dye Color
D3S1358	3p	TCTA (TCTG)1-3(TCTA)n	114-142	5-FAM	Blue
VWA	12p12-pter	TCTA (TCTG)3-4(TCTA)n	157-197	5-FAM	Blue
FGA	4q28	(TTTC)3TTTTTTCT (CTTT)n CTCC (TTCC)2	219-267	5-FAM	Blue
Amelogenin	X:p22.1-22.3	-	107	JOE	Green
	Y:p11.2	-	113	JOE	Green
D8S1179 (=D6S502)	8	(TCTR)n	128-168	JOE	Green
D21S11	21	(TCTA)9(TCTG)n[(TCTA)3 TA(TCTA)3CA(TCTA)2 TCCATA](TCTA)n	189-243	JOE	Green
D18S51	18q21.3	(AGAA)n	273-341	JOE	Green
D5S818	5q21-31	(AGAT) n	135-171	NED	Yellow
D13S317	13q22-31	(GATA) n	206-234	NED	Yellow
D7S820	7q11.21-22	(GATA) n	258-294	NED	Yellow

Since the analysis would be extremely useful for researchers, the UCCC DNA Sequencing & Analysis Core offers this service to the research community at a much lower price.

Submitters should purify total DNA from desired cell lines (see below) and bring the samples (in water or 10 mM Tris-HCl, pH 8) to the Core. The Core will confirm the quantification of the DNA by gel electrophoresis, PCR amplify the different loci described in Table 1 by multiplex PCR, electrophorese the products on (1) an agarose gel to confirm the PCR reaction worked, and (2) an automated sequencer to determine which specific alleles are present. The actual cost for the reagents is $20/PCR reaction. The total cost per sample will be $40-75 (depending on affiliation), which includes analysis of the results.

Working with different investigators, we have characterized numerous samples of commonly used human cell lines, which have also been characterized by cytogenetics whenever possible. We currently retain on file the various results obtained by different investigators, in a shared database, in order to spot possible abnormalities that might arise. This is an underapreciated, but very a useful service and database to the research community which can be used to avoid perfoming experiments on cell lines that have become contaminated.

References for DNA Profiling

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DNA PURIFICATION PROTOCOL FOR DNA PROFILING

There are numerous protocols for isolating DNA from human cells that are available in the literature and from several companies. If customers have already a protocol with which they are comfortable and it produces DNA satisfactory for PCR amplification, we recommend they continue to use it.

Alternatively, customers can purchase suitable kits commercially. Two excellent commercial kits for high yields of DNA are the Epicentre Master Pure Total DNA Purification kit (cat. no. MPCS 20226 @ 1-800-284-8474) and the Gentra Systems Generation Capture Column kit (Cat. No. GC-050, GC-0300, GC-1200). The simplest to use is the Gentra Generation Capture Column kit; however, it requires a heating block that can heat the columns to 100-105 °C. The Epicentre kit gives the highest yield, but has more steps.

Another simple procedure from Epicentre is to use their Catch-All Sample Collection Swabs with their BuccalAmp DNA Extraction kit (BQ0901S) to collect cells by swabbing a flask or plate of cells and extracting the DNA. They claim one can obtain enough cells to extract DNA for numerous PCR reactions, but quantification of the DNA may be difficult.

The simplest non-commercial method for isolating DNA is one of the easy variants of the salting-out procedure of Miller et al. (1988). Below is a modified salting-out procedure originally published by Gemmell and Akiyama (1996) that customers may try. This procedure may be scaled-up.

In any case, we do not recommend the very simple method of Parodi et al. (2002), because it extracts DNA in to PCR buffer and probably produces very low amounts of DNA that cannot accurately be quantified; but it can be used for screening for interspecies contamination by their method.

Whichever method is used to extract DNA, it is critical that the DNA concentration be estimated, diluted, and prepared for analysis as described below.

DNA Extraction by Salting Out

- Suspend a sample of tissue (100 mg), 100 microliter of cell suspension from a growth flask (e.g., T25), or 100 microliter of whole blood in 300 microliter of digestion buffer (100 mM NaCl, 50 mM Tris-HCl, 1% SDS, 50 mM Na_3EDTA, pH 8.0) in a 1.5 mL microcentrifuge tube.
- Add proteinase K solution (10 mg/mL) to a final concentration of 100 micrograms/mL. Incubate the samples for 2 hours at 50°C followed by overnight at 37°C.
- Add 300 microliter 5 M LiCl to each tube (poorer alternatives are: 2.5 M $NaClO_4$ or 5 M NaCl). Mix thoroughly by inversion for 1 min (NOT by vortexing).
- Add 600 microliter chloroform or chloroform:isoamyl alcohol (24:1) mixture and place on a rotating wheel for 30 min at room temperature (i.e., for gentle mixing and extraction of protein).
- Spin the 1.5 mL microcentrifuge tube for 15 min at max speed to extract the protein.
- Transfer the supernatant with a wide bore pipettor tip (or one cut off with a new sterile razor blade).
- Add 2.0 volumes of room temperature absolute ethanol and mix gently by inversion. The DNA should precipitate as gelatinous strands, which can be collected by two methods below.

Best Alternative Collection Method

- Collect the DNA strands using either a sterile, sealed, and curled pipettor tip (e.g., 200 microliter) or a sterile, unplugged, sealed, and curled Pasteur pipette. Either of these tools can be made by melting the ends in a bunsen burner flame to seal them and cause the end to curl slightly.
- Transfer the DNA to a microcentrifuge tube containing 0.5 mL of 70 % ethanol and jiggle it to rinse the DNA. Lift the DNA out of the ethanol and press out the excess liquid against the side of the microcentrifuge tube.
- Finally transfer the DNA to a microcentrifuge tube containing 200 microliter of Low TE (10 mM Tris-HCl, 0.2 mM Na_3EDTA, pH 7.5) and allow the DNA to dissolve at 37°C for 2 hours or 10 min at 65°C, and or at 4°C overnight, which ever works best and is most convenient.

Quick Alternative Collection Method

- Collect the DNA strands by centrifuging the DNA containing solution at ≈ 12,000 rpm in a microcentrifuge for 15 min at room temperature.
- Afterwards, decant the supernatant, add 1 mL of 70% ethanol (room temperature), and gently mix by inversion to resuspend the pellet.
- Centrifuge the tube for 5 min at max speed to collect the DNA.
- Decant and/or pipette off the supernatant, spin to collect any liquid off the tube walls and remove the supernatant by pipetting, avoiding the pellet.
- Allow the pellet to air dry for 10 min with the lid open either on the bench or by centrifugation with the lid open.
- Add 200 microliter of Low TE (10 mM Tris-HCl, 0.2 mM Na_3EDTA, pH 7.5) and allow the DNA to dissolve as above (may require incubation overnight).

DNA Quantification and Sample Preparation Methods

Since excess DNA reduces the yield of longer PCR fragments in this assay, accurate DNA quantification is critical for the optimal multiplex amplification of the 10 STR loci. Therefore please follow the sample preparation protocol below.

Method 1 - UV Absorbance (not the most reliable if RNA is present in the sample).

- [A260nm - A320nm] x [50 microgram DNA/mL] x Absorbance units] x [1/dilution factor] = microgram DNA / microliter
- DNA purity quotient = (A260nm - A320nm)/(A280nm - A320nm)

Method 2 - Agarose Gel electrophoresis / Ethdium bromide (0.2-0.3 g / microliter) in 1X TAE or 0.5-1.0X TBE)

- Compare aliquots of the DNA solution with a set of either mass ladder standards of DNA or samples of calf-thymus (CT) DNA dissolved in Low TE. Load 50, 100, 200, 400, 1,000 ng of CT DNA for comparison and dilute the purified DNA in Low TE to a concentration to lie within this range as estimated by absorbance at 260 nm. This gel electrophoresis will confirm whether the DNA is essentially free of RNA. Running the gel for different times and photographing it will help in the quantification of the large DNA when comparing to the mass ladders (e.g., Gene Choice DNA Ladders I or II {#65-600, 65-602, 65-604, 65-606} from PGC Scientifics [1-800-424-3300] or High DNA Mass ladder {Gibco-BRL 10496-016} from Invitrogen [1-800-8286686]). The Gene Choice Mass Ladder I is available for purchase from the UCCC DNA Sequencing & Analysis Core.

DNA Samples

Prepare three tubes of DNA as follows:

- One tube should contain 10 microliters of an undiluted sample of your DNA preparation. Estimate the DNA concentration estimated by the above methods.
- A second sample containing 100 microliters is made by diluting the first sample to a concentration of 1 nanogram/microliter in either water or 10 mM Tris-HCl pH 8.0 (Note: no EDTA).
- The third tube is a duplicate dilution of the DNA to 1 nanogram/microliter of which you will use 2-5 microliters to test whether the DNA works well as a PCR template in a 25 microliter PCR reaction. Choose a locus to amplify that you know amplifies well in your hands.

The Core will use the first sample (undiluted sample) to run on an agarose gel for confirmation of your quantification. The second sample (approximately 1 nanogram / microliter and which has not been opened after having been made) will be used by the Core for the multiplex PCR of the different STR loci.

Important Notes

- Please, remember to use PCR Clean conditions for the preparation of your DNA, including tubes, water solutions, etc.
- When submitting a sample for analysis, please place the 1.5 mL microcentrifuge tubes containing the DNA samples in two separate fresh plastic bags (one for the concentrated stocks and the second for the dilute DNAs)and labeled with the DNA concentration. The bags may be obtained from the DNA Sequencing Core. This is done to minimize the potential of DNA contamination by other Core DNAs.

References for DNA Extraction Procedure

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