BioSensing BioActuation BioNanotechnology Summer Institute 2012

University of Illinois at Urbana-Champaign
July 30 – August 10, 2012

MOLECULAR BIOLOGY LAB MODULE
Location: 3375 Micro and Nanotechnology Lab (MNTL)
Lead Instructor: Susan Steenbergen, Pathobiology
Lab Assistant: Bahaa Fadl-Alla, Pathobiology

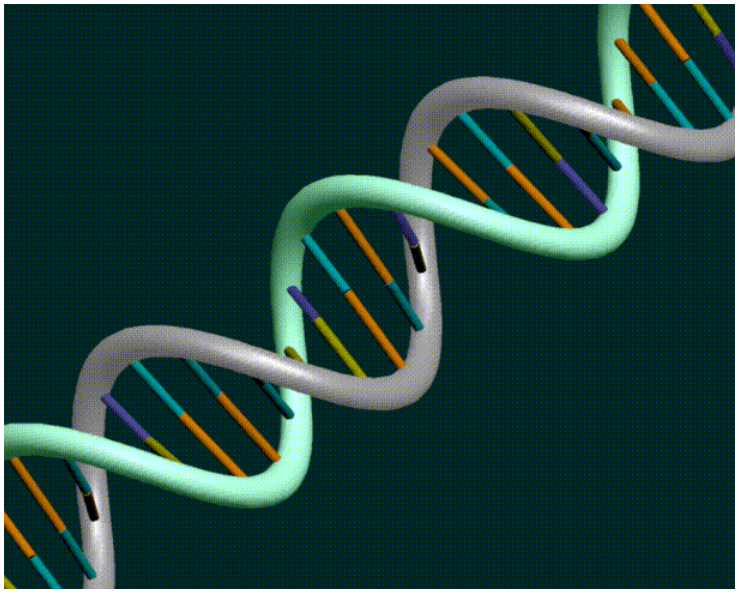
Purpose and Expected Outcome:

In this lab module you will:

- Learn how to isolate genomic DNA from gram negative bacteria
- Perform site-directed mutagenesis on the GFP gene by PCR
- Purify total RNA
- Run qRT-PCR reactions to demonstrate how this method is used to quantify the amount of message present in a sample

Please read the three selected articles taken from Current Protocols in Molecular Biology before attending the lab module sessions:

1. CHAPTER 2 - Preparation and Analysis of DNA
2. CHAPTER 15 - The Polymerase Chain Reaction
3. CHAPTER 4 - Preparation and Analysis of RNA



ISOLATION OF GENOMIC DNA FROM GRAM NEGATIVE BACTERIA

You will be given a 1.5 ml microcentrifuge tube containing a pellet of *Escherichia coli*, strain BW30270. This strain is a K-12 or laboratory strain of *E.coli*. The pellets are made up of cells from 3 mls of overnight growth in a rich medium.

1. Add 600 µl of Nuclei Lysis Solution. Gently pipet up and down until the cells are resuspended.
2. Incubate at 80°C for 5 minutes to lyse the cells; then cool to room temperature.
3. Add 3µl of RNase Solution to the cell lysate. Invert the tube 2–5 times to mix.
4. Incubate at 37°C for 30 minutes. Cool the sample to room temperature.
5. Add 200 µl of Protein Precipitation Solution to the RNase-treated cell lysate.
6. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate, be sure that the two solutions have completely mixed. Do not over-vortex or you risk shearing the chromosome.
7. Incubate the sample on ice for 5 minutes. Centrifuge at 13,000 rpm for 10 minutes.
8. Transfer the supernatant containing the DNA to a clean 1.5ml micro-centrifuge tube containing 600µl of room temperature isopropanol. Do not carry over any flecks of precipitate.
9. Gently mix by inversion until the thread-like strands of DNA form a visible mass. If you do not see threads but a general whitish appearish mix well and continue.
10. Centrifuge at 13,000 rpm for 5 minutes.
11. Carefully pour off the supernatant and drain the tube on clean absorbent paper. Add 600 µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA pellet.
12. Centrifuge at 13,000 rpm for 2 minutes. Carefully aspirate the ethanol from the tube with a pipet. Be careful not to suck up your pellet!
13. Drain the tube on kimwipes and allow the pellet to air-dry for 10–15 minutes.
14. Add 100µl of sterile water to the tube and rehydrate the DNA.

SITE-DIRECTED MUTAGENESIS BY PCR AMPLIFICATION

The first step in the mutagenesis of the *gfp* gene is to methylate the plasmid which carries the gene. This plasmid, pGLO, has been propagated in *E. coli* and then purified. We have done the methylation for you due to time constraints but the procedure is listed below. The methylase used transfers methyl groups from S-adenosylmethionine to cytosine residues occurring next to guanine. When DNA is methylated in this way, and then transformed into a wild-type strain of *E. coli* the DNA will be degraded. Thus after amplification of the plasmid with mutagenic primers, resulting in mutagenized plasmid that is not methylated, only this new plasmid DNA will be replicated in the cell.

Methylation reaction

Mix together:

- 100 ng of the pGLO plasmid
- 1.6 μl of 10X methylation buffer
- 1.6 μl of 10X S-adenosylmethionine (SAM)
- 1 μl DNA methylase
- water to 16 μl total volume

Incubate at 37°C for 1 hour.

<u>YOU WILL BEGIN AT THIS STEP</u>

Mutagenesis reaction

Mix together:

- 4 μl methylated plasmid
- 1 μl of pGLO primer mix
- 45 μl PCR Supermix High Fidelity

Cycling parameters:

1. 94° C for 2 min.
2. 94° C for 30 sec.
3. 55° C for 30 sec.
4. 68° C for 6 min.
5. back to step 2 for 20 more cycles
6. 68° C for 10 min.

After amplification is complete remove 17 μl of the PCR reaction to a clean microfuge tube, add 3 μl of tracking dye and run on an agarose gel to verify amplification. Next, 4 μl of the reaction is transformed into *E. coli* DH5α and plated on a rich media containing ampicillin and arabinose. The ampicilliin selects for the plasmid and the arabinose induces the transcription of the GFP gene. It is important to keep the cells on ice and chilled until the heat shock in step 4. Never vortex competent cells.

1. Put 4 μl of each ligation reaction in a sterile 1.5 ml tube and place on ice. Thaw competent cells on ice.
2. Transfer 50 μl of competent DH5α into each tube and gently mix by tapping the tube.
3. Incubate tubes on ice for 15 min.
4. Heat shock the cells for 3 min. at 42°C.
5. Return tubes to ice for 2 min.
6. Add 900 ml LB broth and incubate at 37°C for 1 hour.
7. Spin culture, remove most of the media, resuspend pellet in remaining meida and plate on LB + 100μg/ml Ampicillin + 0.6% Arabinose

PURIFICATION OF TOTAL RNA

We will be preparing RNA from PK (pig kidney) cultured cells, both cells that have been infected with the Aujeszky's Disease virus and cells that have not.

1. You will be given 2 samples of 175 μl of lysed tissue culture cells.
2. Add 350 μl SV RNA Dilution buffer. Mix by inverting 3-4 times. Incubate at 70°C for **3** minutes (no longer).
3. Centrifuge for 10 minutes at 13,000 rpm, transfer cleared lysate to a fresh tube.
4. Add 200 μl 95% ethanol and mix well.
5. Assemble a spin basket assembly. Transfer mix from step 4 to the assembly and centrifuge for 1 min. Discard fluid.
6. Add 600 μl of SV RNA wash solution (ethanol added). Centrifuge for 1 min, discard fluid.
7. Apply 50 μl DNase mix to the membrane. Incubate at RT for 15 min.
8. Add 200 μl SV DNase Stop solution, centrifuge 1 min.
9. Add 600 μl SV RNA Wash solution, centrifuge 1 min., discard fluid.
10. Add 250 μl SV RNA wash solution and centrifuge for 5 min. Transfer spin basket to elution tube.
11. Add 100 μl Nuclease-Free Water to membrane. Centrifuge for 1 min. to elute RNA. Place on ice immediately.

REAL TIME PCR REACTIONS

The qRT-PCR reactions we will be running will be done to demonstrate how this method is used to quantify the amount of message present in a sample. We will be amplifying a transcript of the Aujeszky's Disease virus from the RNA we purified above. In order to verify our quantification we will also amplify a "house-keeping" gene, ubiquitin. This gene is expressed at a constant rate in cells and so the amount of amplification products that are made should not change from the infected or uninfected cells. Thus, even though we add equivalent amounts of RNA from infected and uninfected cells to each tube, if the ubiquitin reactions do not give the same result we have a way to normalize the two different RNA samples. We will include 2 controls, a control with no reverse transcriptase added, to insure our amplification products are not coming from DNA, and a no template control, to insure none of the reagents are contaminated. We will use the GoTaq 1-step RT-qPCR system (Promega). The set up of the tubes is shown on the next page.

In order to get in all the controls necessary you will set up 6 tubes in the manner below.

	tube 1	tube 2	tube 3	tube 4	tube 5	tube 6
2 X GoTaq qPCR mix (contains reference dye)	10 μl	10 μl	10 μl	10 μl	10 μl	10 μl
Primer mix	2.5 μl of PRV primers	2.5 μl of PRV primers	2.5 μl of PRV primers	2.5 μl of PRV primers	2.5 μl of ubiquitin primers	2.5 μl of ubiquitin primers
RNA template	5 μl of uninfected	5 μl of infected	5 μl of uninfected (no RT)	5 μl of infected (no RT)	5 μl of uninfected	5 μl of infected
GoScript RT Mix	0.4 μl	0.4 μl	-	-	0.4 μl	0.4 μl
Water	2.1μl (add to a final total volume of 20 μl)	2.1μl	2.5 μl	2.5 μl	2.1μl	2.1μl

Samples will be run in an ABI7000 with the following cycles.

1 cycle of 95° C for 2 min.

40 cycles of 95° C for 15 sec. (denaturation) followed by 60° C for 1 min. (annealing and extension).

After the amplication cycles are complete the machine does a melting curve to insure that the dsDNA being measured is a real amplification product and not due to primer interaction, so called “primer-dimers”.

Good reference sources for Molecular Biology techniques

Molecular Cloning: A Laboratory Manual, Sambrook and Russell, Cold Spring Harbor Press.

Current Protocols in Molecular Biology, Ausubel et al editors, Wiley Interscience

PCR Primer: A Laboratory Manual, Dieffenbach and Dveksler editors, Cold Spring Harbor Press.

Companies for molecular biology products:

Promega Corporation
Invitrogen Corporation
Epicentre
USB, United States Biochemicals, now part of Affymetrix
Stratagene
New England Biolabs
Sigma Life Sciences
GE Healthcare (formerly Amersham)
Applied Biosystems

CHAPTER 2
Preparation and Analysis of DNA

INTRODUCTION

The ability to prepare and isolate pure DNA from a variety of sources is an important step in many molecular biology protocols. Indeed, the isolation of genomic, plasmid, or DNA fragments from restriction digests and polymerase chain reaction (PCR) products has become a common everyday practice in almost every laboratory. This chapter therefore begins with protocols for purification of genomic DNA from bacteria, plant cells, and mammalian cells (*UNITS 2.1-2.4*). These protocols consist of two parts: a technique to lyse the cells gently and solubilize the DNA, followed by one of several basic enzymatic or chemical methods to remove contaminating proteins, RNA, and other macromolecules. The basic approaches described here are generally applicable to a wide variety of starting materials. A brief collection of general protocols for further purifying and concentrating nucleic acids is also included.

The last decade has shown a dramatic departure from the use of traditional DNA purification methods outlined in *UNITS 2.2-2.4*, with a concomitant increase in the use of purpose-specific kits for the isolation and purification of DNA. For example, kits for purification of DNA using pre-made anion-exchange columns packaged with all necessary solutions to lyse the cells and solubilize the DNA are available from many molecular biology companies. A variety of kits based on binding of DNA to glass beads are also available. The uses of both types of kits are discussed in *UNIT 2.1B*.

The use of kits has two main advantages: it saves time and makes the process of DNA purification a relatively easy and straightforward process. The purification of DNA by anion-exchange chromatography (*UNIT 2.1B*) is readily becoming the accepted standard for quick and efficient large-scale (more than 100 μg of DNA) production of DNA from bacteria, mammalian tissue, and plant tissue. In most cases, the cell lysis and solubilization of DNA is relatively unchanged compared to traditional methods, with anion-exchange chromatography columns having replaced labor and time-intensive techniques such as cesium chloride centrifugation for the isolation of relatively pure DNA. Purification kits are usually available in several sizes and configurations, allowing the researcher to have variability concerning the processing and purification of their DNA.

A variety of techniques exist for the isolation of small amounts of plasmid DNA from minipreps and for DNA fragments from restriction digests/PCR products from agarose gels (with removal of unincorporated nucleoside triphosphates, reaction products, and small oligonucleotides from PCR reactions). These are detailed in *UNITS 2.1A, 2.1B, 2.6 & 2.7*. Likewise, kits are available from several molecular biology companies, usually based on silica-gel technology, for each of these applications (*UNIT 2.1B*). As with large-scale DNA isolation and purification, these kits provide a quick and efficient means to recover purified DNA that can be used for subsequent cloning or other modifications.

Virtually all protocols in molecular biology require, at some point, fractionation of nucleic acids. Chromatographic techniques are appropriate for some applications and may be used for separation of plasmid from genomic DNA as well as separation of genomic DNA from debris in a cell lysate (*UNIT 2.1B*). Gel electrophoresis, however, has much greater

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resolution than alternative methods and is generally the fractionation method of choice. Gel electrophoretic separations can be either analytical or preparative, and can involve fragments with molecular weights ranging from less than 1000 Daltons to more than 10^8. A variety of electrophoretic systems have been developed to accommodate such a large range of applications.

In general, the use of electrophoresis to separate nucleic acids is simpler than its application to resolve proteins. Nucleic acids are uniformly negatively charged and, for double-stranded DNA, reasonably free of complicating structural effects that affect mobility. A variety of important variables affect migration of nucleic acids on gels. These include the conformation of the nucleic acid, the pore size of the gel, the voltage gradient applied, and the salt concentration of the buffer. The most basic of these variables is the pore size of the gel, which dictates the size of the fragments that can be resolved. In practice, this means that larger-pore agarose gels are used to resolve fragments >500 to 1000 bp (*UNITS 2.5A & 2.6*) and smaller-pore acrylamide or sieving agarose gels (*UNIT 2.7*) are used for fragments <1000 bp. A protocol for resolution of very large pieces of DNA may also be resolved on agarose gels using pulsed-field gel electrophoresis (*UNIT 2.5B*). Finally, the powerful analytical technique of capillary electrophoresis of DNA (*UNIT 2.8*) may be used to assess the purity of synthetic oligonucleotides, analyze quantitative PCR results, and compare DNA fragment lengths from restriction fragment length polymorphism (RFLP) and variable number of tandem repeat (VNTR) analyses.

Frequently it is desirable to identify an individual fragment in a complex mixture that has been resolved by gel electrophoresis. This is accomplished by a technique termed Southern blotting, in which the fragments are transferred from the gel to a nylon or nitrocellulose membrane and the fragment of interest is identified by hybridization with a labeled nucleic acid probe. Section IV of this chapter gives a complete review of methods and materials required for immobilization of fractionated DNA (*UNIT 2.9*) and associated hybridization techniques (*UNIT 2.10*). These methods have greatly contributed to the mapping and identification of single and multicopy sequences in complex genomes, and facilitated the initial eukaryotic cloning experiments.

Other commonly encountered applications of gel electrophoresis include resolution of single-stranded RNA or DNA. Polyacrylamide gels containing high concentrations of urea as a denaturant provide a very powerful system for resolution of short (<500-nucleotide) fragments of single-stranded DNA or RNA. Such gels can resolve fragments differing by only a single nucleotide in length, and are central to all protocols for DNA sequencing (see *UNIT 7.6*). Such gels are used for other applications requiring resolution of single-stranded fragments, particularly including the techniques for analyzing mRNA structure by S1 analysis (*UNIT 4.6*), ribonuclease protection (*UNIT 4.7*), or primer extension (*UNIT 4.8*). Denaturing polyacrylamide gels are also useful for preparative applications, such as small-scale purification of radioactive single-stranded probes and large-scale purification of synthetic oligonucleotides (*UNIT 2.12*).

Resolution of relatively large single-stranded fragments (>500 nucleotides) can be accomplished using denaturing agarose gels. This is of particular importance to the analysis of mRNA populations by northern blotting and hybridization. A protocol for use of agarose gels containing formaldehyde in resolution of single-stranded RNA is presented in *UNIT 4.9*. The use of denaturing alkaline agarose gels for purification of labeled single-stranded DNA probes is described in *UNIT 4.6*.

Gels and Electric Circuits

Gel electrophoresis units are almost always simple electric circuits and can be understood using two simple equations. Ohm's law, $V = IR$, states that the electric field, V (measured in volts), is proportional to current, I (measured in milliamps), times resistance, R (measured in ohms). When a given amount of voltage is applied to a simple circuit, a constant amount of current flows through all the elements and the decrease in the total applied voltage that occurs across any element is a direct consequence of its resistance. For a segment of a gel apparatus, resistance is inversely proportional to both the cross-sectional area and the ionic strength of the buffer. Usually the gel itself provides nearly all of the resistance in the circuit, and the voltage applied to the gel will be essentially the same as the total voltage applied to the circuit. For a given current, decreasing either the thickness of the gel (and any overlying buffer) or the ionic strength of the buffer will increase resistance and, consequently, increase the voltage gradient across the gel and the electrophoretic mobility of the sample.

A practical upper limit to the voltage is usually set by the ability of the gel apparatus to dissipate heat. A second useful equation, $P = I^2R$, states that the power produced by the system, P (measured in watts), is proportional to the resistance times the square of the current. The power produced is manifested as heat, and any gel apparatus can dissipate only a particular amount of power without increasing the temperature of the gel. Above this point small increases in voltage can cause significant and potentially disastrous increases in temperature of the gel. It is very important to know how much power a particular gel apparatus can easily dissipate and to carefully monitor the temperature of gels run above that level.

Two practical examples illustrate applications of the two equations. The first involves the fact that the resistance of acrylamide gels increases somewhat during a run as ions related to polymerization are electrophoresed out of the gel. If such a gel is run at constant current, the voltage will increase with time and significant increases in power can occur. If an acrylamide gel is being run at high voltage, the power supply should be set to deliver constant power. The second situation is the case where there is a limitation in number of power supplies, but not gel apparati. A direct application of the first equation shows that the fraction of total voltage applied to each of two gels hooked up in series (one after another) will be proportional to the fraction of total resistance the gel contributes to the circuit. Two identical gels will each get 50% of the total voltage and power indicated on the power supply.

Finally, it should be noted that some electrophoretic systems employ lethally high voltages, and almost all are potentially hazardous. It is very important to use an adequately shielded apparatus, an appropriately grounded and regulated power supply, and most importantly, common sense when carrying out electrophoresis experiments.

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CHAPTER 15
The Polymerase Chain Reaction

INTRODUCTION

The polymerase chain reaction (PCR) is a rapid procedure for in vitro enzymatic amplification of a specific segment of DNA. Like molecular cloning, PCR has spawned a multitude of experiments that were previously impossible. The number of applications of PCR seems infinite—and is still growing. They include direct cloning from genomic DNA or cDNA, in vitro mutagenesis and engineering of DNA, genetic fingerprinting of forensic samples, assays for the presence of infectious agents, prenatal diagnosis of genetic diseases, analysis of allelic sequence variations, analysis of RNA transcript structure, genomic footprinting, and direct nucleotide sequencing of genomic DNA and cDNA.

The theoretical basis of PCR is outlined in Figure 15.0.1. There are three nucleic acid segments: the segment of double-stranded DNA to be amplified and two single-stranded oligonucleotide primers flanking it. Additionally, there is a protein component (a DNA polymerase), appropriate deoxyribonucleoside triphosphates (dNTPs), a buffer, and salts.

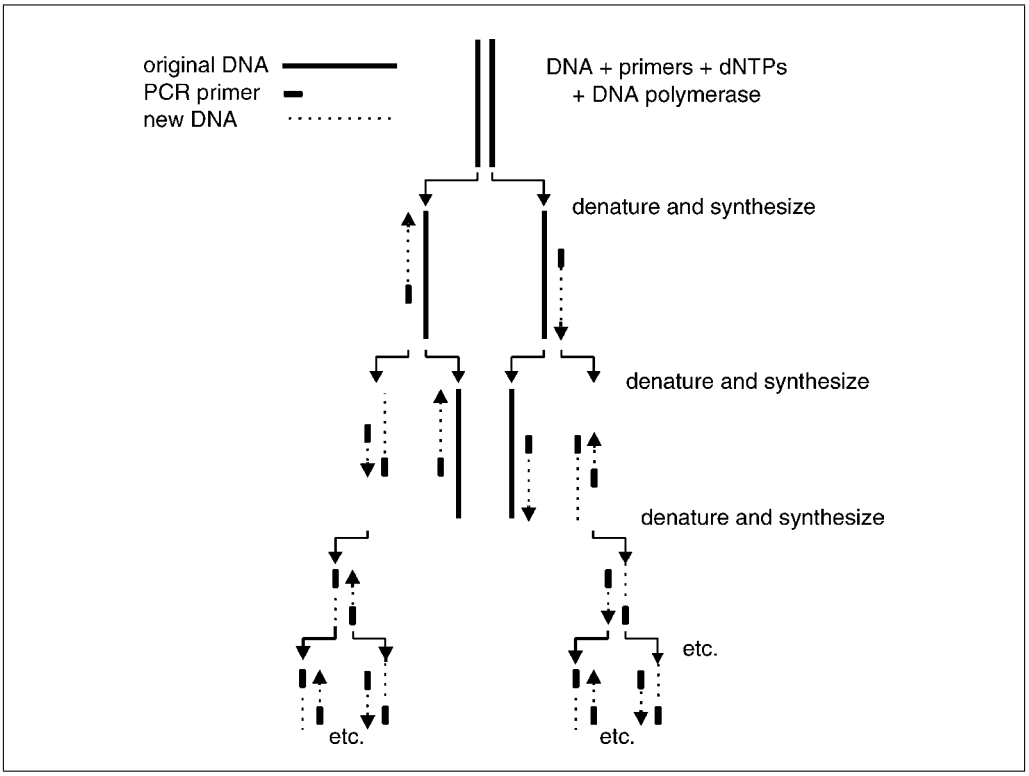

Figure 15.0.1 The polymerase chain reaction. DNA to be amplified is denatured by heating the sample. In the presence of DNA polymerase and excess dNTPs, oligonucleotides that hybridize specifically to the target sequence can prime new DNA synthesis. The first cycle is characterized by a product of indeterminate length; however, the second cycle produces the discrete "short product" which accumulates exponentially with each successive round of amplification. This can lead to the many million-fold amplification of the discrete fragment over the course of 20 to 30 cycles.

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The primers are added in vast excess compared to the DNA to be amplified. They hybridize to opposite strands of the DNA and are oriented with their 3′ ends facing each other so that synthesis by DNA polymerase (which catalyzes growth of new strands 5′→3′) extends across the segment of DNA between them. One round of synthesis results in new strands of indeterminate length which, like the parental strands, can hybridize to the primers upon denaturation and annealing. These products accumulate only arithmetically with each subsequent cycle of denaturation, annealing to primers, and synthesis.

However, the second cycle of denaturation, annealing, and synthesis produces two single-stranded products that together compose a discrete double-stranded product which is exactly the length between the primer ends. Each strand of this discrete product is complementary to one of the two primers and can therefore participate as a template in subsequent cycles. The amount of this product doubles with every subsequent cycle of synthesis, denaturation, and annealing, accumulating exponentially so that 30 cycles should result in a 2^{28}-fold (270 million–fold) amplification of the discrete product.

This chapter consists of protocols that cover some of the more common applications of PCR. For many applications, the first step is simply to get PCR working with a known segment of DNA and a set of primers. Therefore, *UNIT 15.1* presents a basic PCR protocol and ways to optimize it for the sequence of interest.

PCR permits direct sequencing of nucleic acids without requiring cloning, thus avoiding cloning difficulties and artifacts. Several different protocols for preparing PCR products for sequencing using either dideoxy (Sanger) sequencing methods or chemical (Maxam-Gilbert) methods are presented in *UNIT 15.2*. This unit should permit the practitioner to choose a protocol best suited to the problem at hand and to his or her taste.

Several PCR methods have been developed that require knowledge of only a small stretch of sequence (30-40 bases) and add sequence to the ends of amplified molecules to facilitate analyses. One of these, ligation-mediated PCR (*UNIT 15.3*) has broad applications including genomic footprinting and sequencing.

PCR can be used to help clone and manipulate sequences. Various methods for generating suitable ends to facilitate the direct cloning of PCR products are detailed in *UNIT 15.4*. Other protocols for cloning and mutagenesis of DNA using PCR can be found in *UNIT 3.7* and *UNIT 8.5*.

An important application of PCR is to detect RNA transcripts, analyze their structure, and amplify their sequences to permit cloning and/or sequencing. *UNIT 15.5* presents procedures that adapt PCR to RNA templates, via production of a cDNA copy of the RNA by reverse transcriptase (RT-PCR). Anchored PCR, which, like ligation-mediated PCR, requires little knowledge of sequence and makes use of the ends of nucleic acids, is applied in *UNIT 15.6* to analysis of mRNAs.

PCR is frequently used because it is the most sensitive assay for rare sequences. A protocol that not only detects rare DNAs but quantitates them as well is presented in *UNIT 15.7*. The downside of sensitivity is contamination by infinitesimal amounts of unwanted exogenous sequences. Procedures designed to avoid contamination with undesired DNA sequences are emphasized in this unit.

A newer method to quantitate nucleic acids is real-time PCR. This approach, which takes advantage of instrumentation that can measure increases in fluorescence during many amplification reactions simultaneously, provides results much more quickly than older methods. *UNIT 15.8* presents procedures for relative and absolute quantitation of RNA using high-throughput real-time RT-PCR.

Applications of PCR that entail discovery and analysis of differentially expressed genes and assays from single cells can be found in Chapter 25. Other applications of PCR can be found in many other chapters of *Current Protocols in Molecular Biology*, including Chapters 3, 7, 8, 12-14, 16, 21, 22, and 24.

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CHAPTER 4
Preparation and Analysis of RNA

INTRODUCTION

The ability to isolate clean intact RNA from cells is essential for experiments that measure transcript levels, for cloning of intact cDNAs, and for functional analysis of RNA metabolism. RNA isolation procedures frequently must be performed on numerous different cell samples, and therefore are designed to allow processing of multiple samples simultaneously. This chapter begins by describing several methods commonly used to isolate RNA, and concludes with methods used to analyze RNA expression levels and synthesis rates.

The difficulty in RNA isolation is that most ribonucleases are very stable and active enzymes that require no cofactors to function. The first step in all RNA isolation protocols therefore involves lysing the cell in a chemical environment that results in denaturation of ribonuclease. The RNA is then fractionated from the other cellular macromolecules under conditions that limit or eliminate any residual RNase activity. The cell type from which RNA is to be isolated and the eventual use of that RNA will determine which procedure is appropriate. No matter which procedure is used, it is important that the worker use care (e.g., wearing gloves) not to introduce any contamination that might include RNase during work up of the samples, and particularly when the samples are prepared for storage at the final step.

While the RNA isolation protocols describe methods that can be performed using common laboratory reagents, several kits for RNA isolation are commercially available. These kits offer the dual advantage of ease of use and (at least in theory) of reagents that have been tested for effectiveness. These kits frequently work well and are widely used. The disadvantages of using kits are that they are more expensive per sample than isolations that are done using "home made" solutions, and that the kits do not offer flexibility for cell types that require special conditions. The cost disadvantage is frequently outweighed in situations where only a few RNA isolations are performed; however, preparing reagents from scratch can take time, and in the event that any of the reagents are not working properly, troubleshooting will require further time. In situations where numerous samples are routinely processed, significant cost savings can be realized by avoiding the use of kits.

One of the primary uses of RNA isolation procedures is the analysis of gene expression. In order to elucidate the regulatory properties of a gene, it is necessary to know the structure and amount of the RNA produced from that gene. The second part of this chapter is devoted to techniques that are used to analyze RNA. Procedures such as S1 nuclease analysis and ribonuclease protection can be used to do fine-structure mapping of any RNA. These techniques allow characterization of 5′ and 3′ splice junctions as well as the 5′ and 3′ ends of RNA. Both of these procedures, as well as northern analysis, can also be used to accurately determine the steady-state level of any particular message.

After determining the steady-state level of a message, many investigators wish to examine whether that level is set by the rate of transcription of the gene. Alterations

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in steady-state level might also reflect changes in processing or stability of the RNA. The final section of the chapter describes the "nuclear run-off" technique, which determines the number of active RNA polymerase molecules that are traversing any particular segment of DNA. This procedure is used to analyze directly how the rate of transcription of a gene varies when the growth state of a cell is changed.

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