Complementary DNA

A complementary DNA (cDNA) sequencing project was also initiated at the University of New Mexico and Oklahoma State University.

From: Advances in Applied Microbiology, 2002

Related terms:

Polymerase Chain Reaction, Enzymes, Mutation, Gene Expression, Proteins, DNA, RNA, Phosphoproteins, Messenger RNA

View all Topics

Genomics and Gene Expression

David P. Clark, Nanette J. Pazdernik, in Biotechnology (Second Edition), 2016

Hybridization ON DNA Microarrays

Hybridization on a microarray is similar to what occurs during other hybridization procedures, such as Southern blots or Northern blots. All these techniques rely on the complementary nature of nucleic acid bases. When two complementary strands of DNA or RNA are alongside each other, the bases match up with their complement, that is, thymine (or uracil) with adenine, and guanine with cytosine. On a DNA microarray, hybridization is affected by the same parameters as in these other techniques.

How the DNA is attached to the slide can affect how well the probe DNA and target DNA hybridize, especially for oligonucleotide microarrays (Fig. 8.24). The short length of oligonucleotides requires that the entire piece be accessible to hybridize. The length of the spacer between the oligonucleotides and the glass slide optimizes hybridization. An oligonucleotide attached with a short spacer has many of its initial nucleotides too close to the glass and inaccessible to incoming RNA or DNA. Oligonucleotides with longer spacers may fold back and tangle up. Oligonucleotides with medium-sized spacers are far enough from the glass, but not so far as to get tangled. Thus, medium-sized spacers give the best access for hybridization.

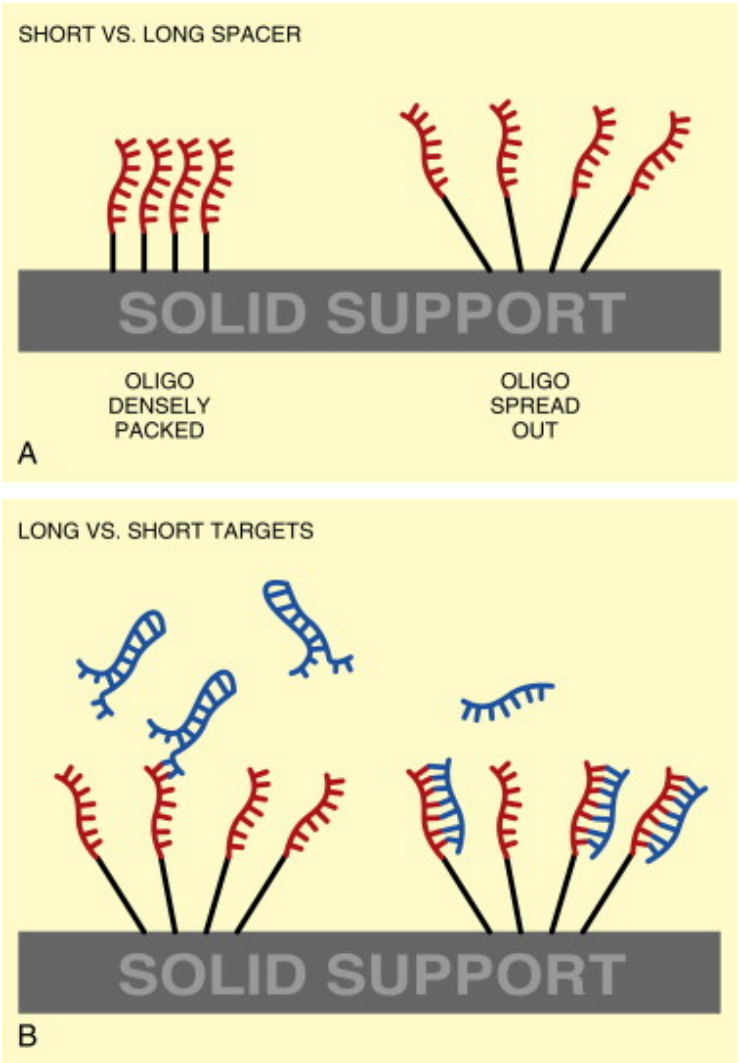

FIGURE 8.24. Length of Spacers and Target Molecules Affect Hybridization on Microarrays(A) When the spacer between the glass slide and oligonucleotide is too short, the oligonucleotides are condensed and not accessible to hybridize. If the spacer region is too long, the oligonucleotides and spacers tangle and fold, preventing optimal hybridization. (B) When the target for hybridization is too long, the target sequences may form hairpins with themselves rather than bind to the array oligonucleotides.

Hybridization of two lengths of DNA (or RNA with DNA) depends on certain sequence features. One important property is the ratio of A:T base pairs to G:C base pairs. G:C base pairs have three hydrogen bonds holding them together, whereas A:T base pairs have only two hydrogen bonds. Thus, more GC base pairs give stronger hybridization. If the sequence has too many A:T base pairs, the duplex may form slowly and be less stable. Another important consideration is secondary structure. If the probe sequence can form a hairpin structure, it will hybridize poorly with the target. If the probe has several mismatches relative to the target, the duplex may not form efficiently. All these issues must be addressed when making an oligonucleotide microarray. Computer programs are available to identify suitable regions of genes with sequences that will produce effective probes.

cDNA arrays are less prone to the problems seen in oligonucleotide arrays. cDNAs are double-stranded, so secondary structures such as hairpins are less likely to be a problem. During a hybridization reaction, cDNA arrays must be denatured either with heat or chemicals, making the probes single-stranded. Then the single-stranded RNA samples are allowed to hybridize on the slide under conditions that promote duplex RNA:cDNA without any mismatches.

Oligonucleotide microarrays must have a sufficient spacer and little secondary structure in order to hybridize with the samples.

> Read full chapter

Recombinant DNA and Biotechnology

John W. Pelley, in Elsevier's Integrated Review Biochemistry (Second Edition), 2012

Complementary DNA

Complementary DNA (cDNA) is synthesized in the laboratory from messenger RNA (Fig. 18-3). cDNA is not genomic DNA, because the transcript of genomic RNA has been processed (i.e., it lacks promoters and introns). The enzyme reverse transcriptase (see Chapter 15) is used to synthesize double-stranded DNA that is a complementary copy of the mRNA. The addition of linker sequences to the end of this DNA, which contain the restriction site, followed by treatment with a restriction enzyme, produces a cDNA preparation with cohesive ends ready for insertion into a vector. A preparation of cDNA represents the genes that were actively being expressed in a cell, an organ, or a whole organism at the time of harvesting and is called a cDNA library.

> Read full chapter

Gastrointestinal Peptides

Celia Chao, Mark R. Hellmich, in Physiology of the Gastrointestinal Tract (Fifth Edition), 2012

6.2.6.1 Cloning

The cDNA sequences for both the rat CCK_1 receptor[128] and dog gastrin receptor-[129] were first reported in early 1992. Later that same year, CCK_2 receptor cDNA clones were isolated from the rat brain and the rat pancreatic acinar carcinoma cell

line, AR4-2J, using a low-stringency hybridization procedure with a CCK_1 receptor probe.[130] Comparison of the dog gastrin receptor and rat CCK_2 receptor cDNA sequences, as well as their binding and signaling properties, demonstrated that they were interspecies homologs of the same receptor subtype. Subsequently, the human CCK_2 receptor cDNA was cloned[131] and its genomic DNA sequence determined.[132]

> Read full chapter

Nucleic Acids and Molecular Genetics

Gerald Litwack PhD, in Human Biochemistry (Second Edition), 2022

Probing Libraries for Specific Genes

A complementary DNA **(cDNA) probe** can be generated from a specific mRNA. The mRNA, encoding a specific protein, is a template. By the action of **reverse transcriptase** and **DNA polymerase**, a cDNA is formed that can be used as a probe to hybridize with a specific gene sequence (Fig. 10.49).

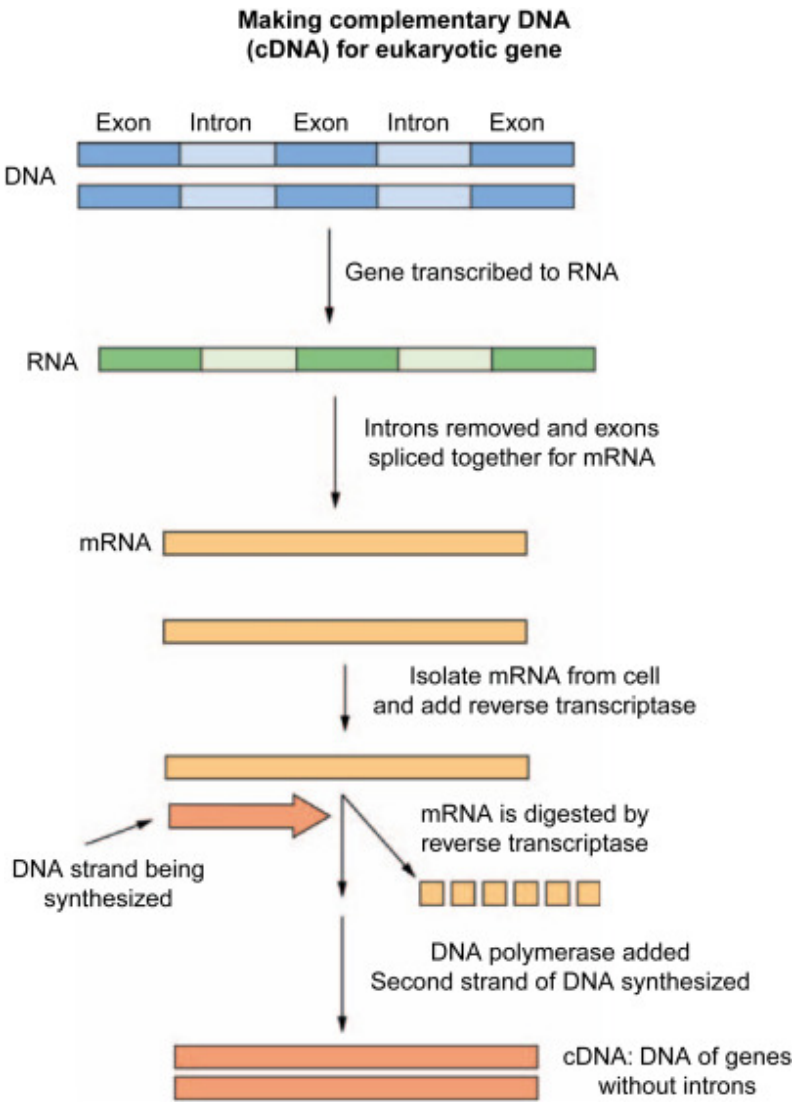

Figure 10.49. Generation of a cDNA from an isolated eukaryotic gene. *cDNA*, Complementary DNA.

Generally, the cDNA probe will be labeled, more recently, with a fluorescent tag that does not interfere with the hybridization reaction. Such a cDNA can be used to probe a library of cDNAs for a complementary sequence, either to find a longer sequence containing more information or to search out a full coding region of the gene.

> Read full chapter

Some Basic Tools: How to Isolate, Cut, Paste, Copy, Measure, Visualize, and Clone DNA

Marjorie A. Hoy, in Insect Molecular Genetics (Fourth Edition), 2019

5.19 Complementary DNA (cDNA) Cloning

cDNA is DNA that is *complementary* to the mRNA transcribed from the gene. Because mRNA is processed, it will lack introns and regulatory elements. cDNA cloning can produce a cDNA library or produce probes for screening genomic libraries.

A **cDNA library** allows the entomologist to clone *only those genes that are active at a specific time or in specific tissues*. Genes that are not transcribed into mRNA will not be represented. Thus, a cDNA library usually contains fewer clones than a genomic library. The gene may occur in a frequency of one in 10^3 or one in 10^4 clones. By contrast, a single-copy gene may be present in a genomic library in a frequency of only one in 10^5 to one in 10^6 clones. A benefit of a cDNA library is that it is possible, if an appropriate expression vector is used, to express a gene in a host such as *E. coli,* insect cells, or yeast. This enables the genetic engineer to produce large amounts of a specific gene product (protein).

The quality of a cDNA library depends on the quality of mRNA used as the template and the fidelity with which it can be **reverse transcribed** into cDNA. Messenger RNA, together with a suitable primer, and a supply of deoxyribonucleoside triphosphates can be converted into a ds DNA molecule with the enzyme **reverse transcriptase** (Figure 5.10).

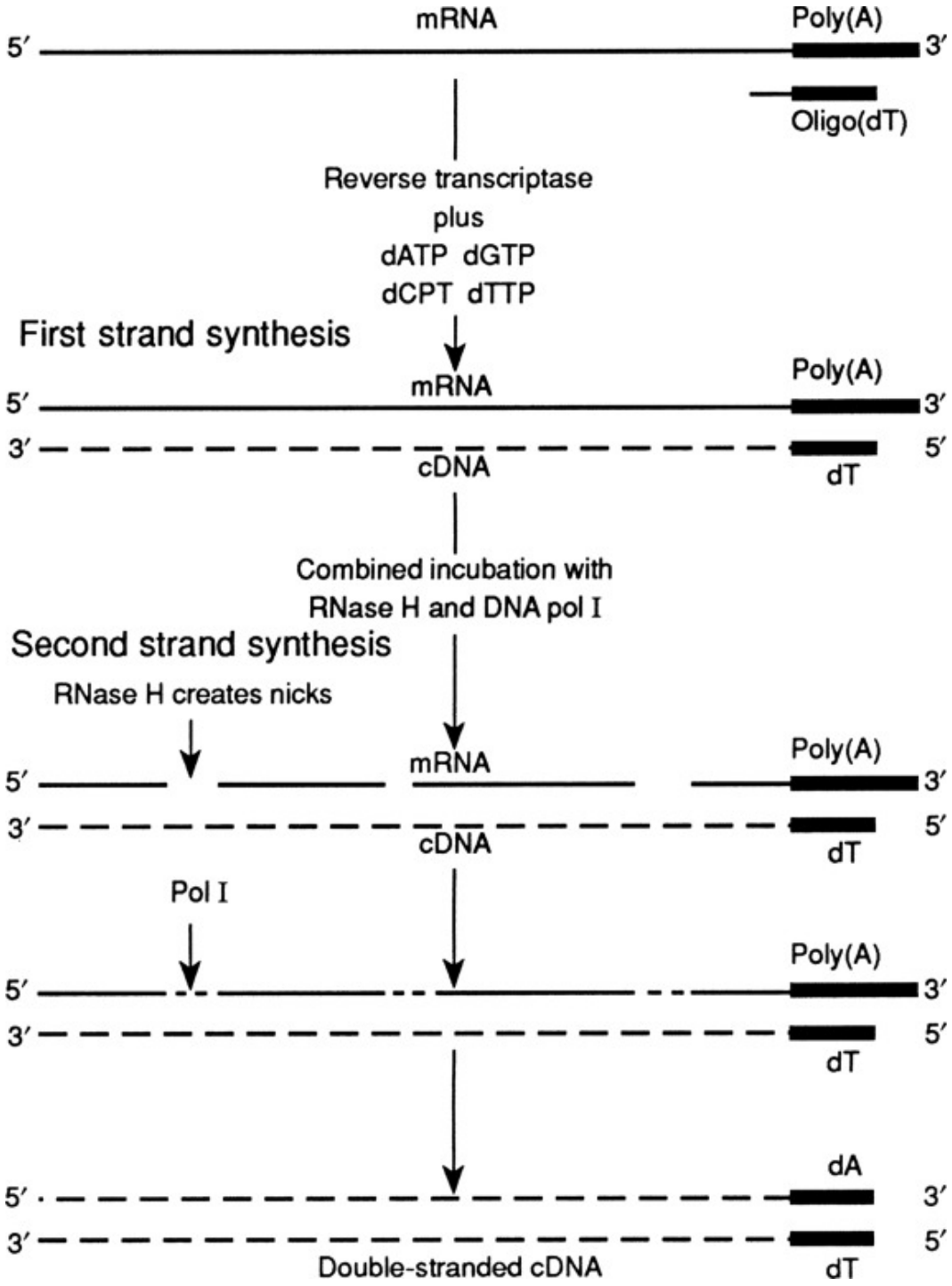

Figure 5.10. cDNA cloning involves two steps. In the first, an oligo (dT) anneals to the poly(A) region of mRNA. The enzyme reverse transcriptase and dATPs, dCTPs, dGTPs, and dTTPs are provided to produce the first cDNA strand. Synthesis of the second strand involves additional incubation with RNAse H and DNA polymerase I. The double-stranded cDNA produced is a complement of the mRNA and thus lacks introns or regulatory sequences.

The cDNA cloning process involves two steps: (1) the first strand of cDNA is produced, and (2) a strand complementary to the first strand is then synthesized, so that a ds DNA molecule is produced. The primer used to synthesize the first DNA strand

is usually an oligonucleotide consisting of deoxythimidine residues (dT) because it can hybridize to the 3□ poly(A) tails of template mRNA and, potentially, give rise to full-length copies of ds DNA. Once the ds cDNA molecule has been synthesized it can then be inserted into a plasmid or phage vector capable of replicating in *E. coli* and, in some cases, of being translated into a protein.

A key to producing cDNA is the enzyme **reverse transcriptase**. Reverse transcriptase is capable of two functions in vitro: a polymerase activity and a ribonuclease H activity. The polymerase activity requires: (1) template RNA hybridized to a DNA primer with a 3□-OH group, and (2) all four dNTPs to synthesize a DNA molecule which is a faithful *complement* of the mRNA.

Cloning a cDNA library is more complex than cloning a genomic library. The goals of the project must be carefully considered. For example, if antibodies will be used to identify clones capable of synthesizing specific peptides, the cDNA should be cloned into expression vectors to produce proteins.

Cloning techniques vary in the type of primer used, the method for second-strand synthesis, and methods for coupling the cDNA to the vector, which can be either a plasmid or □. Commercially available reverse transcriptases can synthesize copies of mRNA sequences > 3 kb long. However, the transcripts often terminate prematurely, making clones containing the 5□-end of the mRNA rare.

Figure 5.10 outlines synthesis of double-stranded cDNA from mRNA. Messenger RNA can be prepared for cDNA cloning by affinity chromatography on oligo (dT) cellulose (or by commercially available kits). The reaction is preceded by a brief heat denaturation of the mRNA to eliminate secondary structure, because reverse transcriptase is inhibited if mRNA exhibits secondary structure. The polyadenylated mRNA, the primer, and the reverse transcriptase are combined. The primer in this case is a short sequence of (dT) residues. The product of the first-strand synthesis is a hybrid of mRNA and the synthesized cDNA. The first strand is used as a template-primer complex to make the second strand of DNA. The enzyme RNase H is used to introduce gaps in the mRNA strand. At the same time, DNA polymerase I uses the primer-template complexes formed by RNase H to synthesize a double-stranded DNA. Once the double-stranded DNA is synthesized, it is inserted into a vector. To insert it into a vector, the synthesized molecule needs to have ends that can be ligated into the vector.

There are about 10,000 *different* mRNA molecules in an average insect cell. At least 200,000 cDNA clones should be generated produce a representative cDNA library. If the desired clone is a single-copy gene, then it will be rare, so powerful screening methods are required to isolate that clone.

Isolating RNA can be more difficult than isolating DNA. Preparation of mRNA requires the absolute elimination of **ribonucleases** (RNases) from glassware, pipets,

tips, and solutions. Anything that might contaminate the reactions with RNase must be eliminated, including hair, dust, and sneezes. Even fingerprints contain enough RNase to degrade your RNA. Furthermore, RNase is a very hardy enzyme and difficult to eliminate. Phenol extraction followed by ethanol precipitation was a common technique for isolating RNA, but various kits designed for that purpose are available now. Once RNA has been isolated, it must be evaluated for quality, often by agarose gel electrophoresis.

> Read full chapter

Expression Arrays: Discovery and Validation

Neal M. Poulin, Torsten O. Nielsen, in Cell and Tissue Based Molecular Pathology, 2009

SPOTTED COMPLEMENTARY DNA MICROARRAYS

cDNA arrays are manufactured from the polymerase chain reaction (PCR) products of bacteriophage cDNA libraries, of which several suppliers maintain extensive collections available in microwell format. Collections are extensively curated and chosen to represent as many unique transcripts as possible, with preference to known transcripts and mapped positions but including uncharacterized and possibly chimeric sequences. cDNAs are long probes, 500 base pairs to 2 kilobases in length, and are generally 3□ biased because of the limitations of reverse transcription.

Probe clones are amplified by PCR within microwell plates, and the products are placed in a denaturing buffer and spotted by a simple robot. The robotics are designed to precisely position an array of micromachined capillary “pens.” Pens are dipped into the cDNA microwells, where they pick up a reproducible quantity of liquid. With submicron precision, the robot then repositions the pens at the appropriate point above the microarray and deposits cDNA by contacting the surface.

Although they have provided extraordinary insights and have enabled rapid progress, cDNA microarrays do have significant disadvantages. cDNA libraries are difficult to maintain, and cross-contamination of clones is common and almost inevitable. Multiple cross-infections and inviable plasmids are commonly observed, and each clone must be validated with every PCR run. Usually, a small fraction of suspect spots is associated with each print.

Another major problem with cDNA arrays is that probe affinities are widely variable across the array; thus, there may be numerous poorly performing probes.

Probe–probe interactions can cause difficulties: probes are composed of double-stranded PCR products, so the antisense strand is present on the array and may contribute to probe–probe hybridizations. Further probe–probe interactions are ascribed to polyadenine–polythymine tracts, which are present in cDNA clones (because of oligo-deoxythymidine [oligo-dt] priming of reverse transcriptase). In addition, cDNA probes are of widely variable affinity because of uncontrolled variations in length and guanine–cytosine content. For these reasons, only relative measures of transcript abundance are practical with these arrays.

The specificity of hybridization to cDNA probes is also not ideal, and significant cross-hybridization has been shown among members of the same gene family. Depending on the specific sequence, this is estimated to be common for genes sharing more than 80% homology over the breadth of the probe.

Spotted cDNA microarrays have nonetheless served as reliable and reproducible hybridization platforms. This largely must be attributed to extensive curation of probe collections and efforts to empirically validate probe response. In particular, when there have been discordant results among array platforms, cDNA results have served as an indispensable reference source. They have also served as an important counterpoint to commercial platforms and have until recently been the only source of publicly available probe sequences.

> Read full chapter

Neurobiology of Steroids

Suresh M. Nair, James H. Eberwine, in Methods in Neurosciences, 1994

Slot-Blot Preparation

Plasmid cDNA clones corresponding to various candidate mRNA sequences are prepared using large-scale (maxipreparation) procedures. Each cDNA clone is linearized using the appropriate restriction enzyme, and stored until further use in 10× SSC buffer. Prior to loading on slot blots, the linearized cDNA in 10 × SSC is heat denatured (85°C for 5 min) to remove secondary structure and 1 μ g of total DNA is loaded per slot under vacuum. The cDNAs are then fixed to the blots by baking them for 4 hr at 80°C under vacuum.

> Read full chapter

Volume II

Shuaib Latif, Daniel J. Garry, in Heart Development and Regeneration, 2010

III.A Complementary DNA (cDNA) Microarrays

Complementary DNA (cDNA) microarrays were the first platform used to rapidly analyze a large number of transcripts from Arabidopsis thaliana (Schena et al., 1995). Since this initial report in 1995, cDNA microarrays have been used with exponentially increasing frequency in studies ranging across a number of species (Fig. 1B). Typically, this platform utilizes PCR to amplify cDNA libraries and the individual amplicons are spotted onto a glass slide using a robotic system to minimize artifacts and efficiently print a number of chips at one time (Fig. 2). The spots containing the PCR products (cDNAs) are approximately 200 microns in diameter, thus allowing 20–40,000 cDNA spots to be placed on one glass microscope slide. The utilization of cDNA libraries allows for the customization of the arrays, which can be produced from engineered cell populations, genetic mouse models or diseased tissue (hearts with congenital or acquired defects) (Hwang et al., 2002; Peng et al., 2002; Zhao et al., 2002; Tabibiazar et al., 2003). Typically, two samples (consisting of total cellular RNA isolated from different cell populations or tissues) are used to generate first strand cDNA, and each sample is then labeled with one or the other of two unique fluorescent dyes (e.g., Cy3 or Cy5). Equal amounts of the Cy3- and Cy5-labeled samples are hybridized to the same chip, thus allowing competitive binding of the fluorescently-labeled cDNAs to the array (Fig. 2). The hybridized chip is then washed, scanned (using a high-resolution laser system) and analyzed for the intensity of the respective fluorescent labels for each spot (Schinke et al., 2003) (Fig. 2).

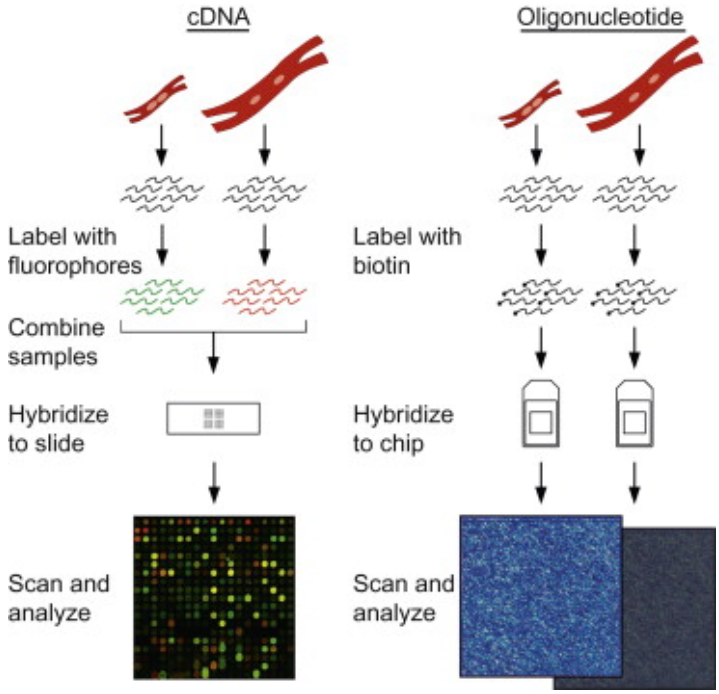

Figure 2. Strategies using cDNA and oligonucleotide arrays for transcriptome analysis. The basic steps using cDNA or oligonucleotide arrays are highlighted to compare

the transcript expression in two samples. Note that the total cellular RNA isolated from two separate samples is hybridized to the same chip for cDNA arrays. The samples competitively hybridize to the chip and result in green, red and yellow (no change) signals. In contrast, two samples can be independently processed and hybridized to two separate chips for the oligonucleotide arrays.

There are a number of advantages of the cDNA array platform (Table 1). The principle advantage is the ability for the investigator to customize the array and directly compare the transcript expression of two separate samples. The cost-per-slide is affordable and, in contrast to other technologies (i.e., differential display and SAGE), is labor-saving (i.e., results can be obtained within days). The limitations of the technology include the amount of RNA that is required, and that only two samples can be compared in any one experiment (Fig. 2; Table 1). Since 1995, the cDNA arrays have been broadly applied for the analysis of gene expression and have been an important technology for studies directed towards an enhanced understanding of cardiac morphogenesis and cardiac repair.

Table 1. Transcriptional Expression Analysis Methods

	Differential display	**SAGE**	**cDNA microarray**	**Oligonucleotide microarray**
Sample comparisons	Any	Any	Two	Any
Amount of starting RNA	5 μg total	5 μg poly-A	1 μg poly-A	1–8 μg
Cost	Low	Medium	High	Medium to high
Complexity of technique	Low	High	High	Medium
Reproducibility	Medium	Medium	Medium	High
Detection of novel genes	Yes	Yes	Possible	No
Global genetic profiling	No	Yes	Yes	Yes
Detection of differential splicing	Yes	No	Possible	Possible
Customization	Yes	Yes	Yes	No

> Read full chapter

The Cell

Tetsuya Watanabe, in Biophysical Basis of Physiology and Calcium Signaling Mechanism in Cardiac and Smooth Muscle, 2018

4.22 Reverse Transcription Polymerase Chain Reaction

Reverse transcription polymerase chain reaction (RT-PCR) is used to make a complementary DNA (cDNA) from the extracted mRNA for finding the specific protein-coding gene or for our cell to acquire the protein encoded in the mRNA. The first step to produce cDNA is to extract mRNA from a tissue and to bind oligo dT primer which is a short single strand of poly-thymine nucleotide onto the poly-A tail of the mature mRNA template. Transcribing the mRNA of our interest into its cDNA is carried out by the enzyme called reverse transcriptase. To initiate the polymerizing reaction, oligo dT primer and all kinds of dNTPs are added in large quantities to a water solution containing of reverse transcriptase and the mRNA. To synthesize the double-stranded cDNA, the template RNA should be digested by an enzyme such as RNase H, or alkali digestion method leaving a single-stranded cDNA copied from the mRNA. Since a single-stranded cDNA is hydrophilic, it tends to loop around itself and forms a hairpin loop at the 3′ end. DNA polymerase can use it as a primer to transcribe the complementary strand. Now we have a double-stranded cDNA which is copied from the mRNA of our interest.

Since cDNA does not contain a promoter and introns, it is different from the genomic DNA. Since messenger RNAs do not last long in the cytoplasm and present in small amounts, the newly synthesized cDNA is in small amounts and should be amplified using traditional PCR. Quantitative RT-PCR is considered as the most powerful, sensitive, and quantitative assay for the detection of mRNA in tissue levels. It is frequently applied to find the specific gene for the defective protein in diseases.

Question 4.9

How do we find a specific gene?

Answer

A DNA copy of mRNA from a tissue can be made by reverse transcriptase. The complimentary DNA is different from the genomic DNA since cDNA does not contain a promoter and introns. A high degree of similarity to the cDNA is strong evidence that the region of a target genome is the protein-coding gene. And from the base sequence, we can determine the structure of the protein.

Question 4.10

How do we make insulin in bacteria?

Answer

To produce human insulin in bacterial host cells, the plasmid that is chosen as a vector should have a bacterial promoter such as a tac promoter. The source DNA should be cDNA which is DNA copy of mRNA for insulin, and it has no introns. Bacteria cannot express the genomic DNA because bacteria cannot splice the RNA. An insertion of a eukaryotic gene into a plasmid and a transfection into *E. coli* cannot

make the host cell to produce the protein encoded in the cDNA since the cDNA lacks the bacterial promoter for RNA polymerase binding, a ribosome-binding sequence, and the bacterial terminator for transcription to end. Commercially available expression vectors have the characteristics of typical vectors such as recognition sequence, origin, and marker genes as well as the extra sequences needed for the foreign cDNA to be expressed in the host cell. The synthesized mRNA will be translated in the ribosome.

When cDNA is prepared using mRNA extracted from beta cells in the pancreas and inserted into an expression vector, bacterial clone that incorporated the vector becomes able to produce insulin. The bacteria are cultured on a piece of filter paper, and the colony which produces insulin can be recognized by the labeled antibody of insulin. Therapeutic quantities of insulin can be made by cloning.

> Read full chapter

RNA

Cornel Mülhardt, E.W. Beese M.D., in Molecular Biology and Genomics, 2007

5.4 Reverse Transcription: cDNA Synthesis

Complementary DNA (cDNA) mandates a change in thinking about the direction of the sequence of DNA to RNA to protein. Viruses can make DNA from RNA; the responsible enzyme is reverse transcriptase. Molecular biologists primarily use cDNA as an initial material for the PCR and for the construction of cDNA banks.

For cDNA synthesis, you pipette RNA and primer together, heat the preparation to 70°C (158°F) to melt the secondary structure of the RNA, and cool it slowly to room temperature so that the primer can hybridize. After that, you add buffer, nucleotides, RNase inhibitors, and reverse transcriptase, and you incubate it for 1 hour at 37°C to 42°C (99°F to 108°F). In the protocol, there is not much that has to be revised, although the components may be altered.

Whether you use total **RNA** or poly A+ RNA depends on the purpose. If the cDNA is to serve as a template for a PCR, total RNA is usually sufficient, because the subsequent amplification is tremendous. If necessary, a small amount of template can be adjusted through the use of several more cycles; the efforts required are substantially less extensive than those required for poly A+ purification. If you are working with mRNA, which is only slightly expressed, you should add poly A+ RNA, and this method also works if the cDNA is to be used as a hybridization probe.

There are many **primers** to choose from. The classic primers are oligo(dT) primers, made up of 16 to 20 thymidines that bind to the poly A+ tail of mRNAs.

Advantage: You can synthesize complete cDNAs, beginning at the poly A+ tail and ending at the 5' end of the mRNA.

Disadvantage: The reverse transcriptases used to synthesize cDNAs have an average length of 1 to 2 kb, whereas mRNAs can easily be 10 kb long. The protein coding portion of interest is usually in the vicinity of the 5' end. As a consequence, it is frequently impossible to demonstrate long mRNAs, or they are poorly demonstrated. In this case, you can take advantage of a random hexamer primer (*random hexamers*), which hybridizes somewhere along the mRNA so that all mRNA segments are represented in the cDNA and the non-mRNA structures. If you are interested in a specific mRNA, you can also make use of a specific primer, although you must frequently optimize the conditions first, or the yields will be smaller than that observed from either of the two other types of primer.

Two **reverse transcriptases** (RTs) challenge one another's positions. The reverse transcriptase from the *avian myoblastosis virus* (AMV-RT) is a DNA polymerase that synthesizes DNA either RNA or DNA dependently. It also demonstrates DNA exonuclease and RNase activities. Its optimal working temperature is about 42°C (108°F), although the enzyme can withstand temperatures of up to 60°C (140°F). The reverse transcriptase from the Moloney murine leukemia virus (MMLV-RT) is an RNA-dependent DNA polymerase, likewise with RNase H activity, which precipitates more weakly than the AMV-RT; the optimal working temperature is about 37°C (99°F), with a maximum of 42°C (108°F). Which of the two you use is a matter of taste. The AMV-RT is frequently preferred, because the RNA is overcome somewhat better by the secondary structures at a higher working temperature, although the MMLV-RT produces longer cDNA transcripts because the mRNAs survive longer due to their lower RNase activity. The development of modified MMLV-RTs represents another step in this direction (e.g., Truescript from AGS, MMLV-RT RNase H Minus from Promega, SuperScript from Gibco BRL), using products that withstand higher temperatures and have no intrinsic RNase H activity, so that it is possible to obtain substantially longer transcripts. In the event of more difficult secondary structures, with which normal reverse transcriptases fail, you can make an attempt with thermostabile polymerases that have reverse transcriptase activity (see Chapter 4, Sections 4.1 and 4.3.12). However, they have substantially smaller yields and are therefore rarely used for standard applications.

You can **quantify** the amount of synthesized cDNA by adding radioactively labeled nucleotide (5 to 10 µCi) to the reaction, removing two 1 µL aliquots (aliquots 1 and 2) before adding reverse transcriptase, and then carrying out the cDNA synthesis. From the completed product, you remove another aliquot (aliquot 3). Aliquot 1 is

thinned 1 to 100, and the total activity in the preparation is determined from 1 μL of this preparation. Aliquots 2 and 3 are precipitated with trichloroacetic acid, and the activity of the pellets is measured; the difference between the values is the quantity of additional activity. The equation is as follows:

> Read full chapter