Chromosome Assignment of Human Genes

by

Ann M. Kays

Department of Biology
Sweet Briar College

April 24, 1996

This senior honors thesis has been awarded high honors.

Robin Lee Davies, First Reader

Joanne Rosinski, Second Reader

Judith Palmer, Outside Reader
Randolph Macon Woman's College

Mapping Genes to Chromosomes

Submitted in partial fulfillment of the requirements for Honors in Biology

Ann Kays
Sweet Briar College
Sweet Briar, Virginia

Table of Contents

Abstract

Two human genes were assigned to chromosomes. Cartilage-derived morphogenic protein 1 (CDMP1-PEN) was assigned to chromosome 20 and asialoglycoprotein receptor 2 (ASGR2) was assigned to one of six different chromosomes using the polymerase chain reaction (PCR) and gel electrophoresis (Chang, Spiess).

PCR is a DNA amplification technique in which a specific sequence is exponentially amplified through the action of a thermostable enzyme that polymerizes complementary nucleotides to a single stranded DNA template to extend primers used to locate the gene by complementary base pairing. Detection is made by analyzing the PCR product using gel electrophoresis, a separation technique in which DNA is caused to migrate through a semi-solid matrix in an electrical current. Chromosomal assignment was made if the primers located the unassigned gene in the chromosomal DNA; exponentially amplified the gene; and produced discernable bands using gel electrophoresis.

Data collected in this research project will be beneficial to the Human Genome Project. The Human Genome Project is a world-wide, collaborative research initiative to produce physical, genetic, and cytogenetic maps of the human genome and, ultimately, identify, sequence, and assign all 50,000 to 100,000 human genes (Collins). Genes successfully assigned to chromosomes will provide researchers with the necessary data to determine the specific location of the gene on the chromosome (Adams).

Introduction

In assigning a gene to a chromosome, the first step is to identify potential genes for assignment must be identified. A list of unassigned genes was obtained from the Genome Data Base and, from this list, the most recent additions to the list were selected (Fasman). Next, these identified genes were checked to see if they had previously been sequenced and, if so, whether there were at least 200 bases before or after the coding sequence (Caskey and Rossiter). Sections containing at least 200 bases before or after the coding sequence were used to select primer pairs by the Whitehead PRIMER Program (PRIMER). Primers selected for synthesis were synthesized using a Pharmacia DNA synthesizer. The synthesized primers were purified by molecular sieve chromatography and precipitated. The concentration of the primers was determined spectrophotometrically.

A preliminary PCR of the unassigned gene was performed to determine whether a primer pair was successful at producing a detectable PCR product and to determine optimal PCR conditions. The preliminary PCR results were analyzed using gel electrophoresis and successful primer pairs completed PCR to produce a band in the gel. Only successful primer pairs were used in chromosome assignment.

Chromosome assignment was made using a somatic cell hybrid mapping panel. Pools of chromosomes were constructed and PCR for the unassigned gene was performed with the pools. Results from chromosomal pool assignment were detected using gel electrophoresis. Once a pool was identified as containing the chromosome with the unassigned gene, then subsequent PCR reactions were performed to assign the gene to a chromosome in the pool. A gene was assigned to a specific chromsome if similar bands were detected for an individual chromosomal DNA PCR reaction and a human DNA PCR reaction.

The data collected in this research project will be beneficial to the Human Genome Project because unassigned genes were assigned to chromosomes. Also, primer pairs that successfully assigned a gene to a chromosome could be used by other researchers working

with specific chromosomes by providing them with the sequence of successful primer pairs. Successful primer pairs could then be used as sequence tagged sites (STSs) to construct a physical map (Davies). A cytogenetic map could also be constructed using yeast artificial chromosomes (YACs) identified by the primer pairs (Adams, Little).

Introduction to the Human Genome Project

The Human Genome Project (HGP) is a world-wide, collaborative fifteen year research initiative developed by the National Institutes of Health (NIH), the Department of Energy (DOE), and the Human Genome Organization (HUGO)(Aldhous). In this project, scientists are working to identify all the genes of the human genome and to develop genetic and physical maps of the human genome and those of other model organisms. Development of these maps includes the determination of the location of the 50,000 to 100,000 human genes (Rose). Ultimately, this project seeks to sequence the entire genome of the human and other model organisms. The data collected in this project will allow for a better understanding of the structure and function of the human genome(Collins).

The NIH and DOE devised specific goals to be met by the Human Genome Project (Green). One of the goals calls for the improvement of mapping technology, in particular, mapping, sequencing, and gene identification. Another goal of the project is to produce genetic-linkage, physical, and cytogenetic maps of the human genome. A genetic-linkage map is a map indicating genetic distances between pairs of linked variable genes and a physical map is a representation of a group of recombinant clones that cover a chromosome from end to end (Rose). A cytogenetic map is the oldest type of map and it indicates the order and spacing of DNA markers along a chromsome relative to the chromosomal banding pattern (Caskey and Rossiter). This type of map is limited to a resolution of approximately ten million base pairs (Rose). Improvement in mapping technology will allow for the production of a more accurate physical map at a higher rate of efficiency (Collins). The goal for the genetic map has already been exceeded and predictions of completing the HGP two years ahead of the scheduled 2005 completion date have been

made (Beardsley).

The information gathered by scientists must be easily accessible to everyone because, as a world-wide collaborative effort, sharing data is critical in disseminating research results. Providing such a database has been a goal of the project and has led to the creation of the Genome Data Base (GDB) at Johns Hopkins University School of Medicine (Fasman). Additionally, the knowledge and understanding which result from the Human Genome Project raises important issues, such as how this information will be used. Thus, the final goal of the Human Genome Project is to address the ethical, legal, and social ramifications of identifying, mapping, and sequencing the entire human genome (Lippman).

Mapping Genes Using PCR and Gel Electrophoresis

In this research project, I worked with Dr. Robin Davies to map unassigned human genes to chromosomes. Genes for assignment were identified using the polymerase chain reaction (PCR) and subsequently analyzed using gel electrophoresis. PCR is a highly specific and sensitive technique in which defined DNA sequences are exponentially amplified through the action of a thermostable DNA polymerase enzyme (Faloona). The amplified product (a piece of DNA of a specific size) is detected via gel electrophoresis (Maniatis).

In order to assign the gene to a chromosome, a somatic cell hybrid mapping panel is required (Zhang). A somatic cell hybrid mapping panel consists of a series of rodent-human cell hybrids, each of which contains a different human chromosome (Figure 1). The unassigned gene was assigned using PCR to examine the human/rodent somatic cell hybrid DNA and detecting the product of PCR with agarose gel electrophoresis to compare bands of the cell hybrids and the human control (Freshney, Zhang). Assignment of a gene to a chromosome was made when the human control PCR product matched the PCR product of one of the hybrids. Matching bands indicated the gene to be located in the human chromsome carried by the somatic cell hybrid (Table 1). The results of successful

gene assignments can then be used to further the Human Genome Project by providing tools for the sub-chromosomal localization of genes.

Polymerase Chain Reaction

In order to assign a gene, a convenient method of gene identification must be available. Identification could be made by synthesizing the gene to be assigned, but it is impractical and too expensive to synthesize 200 to 1,000 bases of a gene. Another alternative would be to identify the gene using a Southern blot with hybrid DNAs to analyze a cDNA or genomic clone (Zhang). This method requires much more time and work because, in order for the gene to be detected, the hybrid DNA must be digested, electrophoresed, blotted, probed, washed, and detected. The cDNA or genomic clone must be labelled for use as a probe. On the other hand, PCR amplification followed by analysis with gel electrophoresis is faster and more straightforward (Zhang). A Southern blot would also require 5μg of DNA, compared to only 5 - 100ng for PCR (Davies). Thus, more time, energy, and DNA is expended in gene identification through a Southern blot than in PCR. Gene assignment by PCR and gel electrophoresis is the most practical method of gene assignment because, in PCR, primers are used to locate the gene and then a DNA polymerase enzyme synthesizes DNA using the located gene as a template (Charlieu). Since PCR is an amplification of the located gene template, less DNA is used than in other techniques, and no additional steps, such as hybridization detection, are required (Faloona, Zhang).

In PCR, a single-stranded DNA is used as a template for a thermostable DNA polymerase enzyme to elongate primers by complementary base pairing of deoxynucleotide triphosphates(dNTPs) in a buffered solution (Mullis). Primers locate and hybridize to a segment of the unassigned gene on the (single-stranded) DNA template. The DNA polymerase enzyme begins at the 3' hydroxyl group of the primer to catalyze the formation of phosphodiester bonds by "stringing" together "free" nucleotides (dNTPs) guided by complementary base pairing to the template strand, in the presence of a stable environment

created by the buffering solution (Rolfs)(Figure 2).

There are three steps to a typical PCR cycle: denaturation at 95°C, annealing at 48°C to 72°C, and extension at 72°C (Mullis). In denaturation, double stranded DNA is separated by breaking the hydrogen bonds connecting the two strands. The high temperature is needed to break the many hydrogen bonds. The separated single strands are each used as a template (Rolfs). Once denaturation is complete, primers are annealed to the single strand DNA template by decreasing the temperature to allow for hydrogen bonds to form between the complementary sites. The annealing temperature is dependent on the length and GC content of the primer; however, 50°C to 60°C is an acceptable range to begin annealing for a primer with approximately 50% GC content (Saiki, "Optimization"). The GC content refers to the percentage of G (guanine) and C (cytosine) and is important because the GC base pair has three hydrogen bonds versus the two hydrogen bonds connecting A (adenine) and T (thymine) and is, therefore, an indicator of DNA stability. Extension is the final step of PCR and refers to the complementary extension of the primer that has been annealed to the template DNA. This step must occur at the highest temperature possible because, by maximizing the extension temperature, incorrectly annealed primers are denatured more readily than correctly annealed primers due to their unstable structure. The temperature of 72°C used in extension is close to the maximum activity temperature of the DNA polymerase enzyme used in PCR (Saiki,"Primer"). The standard time used for extension is one minute for 1000 bases (Saiki,"Optimization"). For both the annealing and extension steps, nonspecific amplification products will result if the temperatures are held for too long. The DNA polymerase enzyme moves across the template strand 3' to 5' so that the synthesized product is made 5' to 3' (Innis).

When these three steps are repeated cyclically in PCR, the complementary strands completed in extension are denatured and separated to be used as templates in the next cycle. Cycling of the reaction makes it important that the melting temperature, the temperature at which half the double strands of DNA has denatured, of the two primers be

similar because the extended primers need to separate from the DNA so that it can also be used as a template (Faloona). This means that new strands are synthesized exponentially because the denaturation provides two template strands and, after one cycle of PCR, double the amount of template strands are available.

Primers

A primer is an 18 to 24 base sequence that signals the beginning site of DNA synthesis to the DNA polymerase enzyme (Sharrocks). Primers are used to identify the gene to be amplified out of the 50,000 to 100,000 genes of the human genome because the many possible combinations of bases in a primer makes it highly specific to the region it will bind. A pair of primers is designed for each gene from the regions 5' or 3' to the coding sequence or from an intron. Primers cannot be selected from the coding sequence because this region codes for a working protein and, is therefore, conserved and the sequence may be seen elsewhere in the DNA (Voet). The primer locates the gene to be amplified by complementary base pairing to each strand of the double stranded DNA at the 3' hydroxyl end, which is the beginning of the region where DNA synthesis is initiated by the DNA polymerase enzyme (Figure 3).

Possible primer pairs for PCR were selected using a computer software program called the Whitehead Primer Program Version 0.5 from the Massachusetts Institute of Technology (PRIMER). Primers selected from the possible primer pairs were synthesized using a computer-controlled Pharmacia DNA synthesizer. Sequences to be synthesized were entered into the computer and were synthesized by a method widely used, the phosphoramidite method (Figure 4)(Voet).

There are two aspects of the chemistry involved in DNA synthesis: anhydrous and protection chemistry. Anhydrous chemistry is responsible for forming the phosphodiester bonds, which make up the backbone of DNA. In anhydrous chemistry, the presence of water in reagents and solvents decreases the coupling efficiency. Coupling efficiency is the rate of success at which bases are "strung" together. Thus, care is taken to keep the

solutions dry by carrying out all work quickly and including drying agents in most reagents. Groups not involved in a reaction are "blocked" by dimethoxytrityl (DMTr) in protection chemistry to insure correct order of reactions and coupling orientation and to maximize the integrity of the final product (Pharmacia).

The first step of the phosphoramidite method of DNA synthesis is detritylation, in which the DMTr group of the nucleotide anchored to a solid support is cleaved to expose the 5' hydroxyl end by treatment with an acid, trichloroacetic acid (TCA). The solid support is an inert polymer encased in a plastic cassette on which DNA synthesis occurs, and the first nucleoside of the 3' end is bound to the support by a succinate bridge. The cleaved DMTr group is washed away and the amount washed away is detected and used to calculate the coupling efficiency. The 3' end of the nucleotide to be coupled to the growing chain is activated by tetrazole and is coupled to the 5' end of the growing chain by forming a phosphite triester bridge in a condensation reaction. Any remaining tetrazole, diisopropylamine, and unreacted nucleotides are washed away. Chains that failed to react are capped by acetylation to prevent the further synthesis of erroneous sequences with incoming nucleotides and to ensure that only the correct sequence is synthesized. Excess capping reagents are washed away. The phosphite triester bridge is oxidized with iodine in the presence of water to the phosphodiester form, thereby completing the coupling of one nucleotide to the growing chain. Once the sequence of interest is completely synthesized, it is cleaved from the solid support by treatment with ammonium hydroxide (Pharmacia).

Template DNA

In this project, PCR conditions were determined for human DNA and, then, the somatic cell hybrid mapping panel DNAs were used (Zhang). These human/rodent somatic cell hybrids are purchased from the Human Genetic Mutant Cell Repository and each contains a different human chromosome (Table 1). The DNA from these cells were grouped into pools that contained a specific number of human chromosomes. Each pool was subjected to PCR to look for a positive identification. A positive identification was

determined when, after electrophoresis and staining with ethidium bromide, bands of the expected size were detected for a pool and matched the band detected for human DNA. The pool with the positive band was broken up into smaller pools containing fewer cell hybrids and a positive identification is again determined. This process was repeated until the gene was assigned to a somatic cell hybrid containing a specific chromosome (Catalog 1992/1993 and 1994/1995).

Gel Electrophoresis

The amplified unassigned gene sequence from PCR was analyzed using gel electrophoresis to determine chromosomal assignment. Gel electrophoresis is a technique that allows for determination of the size of DNA by the separation of molecules in a semi-solid matrix using electric current to cause migration of the electrically charged DNA molecules. The pore size of the matrix is controlled by the number of crosslinks in an agarose gel, which is controlled by agarose concentration, and is used to separate the charged particles based on a desired size (Maniatis). Agarose gels are run on a horizontal slab and the positive electrode is placed furthest away from the wells containing the negatively charged DNA. The DNA migrates through the gel towards the positive electrode, but the speed at which it can travel is limited by its size. Following electrophoresis, the gels are stained using ethidium bromide. Ethidium bromide is used to stain the gel because it intercalates in the DNA and fluoresces under UV light (Figure 5). A single major band for each unassigned gene was detected using this method (Acquaah).

Genes Assigned to Chromosomes

Cartilage-derived Morphogenetic Protein 1

Cartilage-derived morphogenetic protein 1 (CDMP1-PEN) was assigned to the superfamily of transforming growth factor-ß (TGF-ß) by using degenerate primer pairs from the highly conserved carboxyl-terminal region of bone morphogenetic proteins (BMP) in the articular cartilage of newborn calves. Bone morphogenetic proteins belong to the large TGF-ß superfamily, which are a large group of structurally related signaling proteins

released as dimers and then cleaved to their active structure. Many of these proteins have been shown to be important at various stages of embryogenesis and in adult animals. (Chang)

Proteins produced by CDMP-1 have been found at the stage of precartilaginous mesenchymal condensation and in the cartilaginous cores of developing long bones. Another protein known as cartilage-derived morphogenetic protein 2 has been identified and is 82% identical to the carboxyl-terminal TGF-ß domain in CDMP-1. This similarity between CDMP-1 and CDMP-2 defines a new subfamily of proteins. Both genes produce proteins having bone-inductive activity and are preferentially expressed in cartilaginous tissues. Discovery of this novel subfamily is important because CDMP-1 and other BMPs have been demonstrated to be active in early skeletal development and CDMP-2 may be involved in later stages of skeletal formation. This means that these cartilage specific proteins can provide new information in regeneration of cartilage and in studying the cells responsible for production of these proteins (Chang).

Asialoglycoprotein Receptor 2

Asialoglycoprotein receptor 2 (ASGR-2) is one of two receptor proteins, ASGR-1 and -2, which have a 58% protein homology. Two subspecies of ASGR-2 have been identified and they differ by a five-amino acid insertion in the COOH -terminal extracytoplasmic domain (Spiess).

Asialoglycoprotein receptors have been found on mammalian parenchymal hepatocytes and bind desialylated glycoproteins. Glycoproteins are proteins with a covalently bound carbohydrate group and are found in most forms of living things. The functions of glycoproteins are highly varied, including those of enzymes, transport proteins, receptors, hormones, and structural proteins. The asialoglycoprotein receptors bind desialylated glycoproteins and the receptor-ligand complex is taken into the cell and transported to an acidic sorting compartment. The receptor is then recycled back to the surface of the cell and the ligand is degraded by the lysosomes. This activity has been

observed in both mice and humans. Comparison of the human-1 (H-1) and human-2 (H-2) to rat-1 (R-1) and rat-2 (R-2) have shown more homology to exist between H-1 and R-1 and H-2 and R-2 than to each other, suggesting that these genes evolved before the evolutionary separation of humans and rats (Spiess).

Materials and Methods

Identifying Possible Unassigned Genes for Mapping

The Genome Data Base (Fasman) was accessed using Netscape, a world wide web browser, to obtain a current list of unassigned genes. The assignment status of randomly selected new entries on the list was verified by searching for current information on the GDB. Genes selected for assignment had previously been sequenced and contained a minimum of two hundred bases in at least one of their untranslated regions (5' untranslated, 3' untranslated, or introns). An untranslated region of appropriate length (at least 200 bases) was excerpted from each sequence and was placed in a separate computer file for primer selection.

Primer Selection

Primer pairs for each unassigned gene were selected using computer files of the untranslated sequence from the GDB and a primer selection program called the Whitehead Primer Program from the Massachusetts Institute of Technology (PRIMER). The program was used to deermine: which part of the sequence should be amplified; whether possible primers would be compatible to the conditions of the PCR (e.g. annealing temperature); if the primers have a significant degree of complementarity to repeat sequences; whether the primer pairs have a significant degree of complementarity to each other; and whether the PCR product would be of the desired size and GC content. If any repetitive sequences were found, the program was designed to identify them and avoid producing primers in the repetitive sequence, to ensure unique PCR products for each gene. The program produced three possible primer pairs differing in product size range: 100 - 150bp, 150 - 250bp, and 250 - 400bp. Primer pairs selected for synthesis had an even distribution of bases; similar melting temperatures; a 45 - 55% GC content; and mononucleotide chains of less than four bases.

Primer Synthesis

Primers were synthesized using an automated DNA synthesizer, the Gene

Assembler Special/4 Primers. The synthesizer and all reagents for synthesis (Appendix I), were obtained from Pharmacia Biotech. Before synthesis, the argon level of the instrument was checked. The instrument was maintained under pressure with argon to prevent the evaporation and water contamination of the reagents. The pump hose was connected to the instrument. The instrument was purged of reagents remaining in the tubing and a solid support cartridge corresponding to the 3' base of the sequence to be synthesized was placed in the first column. (Unlike DNA synthesis in the cell, automated DNA synthesis proceeds in the 3' to 5' direction.) Next, the pump was calibrated and the new calibration value was only accepted if the new calibration value had a difference of less than 5% from the old calibration value. A proper calibration value is important because the calibration determines when the monitor will measure the detritylation value, which is used to calculate coupling efficiency. Therefore, if the calibration is not correctly adjusted, then the instrument will not calculate the coupling efficiency correctly. Once the instrument was prepared for synthesis the sequence of the primer to be synthesized was entered, edited, described, and saved. Synthesis was initiated and then monitored to insure efficiency.

Cleaving Primer from the Solid Support

When synthesis was completed, the cartridge was removed from the column and placed in a sterile 1.5mL Eppendorf tube. The cartridge was centrifuged at 2500rpm for 1.5 minutes in an Eppendorf centrifuge to remove any remaining acetonitrile. Following centrifugation, the cartridge was transferred to a sterile 1.5mL Eppendorf tube using a sterile 22 gauge / 1.5 inch Becton-Dickinson syringe needle. The tube was tapped to secure the cartridge in the tube. The cartridge was covered with 1mL ammonium hydroxide, sealed with parafilm, and placed in a jar containing ammonium hydroxide overnight (at least eight hours) at room temperature to cleave the synthesized DNA from the solid support.

Primer Purification

The tube containing the cartridge was removed and the cartridge was transferred to

a second sterile 1.5mL Eppendorf tube, saving the ammonium hydroxide remaining in the tube. The cartridge was centrifuged in an Eppendorf centrifuge at 2500rpm for 1.5 minutes to remove ammonium hydroxide. The ammonium hydroxide centrifuged out of the cartridge was combined with the previous ammonium hydroxide saved and the volume was adjusted to 1.0mL using sterile double distilled water.

Primers were purified by molecular sieve chromatography using Pharmacia Biotech NAP-10 columns to remove capped sequences. The top and bottom cap of the column were removed and solution drained off. The column was supported in a vertical position and the gel bed was washed three times with 5mL sterile double distilled water. Once the water had completely entered the gel bed, the sample was poured over the gel bed. The purified sample was eluted with 1.5mL sterile double distilled water and collected into a sterile 1.5mL Eppendorf tube.

Ethanol Precipitation of Primers

The purified sample was aliquoted at a maximum volume of 400µl in sterile 1.5mL Eppendorf tubes. For every 400 µL of purified primer, 40 µL 3M sodium acetate, 4 µL 1M $MgCl_2$, and 935 µL 95% EtOH were added and mixed by inverting the tubes. The tubes were stored for a minimum of two hours at -80°C in a Revco deep freezer to concentrate and purify the primers via precipitation.

Isolation of Primers

The tubes were removed from the -80°C freezer and centrifuged for ten minutes at 14000 rpm in an Eppendorf centrifuge. Following centrifugation, the supernatant was removed and the pellets were rinsed with 1.0mL 70% EtOH in 10mMTris 1mM EDTA, pH 8.0 (TE) and re-centrifuged as above. The supernatant was removed and the pellets were dried in a 600mmHg vacuum and dissolved in 40 µL TE. The concentrated dissolved primers were collected into sterile 1.5mL Eppendorf tubes. The tubes that had contained the pellets were washed with 40 µL TE by transferring from tubes. The TE used for washing was collected into the tube with the concentrated dissolved primer to maximize

yield and to prevent loss of oligonucleotides clinging to the tubes.

Determining the Concentration of Concentrated Primer

The concentration of primer stock solutions was determined by measuring the absorbance (A) of the sample using a Beckman DU-600 spectrophotometer at 260nm. The sample was measured using 1mL of a 1/250 dilution of the concentrated stock. The concentration of the stock solution was determined using the measured absorbance in the following equation (Maniatis):

$$1\ A = 33 mg/mL$$

Measured Absorbance X 33mg/mL = Concentration of Dilution

Concentration of Dilution X 250 dilution factor = Stock Concentration

PCR of Unassigned Genes

Genes were analyzed using PCR, performed in a Power Block I System from Ericomp. Master mixes of the primer pair were made in a sterile Eppendorf tube and contained sterile double distilled water; Gibco 10x buffer $+Mg^{++}$ (1.5mM); Gibco dNTPs (10mM); 100ng/mL of each primer, and Gibco Taq (5U/μL)(Appendix II). The master mix (19μL) was pipeted into a thin wall PCR tube from United Laboratory Plastics. One microliter of 100ng/mL dilution of Sigma (human placental), AHH-1 (human lymphoblast), or Gibco (K562 human transfer cell line) DNA was pipeted into each PCR tube. The positive control used in each reaction contained Gibco DNA and Gibco primers for brain-derived neurotrophic factor.

The tubes were set up for PCR and placed in the thermal cycler and cycled x 30. The program for each reaction was the same except for the annealing temperature. An initial denaturation at 94°C for four minutes was followed by 30 cycles of denaturation at 94°C for 30 seconds; annealing at approximately two degrees below the melting temperature of the primers for 20 seconds; and extension at 72°C for 10 seconds. Final extension was for five minutes at 72°C. Following final extension, the tubes were either held at 4°C for 15 minutes or placed in the freezer until the PCR products were examined

by gel electrophoresis. The stringency of the reaction was adjusted by varying the concentration of Mg^{++} and/or the annealing temperature.

Preparation of Gels for Gel Electrophoresis

All gels used were 1.5% agarose with 5 µL ethidium bromide incorporated into the gel. In a 250mL flask, 1.5g Sigma agarose was combined with 80mL double distilled water and microwaved. Next, 20 mL 5x TBE and 5 µL 10mg/ml ethidium bromide were added.

Examination of PCR Product Using Gel Electrophoresis

The gel was placed in the electrophoresis chamber and approximately 1.8L 1x TBE (10mM tris, pH = 8; 10mM borate; 10mM EDTA) was added. Each 20 µL PCR reaction was augmented with 4 µL of 2.5% bromophenol blue sample buffer, and 22 µL of the PCR product was pipeted into the well. The first, second, and twelfth wells contained DNA markers: bacteriophage lambda DNA digested with *Hin* D III; bacteriophage PhiX 174 DNA digested with *Hae* III; or 1Kb ladder (Appendix III). Gels were run at 180 volts using a Bio-Rad Model 1000/500 Power Supply for thirty minutes and viewed using a Fotodyne Foto/Prep I UV light source. Pictures of the gels were taken with a Fotodyne Polaroid camera and type 667 black-and-white film.

Chromosome Assignment of Gene Using PCR

Twelve pools containing two different chromosomal DNAs from a mapping panel obtained from the NIGMS Human Genetic Mutant Cell Repository were designed for chromosomal assignment. A master mix same as "PCR of Unassigned Genes" was made in a sterile Eppendorf tube. The master mix was mixed by inverting the tube, and 19 µL of the mix was pipeted into each PCR tube. One microliter of the template DNA from one of the twelve pools was added to each tube so that each reaction tube contained DNA from two chromosomes. The positive control contained human DNA (from all chromsomes) and the negative controls were mouse and Chinese hamster DNA from the same mapping panel.

The reaction tubes were placed in the Power Block I System thermal cycler and run through 30 cycles of PCR, according to the same program used to analyze the primers earlier. The PCR products were examined using gel electrophoresis as previously described. Observed bands seen in the gel were examined to determine which pool the gene was observed. PCR was repeated to determine the specific chromosomal location of the gene using separate reaction tubes for each of the two chromosomes present in the positive pool.

Results

Primer Selection and Synthesis

The search of the GDB produced a list of 1697 unassigned genes, and 62 recently submitted genes were selected for potential mapping. Thirty-one of these genes were further selected for mapping because they had previously been sequenced and had a minimum of 200 bases before or after their coding sequence. Nineteen genes were examined using the primer program and twenty-nine primer sequences were selected for synthesis. Twenty-six syntheses were attempted and 16 syntheses were successful (Tables 2 and 3). The success of a synthesis was determined by whether or not the sequence to be synthesized was successfully coupled (Table 4). Eleven of the primers synthesized had coupling efficiency percentages above 90% and most produced a high (above 1000 µg/mL) primer stock concentration; however, the forward primer for TINUR, which had a 100% coupling efficiency, produced the lowest primer stock concentration at 0 µg/mL and the reverse primer for CATR also had a low stock concentration despite its high coupling efficiency percentage. This discrepancy between coupling efficiency percentage and primer stock concentration prevented a direct relationship from being constructed. The three primers that produced low (below 1000µg/mL) primer stock concentrations had below 90% coupling efficiency.

Primers not successfully synthesized were automatically aborted during synthesis. If the cause was a loss of reagents, than synthesis was aborted towards the end of synthesis, with approximately 1 - 3 bases remaining (Table 5). Inspection of Table 5 indicates that for these events the coupling efficiency data was similar to a successful synthesis (Table 6) until the last base in which the Detrit Start and Detrit Value were both zero due to the loss of synthesis solvent. Miscalculation of the amount of amidites, tetrazole, dichloroethane, acetylnitrile, or detritylation solution was due to a discrepancy between the computer monitoring of solvents used and the actual amount available. A number of syntheses were aborted due to detritylation problems. Any syntheses that were

not completed due to low detritylation values were stopped after approximately the third base coupled in an effort to conserve solvents. Synthesis continued despite low detritylation values would waste solvents, since, if no detritylation is occuring, then bases will not be coupled. The detritylation values in this case would drop dramatically after the first coupled base and continue to decrease (Table 7). Synthesis was once aborted due to a break in the pump hose causing a leak. No coupling efficiency data was available because no coupling was made prior to the pump hose break. Pump hose tears were not predictable and, therefore, inspection of the detritylation values was not effective in determining the reason for a stopped synthesis until the pump hose was inspected.

Concentration Determination of Synthesized Primers

Concentration of primers measured at a 250x dilution varied from a high of 4799.0µg/mL (reverse primer for RMSA1-PEN) to a low of 0µg/ml (forward primer for TINUR-LSB). A direct relationship between the average coupling efficiency and the primer stock concentration calculated from the absorbance could not be determined (Table 4).

Preliminary PCR

In order to establish which primer pairs worked and to determine the optimal conditions for PCR, preliminary trials were performed for all primer pairs. The first preliminary PCR was performed for CDMP 1-1 (329bp) and CDMP1-2(205bp), which were prepared from the 5' and the 3' untranslated regions, respectively, of the cartilage -derived morphogenic protein 1 cDNA sequence (Chang)(Figure 6). Wells 1 and 12 contained lambda *Hin* D III and well 2 contained PhiX *Hae* III marker DNAs. Wells 4 through 6 contained human DNA with CDMP1-1 primers and no bands were visible indicating the CDMP1-1 primer pair not to be an assignment candidate. Wells 8 through 10 contained human DNA with the CDMP1-2 primer pair. Bands were visible in all three human DNA PCR reactions and stronger bands were visible for the PCR performed with Gibco and Sigma DNA than with the AHH-1 DNA. The bands visible were 205 bp in

size, which was in agreement with the expected value. The band presence indicated the CDMP1-2 primer pair to be a candidate for assignment.

The second gene sequence to be examined was the asialoglycoprotein receptor 2 (ASGR2) cDNA sequence from the 3' untranslated region (ASGR2-2)(Spiess). The first preliminary PCR for this gene was performed using the primer pair ASGR2-2 with a 58°C annealing temperature (Figure 7). Wells 1 and 7 contained lambda *Hin* D III and well 2 contained PhiX *Hae* III marker DNAs. Wells 4 through 6 contained ASGR2-2 with human DNA. The expected PCR product size was 211bp, no band was detected, rendering ASGR2-2 unusable in assignment.

The next preliminary PCR for this gene was performed using the primer pair ASGR2-1 (Figure 8). Wells 1 and 12 contained lambda *Hin* D III DNA and well 2 contained PhiX *Hae* III marker DNAs. Wells 4 through 6 contained ASGR2-1 with human DNA. Faint bands of 146bp were only visible in wells 4 and 5, which were Gibco and Sigma human DNA. The faintness of the band indicated the PCR to be too stringent.

A second preliminary PCR for ASGR2-1 was performed and the stringency of the reaction was reduced by decreasing the annealing temperature to 57°C (Figure 9). Wells 1 and 10 contained lambda *Hin* D III DNA and well 2 contained PhiX *Hae* III marker DNAs. Wells 3 through 5 contained ASGR2-1 with human DNA. PCR with Gibco DNA in well 3 did not produce a distinct band, but a slight smear instead. The strongest band was produced in well 4 which contained the PCR with Sigma DNA. The weakest band was produced in the PCR using the human DNA from the mapping panel. The gel identified ASGR2-1 as the primer pair to be used in chromosomal assignment of this gene and also demonstrated that annealing at 57°C produced stronger signals than annealing at 58°C. Annealing at 57°C also increased the amount of background DNA present at lower molecular weights, which can be identified by the faint bands of smaller size in Figure 9.

The third gene sequence to be examined was the regulator of mitotic spindle assembly 1 (RMSA). The primer pairs were prepared from the 3' untranslated region of

the RMSA cDNA (Margalit, Yeo). The first preliminary for RMSA was performed at an annealing temperature of 57.5°C (Figure 9). Well 1 contained lambda *Hin* D III DNA and well 2 contained PhiX marker DNAs. Wells 4 through 6 contained RMSA with human DNA. A very faint 249bp band was visible in well 4 which contained Sigma DNA.

Chromosomal Assignment

Cartilage-derived morphogenic protein 1

Initially, chromosome assignment of CDMP1 was attempted using six different pools of chromosomal DNA (Table 8). Each pool contained DNA from 4 - 5 different chromosomes. Assignment using these pools was not successful because no band was detected for any of the pools (Figure 10). The positive PCR control and the positive control using human DNA each produced a band, indicating the PCR to have been a successful reaction. No bands were detected in the mouse and Chinese hamster negative controls. Since no bands were detected, the pool sizes were decreased and the number of pools were increased in order to increase the concentration of the chromosomal DNA in the cell.

Chromosome assignment for CDMP1 was next attempted using 12 different pools of chromosomal DNA, each pool containing DNA from only two different chromosomes (Table 9). A faint band was detected in the PCR product of pool 2, a pool which contained DNA from chromosomes 20 and 21 (Figure 11). PCR was performed for chromosomes 20 and 21 and a band was detected for chromosome 20 (Figure 12). This final result determined the location of CDMP1 to be on the 20th chromosome. Chromosome assignment for CDMP1 was repeated for verification of results using chromosomal DNA from chromsomes 2 and 20. A band was detected for chromosome 20 and no band was detected for chromosome 2 (Figure 13).

Asialoglycoprotein receptor 2

Chromosome assignment of ASGR2 using 12 different pools of chromosomal DNA at 57°C with a 1.50mM Mg^{++} concentration produced faint bands (Figure 14).

Wells 1 and 2 contained 1Kb ladder marker DNA and well 12 contained lambda *Hin* D III DNA. Faint bands were detected in wells 6, 7, and 8, which contained pools 4, 5, and 6. According to this gel, the gene was located on chromosome 17, 16, 15, 14, 13, or 12.

Assignment of the gene to a chromosome was attempted using chromosomal DNA from 17, 16, 15, 14, 13, and 12 at 1.50mM Mg^{++}, but no bands were detected and the reactions were therefore unsuccessful (Figure15). Chromosome assignment was again attempted at a decreased stringency by increasing the concentration of Mg^{++}. Parallel reactions were run at 1.75mM and 2.00mM Mg^{++} and the PCR products were run in a longer gel to allow for more separation between the PCR product bands and the background DNA. Assignment at an increased Mg^{++} concentration was unsuccessful because no bands were observed (Figures 16 and 17).

Discussion

Chromosome Assignment

Cartilage-derived morphogenetic protein 1

The cartilage-derived morphogenetic protein 1 gene was determined to be on chromosome 20. The band for chromosome 20 is faint and none was present for chromsome 21. The assignment was repeated to demonstrate that the assignment was reproducible. Although the bands observed for this gene were faint, they were detected in the gel and indicated that, using the primers for the gene, PCR did occur. The band signal for CDMP1 could be increased by further decreasing the stringency of the reaction using a decreased Mg^{++} concentration.

The primer pairs developed for CDMP1 would be useful in the assignment of CDMP1 to the physical map of chromosome 20. Further testing of this gene should include cytogenetic mapping; ideally, with a YAC containing this gene. Cytogenetic mapping would be performed using fluorescence *in situ* hybridization and should give a signal on the appropriate chromosome (Caskey and Rossiter).

Determination of the chromosome assignment of this gene would be significant because it is a new subfamily of proteins. Chromosomal determination of CDMP2 would now be interesting to determine if this subfamily is inherited together. In the future, this subfamily could be used in gene therapy to treat arthritis.

Asialoglycoprotein receptor 2

The gene for asialoglycoprotein receptor 2 was determined to be located on either chromosome 17, 16, 15, 14, 13, or 12. A specific chromosome assignment was attempted at three different Mg^{++} concentrations, but was unsuccessful. Further testing of this primer pair is necessary to better optimize the PCR conditions and continue chromosome assignment.

Preliminary PCR

Asialoglycoprotein Receptor 2

A primer pair for PCR of asialoglycoprotein receptor 2 was successfully identified. The signal produced by the band was weak, decreasing the stringency of the reaction by decreasing the annealing temperature produced a stronger band. As the annealing temperature was decreased, the signal observed was stronger, but more background bands were also detected. Therefore, an annealing temperature of 57°C was selected because PCR at this temperature produced a stronger signal with as little background as possible.

Although the primer pair has been shown to work, further testing is necessary to optimize PCR conditions. Determination of a successful primer pair for ASGR2 is significant because it will allow for the gene to be assigned to a chromosome using the primer pair as a sequence tag site to determine the sub-chromosomal location.

Regulator of mitotic assembly 1

A primer pair for the regulator of mitotic assembly 1 (RMSA) gene was determined. Optimal conditions for PCR of this gene must still determined. The PCR reaction for this gene may be optimized by adjusting the annealing temperature and the concentration of Mg^{++}. Once conditions for PCR have been determined, the chromosomal location of this gene could be determined. The primer pairs could then be used as an STS to determine the sub-chromosomal location of the gene.

Trouble Shooting

Primer Synthesis

The main problem encountered in primer synthesis was the lack of detritylation. Problems with detritylation were first encountered when a new bottle of acetonitrile was added to the instrument. Detritylation problems are typically indicative of a water contamination of the system and/or solvents. The instrument was flushed on three separate occasions by back pumping acetonitrile through the system.

Since these problems began after the new bottle of acetonitrile was connected to the

instrument, it is probable that the acetonitrile may not have been properly dried. If this was the case, then the water contamination caused by the wet acetonitrile may have been made worse each time new amidites were dissolved and connected to the instrument because they were dissolved in the same wet acetonitrile. Therefore, flushing the instrument may have perpetuated the problem by spreading wet acetonitrile.

In the future, solvents that have molecular sieves added to them may be dried more thoroughly by taking better precautions. Prior to adding the sieves, the sieves could be heated to approximately 60°C to insure their dry state. After adding the heated sieves, the bottle connections on the instrument should be checked to assure that no debris is found in the sealing ring; this should prevent any leaks. The solvents should be connected to the instrument and the pressurized argon should be allowed to flow into the bottles to displace the air introduced by connecting the bottles. The new solvents should be left over night on the instrument and, just before initiating synthesis, the pressurized argon should be allowed to flow into the solvents again.

In the future, if detritylation is not happening in DNA synthesis, then a different trouble-shooting approach would be more efficient in the use of time and money. A new bottle of acetonitrile should be prepared as indicated above. New amidites should be prepared using the new bottle of acetonitrile. This acetonitrile should aso be used to flush the instrument to remove any remaining water.

Concentration Determination of Synthesized Primers

Problems were encountered in determining the absorbance of primers using the Beckman DU-600 spectrophotometer. The spectrophotometer was producing absorbance readings that varied each time a primer absorbance was measured so that data could not be replicated. Next, the spectrophotometer began reading negative absorbance readings; meaning, the TE blank was absorbing more than the primer samples.

In order to verify the working order of the spectrophotometer, a series of dilutions of a known concentration of sheared salmon sperm DNA was used. The purpose of this

assay was to produce absorbance readings that decreased as the concentration decreased if there were no problems with the instrument. Some variation was seen, but, overall, a relative decrease was seen. The spectrophotometer in the chemistry department was used to confirm the absorbance readings of the primers measured by the biology spectrophotometer. Since no problem with the instrument was ascertained, it is likely that the discrepancy in concentration readings was due to incorrect storage of the primers. The primers were being stored in the refigerator at 4°C, but should have been stored in the freezer at 20°C. Degradation may have occurred as a result.

Automated Thermal Cycler

In the first PCRs performed using the automated thermal cycler, the instrument failed to begin final extension at 72°C for 4 minutes. The technician for the company was contacted and he suggested changing the final extension temperature to 71.9°C because the last step prior to final extension was also at 72°C; therefore, the computer may have had a problem with distinguishing between the two steps due to their same temperatures. The "overshoot low" control on the thermal cycler was also lowered to permanently correct the problem.

Conclusion

The two human genes, cartilage-derived morphogenic protein 1 and asialoglycoprotein receptor 2, were assigned to chromosomes. CDMP1 was assigned to chromosome 20 and ASGR2 was located to three different chromosomal pools using physical mapping techniques. Cytogenic mapping using fluorescence *in situ* hybridization should be performed for CDMP1 to determine the chromosomal band location of the gene (Caskey and Rossiter). Further testing of the Mg^{++} concentration and annealing temperature of ASGR2 is necessary to optimize the PCR conditions in order to determine the exact chromosome location of the gene. Once located to a chromosome, the primer pair may be used by others to determine its specific location along the chromosome (Davies). A possible primer pair for regulator of mitotic spindle assembly 1 was determined. Further testing of RMSA is required to optimize its PCR conditions and to assign the gene to a chromosome.

Researchers working on the Human Genome Project may use the primer pairs and chromosome assignments determined to further their work. The primer pairs and chromosome assignments would provide others with the information necessary to perform further mapping of the three genes evaluated here.

Table 1 - Somatic Cell Hybrid Mapping Panel from NIGMS Human Genetic Mutant Cell Repository by fusing human and rodent cells. In cell lines such as GM/NA10791 and GM/NA10888, the chromosome is present in all of the cells.Not all genes are present at 100%, for example in the cell line GM/NA11418, only 88% of the cells contain chromosome 14. A percentage of cells in lines GM/NA07299 and GM/NA10478 both contain two different chromosome.(Catalog, 1992/1993)

NIGMS HUMAN/RODENT SOMATIC CELL HYBRID MAPPING PANEL #2

PERCENTAGE OF CELLS WITH HUMAN CHROMOSOMES

CHROMOSOME#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
HYBRID/DNA																								
GM/NA07299	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	85	0
GM/NA10826B	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10253	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10115	0	0	0	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10114	0	0	0	0	93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10629	0	0	0	0	0	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10791	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10156B	0	0	0	0	0	0	0	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10611	0	0	0	0	0	0	0	0	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10926B	0	0	0	0	0	0	0	0	0	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10927A	0	0	0	0	0	0	0	0	0	0	92	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10868	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA10898	0	0	0	0	0	0	0	0	0	0	0	0	92	0	0	0	0	0	0	0	0	0	0	0
GM/NA10479	0	0	0	0	0	0	0	0	0	0	0	0	0	88	0	0	0	0	0	0	0	0	0	0
GM/NA11418	0	0	0	0	0	0	0	0	0	0	0	0	0	0	88	0	0	0	0	0	0	0	0	0
GM/NA10567	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0	0	0	0
GM/NA10498	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96	0	0	0	0	0	0	0
GM/NA11010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	72	0	0	0	0	0	0
GM/NA10449	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96	0	0	0	0	0
GM/NA10478	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	76	0	0	0	0
GM/NA10323	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	86	0	0	0
GM/NA10888	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0
GM/NA06318B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
GM/NA06317	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96

Table 2 - Name of the genes primers were synthesized for; gene symbol; and PCR designation.

Name of Gene	Gene Symbol	PCR Designation
cartilage-derived morphogenic protein 1	CDMP1-PEN	CDMP1-1
		CDMP1-2
asialoglycoprotein receptor 2	ASGR2	ASGR2-1
		ASGR2-2
tumorigenic conversion associated gene	CATR1-LSB	CATR1-LSB
transcriptionally inducible nuclear receptor	TINUR-LSB	TINUR-LSB
regulator of mitotic spindle assembly 1	RMSA1-PEN	RMSA1-PEN
hepatoma-derived growth factor (heparin-binding)	HDGF-LSB	HDGF-LSB
cytochrome c oxidase subunit VIa polypeptide 1	COX6A1	COX6A1

Table 3 - PCR designation, sequence, primer type, 5' / 3' to coding sequence, and product length of primers synthesized. Annealing temperature of successful primer pairs.

Gene Symbol	Sequence 5' to 3'	Forward / Reverse Primer	5' / 3' to Coding Sequence	Product Length (bp)	Annealing Temp. (Celsius)
CDMP1-1	TGGCAGGAGC ATCTACACAG	forward	3'	329	N / A
	GGAAGTCACC AGGCACAAAT	reverse			
CDMP1-2	TGCACGTCTG GATACGAGAG	forward	5'	205	58
	TGACACCAAA GAGAACAGCG	reverse			
ASGR2	TTCCTACGCC ATTGAAGAGG	reverse	3'		
ASGR2-1	CACTGAGACA GGGGTATGGG	forward		146	57
ASGR2-2	TAACCCATAC CCCACACCTG	forward		211	N/A
CATR1-LSB	CTCTAGGTAT CAGTGGGGCG	reverse	3'	216	N / A
	TTTCCAAGGG CCAATCTATG	forward			
TINUR-LSB	CTGAACTGCA ACAACCAAGC	reverse	3'	303	N / A
	CAGAGATAGC CGTGTGAGCA	forward			
RMSA1-PEN	GTTGTAGCAG GAAAGCAGCC	forward	3'	249	57.5
	TACCCCCACC CAAAGTCATA	reverse			
HDGF-LSB	TCATCAAGAG AATTTGGGGC	forward	3'	313	N / A
COX6A1	TTTAAGCCAT CTCCTGCCAC	reverse	3'	329	N / A

Table 4 - Coupling efficiency and concentration of primers synthesized.

PCR Designation	Forward / Reverse Primer	Coupling Effiency(%)	Primer Stock Conc(µg/mL)
CDMP1-1	forward	100	2251.4
	reverse	100	2856.9
CDMP1-2	forward	99.1	2932.9
	reverse	93.6	4131.6
ASGR2	reverse	100	4115.1
ASGR2-1	forward	100	1360.4
ASGR2-2	forward	86.4	614.60
CATR-LSB	forward	72.0	154.60
	reverse	94.4	830.78
TINUR-LSB	forward	100	0
	reverse	65.8	12.458
RMSA1-PEN	forward	100	4490.3
	reverse	99.7	4799.0
HDGF1-LSB	forward	100	4079.0
COX6A1	reverse	76.8	42.157

Table 5 - coupling efficiency data from DNA synthesis interrupted by loss of solvents.

Position	Base	Detrit Start	Detrit Value
20	C	0.44	81
19	A	0.44	211
18	C	0.44	187
17	C	0.45	175
16	G	0.42	26
15	T	0.45	156
14	C	0.45	144
13	C	0.45	135
12	T	0.46	172
11	C	0.45	135
10	T	0.46	142
9	A	0.45	141
8	C	0.46	126
7	C	0.46	128
6	G	0.42	28
5	A	0.46	124
4	A	0.45	135
3	T	0.46	117
2	T	0.46	1
1	T	0.00	0

Table 6 - Coupling efficiency data from DNA synthesis not detritylating.

Position	Base	Detrit Start	Detrit Value
20	A	0.44	65
19	A	0.41	11
18	C	0.43	4
17	A	0.43	6
16	C	0.43	3

Table 7 - Typical coupling efficiency data for successful DNA synthesis.

Position	Base	Detrit Start	Detrit Value
20	C	0.49	88
19	C	0.48	143
18	G	0.46	121
17	A	0.48	151
16	C	0.49	152
15	G	0.48	131
14	A	0.49	163
13	A	0.51	162
12	A	0.51	170
11	G	0.50	137
10	G	0.50	137
9	A	0.52	160
8	C	0.54	157
7	G	0.53	135
6	A	0.55	164
5	T	0.58	165
4	G	0.54	139
3	T	0.57	164
2	T	0.57	166
1	G	0.55	140

Table 8 - Initial 6 pools used in chromosome assignment.

Pool	Chromosome	Initial Conc (ng/µl)
1	5	371ng/µl
	2	368
	22	376
	13	377
	17	370
2	Y	358
	X	366
	12	364
	6	355
	11	357
3	4	343
	1	352
	19	326
	7	327
4	8	311
	21	314
	20	313
	10	323
5	3	310
	18	283
	15	285
	14	292
6	16	330
	9	351

Table 9 - 12 pools used in chromosome assignment.

Pool	Chromosome	Initial Conc
1	X	366ng/µl
	22	376
2	21	314
	20	313
3	19	326
	18	283
4	17	370
	16	330
5	15	285
	14	292
6	13	377
	12	364
7	11	357
	10	323
8	9	351
	8	311
9	7	327
	6	355
10	5	371
	4	343
11	3	310
	2	368
12	Y	358
	1	352

Fig. 1 - Human rodent somatic cell hybrid showing chromosomes

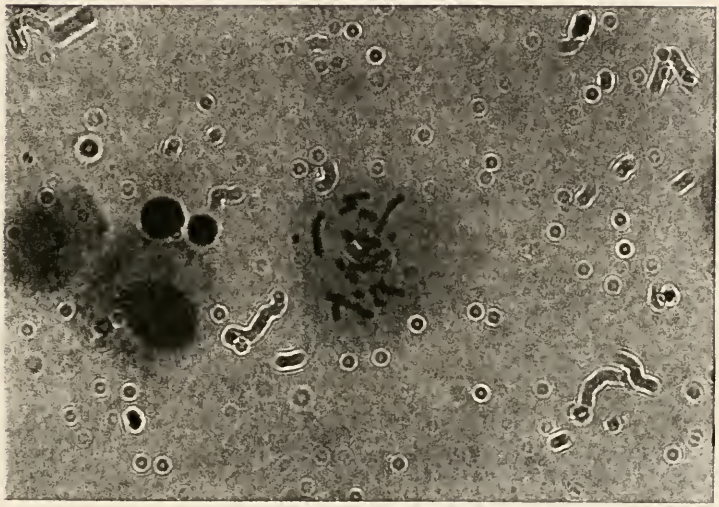


Fig. 2 - Diagram of the polymerase chain reaction(Cooper)

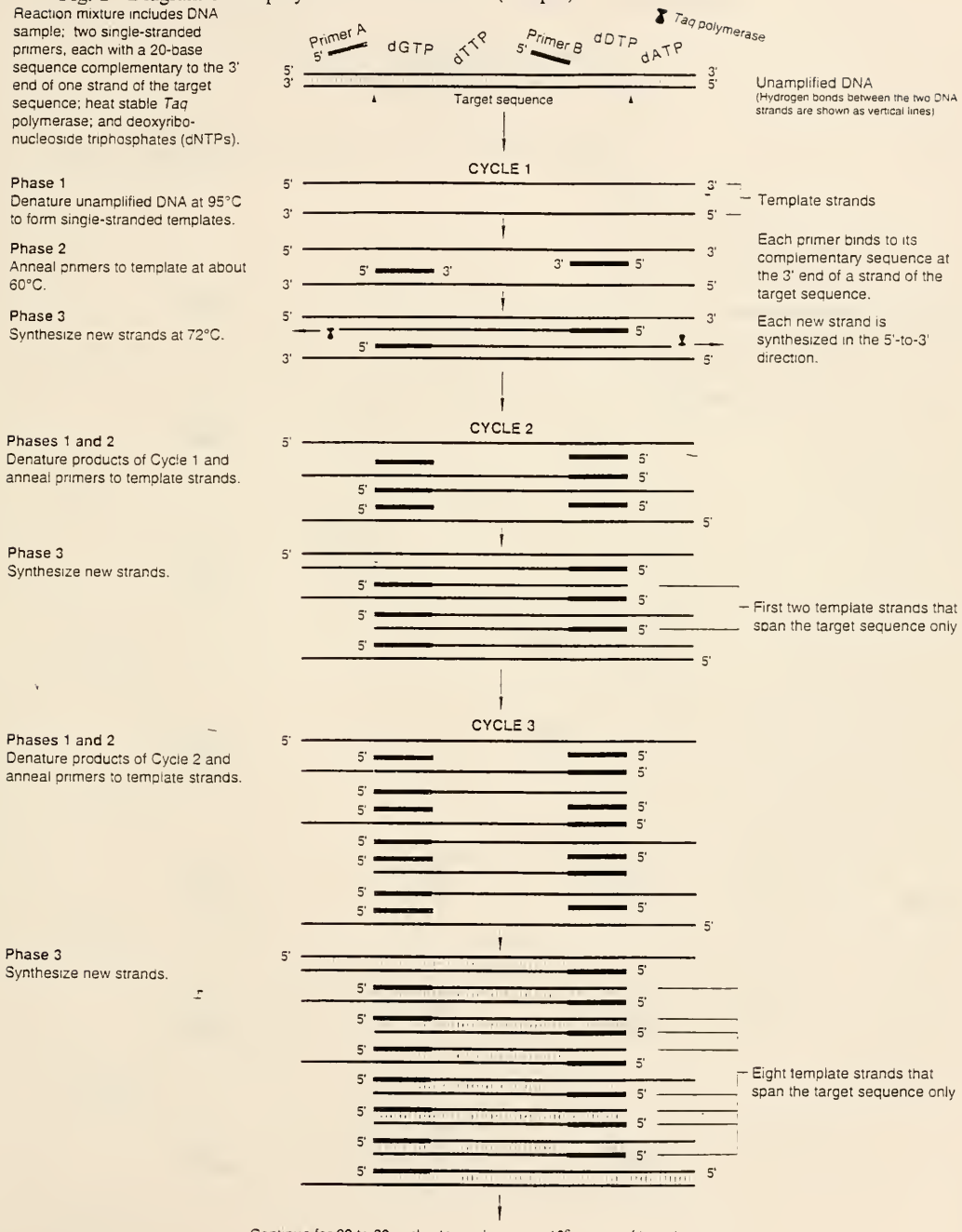


Fig. 3 - Primers bound at the 3' hydroxyl end of the template strand.(Rolfs)

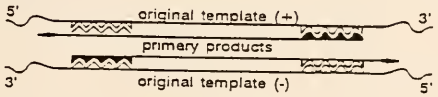


Fig. 4 - Phosphoramidite method of DNA synthesis(Pharmacia)

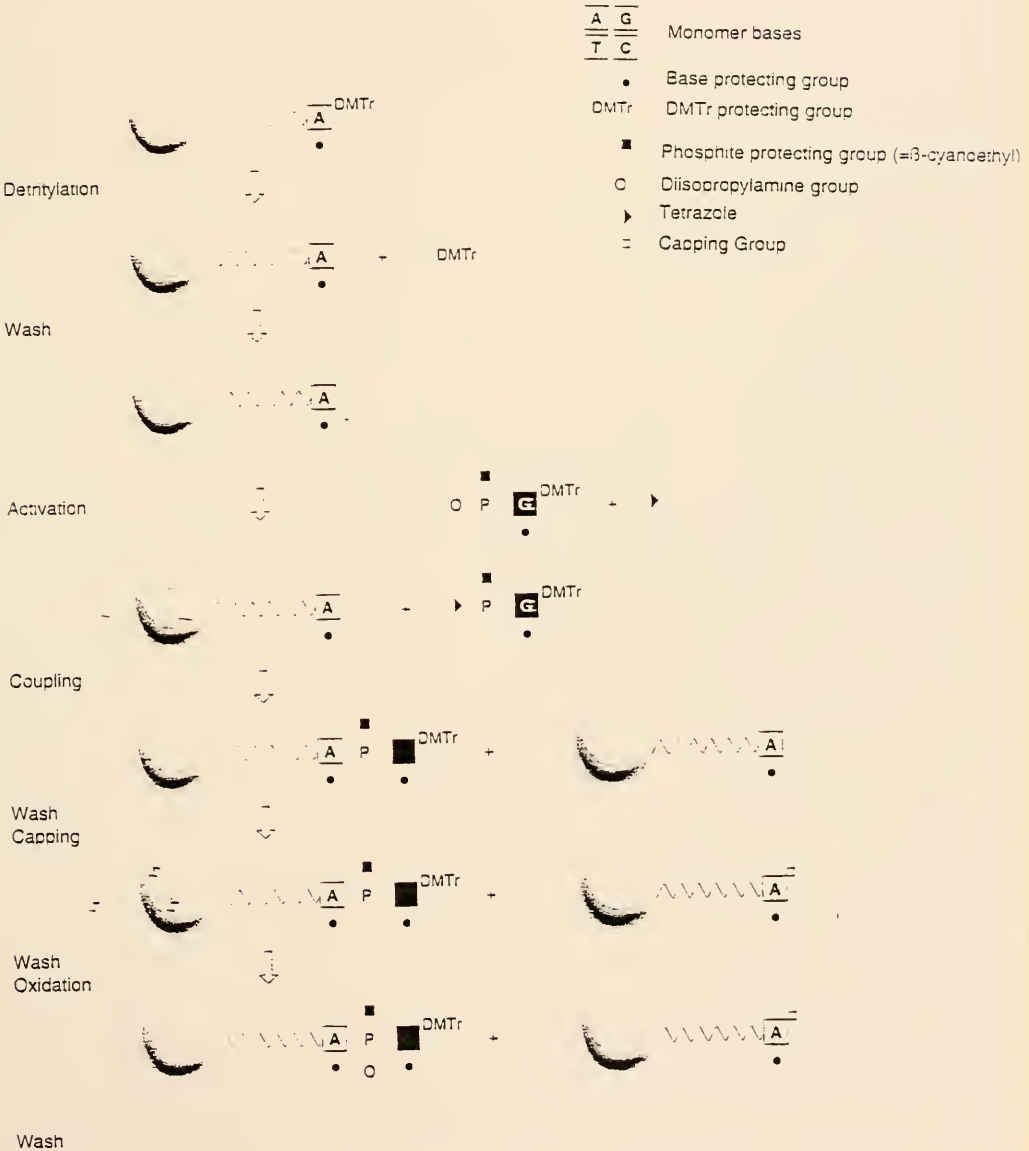


Fig. 5 - Ethidium ion intercalates with DNA.

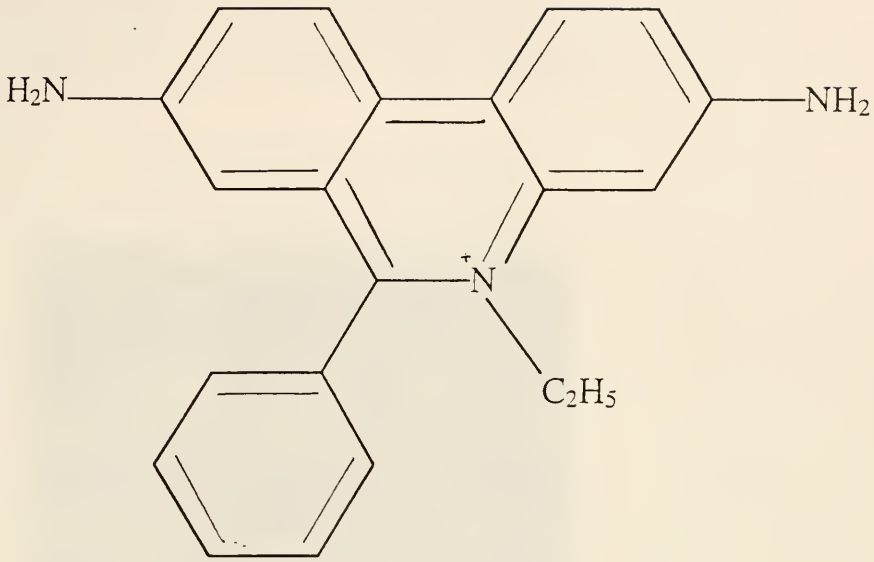


Fig. 6 - Preliminary assignment for CDMP1-1 (329bp) and CDMP1-2 (205bp). CDMP1-1 was determined unusable and CDMP1-2 was shown to be a productive primer pair. Faint band detected in well 4 for pool 2 containing chromosomes 20 and 21. Lanes 1 and 12 = lambda *Hin* D III DNA. Lane 2 = PhiX *Hae* III DNA. Lane 3 = TE and CDMP1-1. Lane 4 = Gibco DNA and CDMP1-1. Lane 5 = Sigma DNA and CDMP1-1. Lane 6 = AHH1 DNA and CDMP1-1. Lane 7 = TE and CDMP1-2. Lane 8 = Gibco DNA and CDMP1-2. Lane 9 = sigma DNA and CDMP1-2. Lane 10 = AHH1 DNA and CDMP1-2. Lane 11 = Gibco Primer mix and DNA.

Fig. 7 - Preliminary PCR for ASGR2-2 (211bp). No bands detected determining ASGR2-2 to be an unusable primer pair. Lane 1 and 7 = lambda *Hin* D III DNA. Lane 2 = PhiX *Hae* III DNA. Lane 3 = TE and ASGR2-2. Lane 4 = Gibco DNA and ASGR2-2. Lane 5 = Sigma DNA and ASGR2-2. Lane 6 = AHH1 and ASGR2-2.

Fig. 8 - Preliminary PCR of ASGR2-1 (146bp) indicating it to be a successful primer pair. Faint band detected in lanes 4 and 5. Lanes 1 and 12 = lambda *Hin* D III DNA. Lane 2 = PhiX *Hae* III DNA. Lane 3 = TE and ASGR2-1. Lane 4 = Gibco DNA and ASGR2-1. Lane 5 = Sigma DNA and ASGR2-1. Lane 6 = Mapping panel human DNA and ASGR2-1. Lane 7 = TE and CDMP1-1. Lane 8 = Gibco DNA and CDMP1-1. Lane 9 = Sigma DNA and CDMP1-1. Lane 10 = Mapping panel human DNA and CDMP1-1. Lane 11 = Gibco primer mix and DNA.

Fig. 9 - Preliminary PCR of ASGR2-1 (146bp) with decreased stringency by decreasing the temperature and preliminary PCR of RMSA (249bp). Lane 1 and 12 = lambda *Hin* D III DNA. Lane 2 = PhiX *Hae* III DNA. Lane 3 = TE and RMSA. Lane 4 = Sigma DNA and RMSA. Lane 5 = Mapping panel human DNA and RMSA. Lane 6 = Gibco DNA and RMSA. Lane 7 = TE and ASGR2-1. Lane 8 = Sigma DNA and ASGR2-1. Lane 9 = Mapping panel human DNA and ASGR2-1. Lane 10 = Gibco DNA and ASGR2-1. Lane 11 = Gibco primer mix and DNA.

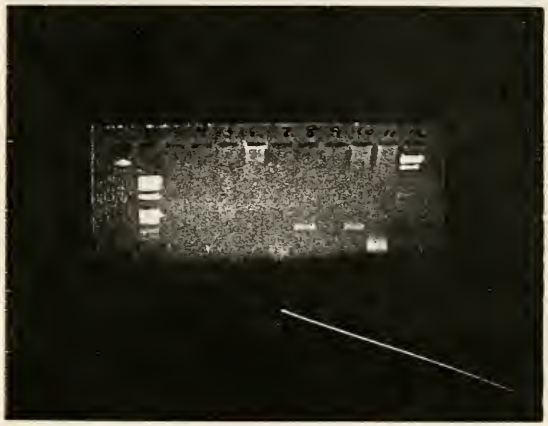


Fig. 10 - Unsuccessful chromosome assignment of CDMP1-2 (205bp). No bands detected. Lanes 1 and 12 = lambda *Hin* D III DNA. Lane 2 = PhiX *Hae* III DNA. Lanes 3 - 6 = Pools 1 - 6 and CDMP1-2. Lane 7 = Mapping panel human DNA and CDMP1-2. Lane 8 - Mapping panel mouse DNA and CDMP1-2. Lane 9 = Mapping panel Chinese hamster DNA and CDMP1-2. Lane 10 = TE and CDMP1-2. Lane 11 = Gibco primer mix and DNA.

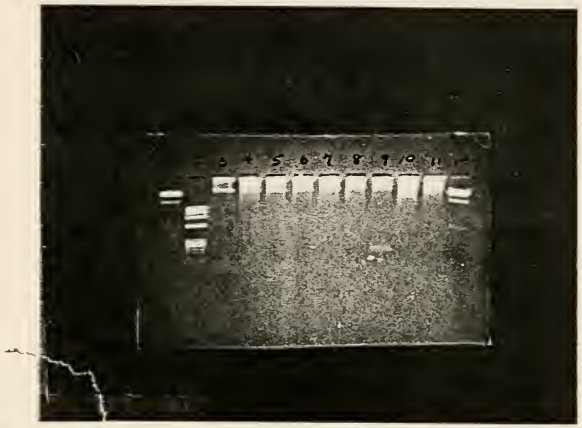


Fig. 11 - Chromosomal pool assignment of CDMP1-2 (205bp) to pool 2, containing chromosomes 20 and 21. Lane 1 and 12 = lambda *Hin* D III DNA. Lane 2 - phiX *Hae* III DNA. Lane 3 = Pool 1. Lane 4 = Pool 2. Lane 5 = Pool 3. Lane 6 = Pool 4. Lane 7 = Pool 5. Lane 8 = Pool 6. Lane 9 = Pool 7. Lane 10 = Pool 8. Lane 11 = Pool 9.

Fig. 12 - Chromosome assignment of CDMP1-2 (205bp) to chromosome 20. Lanes 1 and 11 = lambda *Hin* D III DNA. Lane 2 = PhiX *Hae* III DNA. Lane 3 = chromosome 20 and CDMP1-2. Lane 4 = chromosome 21 and CDMP1-2. Lane 5 = TE and CDMP1-2. Lane 6 = Sigma DNA and CDMP1-2. Lane 7 = Mapping panel human DNA and CDMP1-2. Lane 8 = Mapping panel mouse DNA and CDMP1-2. Lane 9 = Mapping panel Chinese hamster DNA and CDMP1-2. Lane 10 = Gibco DNA and CDMP1-2.

Fig. 13 - Chromosome assignment of CDMP1-2 (205bp) to chromosome 20. Lanes 1 and 10 = lambda *Hin* D III DNA. Lane 2 = 1Kb ladder. Lane 3 = TE and CDMP1-2. Lane 4 = chromosome 2 and CDMP1-2. Lane 5 = chromosome 20 and CDMP1-2. Lane 6 = Mapping panel human DNA and CDMP1-2. Lane 7 = Mapping panel mouse DNA and CDMP1-2. Lane 8 = Mapping panel Chinese hamster DNA and CDMP1-2. Lane 9 = Gibco primer mix and DNA.

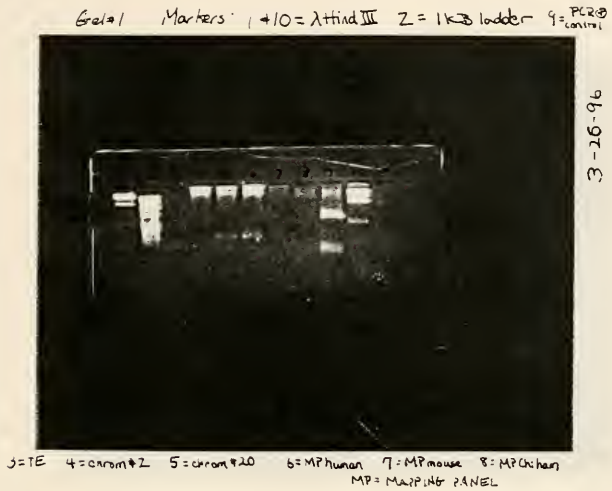


Fig. 14 - Chromosomal pool assignment of ASGR2-1 (146bp). Faint bands detected in lanes 6, 7, and 8. Lanes 1, 2, 13, and 14 = 1Kb ladder. Lane 12 and 21 = lambda *Hin* D III DNA. Lane 3 = Pool 1. Lane 4 = Pool 2. Lane 5 = Pool 3. Lane 6 = Pool 4. Lane 7 = Pool 5. Lane 8 = Pool 6. Lane 9 = Pool 7. Lane 10 = Pool 8. Lane 11 = Pool 9. Lane 15 = Pool 10. Lane 16 = Pool 11. Lane 17 = Pool 12. Lane 18 = Mapping panel human DNA and ASGR2-1. Lane 19 = Mapping mouse DNA and ASGR2-1. Lane 20 = Mapping panel Chinese hamster DNA and ASGR2-1.

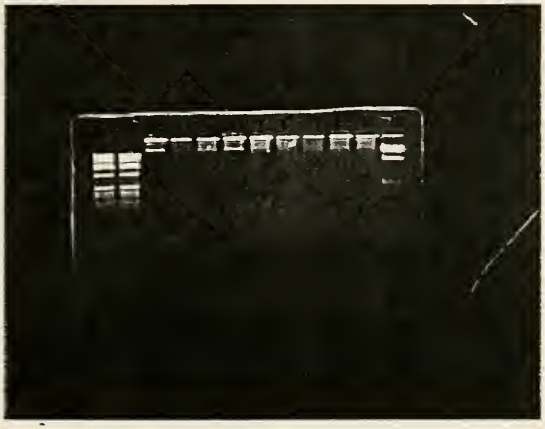


Fig. 15 = Unsuccessful chromosome assignment of ASGR2-1 (146bp) at 1.50mM Mg^{++} concentration. Lane 1 = lambda *Hin* D III DNA. Lane 2 and 12 = 1Kb ladder. Lane 3 = chromosome 17. Lane 4 = chromosome 16. Lane 5 = chromosome 15. Lane 6 = chromosome 14. Lane 7 = chromosome 13. Lane 8 = chromosome 12. Lane 9 = Mapping panel human DNA. Lane 10 = Mapping panel Chinese hamster DNA. Lane 11 = PCR positive control.

Fig. 16 - Chromosomal assignment of ASGR2-1 (146bp) with further decreased stringency by increasing the concentration of Mg^{++} to 1.75mM. No bands detected. Lane 1 and 11 = 1Kb ladder. Lane 2 = lambda *Hin* D III DNA. Lane 3 = chromosome 17. Lane 4 = chromosome 16. Lane 5 = chromosome 15. Lane 6 = chromosome 14. Lane 7 = chromosome 13. Lane 8 = chromosome 12. Lane 9 = Mapping panel human DNA. Lane 10 = Mapping panel Chinese hamster DNA.

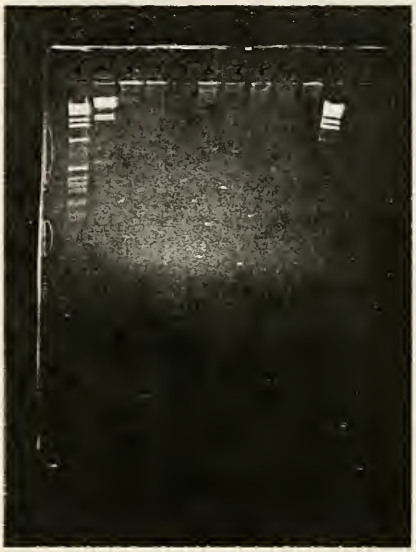
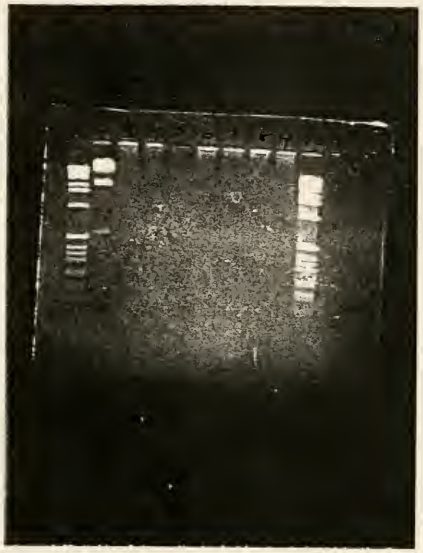


Fig. 17 = Unsuccessful chromosome assignment of ASGR2-1 (146bp) at 2.00mM Mg^{++} concentration. No bands detected. Lane 1 and 10 = 1Kb ladder. Lane 2 = lambda *Hin* D III DNA. Lane 3 = chromosome 17. Lane 4 = chromosome 16. Lane 5 = chromosome 15. Lane 6 = chromosome 14. Lane 7 = chromosome 13. Lane 8 = chromosome 12. Lane 9 = Mapping panel human DNA.



Appendix I

Reagents for DNA synthesis were all purchased from Pharmacia Biotech:

tetrazole
dG beta-Cyanoethyl Phosphoramidite
dC beta-Cyanoethyl Phosphoramidite
T beta-Cyanoethyl Phosphoramidite
dA beta-Cyanoethyl Phosphoramidite
Acetonitrile
Detritylation
Dichloroethane
Capping A and B
Oxidation

Appendix II

For preparing five 20µl PCR reactions, the master mix would contain (all purchased from Gibco):

81µL of sterile double distilled water
10µL 15mM Mg^{++} buffer solution(final conc. = 1.5mM)
2µL 10mM dNTP(final conc. = 0.2mM)
1µL 100ng/µL reverse primer(final conc. = 0.1mL)
1µL 100ng/µL forward primer(final conc. = 0.1mL)
0.5µL 5U/µL *Taq* DNA polymerase(final conc. = 2.5 units)
1µL DNA(final conc. = 200ng or 2 x 10^4 molecules)

Appendix III

Lambda DNA *Hin* D III Digest

Fragment #	# of Base Pairs	Daltons
1	23,130	15.0×10^6
2	9,416	6.12×10^6
3	6,557	4.26×10^6
4	4,361	2.83×10^6
5	2,322	1.51×10^6
6	2,027	1.32×10^6
7	564	0.37×10^6
8	125	0.08×10^6

PhiX *Hae* III Digest

Fragment #	# of Base Pairs	Daltons
1	1353	8.79×10^5
2	1078	7.01×10^5
3	872	5.67×10^5
4	603	3.92×10^5
5	310	2.02×10^5
6a	281	1.83×10^5
6b	271	1.76×10^5
7	234	1.52×10^5
8	194	1.26×10^5
9	118	0.767×10^5
10	72	0.468×10^5

1Kb ladder

Fragment #	# of Base Pairs
1	12,216
2	11,198
3	10,180
4	9162
5	8144
6	7126
7	6108
8	5090
9	4072
10	3054
11	2036
12	1636
13	1018
14	506
15	517
16	396
17	344
18	208

References

Acquaah, George. Practical Protein Electrophoresis for Genetic Research. Portland, OR: Dioscorides Press. 1992.

Adams, M.D. "Complementary DNA sequencing: expressed sequence tags and human genome project." Science, vol. 252: 1651 - 6. 1991

Aldhous, P. "Human genome project. New office for HUGO in Soviet Union[new]." Nature, vol. 351: 683. 1991.

Aldhous, P. "Human genome project. Tit for tat on patents?[news][see comments]" Nature, vol. 353: 785. 1991.

Anderson, C. and P. Aldhous. "Human genome project. Still room for HUGO?[news]." Nature, vol. 355: 4-5. 1992.

Baumiller, R.C. "Teaching about the human genome project." AmJ. Hum Genet, vol. 49: 501-2. 1991.

Beardsley, Tim. "Trends in human genetics: Vital data." Scientific American, March issue: 100-105. 1996.

Billings, P.R. "Promotion of the human genome project [letter; comment]." Science, vol. 250: 1071. 1990.

Bodmer, W.F. "Cancer genetics and the human genome." Hosp Pract, vol. 26: 101-6, 11-2, 117. 1991.

Brody, H.. "Ethics, technology, and the human genome project." J Clin Ethics, vol. 2: 278-81. 1991.

Caskey, C.T. & B.J. Rossiter. "The human genome project. Purpose and potential." J Pharm Pharmacol, vol. 44 Suppl 1: 1948-204. 1992.

Caskey, C.T. and R.G. Worton. "ASHG human genome committee report. The human genome project: implications for human genetics." Am J Hum Genet, vol. 49: 687-91. 1991.

Cavalli-Sforza, L.L. "Call for a worldwide survey of human genetic diversity: a vanishing opportunity for the Human Genome Project." Genomics, vol. 11: 490-1. 1991.

Catalog of Cell Lines. Human Genetic Mutant Cell Repository. 1992/1993.

Catalog of Cell Lines. Human Genetic Mutant Cell Repository. 1994/1995.

Chang, Steven C. "Cartilage-derived morphogenetic proteins." The Journal of Biological Chemistry, vol. 269, no. 45: 28227-28234. 1994.

Charlieu, Jean Paul. "PCR as a technique used daily in molecular biology." PCR Technology: Current Innovations. CRC Press, Inc, 1-4. 1994.

Clarkson, John M. and Benjamin D. Cobb. "A simple procedure for optimising the polymerase chain reaction (PCR) using modified Taguchi methods." Nucleic Acids Research, vol. 22, no. 18: 3801-3805. 1994.

Collins, Francis and David Galas. "A New Five Year Plan for the U.S. Human Genome Project." Science, vol. 262: 43-46. 1993.

Cooper, N.G.(edt.) The Human Genome Project: Deciphering the Bluepreint of Heredity. Mill Valley, CA: University Science Books. 1994.

Cox, D.R. "Assessing mapping progress in the Human Genome Project." Science, vol. 265: 2031-2032. 1994.

Cui, K.H. "Genome project and human reproduction." Hum Reprod, vol. 10: 1275-1279. 1995.

"Current trends in mapping human genes." FASEB J, vol. 5: 12-20. 1991.

Cuticchia, A.J. "Managing all those bytes: the Human Genome Project[see comments]." Science, vol. 262: 45-8. 1993.

Davies, Robin. "Physical mapping of the human ELA1 gene between D12S361 and D12S347 on chromosome 12q13." Genomics, vol. 29: 766 - 768. 1995.

`Dausset, J. H. Cann, D. Cohen, M. Lathrop, J-M Lalouel, R. white. "Centre d'Etude du Polymorphisme Humain (CEPH): Collaborative genetic mapping of the human genome." Genomics., vol. 6, no. 3: 575-577. 1990.

Dionne, CA. R. Kaplan, H. Senanez, S.J. O'Brien, M. Jaye. "Chromosome assignment of the oncogene FGF-5 to human chromosome 4." Biotechniques, vol. 3, no. 2: 190-194. 1990.

Dulbecco, R. "Co-chairman's remarks: the human genome project and cancer." Gene, vol. 135: 259-60. 1993.

Durfy, S.G. "Ethics and the Human Genome Project." Arch Pathol Lab Med, vol. 117: 466-9. 1993.

Edgington, S.M. "Breaking open the bottlenecks in genomic DNA sequencing. should biotechnology become a stronger player in shaping the human genome project's overall development." Biotechnology(NY), vol. 11: 39-42. 1993.

Engel, L.W. "The Human Genome Project. History, goals, and progress to date." Arch Pathol Lab Med, vol. 117: 459-65. 1993.

Engelking, C. The human genome exposed: a glimpse of promise, predicament, and impact on practice." Oncol Nurs Forum, vol. 22: 3-9. 1995.

Erlich, Henry A., edt. Current Communications in Molecular Biology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 1989.

Faloona, Fred A. and Kary B. Mullis. "Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction." Methods in Enzymology, vol. 155: 335-350. 1987.

Fasman, Kenneth H. "The GDB Human Genome Data Base anno 1994." Nucleic Acids Research, vol. 22, no 17: 3462-3469. 1994.

Flower, M.J. and D. Heath. "Micro-anatomo politics: mapping the Human Genome Project." Cult med Psychiatry, vol. 17: 27-41. 1993.

Freshney, R. Ian. Culture of Animal Cells: A Manual of Basic Technique. New York, NY: Wiley-Liss, Inc. 1987.

Garver, K.L. and B. Garver. "The Human Genome Project and eugenic concerns." Am J Hum Genet, vol. 54: 148-58. 1994.

"Genome diversity alarms." Nature, vol. 377: 372. 1995.

"Genome programmes: an opportunity for biology." Biomed Pharmacother, vol. 48: 183-190. 1994.

Goodfellow, P.N. "Human genome project. Variation is now the theme[news; comment]." Nature, vol. 359: 777-8. 1992.

Gottesman, M.M. and F.S. Collins. "The role of the human genome project in disease prevention." Prev Med, vol. 23: 591-594. 1994.

Green, E.D. and R.H. Waterston. "The human genome project. Prospects and implications for clinical medicine[see comments]." JAMA, vol. 266: 1966-75. 1991.

Gros, F. "Co-chairman's remarks: Jim Watson: the double helix, messenger RNA and the Human Genome Project--a personal view." Gene, vol. 135: 305-8. 1993.

Guyer, M.S. & F.S. Collins. "How is the Human Genome Project doing, and what have we learned so far?" Proceedings of the National Academy of Sciences, U.S.A., vol. 92: 10841-10848. 1995.

Guyer, M.S. and F.S. Collins. "The Human Genome Project and the future of medicine." Am J Dis Child, vol. 147: 1145-52. 1993.

Guyer, Ruth Levy and Daniel E. Koshland, Jr. "The Molecule of the Year." Science, vol. 246, no 4937: 1541-1546.. 1989.

Harper, P.S. "The human genome project and medical genetics[editorial]." J. Med Genet, vol. 29: 1-2. 1992.

Healy, B. "Hearing on the possible uses and misuses of genetic information." Hum Gene Ther, vol 3: 51-6. 1992.

Hoffman, E.P. "The Evolving Genome Project: current and future impact." Am J Hum Genet, vol. 54: 129-36. 1994.

Holtzman, N.A. "Discovery, transfer, and diffusion of technologies for the detection of genetic disorders. Policy implications." Int J Technol Assess Health Care, vol. 10: 562-572. 1994.

"The Human Genome Project and patents[letter]." Science, vol. 254: 1710, 1712. 1991.

Ikawa, Y. "Human genome efforts in Japan." FASEB J, vol. 5: 66-9. 1991.

Innis, Michael A., edt. PCR Protocols: A Guide to Methods and Applications. San Diego, CA: Academic Press, Inc. 1990.

Jordan, E. "The Human Genome Project: where did it come from, where is it going?[editorial]." Am J Hum Genet, vol. 51: 1-6. 1992.

Juengst, E.T. "Human genome research and the public interest: progress notes from an American science policy experiment." Am. J. Hum. Gen, vol. 54: 121-8. 1994.

Knoppers, B.M. and R. Chadwick. "The Human Genome Project: under an international ethical microscope." Science, vol. 265: 2035-6. 1994.

Korenberg, J.R. and D.L. Rimoin. "Medical genetics." JAMA, vol. 273: 1692-1693. 1995.

Kozyavkin, S.A. "DNA enzymology above 100 degrees C. Topoisomerase V unlinks circular DNA at 80-122 degree C." J Biol Chem, vol. 270: 13593-13595. 1995.

Lippman, A. "Led (astray) by genetic maps: the cartography of the human genome and health care." Soc Sci Med, vol. 35: 1469-76. 1992.

Little, P. "Human Genome Project: Mapping the way ahead[comment]." Nature, vol. 359: 367-8. 1992.

Macer, D. "The 'far east' of biological ethics." Nature, vol. 359: 770. 1992.

Mac Kay, C.R. "Discussion points to consider in research related to the human genome." Hum Gene Ther, vol. 4: 477-95. 1993.

Maniatis, T. Molecular Cloning: A Laboratory Manual. Plainview, NY: Cold Spring Harbor Laboratory Press. 1989.

Margalit, H. "A complete Alu element within the coding sequence of a central gene." Cell, vol. 78: 173-174. 1994.

Marshall, E. "Human genome project. Emphasis turns from mapping to large-scale sequencing[news]" Science, vol. 268: 1270-1271. 1995.

Matsubara, K. and K. Okubo. "cDNA analyses in the human genome project." Gene, vol. 135: 265-74. 1993.

McInerney, J.D. "The Human Genome Project relevant to genetics education in high school." Am J. Hum Genet, vol. 52: 235-8. 1993.

Molitor, T.W. "Polymerase chain reaction (PCR) amplification for the detection of porcine parvovirus." J Virol Methods, vol. 32: 201-11. 1991.

Muller-Hill, B. "The shadow of genetic injustice." Nature, vol. 362: 491-2. 1993.

Mullis, KB. "The polymerase chain reaction: Why it works." Current Communications in Molecular Biology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 237-243. 1989.

Murray, J.C. "A comprehensive human linkage map with centimorgan density. Cooperative Human Linkage Center(CHLC)." Science, vol. 265: 2049-2054. 1994.

Olson, M.V. "The human genome project." Proceedings of the National Academy of Sciences, U.S.A., vol. 90: 4338-44. 1993.

Parker, L.S. "Bioethics for human geneticists: models for reasoning and methods for teaching." Am J Hum Genet, vol. 54: 137-47. 1994.

Pearson, M.L. & D. Soll. "The Human Genome Project: a paradigm for information management in the life sciences." FASEB J, vol. 5: 35-9. 1991.

Pharmacia Biotech. Gene Assembler Special/4 Primers: User Manual.

PRIMER. The Whitehead Institute for Biomedical Research. Nine Cambridge Center Cambridge, Massachusetts 02142.

"Project to sequence human genome moves on to the starting blocks." Nature, vol. 375: 93-4. 1995.

Rolfs, Arnold. PCR: Clinical Diagnostics and Research. New York, NY: Springer-Verlag, 1-18. 1992.

Rose, EA. "Applications of the polymerase chain reaction to genome analysis." JASEB J., vol. 5, no. 1: 46-54. 1991.

Rossiter, B.J. and C.T. Caskey. "The human genome project." Clin Obstet Gynecol, vol. 36: 466-75. 1993.

Sachs, B.P. and B. Korf. "The Human Genome Project: implications for the practicing obstetrician." Obstet Gynecol, vol. 81: 458-62. 1993.

Saiki, Randall K. "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase." Science, vol. 239: 487-491. 1988.

Saiki, Randall K. "Optimization of the polymerase chain reaction." Current Communications in Molecular Biology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 25-29. 1989.

Sawicki, M.P. "Human Genome Project." Am J Surg, vol. 165: 258-64. 1993.

Shapiro, L.J. "Human genome project." West J Med, vol. 158: 181. 1993.

Sharrocks, Andrew D. "The design of primers for PCR." PCR Technology: Current Innovations. CRC Press, Inc, 5-10

Spiess, Martin. " Sequence of a second human asialoglycoprotein receptor: Conservation of two receptor genes during evolution." Proceedings of the National Academy of Sciences, USA, vol. 82: 6465-6469. 1985.

Swinbanks, D. "Human genome. Japan's project stalls[news]." Nature, vol. 349: 360. 1991.

Swinbanks, D. "Japan's human genome project takes shape[news]" Nature, vol. 351: 593. 1991.

Szybalski, W. "From the double-helix to novel approaches to the sequencing of large genomes." Gene, vol. 135: 279-90. 1993.

van Ommen, G.J. "Advances in genome research and molecular diagnostics." Keio J Med, vol. 43: 211-213. 1994.

Voet, Donald and Judith G. Voet. Biochemistry, 2nd edition. New York, New York: John Wiley & Sons, Inc. 1995.

Whittaker, L.A. "The implications of the Human Genome Project for family practice[see comments]." J Fam Pract, vol. 35: 294-301. 1992.

Wright, PA and D. Wynford-Thomas. "The polymerase chain reaction: Miracle or mirage? A critical review of its uses and limitations in diagnosis and research." J. Pathol., vol. 162, no. 2: 99-117. 1990.

Yeo, Jing-Ping. "A new chromosomal protein essential for mitotic spindle assembly." Nature, vol. 367: 288-291. 1994.

Zhang, Ji. K. Derriendt, P. Marynen, H. VanDen Berghe, J-J. Cassiman. "Chromosome mapping using polymerase chain reaction on somatic cell hybrids." Cancer Genet. Cytogenet, vol. 45, no. 2: 217-221. 1990.

Zinder, N.D. "Patenting cDNA 1993: efforts and happenings (Human Genome Project; NIH: US PTO; onco-mouse; European Patents)." Gene, vol. 135: 295-8. 1993.

Acknowledgments

Thank you to Dr. Davies for all of your time, energy, enthusiasm, and patience, without whom which none of this would have been possible.

Thank you to Dr. Rosinski for all of your advise, comments, and patience in the production of this thesis.

Thank you to Dr. Granger for your help with the temperamental DNA synthesizer and your patience with biochem assignments.

Sweet Briar Honor Pledge

I pledge that I will guarantee the validity of my word, maintain absolute honesty in my work, and respect the property of others. Realizing that these standards are an integral part of life at Sweet Briar, I hereby assume my obligation to uphold them. I will report myself and ask others to report themselves for any infraction of this pledge.