Genetics in the Laboratory

National DNA Day
April 25, 2008

When do we need genetics in the laboratory?

- Forensic testing: testing DNA from biological specimens found at a crime scene
- Diagnostic testing: to determine the presence or absence of a genetic factor causing disease in an individual
  - Prenatal testing: a subset of diagnostic testing
- Carrier testing: to determine if an individual is a *carrier* of a recessive condition which may be passed on to their offspring
- Research testing: to discover the mechanisms by which genes and proteins operate for application in clinical practice
- Many others!
  - Paternity testing
  - Infertility testing
  - Monitoring of the progression of some cancers or hematological disorders

Utilize many, many different kinds of tests and techniques to get results

- Including . . .
  - DNA extraction
  - Karyotyping
  - FISH analysis
  - Gel electrophoresis
  - Mass spectrometry
- And many, many others

DNA Extraction

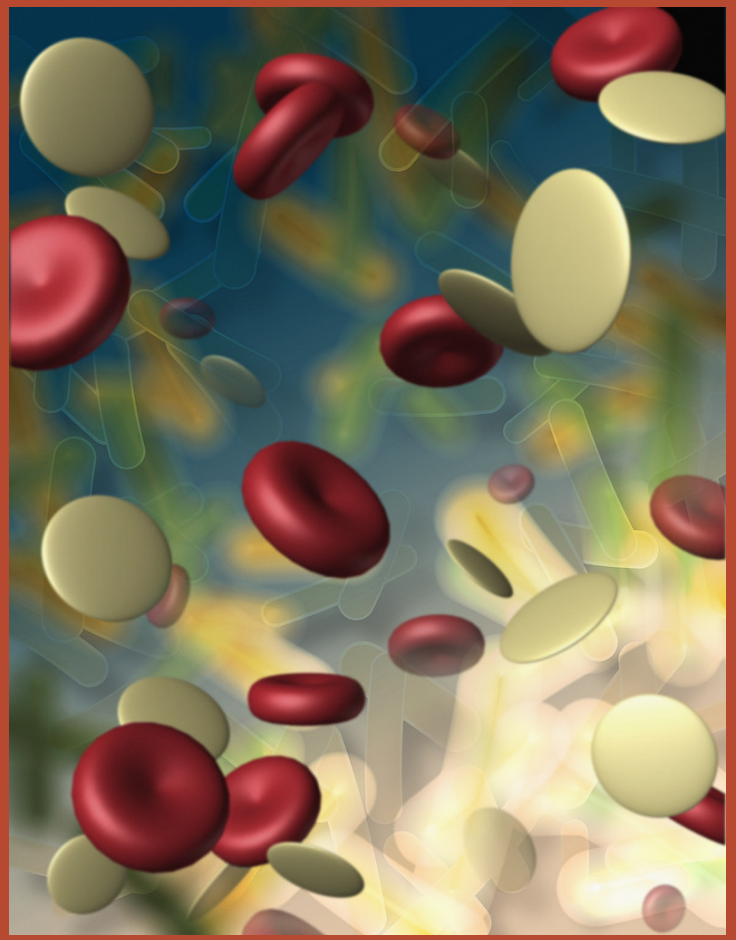
- Goal is to isolate DNA from a sample of tissue
- Need to get DNA on its own away from proteins and other components of a cell
- Samples can be from a cheek swab, a blood spot, or any other sample from which cells can be easily removed
- May need to isolate human DNA in order to:
  - Perform genetic testing
  - Identify a body
  - Perform forensic analysis

Isolating DNA from blood

- Where is the DNA located in the blood?

In the cells!

- Specifically, in the nuclei of cells
- But wait a minute! A red blood cell doesn't have a nucleus!
- So we have to get the DNA from a white blood cell



The DNA is in the nucleus of the white blood cell

- How do we get the white blood cells separated from the rest of the blood?

Spin the blood

- White blood cells are heavier than red blood cells, especially if the red blood cells are broken up first
- So when you put blood in a test tube, break up the RBCs with solution, then centrifuge the tube, the white blood cells all sink to the bottom of the tube, forming a pellet
- Remove the pellet and you've got your white blood cells

The DNA is in the nucleus of the white blood cell

- We’ve got the white blood cells
- How do we get the DNA out of the cell?

Break it up!

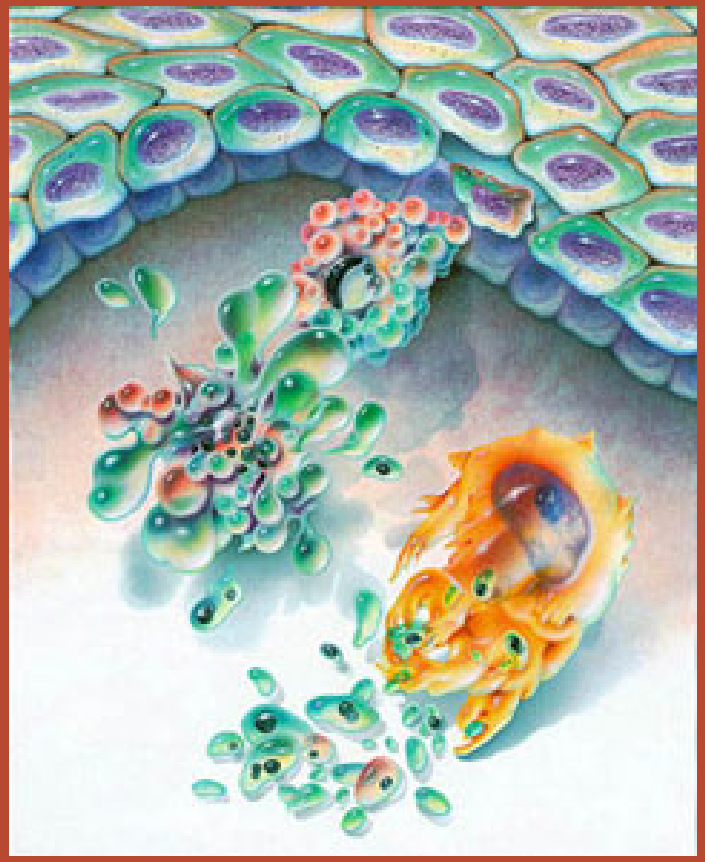


Photo courtesy of Lawrence Berkeley National Laboratory

- Add a *lysis* solution to the pellet
- Then centrifuge again
- The cell proteins will move towards the bottom of the tube and form a new pellet
- The DNA will remain floating around in the liquid (called the *supernatant)*

The final steps

- Add isopropanol to the tube to make the DNA appear in the solution
- Centrifuge AGAIN
- DNA will be in the pellet at the bottom of the tube
- Wash the DNA with ethanol
- Preserve it in buffer solution
- The DNA is ready!

The DNA is ready for testing

- So now what do I do with it?
- It all depends on the question you're trying to answer
- Chances are, you're going to need to prepare the DNA even further before you can begin to analyze it
- Each laboratory technique requires its own DNA preparation in order to use it properly

When testing is needed

- A baby is referred to a geneticist because she has a small head size and is “floppy”
  - “Floppy” meaning she seems very weak and loose in her movements
- Geneticist examines her and notices her facial features have a distinct, different appearance
- The geneticist also notices when the baby cries it kind of sounds like a cat yowling
- The geneticist takes a sample of blood and sends it to the lab, asking for an analysis of the chromosomes

Chromosome Analysis

- When the lab receives the specimen, the lab technologist extracts the chromosomes from the cells
  - Goal is to extract the chromosomes while the cell is in the metaphase stage of mitosis; this is when the chromosomes are easiest to study
- Uses a special dyeing technique on the chromosomes so bands appear
  - Banding patterns are different for each of the 23 chromosome pairs, making it much easier to tell which chromosome is a 1, a 2, an X, and so on
- Once the chromosomes can be distinguished from one another, the lab tech arranges them into a *karyotype*, or picture of the chromosomes, and looks for abnormalities.

The karyotype of the little girl comes out looking like this. Notice anything unusual?

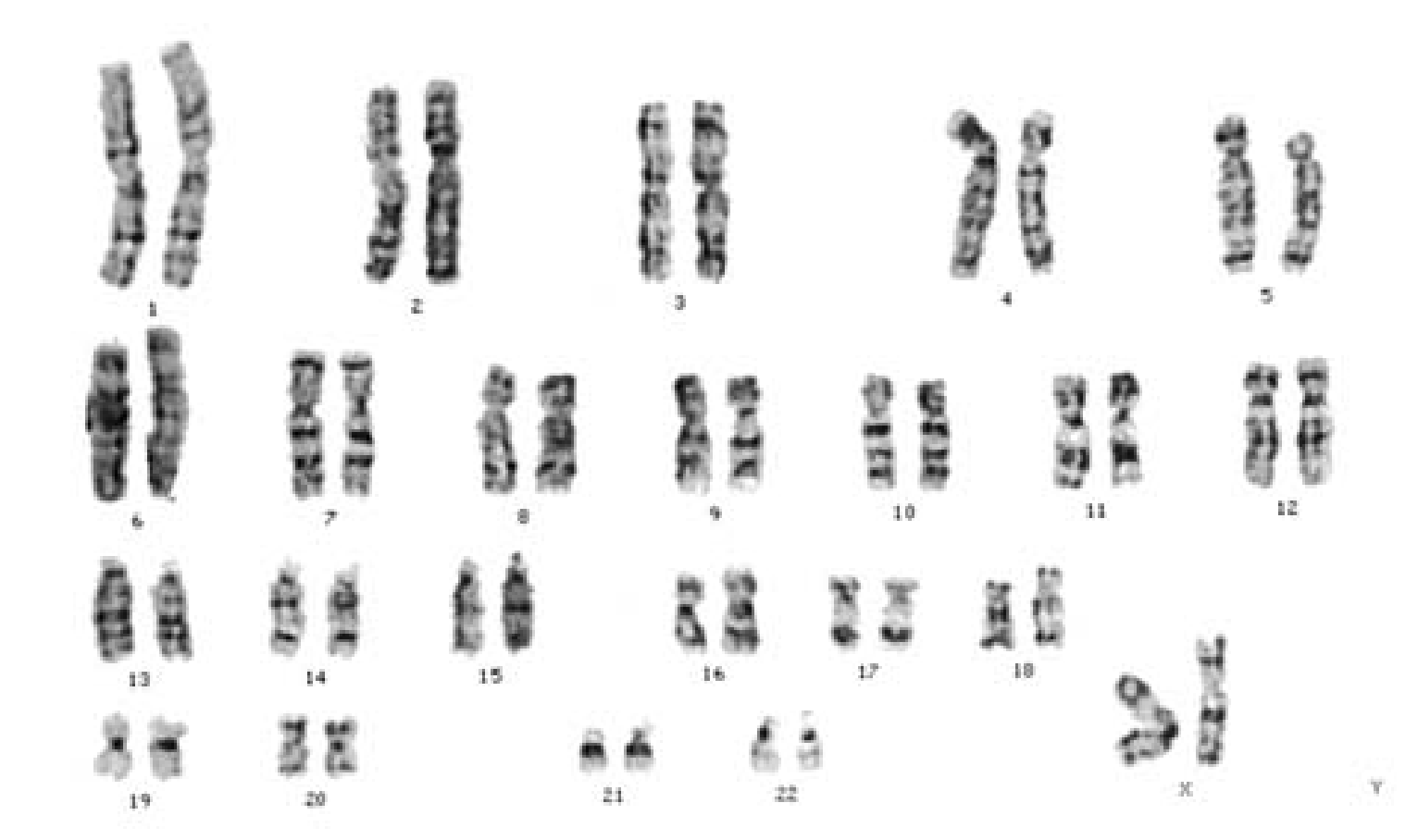


Photo courtesy of London's Global University Medical School

Part of the short arm of chromosome 5 is missing

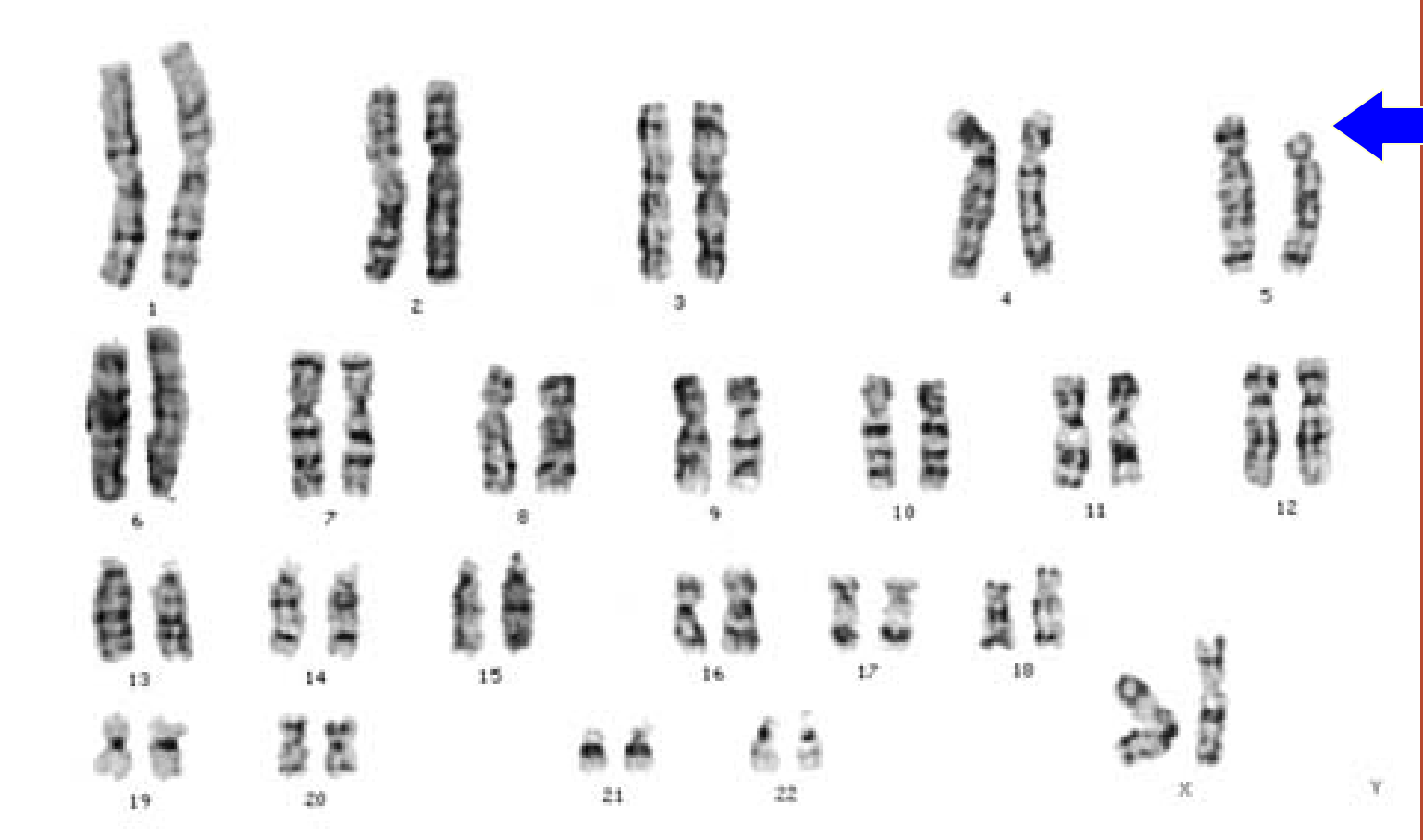


Photo courtesy of London's Global University Medical School

Cri du Chat syndrome

- Cri du chat syndrome literally means “cry of the cat” syndrome
- Also known as 5p- syndrome as a small portion of the p arm (short arm) on chromosome 5 is always missing
- Characterized by developmental delay, mental retardation, distinct facial features, low birth weight, a small head circumference, hypotonia (“floppiness” due to muscle weakness) and a cat-like cry
- Diagnosis is usually suspected by the doctor, then confirmed by the analysis of chromosomes

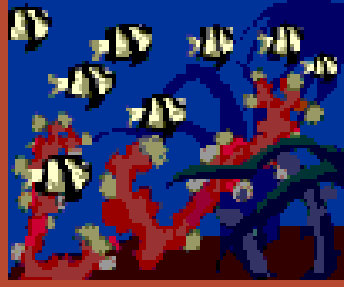
The person analyzing the karyotpe would most likely be a cytogenetic technologist

- Cytogenetics involves the study of chromosomes and their relationship to disease
- Involved in prenatal diagnosis, diagnosis of chromosomal abnormalities in individuals and families, evaluation of cancers and bleeding disorders
- Process specimens, perform karyotyping, might perform FISH

Cytogenetic Technologist

- Average national salary: about $42,000-$50,000/year
- Requirements:
  - Bachelor's degree in biology, biochemistry, molecular biology, or similar (about 4 years)
  - A post-graduate degree (1-2 years) OR 1 year of on-the-job training
  - Certification as a Clinical Laboratory Specialist in Cytogenetics
  - Some states may require licensure

Another cytogenetic technologist task: FISH



- FISH stands for fluorescent in situ hybridization
- FISH allows lab techs to work on cells that are not dividing. This is useful because most of the time, the cells in our bodies are not in mitosis or meiosis.
- FISH also allows lab techs to map an individual's DNA and detect smaller abnormalities not typically visible when looking at a chromosome
  - Example: small mutations involving only one or two genes

How does FISH work?

- First, you have to have an idea what you're looking for
- If you suspect a certain area of DNA is involved, and you know the sequence of that DNA, you can create a *probe*
- The probe is a single-stranded piece of DNA which will match the sequence of DNA you are interested in and will be able to bind to it
- The probes are made fluorescent so they will be easy to spot once they bind
- If a probe binds, the lab technician will know where that DNA sequence is located (or if it is even present!)

FISH

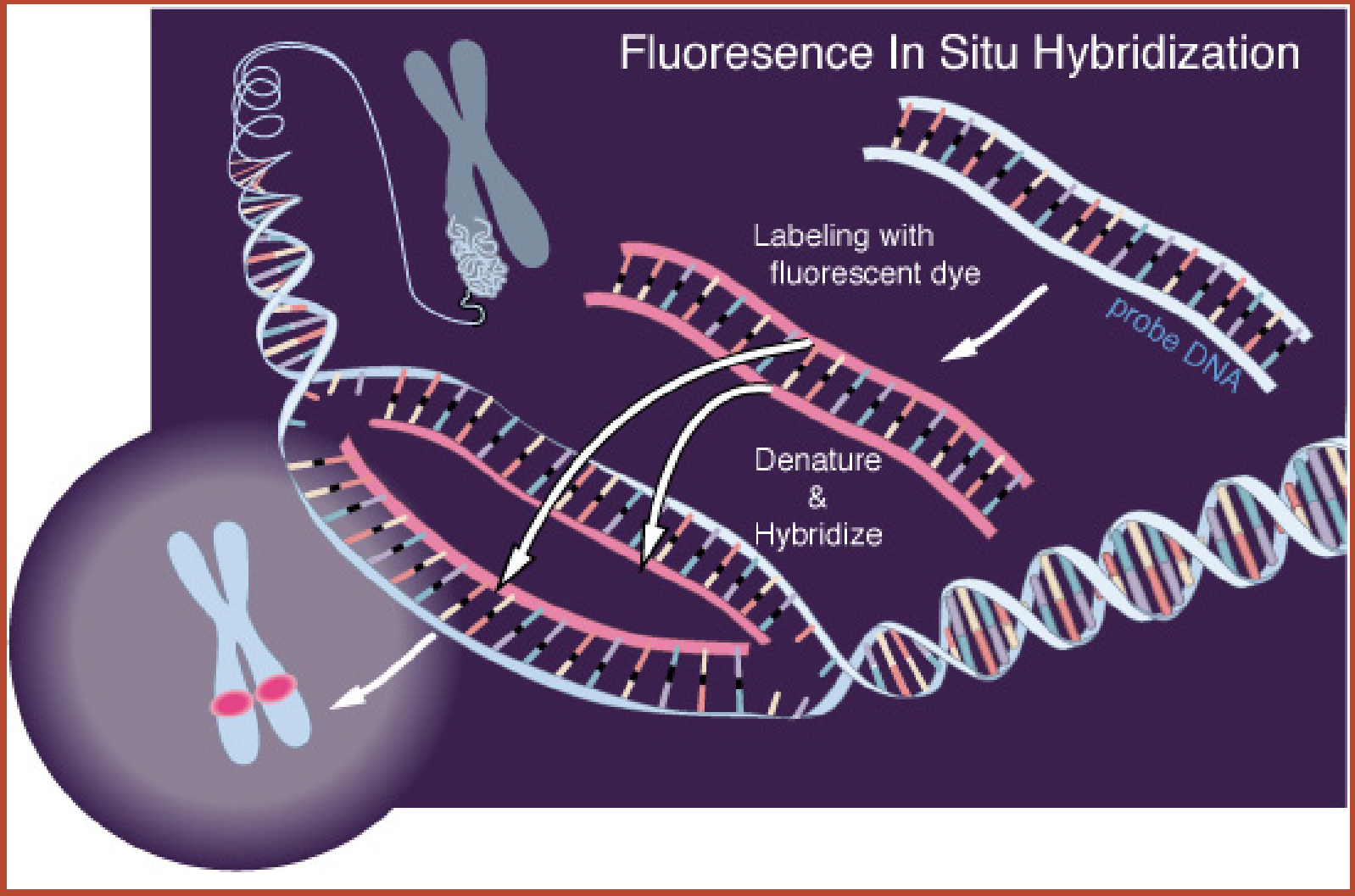
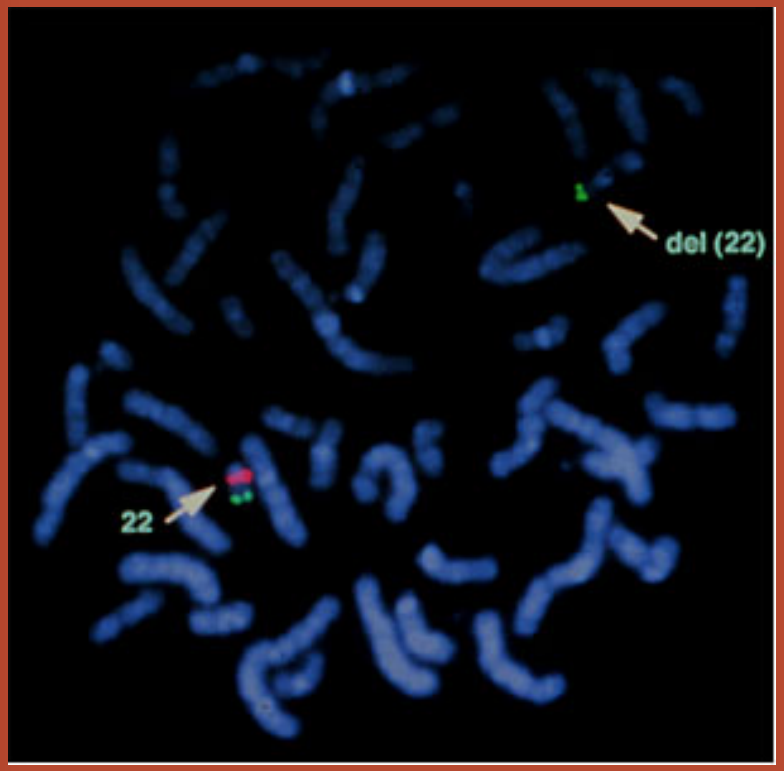


Photo courtesy of the National Human Genome Research Institute

Clinical Example

- A geneticist sees an 8-year-old girl who has been having learning difficulties in school
- Her pediatrician has noted that she has “unusual facial features”
- When the girl was born, she had a cleft palate which was repaired
- The geneticist first asks for a cardiac evaluation. The results show the girl has a heart defect.
- The geneticist is suspicious for a certain condition and orders FISH analysis

Photo courtesy of 22q11.2 Deletion Syndrome Foundation



Pink = 22q probe Green = control

- The laboratory performed FISH using a probe which binds to a region on the q arm (long arm) of chromosome 22
- One of two probes bound to the DNA, one didn't
- The lab tech concluded the probe didn't bind because the region of 22q was missing in this little girl

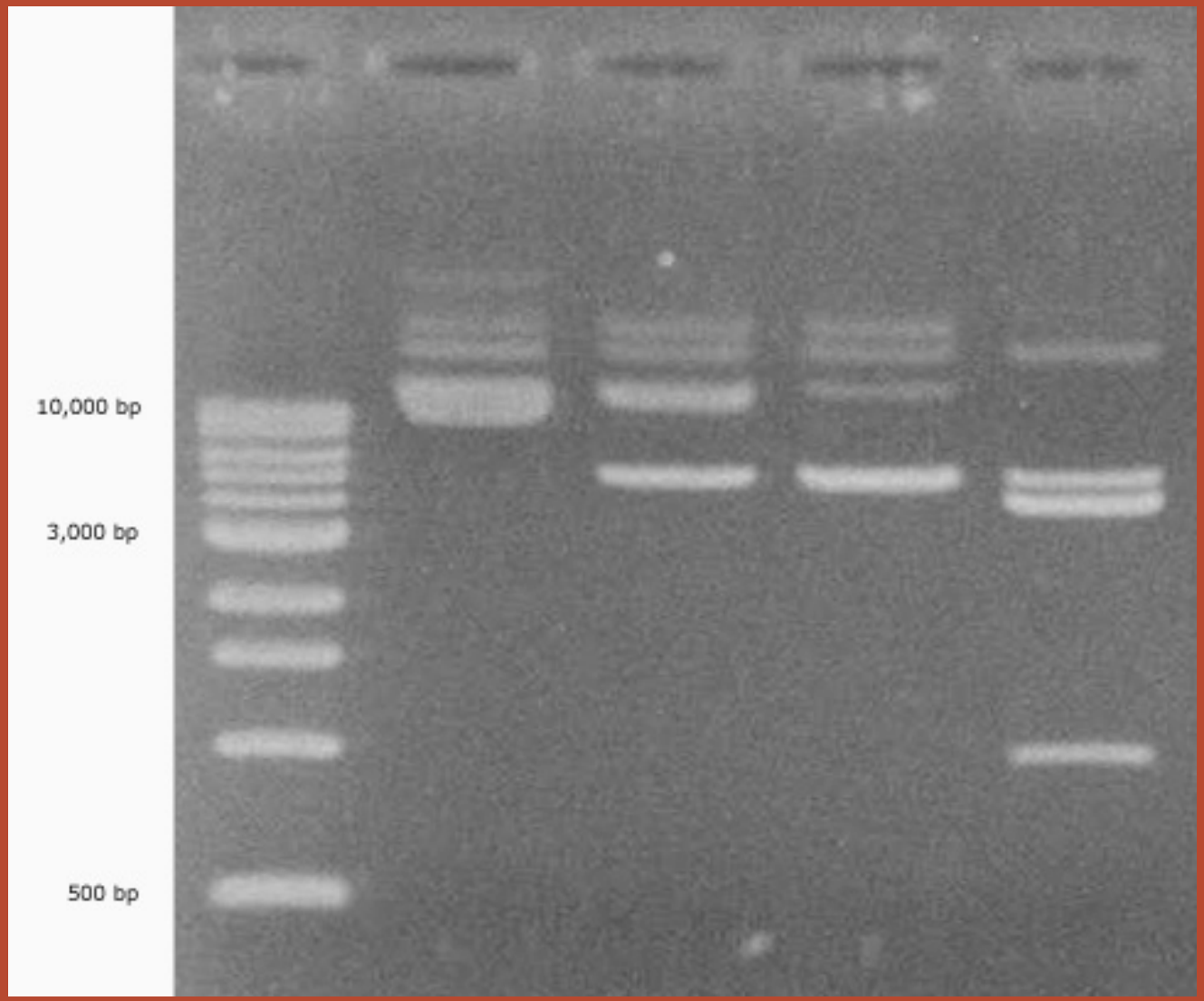
- She could now be given a diagnosis of 22q deletion syndrome

- All the characteristics noted in this child (heart defect, cleft palate, learning difficulties, unusual facial features) are due to the genes in this region of 22q being absent

Another lab technique: Gel Electrophoresis

- Lab techs use gel electrophoresis to sort DNA strands by length
  - Can also sort other molecules, like proteins
- Once DNA is isolated, place it at one end of a gel tray which acts like a filter
- 
  Add an electric charge to the gel to make the DNA move
- Longer strands move much more slowly through the gel filter than shorter strands
- When you take the electric charge away, the shorter strands will be further away from the starting point than the longer strands
- You can then stain the gel so the DNA on it is visible to the naked eye

Example of a Gel



Why do we care about separating DNA by length?

- Gel electrophoresis is run for many purposes
- One of the more common purposes for gel electrophoresis today is DNA fingerprinting
  - DNA fingerprinting performed for criminal cases, paternity testing

DNA fingerprinting

- Creating a DNA “fingerprint” relies on the fact that even though the DNA sequence of any two individuals is 99.9% identical, that 0.1% makes a person’s own DNA sequence perfectly unique
- DNA fingerprinting begins with treating a sample of DNA with *restriction enzymes*
- These enzymes break the DNA into fragments
  - Different people have different “breaking points” in their DNA sequence – this is part of the 0.1% of the DNA sequence which is unique in an individaul
  - The number and lengths of DNA fragments will be different for everyone

DNA fingerprinting

- When these fragments of DNA are then placed in a gel and put through the electrophoresis process, the fragments of their DNA will separate by length
- Since everyone will have different sizes and numbers of fragments, the pattern those fragments create as they move down the gel will be unique for each person
- The pattern created on the gel is the DNA “fingerprint”

Applications to forensics

- If a detective has a sample of DNA found at a crime scene, the DNA fingerprint of the person at that crime scene can be created
- If the sample appears to come from the criminal, the detective will be able to match the DNA fingerprint to the DNA fingerprint of any suspect in the case
  - Assuming the suspect submits a sample of their own DNA!

A famous example: OJ Simpson

- Police found blood at the scene where Nicole Brown Simpson and Ronald Goldman were murdered
- A DNA fingerprint created from the DNA in the blood samples matched OJ Simpson's own DNA fingerprint
- The chance the blood was not OJ Simpson's was about 1 in 57 billion
- However, OJ Simpson was acquitted
  - Defense attorneys argued that the lab where the DNA fingerprinting was performed had been sloppy and contamination and mixing of samples was possible. After all, a sample of Simpson's blood submitted by him directly was present in the lab and being tested at the same time the DNA fingerprinting was being done
- This case brought major public attention to the potential for DNA analysis in solving crimes, but also the possible problems

The person performing a gel electrophoresis is likely to be a Molecular Genetics Technologist

- Molecular genetics involves using DNA and RNA to better define the relationship between genetics and personal health
- Involved in prenatal and preimplantation diagnosis, diagnosis of neurological disorders, evaluation of cancers and bleeding disorders, risk assessment for familial cancers, forensics
- Extract DNA from cells & tissues, perform PCR, perform gel electrophoresis, DNA/RNA sequencing, Southern blots and related techniques

Molecular Genetics Technologist

- Average national salary: about $37,000-$60,000/year
- Requirements:
  - Bachelor's degree in biology, chemistry, biochemistry, or similar (about 4 years)
  - A post-graduate degree (1-2 years) OR 1 year of on-the-job training
  - Certification as a Clinical Laboratory Specialist in Molecular Biology
  - Some states may require licensure

Genetic testing without genes

- Not all laboratory tests involve analyzing the DNA or chromosomes directly
- Some tests analyze the gene *products*, like amino acids, proteins, and enzymes
- One such test is known as mass spectrometry

Mass spectrometry

- Mass spectrometers are kind of like scales for molecules – they determine the weight of molecules found in a given sample
- The weight of a molecule is referred to as its *mass*
- Molecules in a sample can be sorted by their mass
- Once sorted, we can not only identify which molecule is present in the sample, but also how much of it is there
- Molecules like amino acids, fatty acids, and organic acids can be identified by mass spectrometry

Newborn Screening

- A special type of mass spectrometry called *tandem mass spectrometry* (MS/MS) is used in the process known as newborn screening
- Newborn screening involves taking a small sample of blood from a newborn infant and sending it to a lab in order to detect certain diseases
  - Newborn screening is performed on *all* newborn infants automatically

Using MS/MS to save babies' lives

- Every state screens for a different number of disorders
  - Wisconsin screens for 47 different diseases
- When a baby's blood sample is sent to the laboratory, it goes through MS/MS
  - As well as several other tests
- MS/MS can determine if a blood sample has too many or too few amino acids, organic acids, or fatty acids
  - This can mean a baby has a disorder like an aminoacidopathy or organic acidemia
- These disorders can lead to symptoms like developmental delay, seizures, coma, or even death
- If a baby is identified as having a disorder early, doctors can start treatment right away to prevent these symptoms and possibly save the baby's life

Newborn screening in action

- A young couple brings their 3-day old baby boy into their pediatrician
- The baby has been fussy, has refused to eat, and seems very tired and limp
- While the baby is in the doctor's office, he starts to vomit and shake
  - All three adults also notice a faint odor, which reminds them of sweaty gym socks
- The doctor instructs the parents to take their son to the emergency room immediately

Newborn screening in action

- As the doctor is preparing to leave for the ER, he gets a call from the newborn screening lab
- The lab has just finished analyzing the baby's newborn screening sample
- The results of MS/MS testing seem to indicate the baby has a condition known as isovaleric acidemia

Isovaleric acidemia (IVA)

- Isovaleric acidemia is an example of an organic acidemia
- Babies with IVA cannot break down proteins properly, so they get very sick as the proteins and amino acids build up in their bodies
- Causes poor feeding, vomiting, seizures
  - Babies with IVA sometimes give off a smell like sweaty feet
  - If untreated, could eventually cause coma or even death
- Easy to treat – allow only very low levels of protein in a person's diet

Newborn screening in action

- Once the pediatrician has been informed of the baby's newborn screen result, he calls the emergency room
- The doctors in the ER are able to begin the proper treatment right away
- The baby boy recovers quickly and will likely not have any harmful side effects from the seizures
- The parents are given the contact information for a doctor who will help them maintain a low-protein diet for their son

The person performing MS/MS in a lab is likely a Biochemical Genetics Technologist

- Biochemical genetics involves the interactions of enzymes and proteins in metabolism and their relationship to disease
- Involved in newborn screening, the detection of inborn errors of metabolism and inherited metabolic disorders, the monitoring of treatment, and distinguishing carriers from non-carriers
- Process specimens, detect genetic markers, analysis using mass spectroscopy, gas chromatography, and high performance liquid chromatography

Biochemical Genetics Technologist

- Average national salary: about $32,000-$58,000/year
- Requirements:
  - Bachelor's degree in biology, chemistry, biochemistry, or similar (about 4 years)
  - Some states may require licensure

Careers in the Laboratory

- There are many opportunities for those interested in pursuing a career in the laboratory, but in general there are three types:
  - Medical Geneticist
  - Laboratory Research Assistant
  - Laboratory Technician

Medical Geneticist

- Conduct research in any area of genetics
- Can counsel but not treat patients; usually work very closely with clinical geneticists
  - Many medical geneticists (PhD) are also clinical geneticists (MD)
- Develop new and better methods to diagnose and treat patients with genetic conditions, improve understanding of the course of a disease and its manifestations

Medical Geneticist

- Average national salary about $60,000-$75,000
  - Higher if directing a laboratory or managing several projects
- Requirements:
  - PhD training in a doctoral program in cytogenetics, molecular genetics, population genetics, etc.
  - Genetics fellowship in a program accredited by the American Board of Medical Genetics
  - Many positions require you also be ABMG certified

Laboratory Research Assistant

- Role is to conduct research under the supervision of a medical geneticist
  - May also supervise laboratory
- Research areas are broad and variable depending on the setting (university, diagnostics lab, forensics lab, etc.) and the interest of the principal investigator

Laboratory Research Assistant

- Salary highly variable depending on setting and experience, can typically expect at least $30,000/year, possibly much higher
- Requirements:
  - Bachelor's degree in biology, biochemistry, molecular biology, or similar (about 4 years)
  - Master's degree in genetics or biological sciences

Laboratory Technician

- Cover all areas of genetics: human and non-human, research and clinical, etc.
- Perform the laboratory tests and perform initial interpretation and analysis of results
- For a career as a lab tech, high school courses should include:
  - Biology, algebra, chemistry, physics, geometry, English, and computer skills
- Three major areas:
  - Cytogenetics
  - Molecular genetics
  - Biochemical genetics

For more information

- Contact the Association of Genetic Technologists:

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