

Main Ideas

- DNA and RNA can be isolated from cells
- **Restriction endonucleases**= cutting DNA at specific recognition sites
- **Gel electrophoresis**= separating nucleic acids according to size
- **Hybridization**= detecting specific DNA/RNA sequences
- **Polymerase chain rxn (PCR)**= amplifying specific DNA sequences
- **Cloning**= isolating and propagating single genes-->whole DNA libraries
- **Sequencing**= determining the nucleotides sequence in DNA and RNA

Lecture Objectives

- 1) Understand the molecular techniques discussed
- 2) Understand how combinations of the shown techniques are used in diagnostics and research

DNA and RNA can be isolated from cells

- Isolating genomic DNA from cells or tissues

- Today we use kits mostly.

1) Lyse cells

- buffer at physiological pH, block DNases, detergent, proteinases

2) Purify DNA (phenol/chloroform extraction)

- pulls the DNA away from the protein, polar DNA goes into the water and protein is changed by the phenol (hydrophobic aa's on inside-->outside) so the protein avoids the water

3) Precipitate DNA (alcohol and salt), dehydrate the DNA

3) Resuspend DNA (H2O or buffer)

- Isolating RNA from cells or tissue

- Pretty much the exact same as DNA, use kits, only diff. is RNA is degraded much more easily

1) Lyse cells: physio pH, block RNases, detergent, proteinases, DNAases

2) Purify RNA (phenol/chloroform extraction)

3) Precipitate RNA (alcohol/salt)

4) Resuspend RNA (H2O or buffer)

Restriction Enzymes: Cutting DNA into smaller pieces

- Bacterial endonucleases (can cut in the middle of a double strand)

- Recog. short **palindromic** (can read the 5-3 seq. the same way on the strand below) DNA seqs

- Can gen. blunt or sticky DNA ends

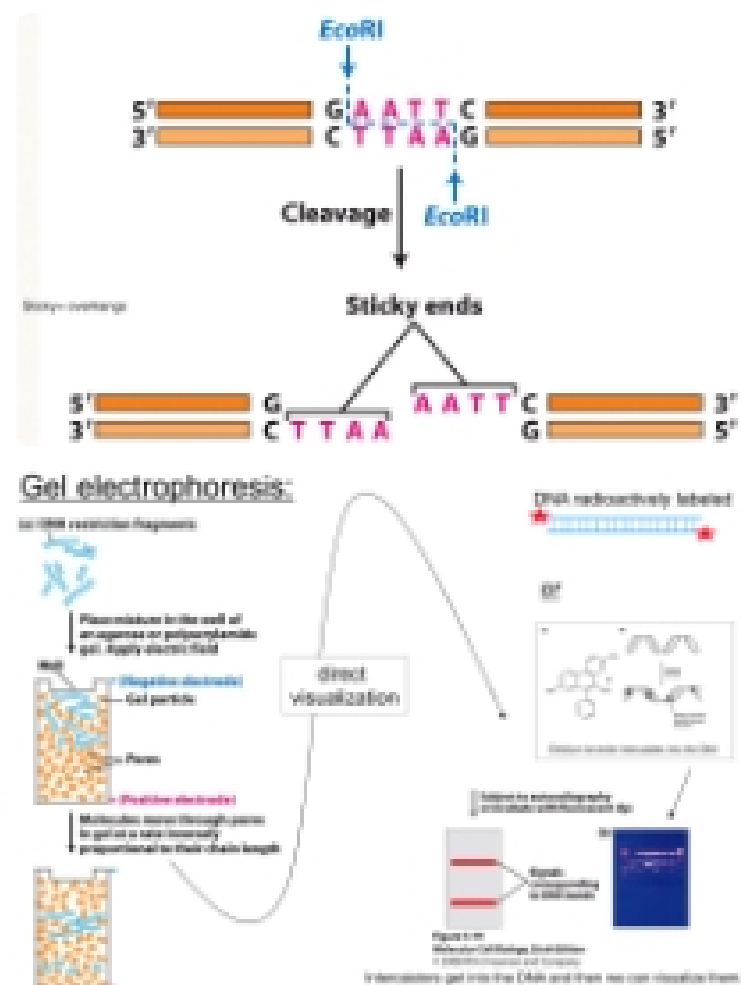
- Type 1-2, most common type 2

- Uses:

- gene cloning and making DNA libraries
- often used w/southern blotting
 - for RFLP= restriction fragment length polymorphism analysis (for pedigree analysis, prenatal diagnosis, and genetic fingerprinting)

Gel Electrophoresis: Separating nucleic acids according to size

- We've cut the DNA and now want to separate it by size
- Most common way to analyze DNA, RNA, and proteins
- Different gel types: agarose and polyacrylamide
- Since DNA/RNA backbone is negatively charged, in an

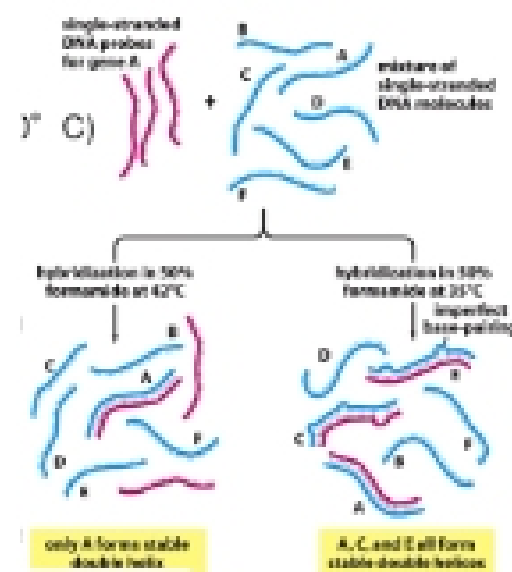


electric current, DNA/RNA moves toward the cathode (+ pole)

- Smaller molecules migrate faster than large one

Hybridization= to identify a specific DNA/RNA seq. in a large mix of DNA/RNA fragments

- Generate a sequence specific probe that's labeled radioactively or chemically (it's complementary to the target sequence).
- Two strands of a DNA double helix melt open (denaturation) upon heat (100c) or high pH (> or = 13)
- Complementary DNA single strands can re-anneal to form DNA double helices when temp or pH is lowered = **Hybridization**
 - need the right conditions (ex. not too cold) for the probe only binds to your target seq. and not other seqs



- 1) Run DNA/RNA on a gel to separate the diff. sized fragments
- 2) Denature DNA (→ ssDNA) → transfer it to a membrane
- 3) Use hybridization methods w/a sequence specific probe

- DNA: Southern

- RNA: Northern

- Uses of Southern blotting:

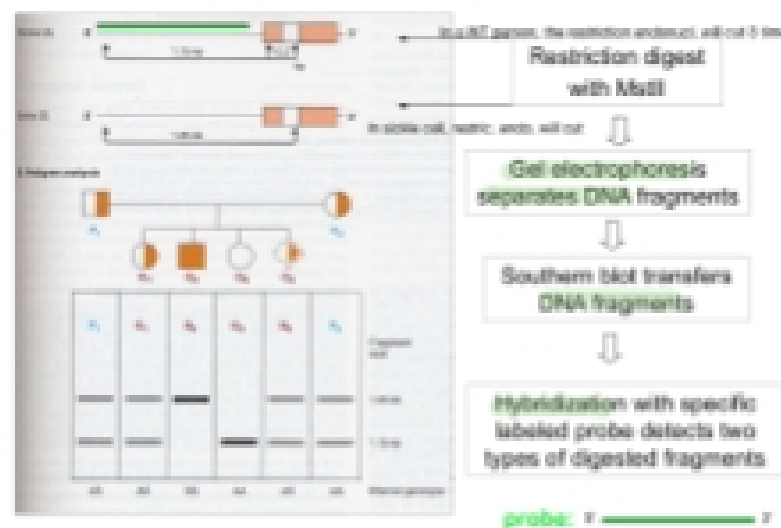
- Identify a single gene and detect seqs in an organism
- analyze genetic patterns
- identify disease assoc. muts, deletions, duplications, and rearrangements
- identify related DNA seqs in the genome
- determined the # of copies of a specific DNA seq. (CNVs, VNTRs, DNA forensics)

- **RFLP**= restriction fragment length polymorphism

Ex. Pedigree analysis

- looking at Sickle cell anemia: looking in a family for the presence of the sickle cell allele
- Only 1 NT change → MstII enzyme recog. CCTNAGG → can't recog. target seq. on Sickle allele

→ you get diff. sized fragments between the WT and the person w/sickle cell. Longer fragment= sickle cell allele, shorter fragment= WT allele



Ex. forensics and paternity test

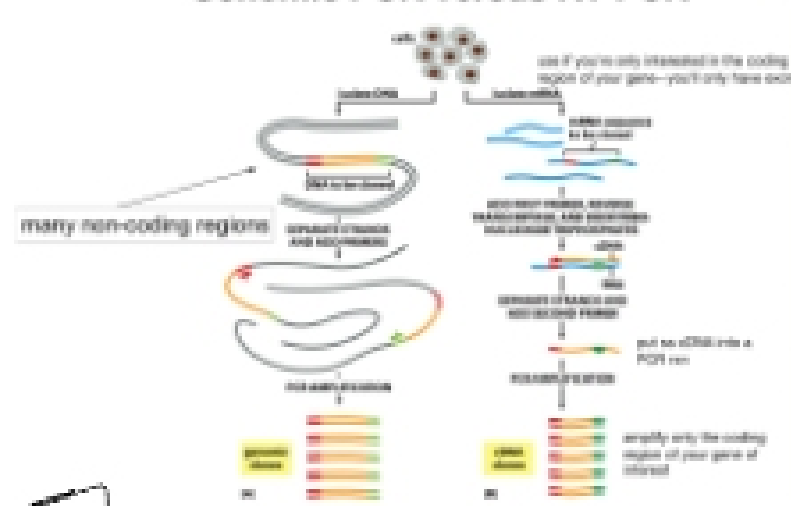
Remember the VNTRs? 17bp

Example: this VNTR can be repeated between 70 and 400 times!

1) restriction digest DNA
2) run samples on gel
3) transfer onto membrane
4) detect with VNTR probe

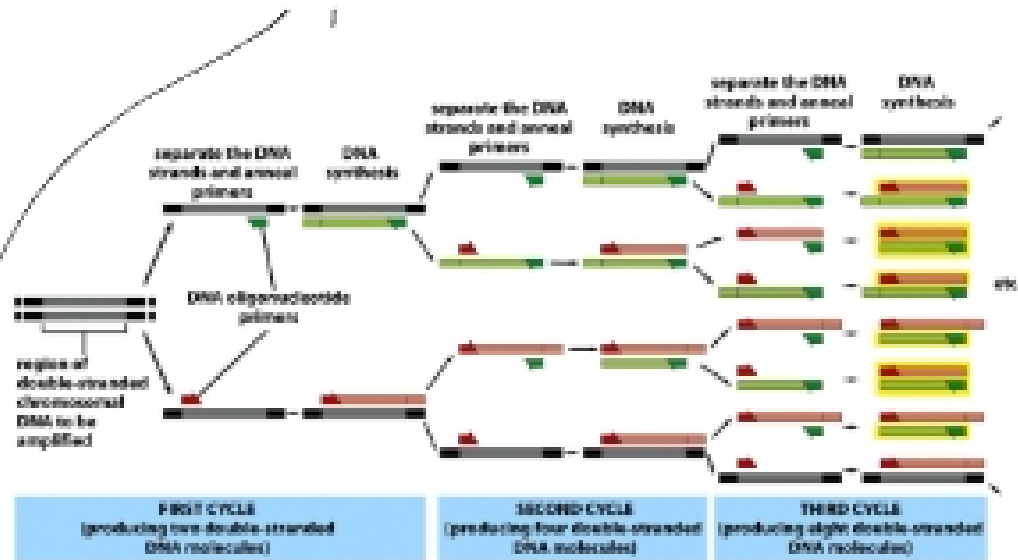
Info: In real life, would pick many VNTR regions

Genomic PCR versus RT-PCR



Polymerase Chain Reaction (PCR): amplifying specific DNA sequences in vitro

- Can use to isolate a gene and multiply it
- Taq DNA polymerase catalyzes PCR (can withstand high heat)
- Genomic PCR vs. RT-PCR
- RT-PCR: amplifying mRNA seqs in vitro



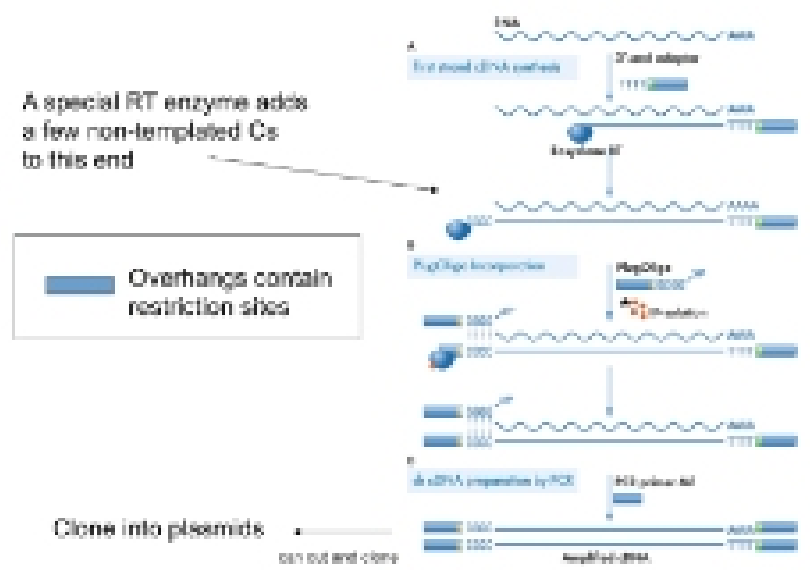
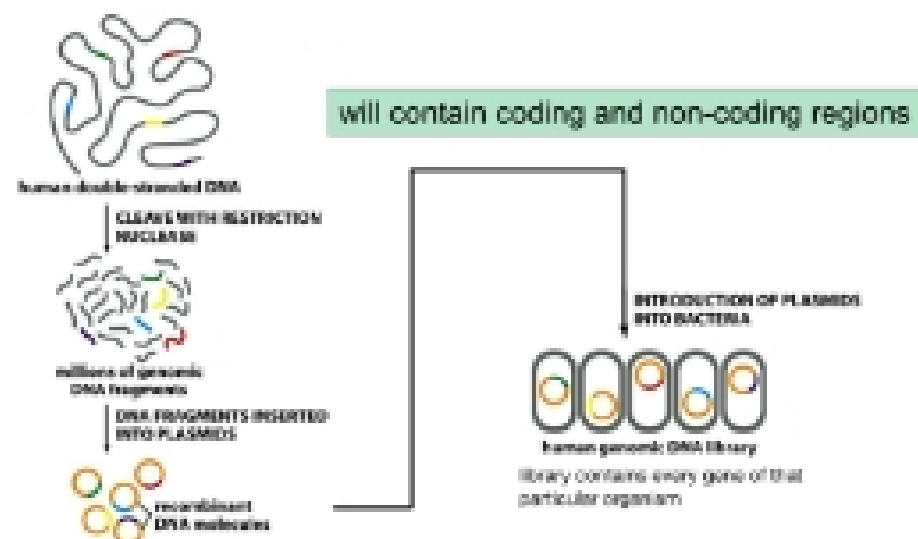
- isolate mRNA from sample (mature mRNA, no introns)
- add Oligo-dT primers (for polyA tail), anneal primer
- use reverse transcriptase to make the complementary cDNA strand
- add RNase H to digest the mRNA
- use the cDNA as a template in the regular PCR rxn, it will be recognized by DNA polymerase and amplified

- Uses: amplify any DNA or mRNA seq. in our genome, clone any DNA or mRNA seq, mutation analysis, analyze variable regions

	"Regular" PCR	RT-PCR
Original source of nucleic acid	genomic DNA	mRNA
PCR template	genomic DNA (double stranded)	cDNA (single stranded)
PCR product	dsDNA	dsDNA
amplified DNA sequence	coding and non-coding DNA	only coding DNA

Cloning a gene/DNA segment

- Need: Cloning vector (plasmid) + DNA segment of interest (insert)
- Take plasmid --> cut w/a restriction enzyme --> get blunt or sticky ends --> digest genomic DNA w/ same restriction enzyme --> add DNA fragment to be cloned and it inserts w/same blunt or sticky ends --> covalently link w/DNA ligase --> recombinant DNA
- To amplify: take bacterial cell + ds recombinant plasmid DNA --> cell culture produces hundreds of millions of new bacteria --> many copies of purified recombinant plasmid isolated from lysed bacterial cells
- To clone a PCR product:
 - use PCR primers with specific restriction enzymes (RE) sequences
 - after PCR, digest PCR products w/RE
 - digest target plasmid w/same REs and ligate
- To generate genomic DNA libraries



to generate cDNA libraries