Hello everybody, hopefully this section is a review if you have taken Genetics lab (Bio 2106). Regardless, this will be a helpful unit both here [there (2106)] and with any other molecular biology/biochem you’ll see down the road!

Remember: the Tutoring Center offers free individual and group tutoring for this Genetics. Our Group Tutoring sessions will be Thursdays from 5:15-6:15 PM at the Sid Rich basement, room 75! You can reserve a spot at https://baylor.edu/tutoring. I hope to see you there!

Keywords: PCR, Blotting, Cloning, Sequencing

**Topic of the Week:** Recombinant DNA Technology and Sequencing (19)

**Recombinant DNA Technology:** techniques which locate, isolate, alter and study DNA

**Hybridization:** a single-stranded (*denatured*) DNA molecule is bound by a complimentary stretch of DNA from a secondary source

**Probes:** molecules that *hybridize* with DNA to identify particular stretches→ *fluorescent*

**Blotting:** various procedures for transferring molecules from a ‘soft’ gel to a harder nitrocellulose film for analysis

- **Northern:** transferring DNA from electrophoresis gel to a harder film
- **Southern:** transferring RNA from electrophoresis gel to a harder film

**Autoradiography:** radiographic probes are added to the transfer film and the films are analyzed by a radiography detector to identify target sequences

- **Western:** transferring proteins via a *polyacrylamide gel electrophoresis* to a nitrocellulose film (another name is immunoblotting)

**Immunoblotting:** for *Western blots*, antibodies with fluorescent tags in solution are flushed over membrane; they bind and identify the target protein (*antigen*)

**In-Situ Hybridization:** hybridizing a section of fluorescent DNA or RNA probe to denatured nucleic acid in a cell that is *fixed to a slide*

→ allows observers to see the specific function/activity of an **active gene** within a live cell.
Polymerase Chain Reaction (PCR): DNA amplification using thermocycling (cycles of changing temperatures)

‘Raw Materials’: buffer solution (KCl or MgCl₂), Taq Polymerase, dNTPs, Template DNA, forward and reverse primer (ie free 3’-OH group)

Reaction: a process repeated ~20-40 times to amplify DNA exponentially

Denaturation (~2min @95°C): separates (denatures) DNA strands at high heat

Annealing (~1min @60°C): primers bind (anneal) to the ssDNA templates

Elongation (~1min @72°C): Taq Pol. adds dNTPs to ssDNA template

Gene Library: a colony of bacteria with another organisms’ genome cloned to plasmids

Genomic Library: library containing the entire genome

cDNA Library: library of all expressed genes as complementary DNA (cDNA) to the mRNA molecules from a cell

Creation: mRNA is bound by reverse transcriptase which makes a DNA-RNA hybrid which is converted to a dsDNA molecule; this molecule is amplified by PCR (aka rtPCR → FYI: also a way to detect SARS-CoV-2 or other RNA viruses)

DNA Sequencing: determining the primary (nucleic acid) sequence of a DNA molecule

Sanger ‘Di-deoxy’ Sequencing: reaction is similar to PCR, but uses 4 separate containers with one of the four types of di-deoxy nucleoside triphosphates (ddNTPs) in addition to dNTPs. This gives the sequence complementary to each DNA strand

These lack a 3’-OH group, so they terminate DNA replication

Each of the four reactions (ddATP, ddGTP, ddTTP, and ddCTP) are placed into separate gels and run in electrophoresis → each ddNTPs has a fluorescent tag

The shortest molecules travel the furthest, so the DNA sequence can be determined by looking at band position from the bottom up [to the wells].

Next-gen Sequencing: faster, cheaper and more efficient → sequencing in parallel

Parallel Sequencing: a category of sequencing that allows the sequence of thousands/millions of DNA bases simultaneously

Illumina Sequencing: special dNTPs with reversible terminators and fluorescent tags are added to a mixture of substrates similar to PCR.

Tagged dNTPs complementary bind to ssDNA templates; this briefly pauses replication. When this tag is excited by a light source, the fluorescent tag flashes and the terminator leaves (allowing the continuation of the process) → left

Really cool FYI, but here’s the full process:
https://www.youtube.com/watch?v=fCd6B5HRaZ8
**Pyro Sequencing:** emulsion PCR captures DNA in beads and bind them in arrays to sequence DNA

**CRISPR-Cas Gene Editing:** please see the included video for info on CRISPR. It's ~7:30 and very informative: [https://www.youtube.com/watch?v=MnYppmstxIs](https://www.youtube.com/watch?v=MnYppmstxIs)

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**Highlight #1: DNA Cloning** (19.2)

**Forward Genetics:** observe a phenotype and determine a gene

**Reverse Genetics:** mutate a gene and watch for the change in phenotype

**Cloning:** a section cut from one DNA molecule by a *restriction digest* and is *ligated* onto a new DNA molecule cut by the same restriction enzyme.

**Restriction Enzymes:** bacterial enzymes that cleave specific “*restriction sites*” that can be used to make double stranded breaks in DNA (note: named as *bacterial species* + the specific strain)

**Sticky Ends:** give single stranded overhangs [or complementary underhangs] → **cohesive**

**Ex. *HindIII***: cuts at → 5’- AAGCTT - 3’

**Blunt Ends:** give flat ends which **cannot** be easily re-joined

**How:** a restriction enzyme will be used to cut a **vector** (a DNA molecule used to carry gene(s)) at the same restriction site as target DNA

**Plasmid Cloning:** DNA from a source is cloned into a circular type of accessory DNA from a bacteria called a *plasmid* (ex. *pUC19*) → must have origin of replication (*ori*), one or more restriction sites, and selectable markers (ex. *Lac Z*, ampicillin resistance [*Amp*R])

**Recall:** what is it called when a DNA molecule from one organism is added to the genome of another? → **Transformation!**

**Transformation:** warming up bacteria makes membranes fluid; they uptake the plasmid

**Screening:** the bacteria are cultured on media containing *X-gal* and *Ampicillin*

**Ampicillin:** all transformed bacteria contain the *Amp*<sup>R</sup> gene, but **untransformed** bacteria will not be able to replicate on this media and will not form colonies

**LacZ:** the non-recombinant (ie. the plasmid without the ligated DNA) plasmid will be LacZ<sup>+</sup> and recombinant plasmid (ie. with ligated DNA) will be LacZ<sup>−</sup>. The surviving (transformed) cultures are treated with X-gal, a substrate analog of β-gal. X-gal turns **blue** when cleaved.

**Blue Colony:** non-recombinant; LacZ<sup>+</sup> = β-gal → cleave X-gal

**White Colony:** recombinant; LacZ<sup>−</sup> = no β-gal; no X-gal cleaved
**Expression Vector:** cloning a sequence into a vector meant to be expressed (ex. producing *insulin* in lab)
→ all sequences needed for transcription and translation are present in the vector (operator, start and stop sites for transcription, shine dalgarno sequence)

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**Highlight #1: Gene Expression Studies (20.4)**

**Microarrays:** a lab technique used to study the expression of many genes simultaneously (RNA)
A chip with many cDNA probes and a reporter will bind to a target mRNA (if present). If the target sequence(s) are present, the individual locations on the microarray will light up to signify the presence of that gene. These can be extracted and sequenced (**RNAseq**)

**RNA Sequencing:** (aka RNAseq) a cell’s RNA content is extracted from a microarray or a similar probing technique. The samples are amplified with **rtPCR** to create many copies of cDNA that can be sequenced, placed into cDNA libraries, etc.

**Reporter Sequences:** The coding region of a gene is replaced with **GFP** (green fluorescent protein). When the gene is expressed, its expression, location in a cell and destination can all be viewed.

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**Week 12 Concept Check:**

1. A group of researchers has extracted proteins from a *neuroblastoma* cell they are studying and need to study specific proteins. What would be a logical step to evaluate the protein content only of the molecules they extracted?
   a. Western Blotting
   b. Eastern Blotting
   c. Northern Blotting
   d. Southern Blotting

2. A DNA vector-cloning experiment is conducted with a non-recombinant vector containing LacZ and *amp*R. Tumor protein 53 (*TP53*) is cloned into the vector. If a cell has been transformed by this vector, how could you determine which are recombinant?
   a. If the colonies are blue, they are recombinant
   b. If the colonies grow on the ampicillin media, they are recombinant
   c. If the colonies are white, they are recombinant
   d. If the colonies do not grow on the ampicillin, they are recombinant

3. Which of these is not required by an expression vector to create cloned proteins?
   a. A TATA box
   b. Operator
   c. RNA binding sequence (*shine dalgarno*)
   d. Start and stop sites for transcription

4. Which of these would be the most helpful to study the expression of a given cancerous mRNA sequence in a given tumor cell?
   a. Western blotting
b. RNAseq  
c. DNA PCR  
d. Immunoblotting  

5. Which of these would be the best for detecting a DNA virus?  
a. Western Blotting  
b. Sanger Sequencing  
c. rtPCR  
d. PCR  

6. Why might reporter sequencing be helpful in studying secreted protein hormones in a small organism?  
a. It helps researchers to follow a protein around the organism and determine its pathways/location or expression  
b. This allows the scientists to identity the mRNA sequence that codes for this protein  
c. It makes cDNA studies unnecessary for that organism  
d. It allows the researchers to see all the proteins produced by the cell  

7. What is the sequence produced from this sanger sequencing gel? (right)  
a. 5’-GACTGCATCGTACTCAGA-3’  
b. 5’-CTGACGTAGCATGAGTCT-3’  
c. 5’-TCTGAGTACGATGCAGTC-3’  
d. 5’-GACUGCAUCGUACUCAGA-3’  

8. Which of these is not needed for PCR  
a. dNTPs  
b. Buffer solution  
c. DNA template  
d. Primers  
e. ddNTPs  

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**THINGS YOU MAY STRUGGLE WITH:**

1. The annealing temperature of DNA is the specific temperature that favors the binding of its complementary strand  
2. In plasmid cloning, the LacZ gene likely has a conditional operator, meaning that host-cell regulation will not inhibit β-gal synthesis, even in the absence of Lactose. Thus, when plated on lactose free media, non-recombinant plasmids will be able to make β-gal and thus will produce the telltale blue-colored colonies when X-gal is present. However, the recombinant plasmid does not produce β-gal at all, giving the white colonies.
CONGRATS; You made it to the end of the resource! Again, group tutoring will be every Thursday from 5:15-6:30 PM. You can reserve a spot at https://baylor.edu/tutoring. I hope to see you there!

Answers:

1. A.
2. C.
3. A.
4. B.
5. D.
6. A.
7. C.
8. E.