Week 12
Genetics: BIO-2306

The concepts this resource covers are the topics typically covered during this week of the semester. If you do not see the topics your particular section of class is learning this week, please take a look at other weekly resources listed on our website for additional topics throughout the semester.

We also invite you to look at the group tutoring chart on our website to see if this course has a group tutoring session offered this semester.

If you have any questions about these study guides, group tutoring sessions, private 30 minute tutoring appointments, the Baylor Tutoring YouTube channel or any tutoring services we offer, please visit our website www.baylor.edu/tutoring or call our drop in center during open business hours. M-Th 9am-8pm on class days 254-710-4135.

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

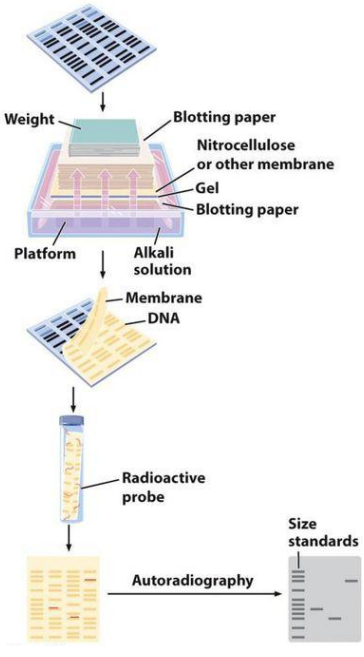
Hybridization: a single-stranded (*denatured*) DNA molecule is bound by a complimentary stretch of DNA from a secondary source

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

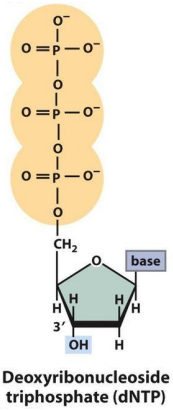
Western: transferring proteins via a *polyacrylamide gel electrophoresis* to a nitrocellulose film (another name is *immunoblotting*)

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 $MgCl_2$), *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 @95ºC)**:** separates (*denatures*) DNA strands at high heat

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

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

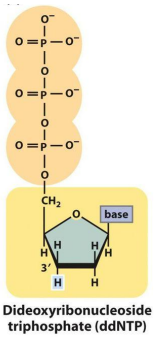
Gene Library: a colony of bacteria with another organisms' genome **cloned** to plasmids

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

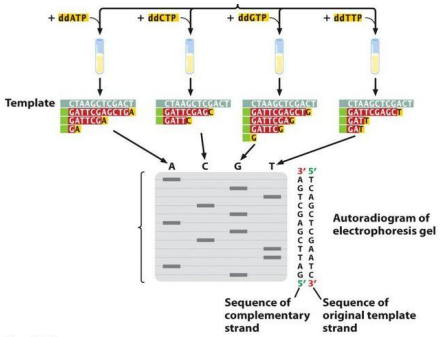


Sanger 'Di-deoxy' Sequencing: reaction is similar to PCR, but uses 4 separate containers with one of the four types of *di-deoxy nucleoside triphosphates* (**ddNTPs**) in addition to dNTPs. This gives the sequence *complementary* to each DNA strand

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

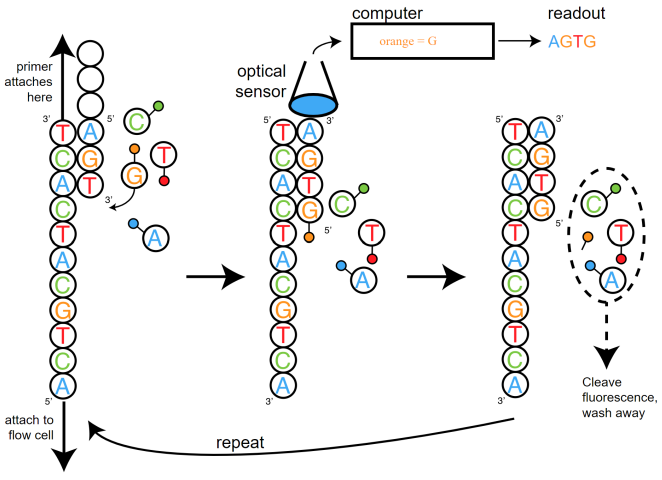
The *shortest* molecules travel the furthest, so the DNA sequence can be determined by looking at band position from the bottom up [to the wells].



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. (*cont. on next page*)



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

---

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.

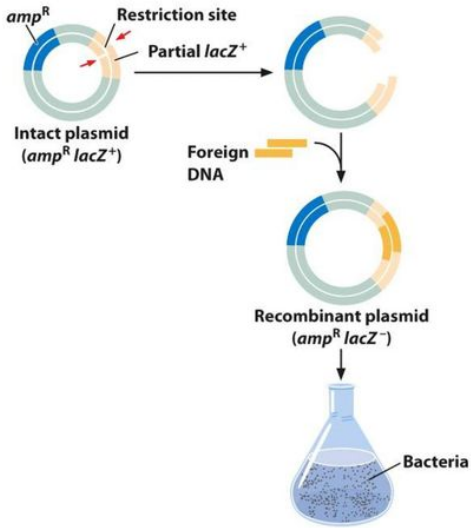
Restriction Enzymes: bacterial enzymes that cleave specific "*restriction sites*" that can be used to make double stranded breaks in DNA (note: named as *bacterial species* + the specific strain)

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

Ex. *Hin*dIII: 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

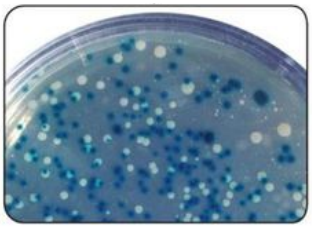


Plasmid Cloning: DNA from a source is cloned into a circular type of accessory DNA from a bacteria called a *plasmid* (ex. *pUC19*) → must have origin of replication (*ori*), one or more restriction sites, and selectable markers (ex. *Lac Z*, ampicillin resistance [Amp^R])

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^R 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 $LacZ^+$ and recombinant plasmid (ie. with ligated DNA) will be $LacZ^-$.

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; $LacZ^+$ = *β-gal* → cleave X-gal

White Colony: recombinant; $LacZ^-$ = 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)

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.

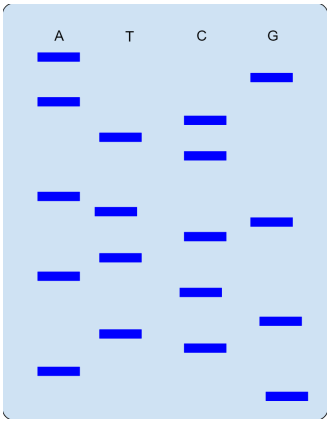
CHECK YOUR LEARNING

Concept Check: *(Answers found on the last page)*

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 <u>not</u> grow on the ampicillin, they are recombinant
3. Which of these is <u>not</u> 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



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! Thanks for checking out these weekly resources! Don't forget to check out our website for group tutoring times, video tutorials and lots of other resources: www.baylor.edu/tutoring!

Answers to check your learning questions are below!

Answers:

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