MOLECULAR CLONING OF A GENE:   
With Recombinant DNA Technology/Genetic Engineering

1. Isolate DNA from cells that contain your gene (eg: if you want genes involved in frog tongue development, you will need to isolate Frog DNA! If you want to study fungal resistance to stress, isolate DNA from that fungus!)
2. What kind of DNA to choose:
   1. **Not discussed in our class: [cDNA** – made from mRNA of expressed genes. Limited but specific collection of DNA. Will not contain any regulatory regions (eg: promoters, enhancers, silencers, introns).]
   2. **Genomic DNA (gDNA)** – fragment ALL DNA isolated from organism. Potentially find any DNA sequence: genes and regulatory sequences, and repetitive DNA too (noncoding, like telomeres and satellite DNA).
3. Choose a VECTOR: small “chromosome” to carry your DNA into cells/organisms of your choice. Plasmids and altered viruses are the most common Genetic Vectors.
   1. Origin of replication – so that transformed cells can copy the vector and your gene you have cloned into it.
   2. Selectable gene marker: such as antibiotic resistance. Eg: ampR gene allows our cells so survive in media with ampicillin antibiotic. So, these cells/colonies that grow MUST contain our vector.
   3. Desirable Characteristics: Polycloning (multiple-cloning) sites where you can cleave the vector and your source dna with any single Restriction Endonuclease/Enzyme from many choices.
   4. Disruptable gene: a “reporter gene” with an easy phenotype to observe. The Polycloning Site is IN this gene, so if we clone a foreign gene into our vector, this reporter gene will be INACTIVATED/Disrupted (eg: **Lac Z+ 🡪 LacZ- [WHITE, not blue, colonies],** or GFP+ 🡪 GFP- [colonies do NOT glow green]). If the reporter/disruptable gene is not inactivated, then you likely have empty vector with no recombinant/foreign gene cloned into it.
4. Once source DNA and Vector DNA (plasmid) are cleaved by the same Restriction Enzyme, they can be LIGATED together (“gene splicing”) and this collection of source-DNA fragments cloned into recombinant vectors can be TRANSFORMED into bacterial (microbial) cells. Colonies of transformed bacterial (eg: E. coli) cells that contain cloned fragments are collectively called your GENE LIBRARY!
5. Screen the Gene Library for the gene you want: Separate fragments on gels, blot to nylon filter, and probe with a related sequence of labeled DNA or RNA (HYBRIDIZATION with a PROBE).
   1. Also: Library colonies can be directly blotted onto a filter, and colonies probed for your gene/sequence of interest.
   2. *NOTE: you must know something about the gene sequence to make a probe – usually by cloning first in a simple organism, or by genetic mapping to a nearby sequence on the chromosome.*
6. Once your gene is discovered by gel blot, colony blot, or even **PCR** with specific DNA PRIMERS, then you can make many copies (**AMPLIFICATION of CLONED DNA**) of the gene by growing the host cells or by more PCR. This **gene can be used to engineer organisms** to get more desirable properties (eg: hardier crops, or gene therapy for patients), \*\*or **the gene product can be made for medical or industrial purposes** (digestive enzymes to break down wastes or to remove stains from clothing; hormones to help patients with metabolic diseases; etc…)