BIS101 F2014 Gene Isolation and Manipulation

Notes

Gene manipulation

In vitro vs. in vivo (vs. in silico)

PCR

What do you need ?

Need primer homologous to sequence of interest. Nucleotides Polymerase that can tolerate high temps (from where ? T. aquaticus)

Work through two rounds of PCR

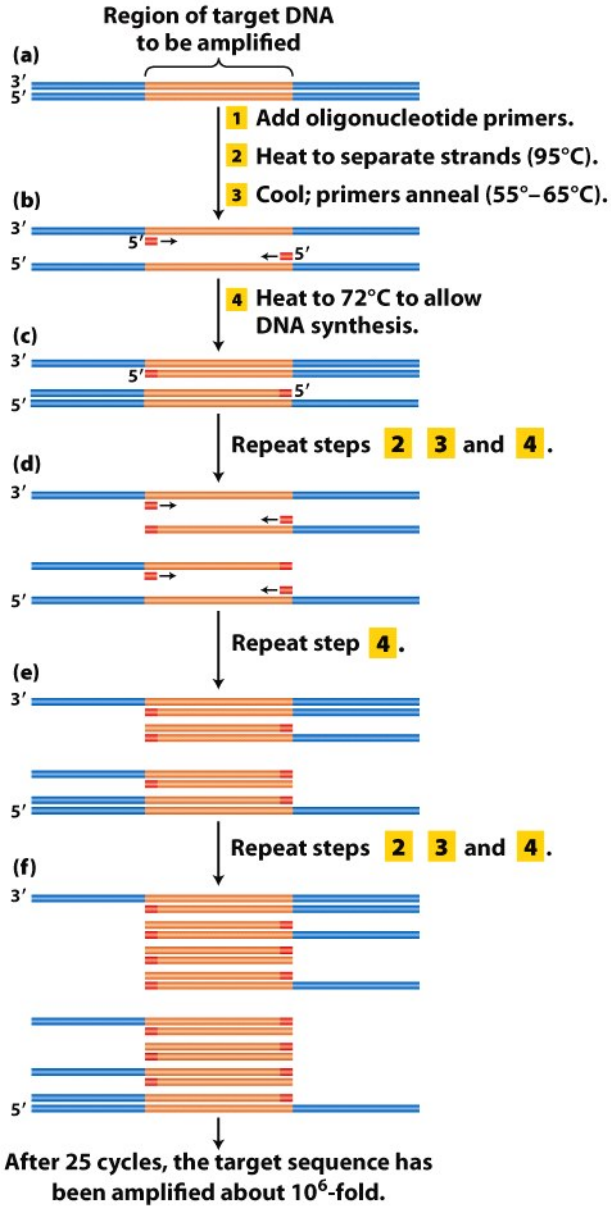


Figure 10-3
Introduction to Genetic Analysis, Tenth Edition

cDNA

Can use reverse transcriptase (talk) to amplify RNA and make cDNA. What would you use as a primer? T's to match poly-A tail.

RT then makes single-strand DNA complement. Then can sequence, transform, etc. Often useful for putting genes in bacteria or other organisms so no need to worry about correct splicing.

Sequencing

Even fancier

Sequencing by synthesis. Illumina. Chop up DNA. Add adapter seq.

Use PCR of adapter to make tons of copies in physical spot on a chip.

Each round, incorporate special bases that block more polymerase and are flourescent. Wash to remove unincorporated. Photo.

Remove 3' block (keep nucleotdie) and dye, repeat.

Draw how image on chip of red-blue-blue-green-red gives sequence.

$2.50 for 500bp of Dideoxy

$2.50 for 75,000,000bp of Illumina (was 50M last year)

Cloning

Restriction enzymes ? . Proteins that cut DNA. Can leave blunt or sticky ends.

e.g. EcoRI GAATTC cuts at first G on both strands (show).

Sequences will have multiple restriction sites. Presence absence of sites can be found by running on gel. Can separate DNA based on size. How ? DNA negatively charged, so can run current and DNA moves through gel., with larger fragments moving slower.

These size fragments can be used as a marker! I do a digest of two individuals and their offsrping, places where they have different restriction sites will show up as different sized bands. Presence/absence of these bands will segregate in offspring, and I can build genetic map.

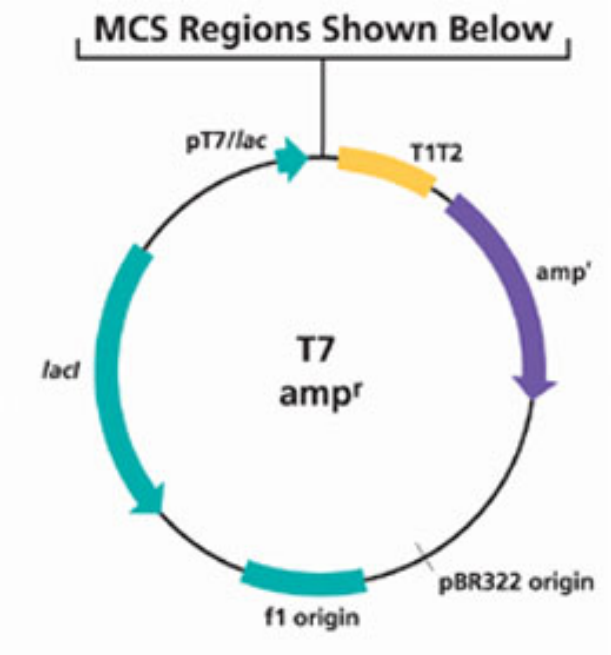
Cloning

Not how to make Jusassic park, but instead process of copying a DNA not by PCR but by making bacteria do it for you.

Insert sequence of interest in plasmid -- circular self-replicating chromosome in a bacteria. Also called a vector.

Useful cloning plasmid needs:

- origin of replication (why?) to replicate in host cell
- restriction sites (why ?)
- reporter (antibiotic resistance) for ? knowing plasmid made it into bacteria
- often will include reporter to break (lacZ). why? ? so you know it has gene in there. lacZ + beta-X-gal makes blue dye product. So white colonies that survive = gene!



MCS is set of restriction sites, where you insert sequence of interest.

Show how sticky-end allows cutting one sequence and inserting into plasmid.

Can work with blunt ends, just less efficient. Both need ligase to connect the sugar-phosphate backbone.

New DNA inserted into bacteria by:

- Transformation: expose to DNA (sometimes heat shock cells so take up DNA more readily)
- Conjugation: exchange of DNA between bacteria
- Transduction: DNA from a bacteriophage ? can be inserted into and taken up by bacteria.

A number of human proteins are engineerd in e. coli: insulin, HGH, erythropoetin (for bicyclists), proteases in laundry

Probing

We can use cloning to make libraries of sequence (cDNA or DNA). But how do we check for presence of sequence of interest.

Can use a petri dish of colonies or gel.

Transfer DNA from gel or plate onto a membrane, and wash the membrane with radioactively labelled sequence of interest. Then expose to X-Ray film (results is **autoradiogram**)and find which band or which colony has sequence. Can also use flourescently tagged DNA and expose to light and photograph.

Draw plate with colonies. Draw which ones show up on radiolabelled film.

Also works on a gel!

Probe for DNA is called a Southern blot (after Dr. Edwin Southern). Probe for RNA is called a Northern (because someone thought it was funny) Probe for DNA uses an antibody, called a Western blot. Could use a non-antibody protein to detect protein-protein interactions. Then a far-western blot. Probe for post-translational modification of protein (phosphoylation etc) called an Eastern blot.

Transformation in eukaryotes

Define: transgene: any gene introduced artificially into a genome. Can be from same species!

Anything with a transgene is a transgenic organism or a GMO.

I have pretty strong opinions on GMOs and happy to talk about them if anyone's interested. But the fact is that there is no good science arguing that any GMO on the market is dangerous. There is good science showing they are useful. That doesn't mean all GMOs will be perfect, and some might mess with other genes (silencing) or knock out genes, some might coneivably have health risks, and some might have ecological consequences. So testing/regulation a must.

Transgene can either replace target gene or insert at dseired locus, or insert **ectopically**

Yeast

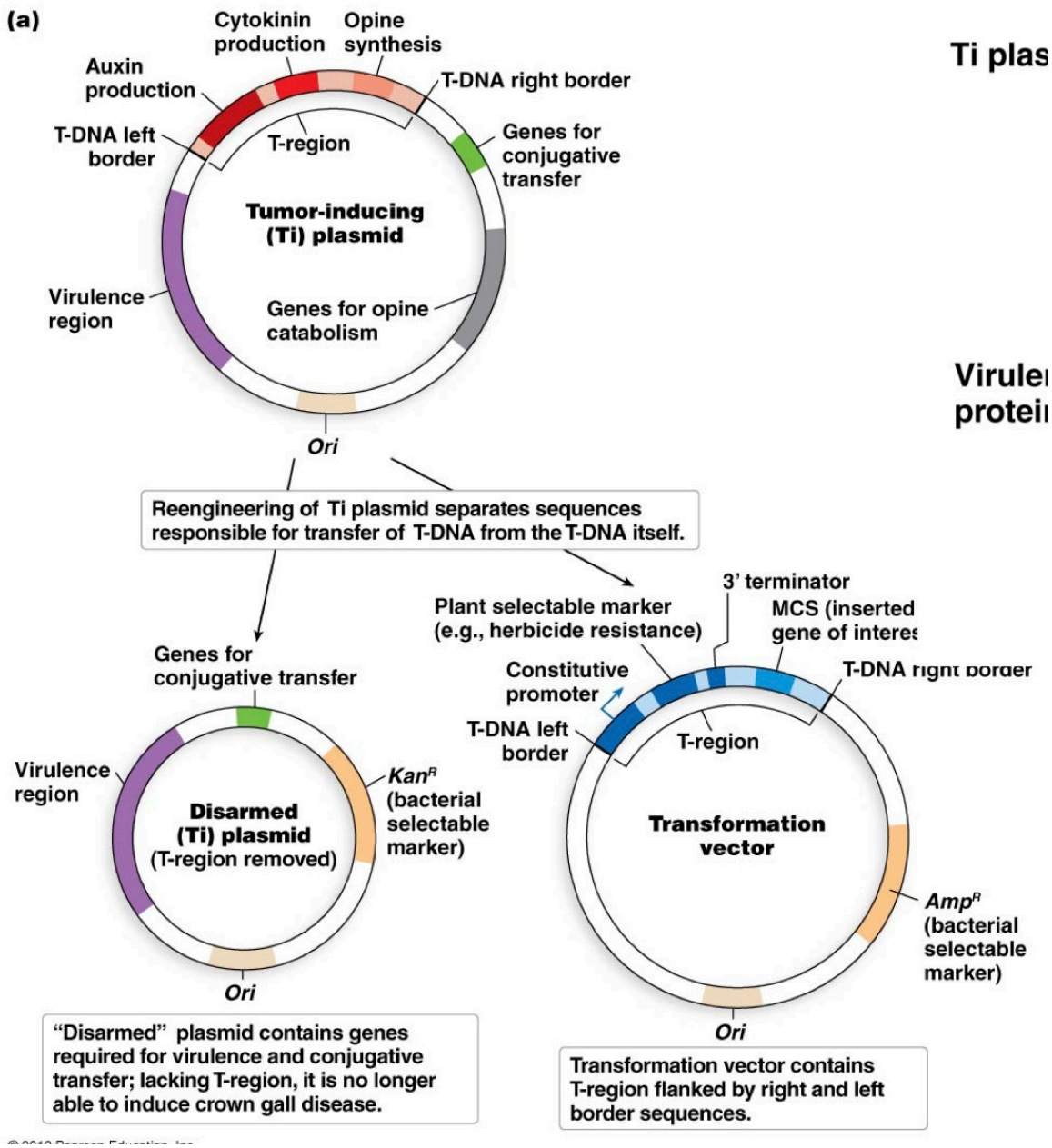
Shuttle vector (can be used in both yeast and e. coli Ori)

- do gene manipulation (cloning, etc.) in bacteria
- move into yeast, get homologous recombination -- replace sequence.
- test function in a eukaryote. has sequences necessary for bacteria (Origin of Rep.), and a yeast Or. Replication and centromere

Plants

Agrobacterium

- T-region, flanked w/ repeats. This region encodes genes that make tumors. multiplies Agro. in plant.
- virulent region & conjugative -> proteins to infect cell, transfer DNA, move to nucleus (prob.)
- make two plasmids
    - one with virulent/conjugative & bacterial selectable marker
    - one with new genes of interest & plant selectable marker (herbicide resistance) (what is **?**) to be sure you have the transformation vector and/or not the other vector



Gene gun: shoot DNA-coated tungsten (or gold) and hope for illegitimate recombination. Rare, so need to do a lot of cells.

Grow up cells in culture -> treat with hormones to get adult plant. Usually hemizygous (?) so need to duplicate.

- round-up ready
- bt toxin

- golden rice (vitamin A)

Animals

Inject into nucleus of fertilized egg cell (or gonads, etc.). In some animals will get incorporated into germline, and progeny will have mutation.

Can also use yeat-like homologous recombination in stem cells. Then insert stem cells into embryo to get transgenic animal.

- knock-out -- destroy locus
- knock-in -- insert something directly into a specific locus

Make libraries of knockouts, knockins for community use.

CRISPR-CAS

a system that evolved in bacteria as a form of defense against viruses, now used for genomic editing.

CAS9 protein: endonuclease ? (cuts DNA)

gRNA: guide RNA: homology to tagert DNA, also scaffolding properties that allow CAS9 to bind an activate

gRNA+CAS9: makes double-strand cut in DNA

DRAW

can be repaired with end joining -- cause deletion

can be repaired with recombination with homologous piece of DNA.

if you insert piece of DNA with new sequence, that new sequence will be incorporated.

Can also bind a transcription factor to inactive (noncutting) CAS9 and target a gene. effect? to upregulate! or just bind to say promoter and downregulate!

Caveats: needs CAS9 and gRNA and DNA replacement all in cell. Hard to do.