Cloning genes by complementation

Readings: Griffiths et al: 7th Ed. Ch. 12 pp 379-382; Ch. 13 pp 408-427, 8th Ed. pp. 351-358
Assigned Problems: 8th Ch. 11: 29, 7th Ch. 12: 12

Concepts:
How can we identify and clone the gene of interest?
1. The isolation of genes proceeds via screening libraries for a gene of interest.
2. A clone with a specific gene may be identified if it is able to complement a host mutation.
3. Most genes in most organisms, especially eukaryotes, cannot be isolated by simple complementation methods.
4. Transgenes can complement host mutations and confirm which gene is mutant.

Genomic DNA libraries – Which clone?
Set of clones with genomic DNA cloned into a vector.
"Shot-gunned" libraries have randomly organized clones.
The problem is which clone, amongst the many, has the gene of interest?
One method, among several that will be presented over the next few lectures, is:
Cloning by complementation of a host mutant is used in microorganisms where many individual clones can be examined easily. Relies on the cloned gene providing the gene product that is absent in the mutant host.
Example: Cloning a gene for an E. coli auxotrophic mutant - mutant in some gene called "A".
E. coli Strain minimal media minimal media+supplement
A- auxotrophic (no growth) growth
A+ growth growth
Goal: Clone the A+ gene, which is the mutant in the A- strain.

A genomic DNA library in a plasmid vector.
1)- Build a genomic DNA library in a plasmid vector
Use wild type DNA (this has A+ gene and not A-) so that some clone among the set will have an A+ gene inserted onto the plasmid vector.
Cut DNA with Restricton enzyme and ligate into vector.
E. coli fragment -> clone in genome Library
2)- Use these recombinant DNA molecules containing foreign (E. coli) DNA insertion into plasmid vector and transform the A- mutant strain E. coli.
3)- Expected results from transformation of A- strain with the library of clones:
diagrams: Select for clone that contains a gene that will complement the A- mutant strain. It should contain the A+ gene.

Numbers
Each plasmid contains ~4.5 Kb of insert DNA and E. coli has ~4.5 Mb DNA in the genome.
Therefore 1000 plasmid inserts = 1 E. coli genome's worth of DNA.
We need to screen the equivalent of 5000 molecules to ensure a 99% chance of finding gene A+.
5000 bacterial clones can be produced easily and screened quickly on one petri dish plate.
Works well with bacterial or yeast hosts and plasmid vectors, but what about higher organisms? -> problems with larger, multi-cellular organisms.
For constructing Genomic Libraries, the probability that any given sequence will be represented can be calculated by the equation.

\[ N = \frac{\ln(1 - P)}{\ln(1 - f)} \]

where \( P \) is the desired probability, \( f \) is the fractional proportion of the genome in a single recombinant, and \( N \) is the necessary number of clones in the library. For example, one might want a 99% probability that a given sequence is cloned from a library of 15kb fragments of mammalian DNA (3 \( \times \) 10^9) bp.

\[ f = \frac{1.5 \times 10^4}{3 \times 10^9} = 0.5 \times 10^{-5} \]

Problems that prevent the use of complementation to screen for genes in higher organisms:

1) Higher organisms have much larger genome size. More than 5 \( \times \) 10^6 plasmids would be needed to screen the human genome.
2) Higher organisms are multicellular and therefore the cloned DNA has to enter all body cells or the germline cells.
3) Auxotrophic mutants are more difficult to find and more difficult to manipulate.
4) Genes are often very large - too large to fit in one vector insert.

**Conclusion:**
Complementation methods cannot be used to screen libraries for genes in higher, multicellular organisms.

However, complementation can be used in higher organisms to confirm that a cloned gene is the correct one since it should be able to "rescue a mutant". Transgenic animals can rescue (change the phenotype to wild type) the mutant phenotype.

Transgenic animals (plants, too)

Transgene - a foreign gene that is introduced into an organism by incorporating it into the host's genome.

**Example Drosophila white+ Transgenes.**

wild type -> white+ gives a red eye colour (pigmented)

mutant -> white- gives a white eye colour (unpigmented)

The white+ gene had been cloned (by non-complementation methods)

It has been introduced into the germline of a white- mutant strain using a "Drosophila vector system".

The Drosophila vector system is shown using the rosy+ gene in Fig 13-22(8th) 20-38(7th)

1) - subclone white+ gene into P element vector
2) - co-inject with helper plasmid into the eggs of mutant white- strain
3) - examine G0 and G1 progeny for red eyed flies

The cloned white+ gene is incorporated into the white- strain genomic DNA and the phenotype of the white- strain, containing the cloned white+ gene, is red eyes.

Gene rescue: the cloned white+ gene is able to complement the white- mutation, and provide definitive proof that the mutation is due to an absence of the cloned gene's product.

Similar transgene systems are available for most model organisms and commercially important species.

Human Analogy - gene therapy
There are many genetic diseases that are due to loss-of-function mutations. If the normal functioning gene can be introduced and compensate (complement) for the mutant genes then the effect of the disease (the mutant phenotype) can be avoided. Methodology for "gene-therapy"
- introduce a normal gene to compensate (complement) for the mutant.
Problem: need to fix somatic cells not germline.
Drosophila white+ gene example above is just "gene therapy" for fruit flies.

A Human Example (Cloning the PKU Gene) p. 362-363 (Fig. 11-22)

Black urine disease (from Garrod’s work)
Shown to be a Mendelian trait.
There was a proposed enzyme deficiency. (HGO)
HGO = Homogentisate 1,2 dioxygenase
Mapped to Chromosome 3
Identified a human gene based on a data base search
Showed the the human gene produced HGO in E. coli.
Mapped HGO gene to chromosome 3
Final confirmation was that the individuals with the disease had a mutation in the gene of interest.