Giving a Scientific Talk

Most of these suggestions are from an excellent book about giving scientific presentations titled *Dazzle 'em with Style* by Robert Anholt published by W.F. Freeman and Co.

1) Identify your audience - Gear your talk so that the audience will understand your presentation. Use a minimum of jargon to express yourself.

2) Keep your presentation to the time allotted, nothing annoys an audience more than a speaker that exceeds the time (plan your talk to be about 80% of the allotted time).

3) Tell them what you are going to tell them, tell them, and then tell them what you told them.

4) Make sure the information you present is accurate.

5) Try to present the information as you are telling a story. Emphasize the parts that are the important and try to convey one major message.

6) The information presented must be logically organized and presented. Make sure that the audience can track with the logic of the talk. The information presented should build on itself as the talk progresses.

7) Some repetition can be good thing. If you have only mentioned an idea once, earlier in the talk, it often helps if you refresh the audience’s memory.

8) Overheads or slides should be simple, clear, presenting one basic point. Make sure that the figure is clearly visible to the whole audience before giving the talk. Do not put too much information on a slide or overhead.

9) Make sure you know what slide or overhead is next. You can keep a cheat sheet of all the slides or overheads so you know what is coming. If you want to present a slide at two different points in the talk, make two copies. Don’t spend time flipping through the slide tray or trying to find that overhead in the pile.

10) Prepare your talk in advance, at least several days before you give it. Rehearse your talk until you are sure what you are going to say. It is often helpful to give a practice talk in front of a trusted colleague to see how another person reacts.

11) Try to talk artificially slowly. Since the tendency is to talk too fast, going purposely slowly will appear to be a normal rate of speech.

12) Try to be enthusiastic about the material - avoid talking in a monotone. Most importantly, speak louder than is natural for you. Make eye contact with the audience, move around a bit, and use a pointer to direct the audiences attention.

13) When answering questions, be pleasant, succinct, and clear. It is often a good idea to repeat the question so everyone can hear it and to give yourself a little time to think. If you do not know the answer to a question do not make something up. It is OK to say “I don’t know” or “that is a good question but we have not looked at that.”
Introns, Exons, and Splicing

Promoter / Enhancer

Primary Transcript

Exon 1  Exon 2  Exon 3  Exon 4  Exon 5

Introns

Splicing and PolyA addition

mRNA or polyA RNA

ATG stop

coding region or open reading frame (ORF)

AAAAAAA_A150

poly A tail

mRNA is Translated

Protein

NH2 COOH
Creating Genomic Libraries

Genomic DNA is purified from almost any tissue. The genomic DNA is double stranded and contains exons, introns, promoters, enhancers, and repetitive DNA.

Limited digestion with a restriction enzyme (usually a 4 cutter)

Pieces of double stranded DNA of lengths from 10 to 40 kilobases are selected and ligated into lambda arms or cosmids.

Ligated DNA is packaged into phage heads used to infect bacteria. A good genomic library will contain all genomic DNA sequences in overlapping pieces.
Creating cDNA Libraries

A pool of mRNA is purified from tissue source (for example brain). The pool of mRNA may be comprised of mRNAs from ~ 50,000 genes. Some genes may produce thousands of mRNAs and others only a few.

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5' ATG 3' stop
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3' --------------- 5'
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reverse transcription

Convert to Double Stranded DNA

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AAAAAAA
TTTTTTTT
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Linker, Ligate

Lambda Arms

Package Ligated cDNA

Each phage contains a single cDNA which was reverse transcribed from one mRNA. When the phage infects a bacteria many copies of the phage will be produced. Since all the copies that result from a single phage are identical it is called a clone. A cDNA library usually contains 1 million different clones - each clone results from copying a single mRNA.
Southern Blot

Double stranded DNA from either tissue (genomic), cDNA, or plasmid DNA is digested with a restriction enzyme or enzymes. It is then run on an agarose gel. Large fragments move more slowly and small pieces move more quickly through the gel. By running known size markers, the size of the fragments can be determined. After running the gel the gel is treated with NaOH to denature the DNA and then the DNA is transferred to a nylon or nitrocellulose membrane. It is then probed with a radiolabeled DNA probe. The band that contains sequence in common with the probe will hybridize to the probe and appear as a dark band on xray film.
Northern Blot

Total or polyA RNA is then run on an agarose gel. Large RNAs move more slowly and small ones move more quickly through the gel. By running known size markers, the size of the fragments can be determined. After running the gel, the RNA is transferred to a nylon or nitrocellulose membrane. It is then probed with a radiolabeled DNA probe. The RNA that contains sequence in common with the probe will hybridize to the probe and appear as a dark band on xray film.
Western Blot

Proteins are heated in a SDS containing buffer that denatures the protein. SDS binds to the length of the protein, making it negatively charged. It is then run on a polyacrylamide gel toward the positive pole. Like RNA and DNA, larger proteins migrate slower through the gel. After electrophoresis, the gel is electroblotted onto a membrane (nitrocellulose, nylon, or PVDF). Proteins immobilized on the membrane are incubated with an antibody (primary antibody) that specifically recognizes a particular protein. In order to visualize the primary antibody-protein complex, another antibody (secondary antibody) that recognizes the primary antibody is added. An enzyme is chemically attached to the secondary antibody. A substrate is added to the blot that allows the secondary antibody-enzyme complex to be visualized.
Phage are plated on a lawn of bacteria. Usually between 100,000 and 1 million plaques are plated.

A nitrocellulose or nylon filter is overlayed on the plate and phage are transferred to the membrane. The DNA on the filter is denatured to make the DNA single stranded.

Radiolabeled single stranded DNA of the gene that you are screening for (the probe) is incubated with the filters and then washed. It will stick to the plaque that contains DNA for that gene. The filters are then exposed to xray film.

Dark spot on film indicates hybridization of probe to plaque.
Membrane and secreted proteins are synthesized on the rough endoplasmic reticulum (RER). They often have a signal sequence (hydrophobic stretch) at the amino end that is cleaved off inside the RER. If the protein has several membrane spanning segments these are threaded through the ER membrane. Amino acid residues facing the inside of the RER will be extracellular while those facing the cytoplasm will be intracellular. Most secreted and membrane proteins are glycosylated in the RER and Golgi as they move through the secretory pathway. Glycosylation only occurs at residues that are destined to be extracellular.