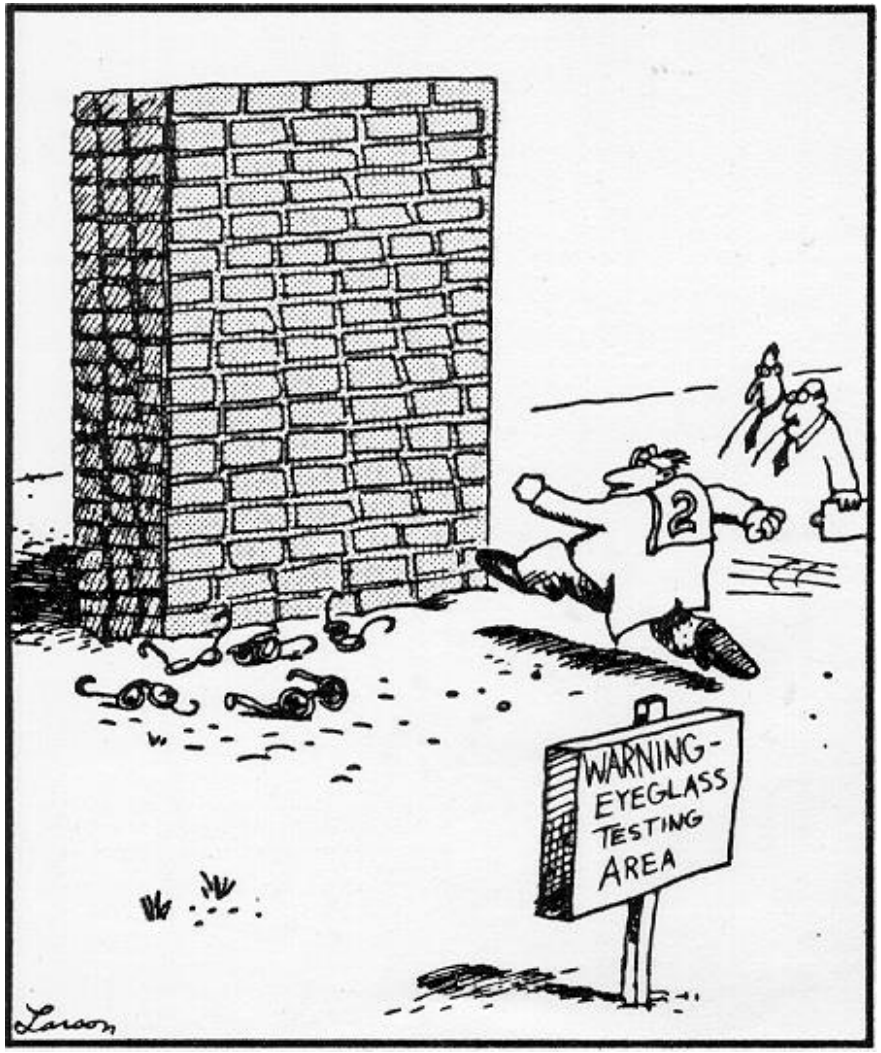
IBC Protocol Reviews (aka Risk Assessment)

It's a Matter of Perspective…



Individuals with different experience will view risk differently

IBCs rely on multiple perspectives to evaluate risk

Challenge of Risk Assessment for rDNA Materials

- Recombinant DNA raises possibility of modifying a host or vector to impart new properties not considered in original risk group classification
- Requires an understanding of the host/vector system involved, the expression construct, and the finished product
  - protection of personnel
  - guidance for containment and work practices

Elements of Risk Assessment

- For rDNA materials must consider:
  - Transgene
    - Effects of the gene product being produced
    - Effect of eliminating a gene product
  - Vector/host system
    - Intrinsic characteristics (risk group)
    - Replication competence
    - Residual viral gene expression
  - End product
    - Possible introduction or increase of virulence

Risk Assessment

Points to Consider

Gene Product Effects

- **Local effects**
  - **Protein may have deleterious effects on the cell it is expressed in, but can't spread**
    - **Ion channels, enzymes**
- **Systemic effects**
  - **Secreted protein that can disseminate and exert an effect on otherwise unmodified cells**
    - **Cytokines, growth factors**
  - **A protein that modifies a cell so that this cell becomes a threat**
    - **Oncogenes**

Loss of Gene Product

- RNAi used to knock-down expression of a normal cellular protein
- Will the effect of this change be:
  - Local? (i.e. eliminating an enzyme in a metabolic pathway)
  - Disseminated? (i.e. eliminating a protein that regulates growth control)
- Off-target effects

Bacterial Vector Considerations

- **Basic characteristics of organism**
  - **Pathogenicity**
  - **Ability to persist in host**
- **Mobile Genetic Elements**
  - **Plasmids**
  - **Insertion sequences and transposons**
  - **Bacteriophages**
  - **Ability to shuttle virulence factors**

Viral Vector Considerations: General

- Pathogenicity of parental virus
- Cytopathogenicity of vector
- Scale-up considerations
- Requirements for specialized facilities
- Training requirements

Viral Vector Considerations: Efficiency and Persistence

- **Efficiency**
  - **In vitro *vs* in vivo delivery**
  - **Tissue tropism**
  - **Inactivation by complement**
- **Persistence**
  - **Integration or persistence of transgene**
  - **Immune response to vector**
- **Concern: ability to prolong effects of accidental exposure**

Viral Vector Considerations: Route of Transmission

- **Blood-borne**
  - **Lentivirus**
- **Direct contact**
  - **vaccinia**
- **Respiratory**
  - **Adenovirus, adeno-associated virus**
- **Containment *vs* work practices**
  - **Does containment really help, or would eliminating sharps accomplish more**

Viral Vector Considerations: Host Range

- Based on human pathogens
  - Replication incompetent
  - Viral gene products in vector
- Non-pathogenic viruses
  - Adeno-associated virus
- Based on non-human pathogens
  - Reduced pathogenicity (vaccinia, avipox)
  - Non-pathogenic (baculovirus)
- Tropism and host range
  - Change in cell type or species affected

Viral Vector Considerations: Replication Competence

- **Replication competent**
  - **Vaccinia**
  - **Baculovirus**
- **Replication competent but crippled**
  - **Alphaviruses**
- **Replication incompetent**
  - **Retrovirus, lentivirus, adeno-associated virus**
  - **Adenovirus (early generations), herpesvirus**

End Product Concerns

- Increased risk over vector alone
  - Introduction of virulence factors
    - Toxins, antibiotic resistance
    - Increased ability to evade immune system
  - Efficient delivery of a product with disseminated effects
- Reconstitution of replication competence
- Concern: creation or restoration of pathogenicity

Host/Vector Interactions

- **Evade/defeat host immune system**
  - **Limited exposure to immune system**
  - **Latency**
  - **Gene products to suppress immune response or interfere with immune recognition**
- **Adherence to host cell**
  - **Surface protein on agent recognizes cell-surface molecule (generally protein)**
  - **Responsible for tropism and host range of agent**

Host/Vector Interactions

- Penetration
  - Cells take up agent (result of binding, phagocytosis)
  - Fusion proteins, nuclear localization signals
- Colonization and multiplication
  - Attachment factors (capsules, pili)
  - Virulence factors (toxins, antibiotics)
  - Commandeer host cell metabolism
- Spread
  - Escape from cell, access to circulation

What is Low Risk?

- Some rDNA work is reasonable to do at BSL1 level
- Things to consider
  - Exposure of user to vector system/cDNA
    - Route of infection
    - Effect of gene product
    - Persistence in host
  - Environmental release
    - Persistence in the environment
    - Nothing gets out alive!

Gene Expression

- At the core of any rDNA experiment is the need to express a protein or a shRNA to knock down expression
- What are the possible effects of this expression?
  - Let's assume the risks are low…
- What type of system will be used to express this construct? What are the risks of the finished product?

Propagation of DNA in *E. coli* K-12

- **Much rDNA work in K-12 is considered low risk (exempt)**
  - **Non-pathogenic for humans**
  - **Does not persist in host (no attachment)**
  - **Oral route of infection**
  - **Bacterial promoters are different than higher order systems**
- **Certain gene products can raise concerns**

Protein Expression in the Baculovirus-Insect Cell System

- Basically an expression cassette but moved into a viral vector
- Baculovirus is considered a low risk viral vector
  - Not replication in mammalian cells
  - No known pathogenicity in humans
  - Form of virus commonly used in lab is not infectious for natural host (insect larvae)
- Always a good idea to inactivate recombinant materials for disposal

What Factor Can Escalate Risk?

- **Cascade of infection**
  - **Evade/defeat host immune system**
  - **Adherence to host cell**
  - **Penetration**
  - **Colonization and multiplication**
  - **Spread**
- **Gene products derived from either host or vector systems that are involved in, or could enhance these processes can be concerns in rDNA work**

Production of Toxin in *E. coli* K-12

- **Simple gene expression cassette in *E. coli***
- **Addition of possible virulence factor**
  - **What are the effects of the toxin**
  - **LD_{50} data if available**
  - **Possible implications for oversight groups**
- **Nature of the transgene causes a reexamination of risk of *E. coli* expression**

Protein Expression in Mammalian Cells using Adenoviral Vectors

- Again - just an expression cassette
- The vector: based on a human pathogenic virus
  - Risk factors of starting virus
  - How has vector been disabled
  - Chance of replication competent virus
  - Re-examine the transgene risk
- Nature of the vector can change the risk assessment of the experiment