Institutional Biosafety Committees

Purpose & Objectives

UNIVERSITY of WASHINGTON

Presented by:

Diversified Laboratory Sciences, Inc.

July 1, 2009

Introduction

The Project Team

- **Jeff Owens, MPH, CSP, CBSP**
  Biosafety & Biosecurity Consultant
- **Patrick Condreay, PhD**
  Biosafety Consultant

DLS
LABORATORY
CONSULTANTS

Purpose

Design and present training for the University of Washington Institutional Biosafety Committee (IBC) members that meets the requirements of the *NIH Guidelines* and prepares them for expanded efforts in the review of Recombinant DNA (rDNA) applications.

Objectives

- Provide a basic introduction to rDNA research for IBC members who do not regularly work with rDNA.
- Review the roles and responsibilities of the IBC in oversight of rDNA research, including the various types of research covered by the NIH Guidelines, the biosafety levels of the Guidelines, and the typical review and approval processes that apply to various forms of rDNA research, exclusive of University policies and procedures.
- Apply practical guidance for the review of research applications utilizing general research examples and case studies as well as specific University principal investigator applications provided by the University Biosafety Officer and IBC Chair.

DLS LABORATORY CONSULTANTS

Agenda

- Definitions
- Biosafety oversight
- Research involving recombinant DNA
- Responsibilities under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*
- IBC protocol review

Definitions

Definitions

Biohazard:

An agent of biological origin that has the capacity to produce harmful effects on humans; i.e. microorganisms, toxins and allergens derived from those organisms, and allergens and toxins derived from plants or animals.

Definitions

Biosafety:

Applying a combination of laboratory practices and procedures, laboratory facilities, and safety equipment when working with potentially infectious microorganisms.

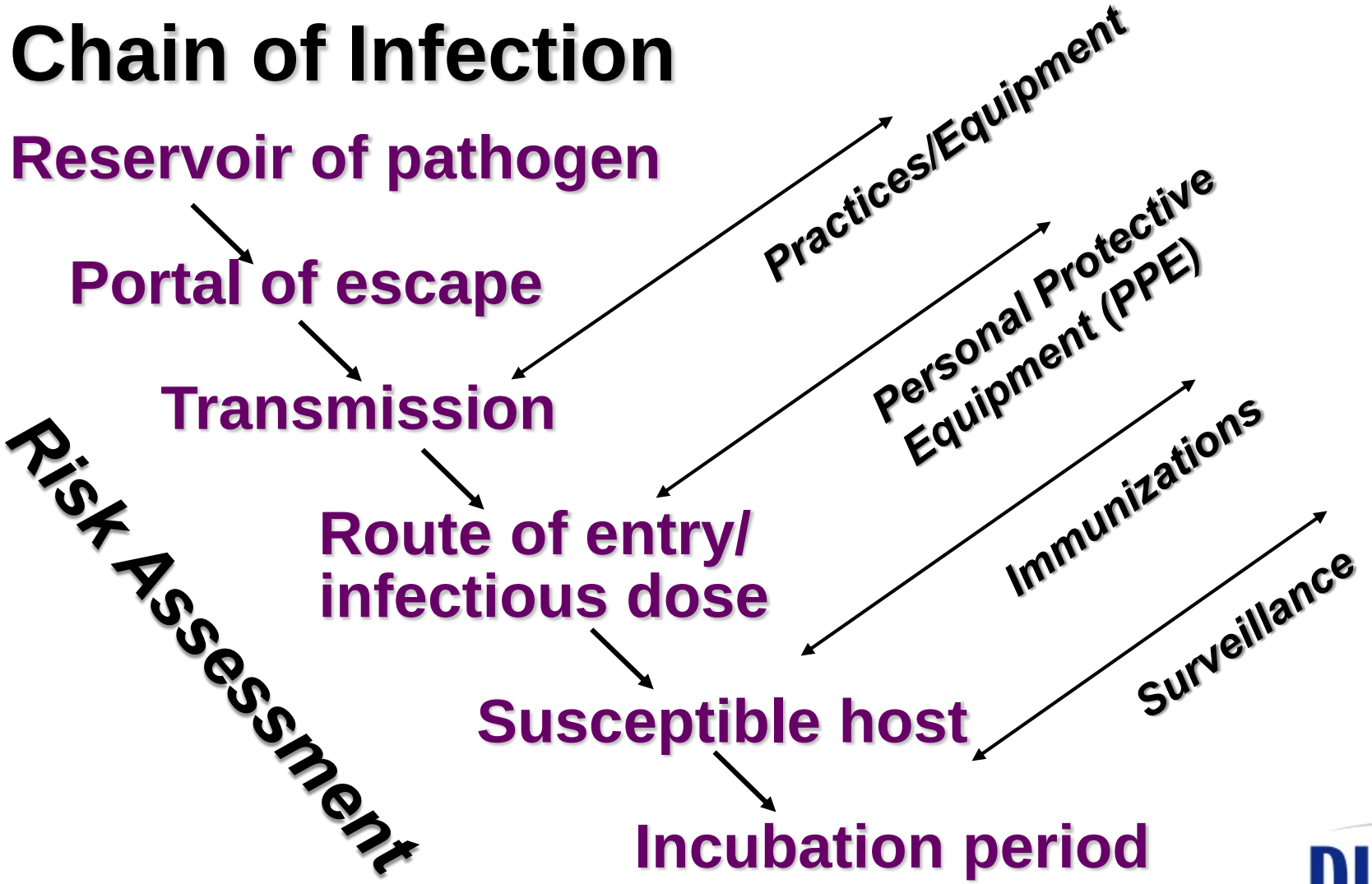
Definitions

Risk Assessment:

Addressing laboratory activities involving infectious or potentially infectious material and implementing measures to reduce the worker's and environment's risk of exposure to an agent to an absolute minimum.

DLS LABORATORY CONSULTANTS

Risk Assessment



Definitions

Biosecurity:

Protection of high-consequence microbial agents and toxins, or critical relevant information, against theft or diversion by those who intend to pursue intentional misuse.

Biosecurity vs. Biosafety

- **Biosecurity** refers to ensuring the security of biological materials to prevent theft, illicit use, or release
- **Biosafety** focuses on reducing exposure to and release of biological materials
- Both involve conducting a **risk assessment** to mitigate risks

Definitions

Select Agents:

Pathogens and toxins considered to have the potential to pose a severe threat to human, animal, or plant health and safety.

- **Viruses**
- **Bacteria**
- **Fungi**
- **Toxins**

Definitions

Responsible Official (RO) and Alternate Responsible Official (ARO)

An individual designated by the entity, which acts on their behalf and has the authority and control to ensure compliance with the regulations

- **Approved by the Department of Justice**
- **Familiar with regulation requirements**

Definitions

Recombinant DNA Molecules

Under the current *NIH Guidelines*, these are molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from their replication.

Definitions

NIH Guidelines for Research Involving Recombinant DNA Molecules

(NIH Guidelines)

A document created in 1976 that outlines principles for the safe conduct of research employing recombinant DNA technology. The NIH Guidelines detail practices and procedures for the containment of various forms of recombinant DNA research, for the proper conduct of research involving genetically modified plants and animals, and for the safe conduct of human gene transfer research.

DLS LABORATORY CONSULTANTS

Biosafety Oversight

Biosafety Program Elements

- Organization
- Biosafety Manual (general and lab-specific)
- Registration and Inventory Control
- Risk Assessment and Control of Biohazards
- Biosafety/IBC Committee
- Biosafety Training
- Emergency Response
- Medical Surveillance
- Auditing Program
- Documentation

DLS LABORATORY CONSULTANTS

External Oversight

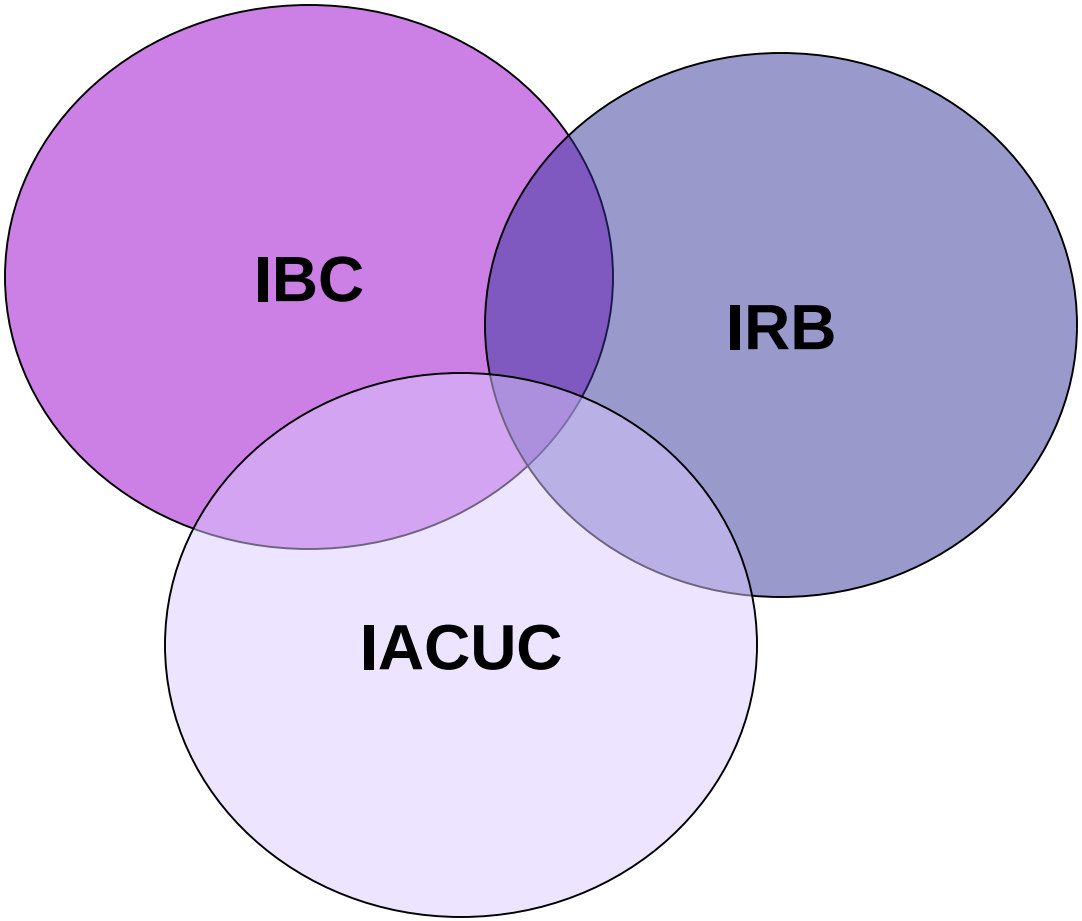
- National Institutes of Health (NIH)
  - Office of Biotechnology Activities
- Centers for Disease Control (CDC)
- Occupational Safety & Health Administration (OSHA)
- Environmental Protection Agency (EPA)
- US Dept of Agriculture (USDA)
- US Dept of Justice
- US Dept of Transportation
- US Dept of Commerce
- World Health Organization (WHO)
- Community Activist Groups

Internal Oversight

- Institutional Biosafety Committee (IBC)
- Biosecurity and Biosafety Programs
- Emergency Response Plan
- Standard Operating Procedures (SOP's)
- Laboratory Inspections (internal and external; CDC, USDA, AAALAC, etc.)
- Training and Documentation
- Institutional Animal Care and Use Committee (IACUC)
- Institutional Review Board (IRB)

DLS LABORATORY CONSULTANTS

Institutional Committees



IBC's, IACUC's, and IRB's

- **Relationship not prescribed in the *NIH Guidelines***
- **Institutions should determine best way for these committees to interact and share information**

Introduction to Research Involving rDNA Molecules

Objectives

- Understand basic processes involved in gene expression
- Become familiar with characteristics and construction of recombinant gene transfer vectors
- Learn to recognize the characteristics of gene transfer systems that have implications for their safe use

DLS
LABORATORY
CONSULTANTS

Outline

- Gene Expression
- Recombinant Gene Expression
- Gene Non-Expression
- Viral Vector Technology

DLS
LABORATORY
CONSULTANTS

Gene Expression

DLS
LABORATORY
CONSULTANTS

Molecular Biology

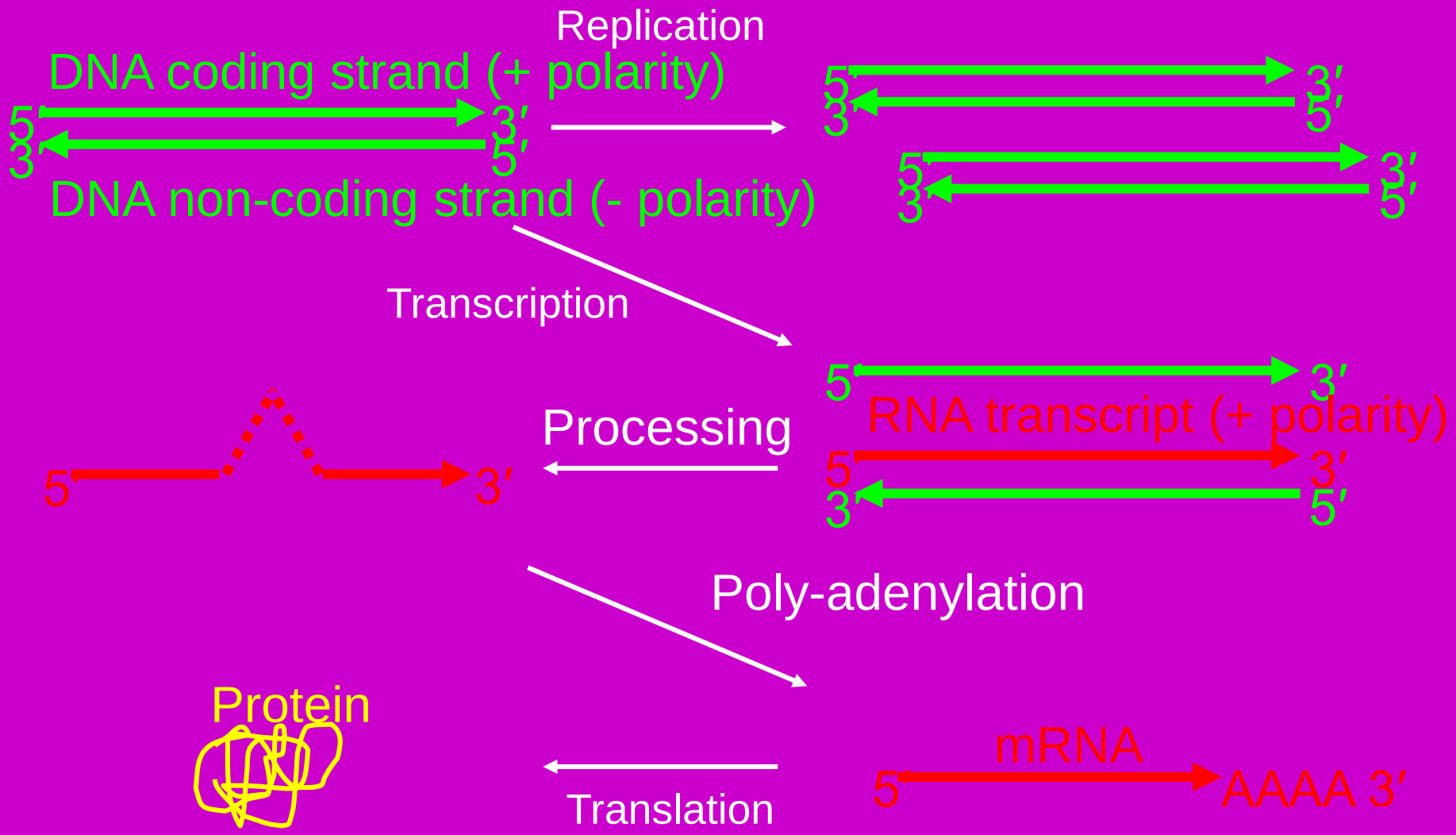
- An attempt to explain cellular processes by understanding the interactions of the molecules involved
- Requires manipulation of genes and their expression in different situations
- Recombinant DNA and gene transfer are enabling technologies for molecular biology

DLS
LABORATORY
CONSULTANTS

Basic Processes of Gene Expression

- **Replication**
  - synthesis of an exact duplicate nucleic acid (maintenance of the genetic information)
- **Transcription**
  - making an RNA copy of a DNA molecule
- **Translation**
  - converting RNA sequence into an amino acid sequence

The Flow of Genetic Information

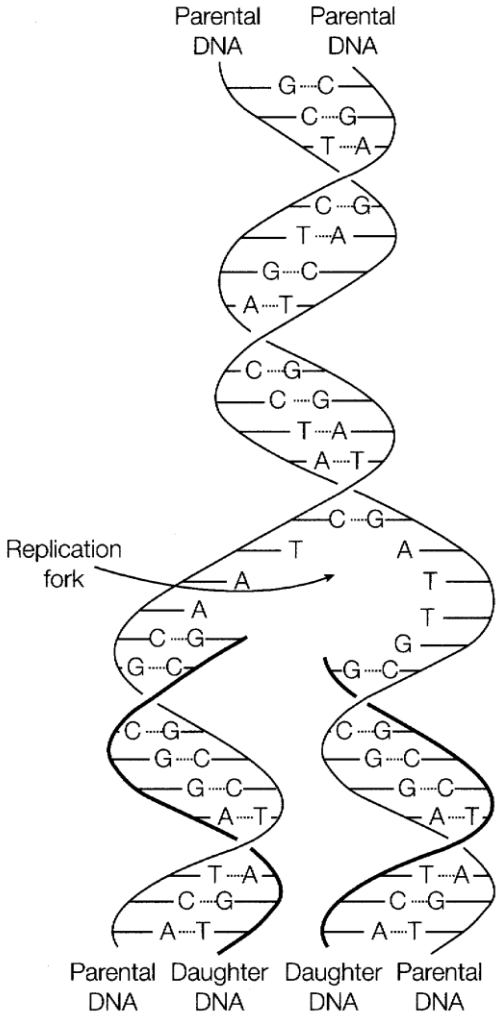


Deoxyribonucleic Acid (DNA)

- Genetic material for most organisms
- Long strands of nucleotide “bases”
  - Four different bases (A, G, C, T)
  - Uniqueness due to specific sequence of bases
  - Two strands associate through hydrogen bonds between complementary bases
  - A bonds with T, C bonds with G

DLS LABORATORY CONSULTANTS

DNA Replication



- **Because of base pairing, sequence of bases on one strand determines sequence of the other strand**
- **Each strand in a molecule can be a template to make a copy of the molecule**

Ribonucleic Acid (RNA)

- There are some subtle chemical differences between DNA and RNA
- Genetic material for some viruses
- Messenger RNA (mRNA): an information exchange molecule
- Other RNAs
  - Protein synthesis (rRNA, tRNA)
  - Catalysis (RNAase P)
  - Regulatory functions (miRNA)

DLS LABORATORY CONSULTANTS

Messenger RNA (mRNA)

- An information exchange molecule
  - The information is stored in the sequence of bases in the strand
- mRNAs are translated to make a protein
- In eukaryotic cells a messenger RNA codes for only one protein

DLS LABORATORY CONSULTANTS

Reading Frame

- Sequence of bases in mRNA is read in groups of three for translation
  - Referred to as codons
- A sequence can be parsed into codons in three ways:

  AGC TAG CTA GCT AGC TAG CTA

  A GCT AGC TAG CTA GCT AGC TA

  AG CTA GCT AGC TAG CTA GCT A
- This is referred to as reading frame

DLS LABORATORY CONSULTANTS

The Genetic Code

UUU	phe	CUU	leu	AUU	ile	GUU	val
UUC	phe	CUC	leu	AUC	ile	GUC	val
UUA	leu	CUA	leu	AUA	ile	GUA	val
UUG	leu	CUG	leu	**AUG**	**met**	GUG	val
UCU	ser	CCU	pro	ACU	thr	GCU	ala
UCC	ser	CCC	pro	ACC	thr	GCC	ala
UCA	ser	CCA	pro	ACA	thr	GCA	ala
UCG	ser	CCG	pro	ACG	thr	GCG	ala
UAU	tyr	CAU	his	AAU	asn	GAU	asp
UAC	tyr	CAC	his	AAC	asn	GAC	asp
UAA	***	CAA	gln	AAA	lys	GAA	glu
UAG	***	CAG	gln	AAG	lys	GAG	glu
UGU	cys	CGU	arg	AGU	ser	GGU	gly
UGC	cys	CGC	arg	AGC	ser	GGC	gly
UGA	***	CGA	arg	AGA	arg	GGA	gly
UGG	trp	CGG	arg	AGG	arg	GGG	gly

- **The code is *degenerate***
  - **one amino acid can be encoded by more than one codon**
- **Particular codons are used to designate the beginning and end of a reading frame**

DLS LABORATORY CONSULTANTS

Proteins

- Long linear polymers of molecules called amino acids
- Information for synthesis of proteins is contained in the nucleic acid
- Proteins have a variety of functions
  - Catalysis of chemical reactions (enzymes)
  - Structural (histones, cytoskeletal proteins)
  - Regulatory (transcription factors, growth factors)

Post-Translational Modification

- **Proteins can be modified after their initial synthesis**
  - **Cleavage**
  - **Glycosylation**
  - **Phosphorylation**
  - **Addition of lipids, nucleotides**
  - **Acetylation, methylation, hydroxylation**
- **Alters function, targets proteins, regulates interactions, *etc***
- **Driver for gene expression in different systems**

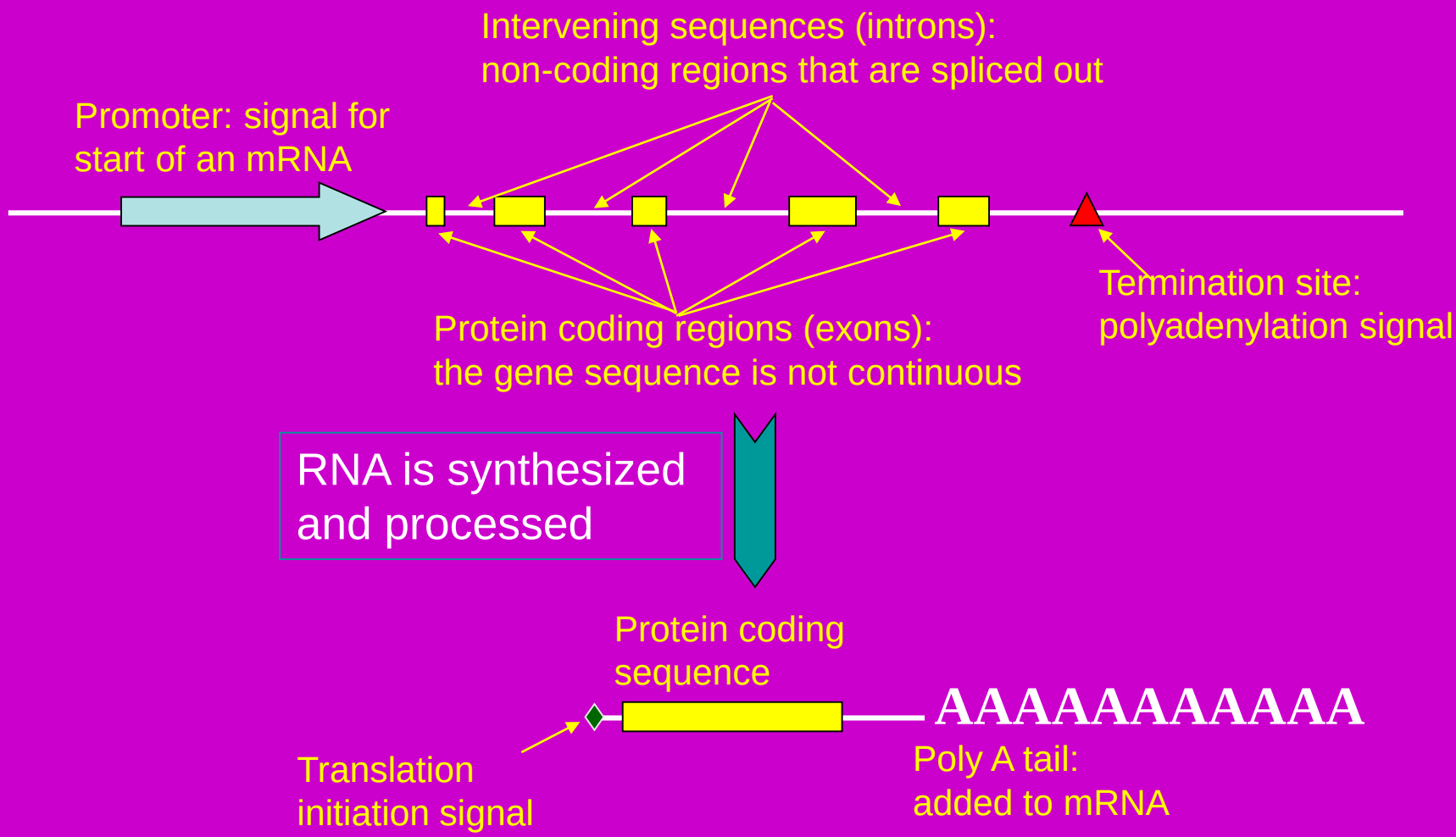
DLS
LABORATORY
CONSULTANTS

What Is A Gene?

- Region of nucleic acid that contains the base sequence information to encode a protein (or RNA)
- In eukaryotic cells coding regions are often interrupted with non-coding regions that have to be removed in mRNA
  - Called intervening sequences or introns
- Genes are preceded by a promoter
  - Starting signal for the synthesis of an mRNA

DLS LABORATORY CONSULTANTS

Gene Structure in Mammalian Cells



Recombinant Gene Expression

Recombinant Gene Expression

- **Over-expression for purification**
  - **Bacterial expression (*E. coli*)**
  - **Insect cell expression (baculovirus)**
  - **Mammalian cell expression (vaccinia virus)**
  - **Generally done in cultured cells**
- **Expression in a cell to exert an effect**
  - **Tailored to the cell system being studied**
  - **Can be in cultured cells or *in vivo***

Applications of rDNA

- **Research tools**
  - **Protein production**
  - **Transient expression studies**
  - **Viral replicons**
  - **Stable cell lines**
  - **Transgenic animals**
  - **Knock-down/knock-out animals**
- **Gene therapy**
- **Transgenic animals for protein production**

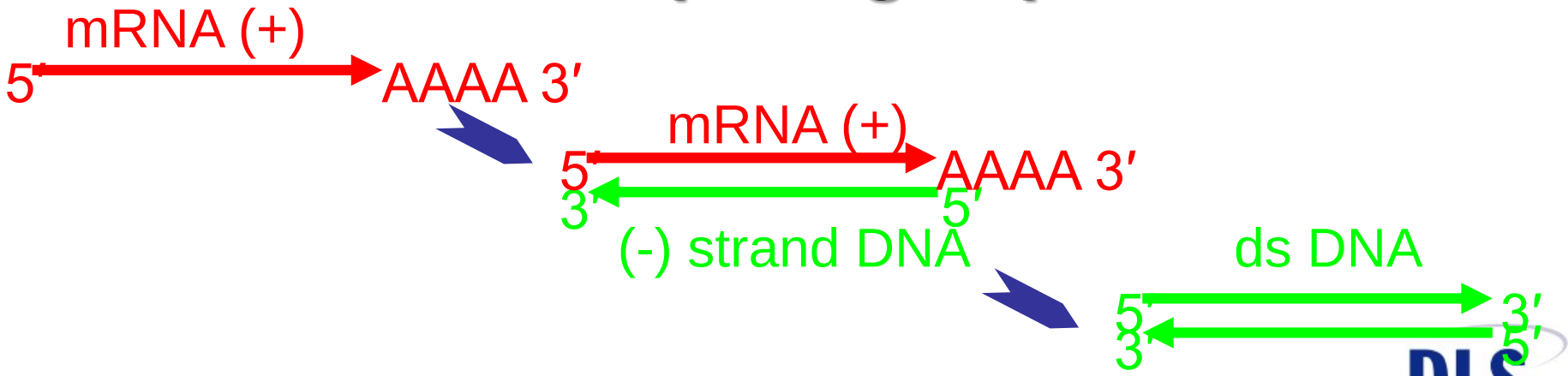
Applications of rDNA

- **Pharmaceutical compound screening**
  - **Biochemical assays**
  - **Cell-based assays**
- **Vaccine production**
  - **Sub-unit vaccines**
  - **Virus-like particles**
- **Transgenic plants**
  - **Crop improvement**
  - **Disease/pest-resistant plants**

DLS LABORATORY CONSULTANTS

What is cDNA?

- **cDNA: a DNA copy of an mRNA containing the protein coding domain of a gene**
- **Generally do not use genomic DNA clones for recombinant gene expression due to size and splicing requirements**



Expression Cassettes

- **Minimum requirements for constructing a recombinant gene for expression:**
  - **Promoter**
  - **cDNA (transgene)**
  - **Termination signal (poly A site)**

Expression cassette

Escherichia coli K-12

- ***E. coli*** **is a normal resident of the intestinal tract**
- **The K-12 isolate**
  - **Ineffective at colonizing the human gut**
  - **Much rDNA work with K-12 considered lower risk**
  - **Many common laboratory strains derived from K-12**
- **Useful tool in molecular biology**
  - **Easy to propagate in artificial media**
  - **Short generation time**
  - **Can easily generate large numbers of cells**

DLS LABORATORY CONSULTANTS

Plasmid DNA

- Small (~2-20 kbp) circular DNA molecules
  - Replicate in bacterial cells independently of the host cell chromosome
  - Carry genes which render host resistant to antibiotics
  - Some plasmids can be swapped among different species of bacteria (“Broad host range”)
- Are exploited for use as cloning and gene expression vectors

DLS LABORATORY CONSULTANTS

How Do We Exploit Plasmids?

- **Restriction enzymes**
  - **Recognize and cut DNA at specific sequences**
- **Pieces of cut DNA can be mixed and re-sealed to form new plasmids.**
- **Large amounts of new plasmids can be made in *E. coli*.**

Why is *E. coli* So Important?

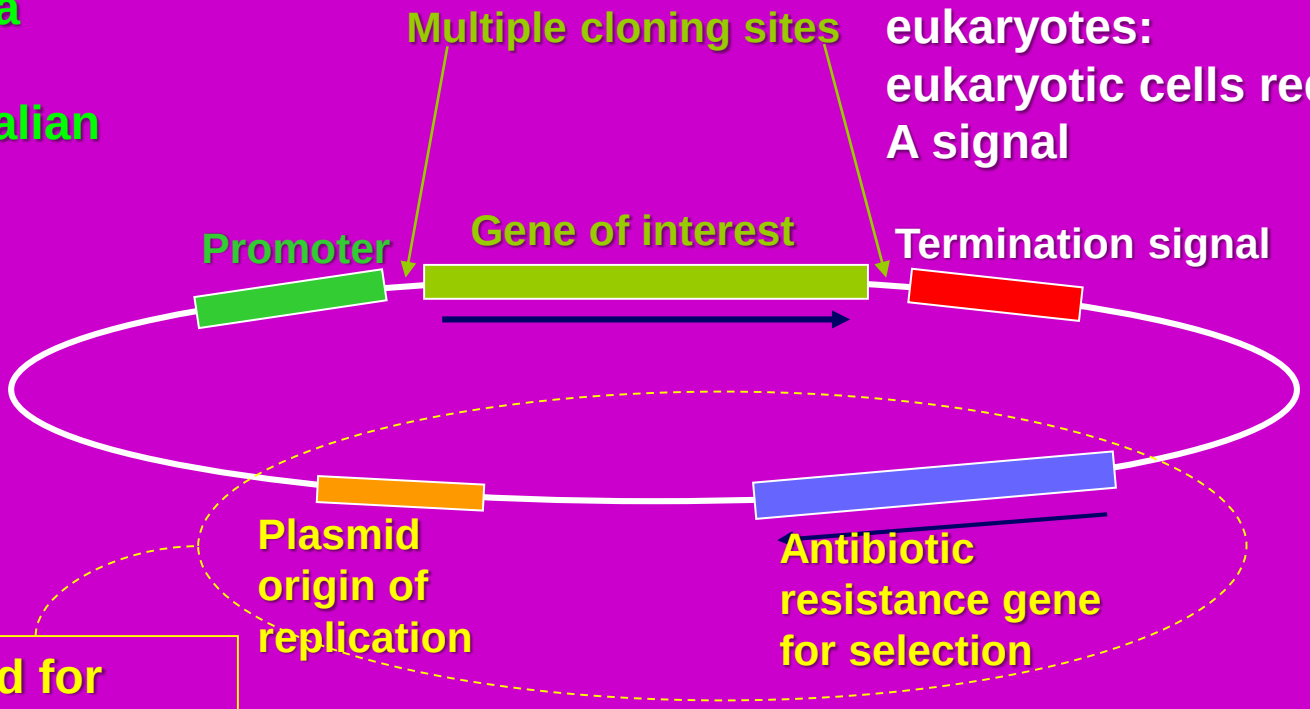
- **Transcriptional regulation is well-understood**
- **Potential for very high expression levels**
- **Excellent plasmid DNA factory**
  - **Easy to introduce plasmid DNA into cells**
  - **Easily cultured to high cell densities**
  - **Plasmid DNA is easily isolated from cells**

DLS
LABORATORY
CONSULTANTS

Elements of a Basic Expression Vector

Promoters are tailored to the type of cell:
bacteria
yeast
mammalian
plant
insect

Termination signals are tailored to prokaryotes or eukaryotes:
eukaryotic cells require a poly A signal



Needed for growth in *E. coli*

Gene Delivery Technologies for Eukaryotic Cells

- **Chemical transfection**
  - **Calcium phosphate**
  - **Liposomes**
- **Electroporation**
- **Micro-injection**
- **Ballistic barrage**
- **Virus-like particles (VLP)**
- **Bacteria/bacteriophage**
- **Viral-mediated**

Chemical methods

Physical methods

Biological methods

DLS LABORATORY CONSULTANTS

Transient Gene Expression

- **Plasmids transfected into cells express proteins for a short time (1 to 3 days)**
- **If there are no elements on a plasmid to allow DNA to be maintained or replicated, the input DNA is degraded and diluted out of the culture by cell division**
- **When a plasmid is transfected into cells for short-term expression, it is referred to as transient gene expression**

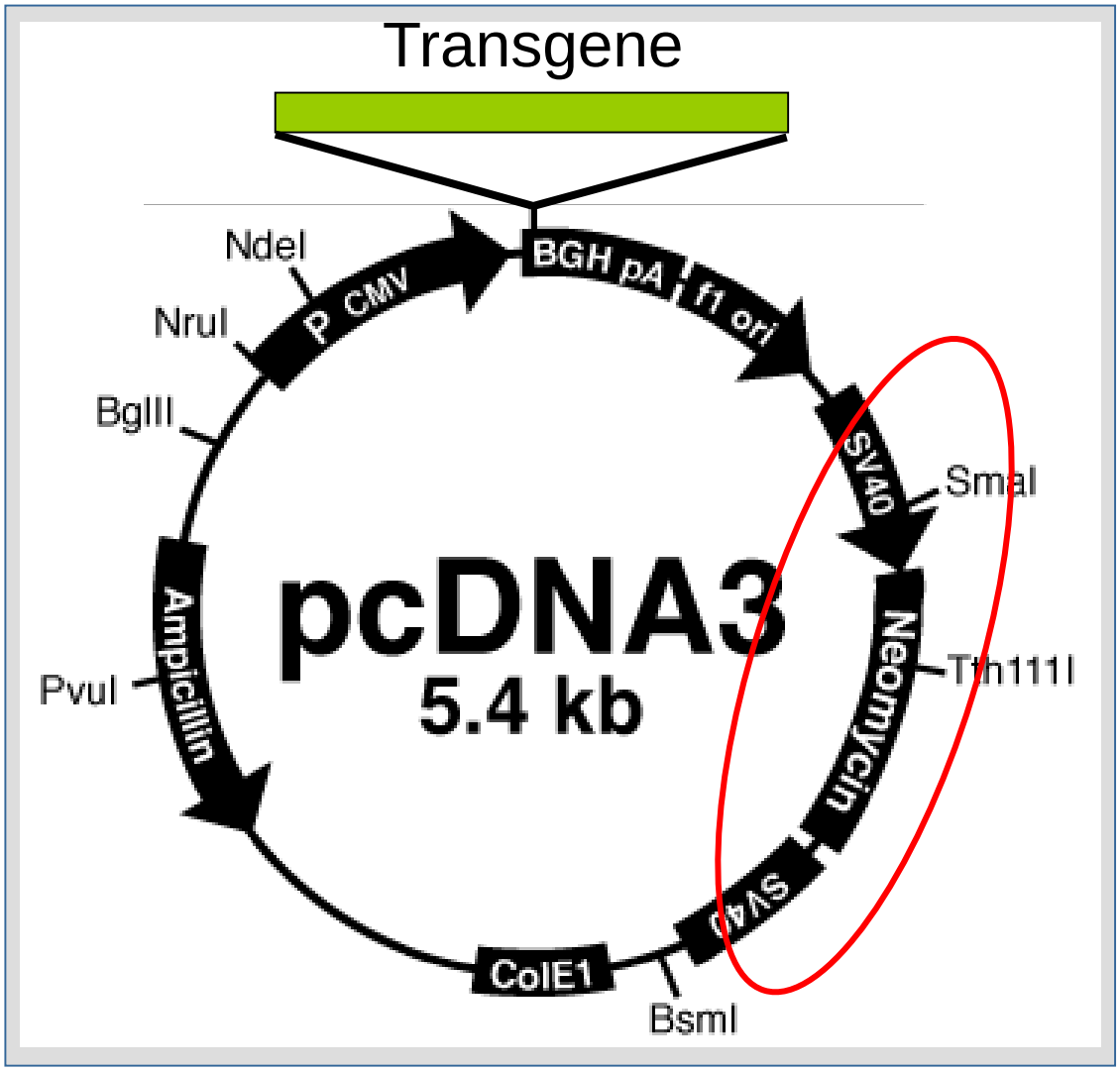
DLS LABORATORY CONSULTANTS

Stable Expression

- **Long-term expression requires maintenance of input DNA**
  - **Cassette that expresses a protein that inactivates a toxic drug (dominant selectable marker)**
  - **Enables cells to survive in presence of drug**
  - **Allows selection of rare cells that have recombined plasmid DNA into their genome**
- **These are referred to as stable cell lines or stably transfected cells**

DLS LABORATORY CONSULTANTS

Expression Vector for Stable Expression



The neomycin phosphotransferase gene inactivates a toxic drug (G418 or geneticin)

Plasma DNA Delivery

Advantages

- Few limitations on DNA size
- Non-infectious
- Chemically defined
- Lack of immune response

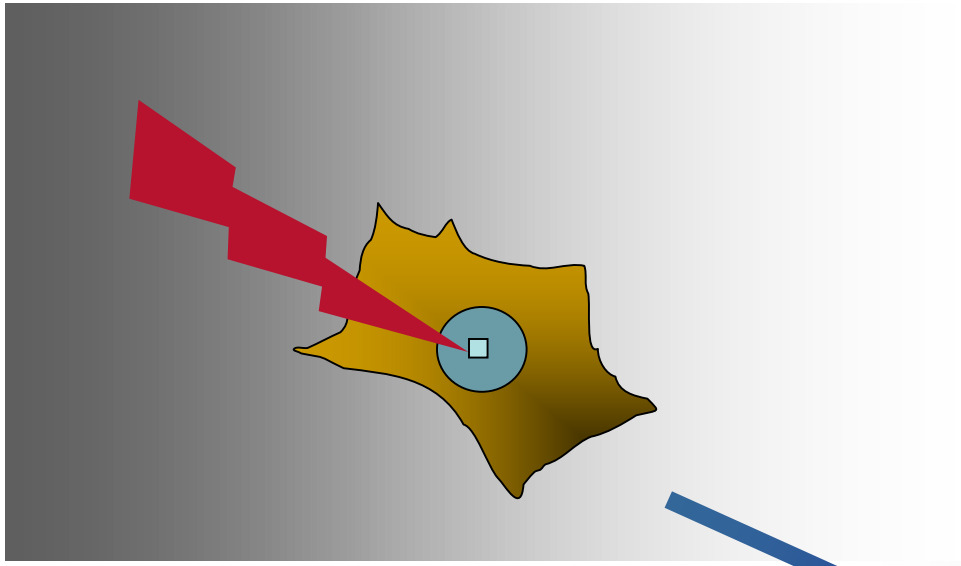
Limitations

- Inefficient delivery
- Low expression levels
- Short term expression
- Toxicity
- Different methods for different cells

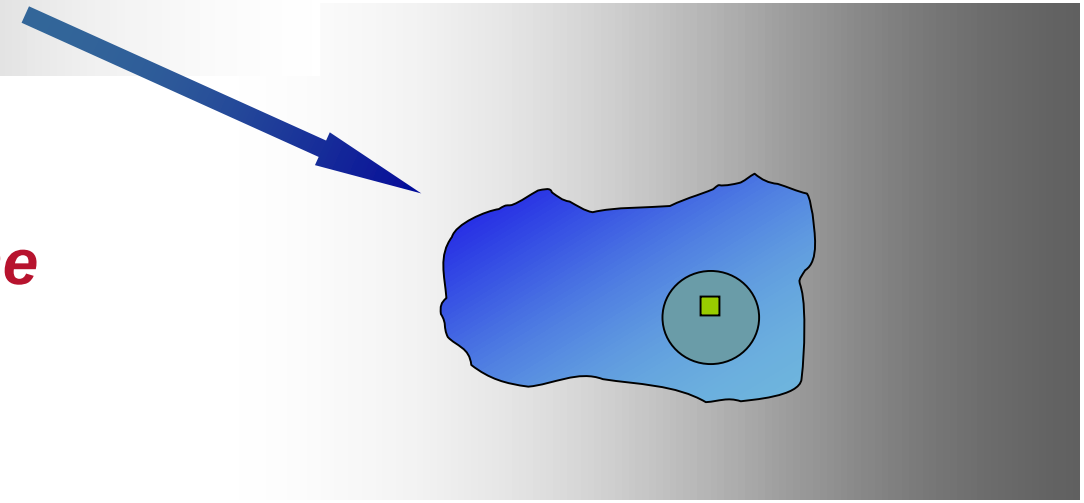
DLS LABORATORY CONSULTANTS

Gene Non-Expression

Knocking Out/Down Expression of a Gene



Look for some observable phenotype in the altered cell.

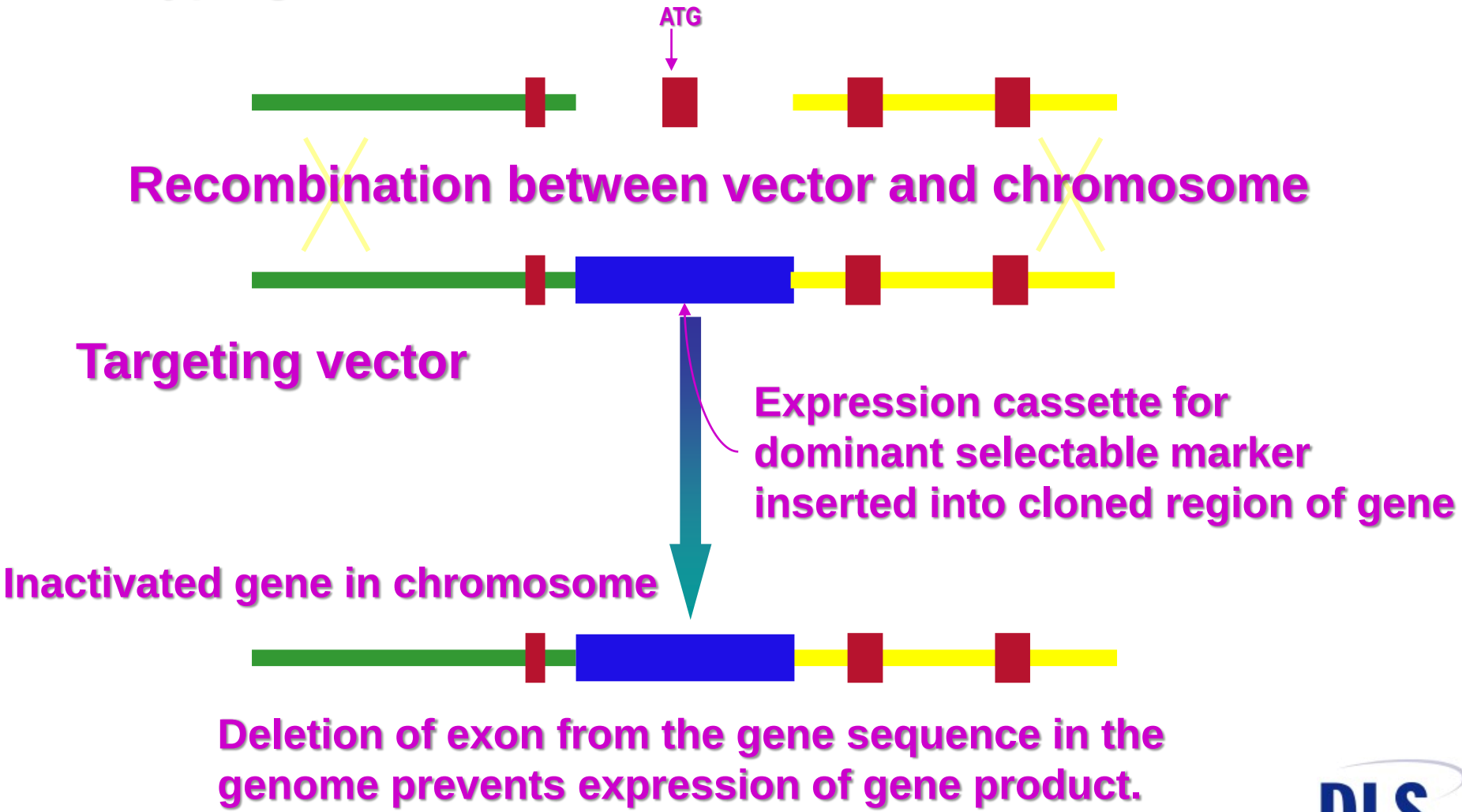


Engineer the cell so that a particular gene will not be expressed.

Applications of Non-Expression

- Elucidation of protein function
- Target identification
- Target validation
- Therapeutics

Knocking Out a Gene



RNA Interference

- **Uses small, double stranded RNAs to silence expression of a gene product**
- **Utilizes a normal host response to dsRNA and a normal cellular post-transcriptional regulatory machinery that is found in organisms ranging from yeast to mammalian species**

Shoot the Messenger

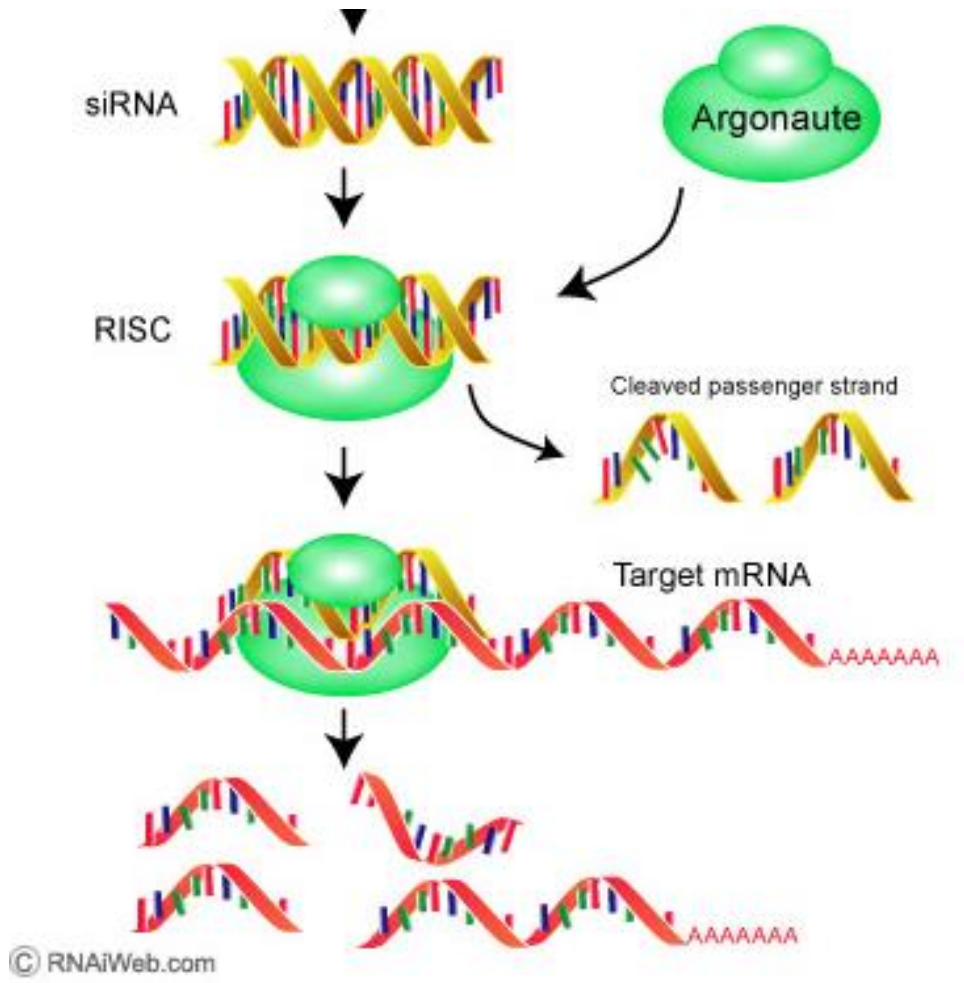
RNAi causes a specific mRNA to be degraded so that protein expression is reduced or eliminated.

MicroRNAs (miRNA)

- Endogenous RNAs that post-transcriptionally regulate a number of cellular pathways
- Thought to regulate as much as one third of human mRNAs
- miRNA action can result in a number of fates:
  - Degradation
  - Inhibition of translation factors
  - Physical block of ribosomes
  - Sequestration into P-bodies

DLS LABORATORY CONSULTANTS

How Does RNAi Work?



- dsRNA is introduced into a cell that is identical to a sequence in the target mRNA
- The RNA-induced silencing complex (RISC) binds to this guide sequence
- The complex finds the mRNA sequence that is complementary to the guide sequence and cuts it so that it can not be translated

DLS LABORATORY CONSULTANTS

RNAs Used to Induce RNAi

- siRNA: short interfering RNA
  - Small dsRNAs that are processed to induce RNAi
  - Chemically synthesized
  - RNA processing induces interference better than pre-processed RNA
- shRNA: short hairpin RNA
  - Mini-genes encoding RNA with complementary regions separated by a stretch of nucleotides
  - Placed into expression cassettes
  - Once transcribed form structures called hairpins

DLS LABORATORY CONSULTANTS

shRNA Expression Vector

Example Target Sequence (AA plus 19 nt)

5'~~~AA GTCAGGCTATCGCGTATCG~~~3'

Annealed Hairpin siRNA Template Insert (order these 2 oligonucleotides)

*BamH*I	Sense Strand	Loop	Antisense Strand	RNA pol III Terminator	*Hind* III
5'–GATCC	GTCAGGCTATCGCGTATCG	TTCAAGAGA	CGATACGCGATAGCCTGAC	TTTTTTGGAAA–3'	
3'–G	CAGTCCGATAGCGCATAGC	AAGTTCTCT	GCTATGCGCTATCGGACTG	AAAAAACCTTTTCGA–5'	

*G
C

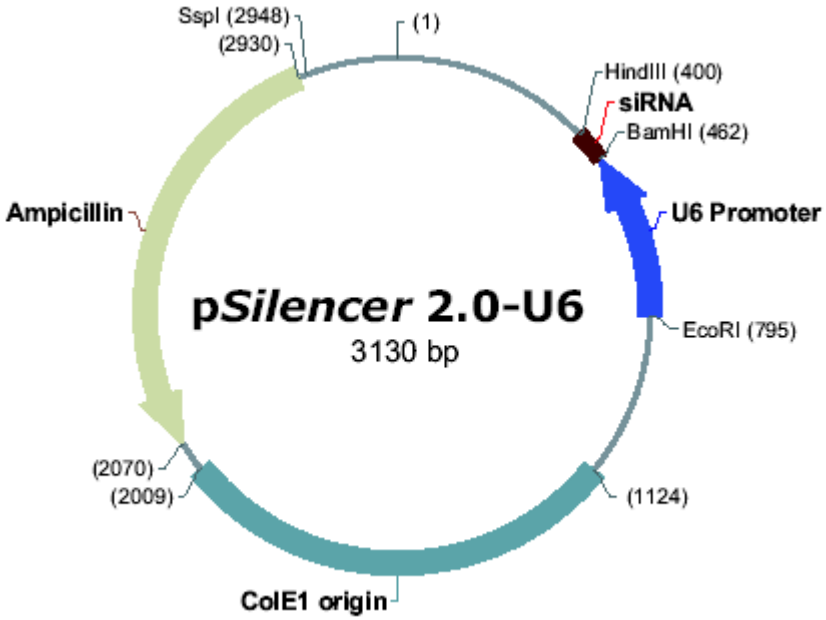
Hairpin siRNA Structure

sense sequence

5'–GUCAGGCUAUCGCGUAUCG U U C A A

3'–UU CAGUCCGAUAGCGCAUAGC A G A G

antisense sequence



Introduction of RNAs

- siRNAs
  - Chemical transfection (i.e. liposomes)
  - Microinjection (embryos)
  - Ballistic barrage
  - Ingestion (in nematodes)
- shRNAs
  - Chemical transfection
  - Ballistic barrage
  - Viral vectors: particularly for *in vivo* applications

Viral Vector Technology

Viruses

- Obligate, intracellular parasites; unable to grow and divide on their own
- Have a variety of methods to commandeer the functions of the host cells to direct their own replication scheme, often at the expense of the host cell
- We can harness the power of these organisms to deliver nucleic acids to turn them into gene delivery tools

DLS
LABORATORY
CONSULTANTS

Why Use Viral Vectors?

- **Efficiently condense, package, and deliver nucleic acids to cells**
- **Large knowledge base about viral systems**
- **Vectors are well-engineered**
- **Relatively easy to generate and renew**
- **Infect a wide variety of cell types**
- **Potential for *in vivo* gene delivery**

Viruses to Vectors: Replication Incompetent Viruses

- Pathogenicity of viruses is disrupted by disabling ability to replicate in target cells
- Accomplished by deletion of genes providing necessary replication functions from the viral genome
- Introduction of these genes along with the defective vector into cells results in synthesis of vector genomes and packaging of the defective genomes into virus particles

DLS LABORATORY CONSULTANTS

Cis-Acting Elements

- This refers to elements that are present on the same piece of DNA or RNA that is being acted upon
  - Can not function for a separate piece of DNA/RNA
- They are generally nucleic acid sequences that function as recognition signals for proteins

Trans-Acting Elements

- **This refers to elements that are able to act upon a different piece of DNA or RNA than the one that they are present on**
- **These are generally proteins that can be encoded on one piece of nucleic acid; once expressed they act upon another piece of nucleic acid**

Viral Vector Elements

***trans*-acting elements**

- **Replication proteins**
  - **Polymerases**
  - **Proteases**
  - **Replicases**
- **Structural proteins**
  - **Capsid proteins**
  - **Envelope proteins**

***cis*-acting elements**

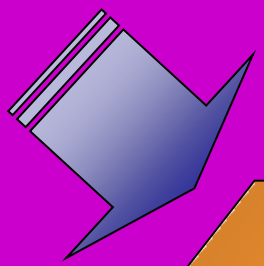
- **End repeats**
  - **ITR**
  - **LTR**
- **Packaging signals**
- **Regulatory sequences**

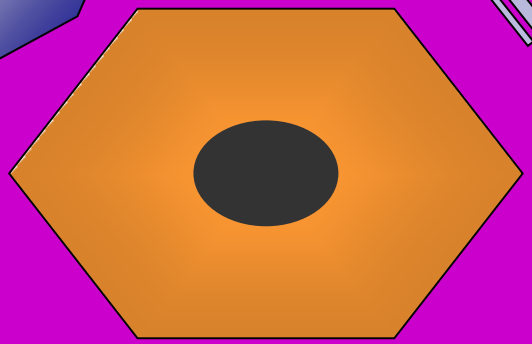
DLS LABORATORY CONSULTANTS

Making a (Generic) Viral Vector

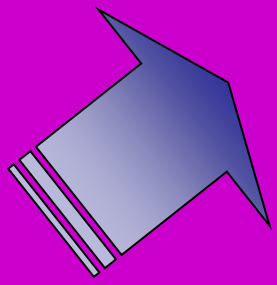
Defective vector genome with transgene expression cassette

Plasmids with viral replication/packaging genes





Host cell



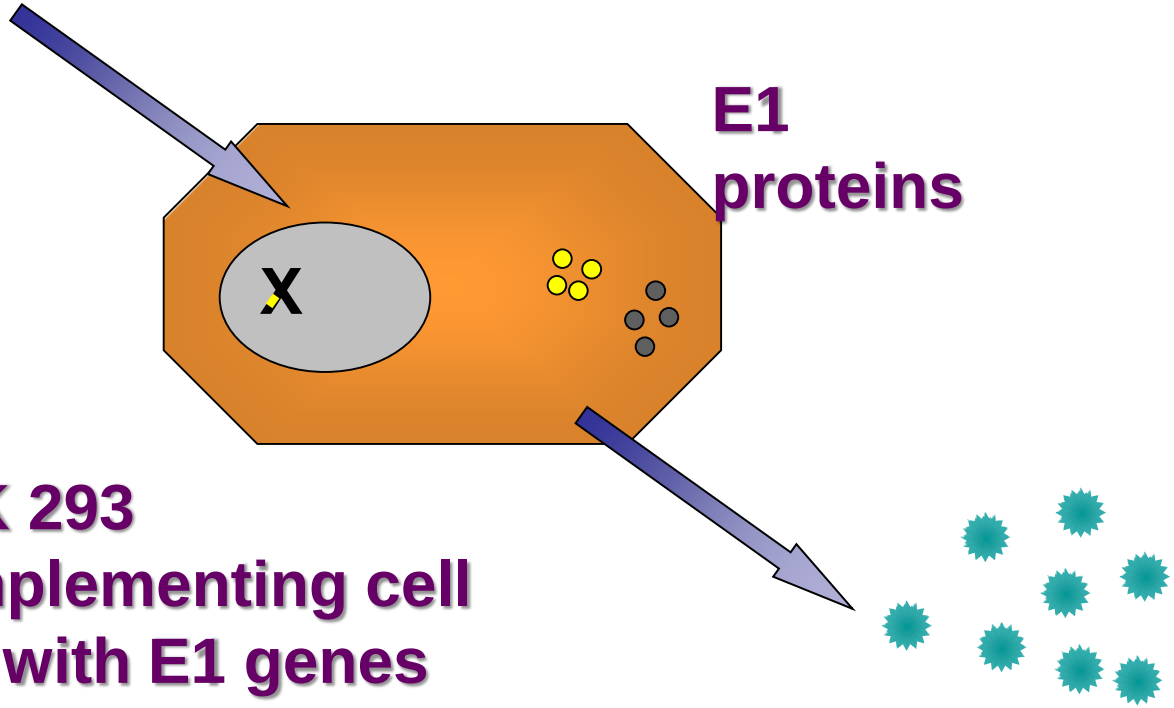
Viral vector particles

Complementing Cell Lines

- **Complementation: Providing *trans*-acting functions to rescue a nucleic acid that is missing, or mutant in, those functions**
- **Stably transfected cells expressing a gene product necessary for virus replication allow growth of viruses deleted for that gene**
- **Example:**
  - **HEK293 cells express Ad E1 and complement E1-deleted adenoviruses**

Using Complementing Cell Lines

Adenoviral vector with E1 region deletion



E1 proteins

HEK 293 complementing cell line with E1 genes incorporated in genome

Adenoviral vector particles

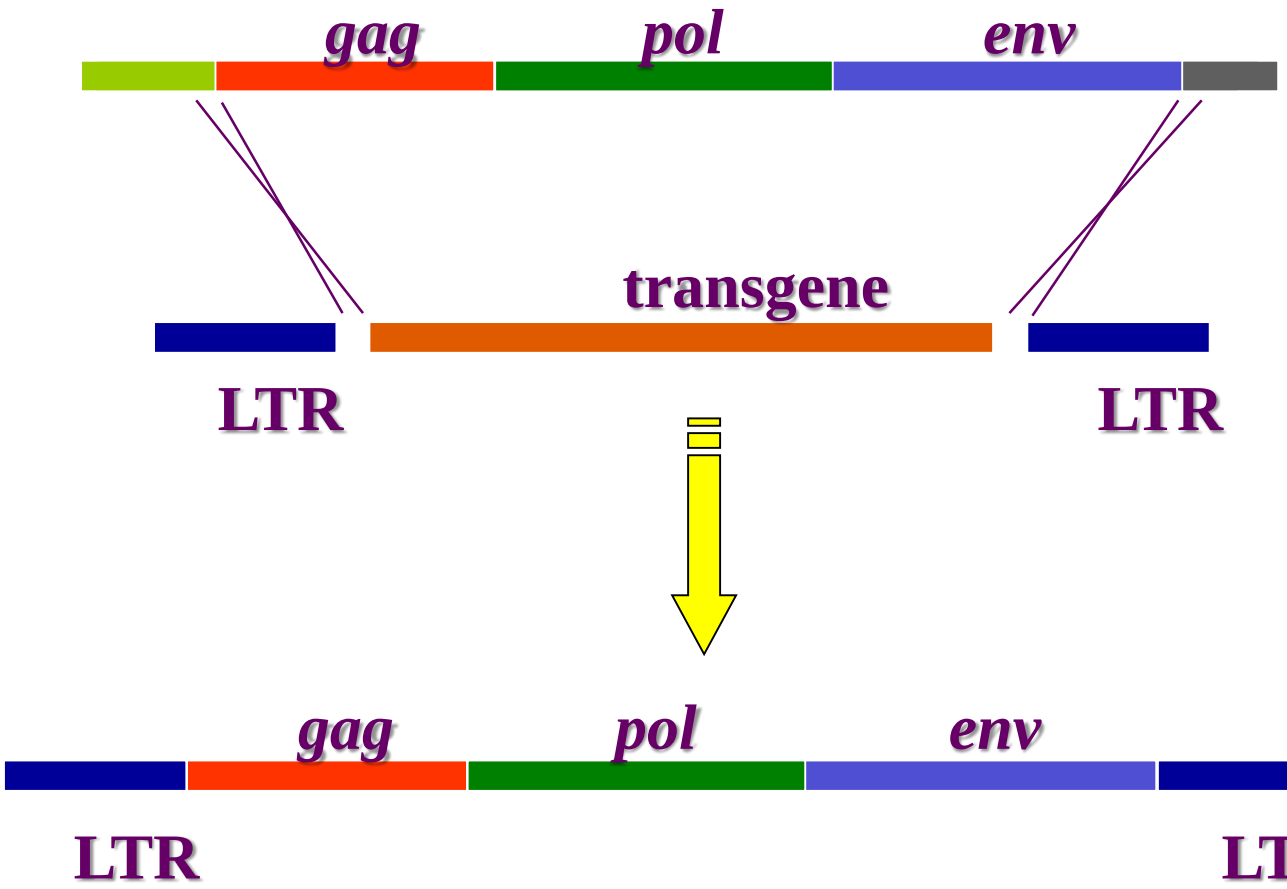
DLS LABORATORY CONSULTANTS

Reconstitution of Replication Competent Virus (RCV)

- During amplification and packaging of viral vectors the possibility exists to re-acquire genes necessary for growth
- Makes virus replication competent again
- Restores pathogenicity of virus

DLS LABORATORY CONSULTANTS

Reconstitution of RCV



Trans-acting genes

Retroviral vector genome

Replication competent retrovirus

Why is This a Problem?

- **Reconstitution is a rare event**
- **Contamination is generally at a low level**

However:

- **Potential for amplification in culture**
- **Pathogenicity *in vivo***
  - **Replication incompetent virus will only have localized effects**
  - **Replication competent virus can disseminate**

DLS LABORATORY CONSULTANTS

Engineering Safety Into Vectors

- **Most strategies to avoid RCV reconstitution are attempts to reduce recombination**
  - **Putting replication genes on different DNA constructs (split genomes)**
  - **Elimination of viral genes**
  - **Removal of viral regulatory regions**
  - **Production as transient single batch rather than continuous culture**
  - **Pseudotyping of virus**

Generations of Vectors

- **Engineering safety into viral vectors is iterative**
  - **New concerns arise through use of vector**
  - **Modifications are made to address the concern**
  - **May take more than one round**
  - **Referred to as 1st, 2nd, etc generation vectors**
- **Examples: Adenoviral vectors, lentiviral vectors**

Responsibilities Under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*

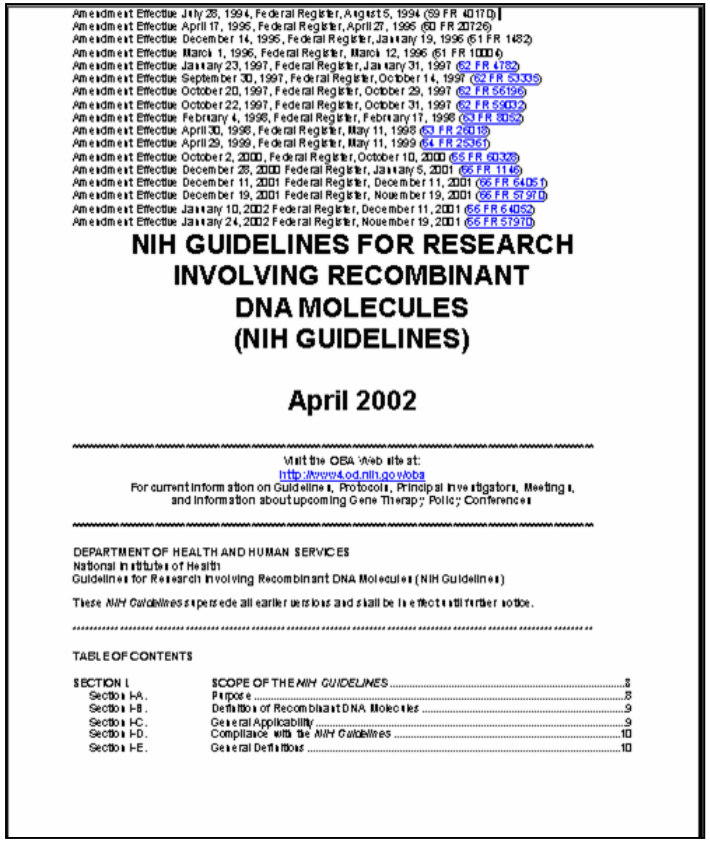



DLS LABORATORY CONSULTANTS

What are the NIH Guidelines?

- **The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) detail procedures and practices for the containment and safe conduct of various forms of recombinant DNA research, including research involving genetically modified plants and animals, and human gene transfer.**

NIH Guidelines for Research Involving Recombinant DNA Molecules



Amendment Effective July 28, 1994, Federal Register, August 5, 1994 (59 FR 40170)
Amendment Effective April 17, 1995, Federal Register, April 27, 1995 (60 FR 20726)
Amendment Effective December 14, 1995, Federal Register, January 19, 1996 (61 FR 1482)
Amendment Effective March 1, 1996, Federal Register, March 12, 1996 (61 FR 10004)
Amendment Effective January 23, 1997, Federal Register, January 31, 1997 (62 FR 4782)
Amendment Effective September 30, 1997, Federal Register, October 14, 1997 (62 FR 53335)
Amendment Effective October 20, 1997, Federal Register, October 29, 1997 (62 FR 56196)
Amendment Effective October 22, 1997, Federal Register, October 31, 1997 (62 FR 64091)
Amendment Effective February 4, 1998, Federal Register, February 17, 1998 (63 FR 8052)
Amendment Effective April 30, 1998, Federal Register, May 11, 1998 (63 FR 26018)
Amendment Effective April 29, 1999, Federal Register, May 11, 1999 (64 FR 25361)
Amendment Effective October 2, 2000, Federal Register, October 10, 2000 (65 FR 60328)
Amendment Effective December 28, 2000 Federal Register, January 5, 2001 (66 FR 1146)
Amendment Effective December 11, 2001 Federal Register, December 11, 2001 (66 FR 64051)
Amendment Effective December 19, 2001 Federal Register, November 19, 2001 (66 FR 57970)
Amendment Effective January 10, 2002 Federal Register, December 11, 2001 (66 FR 64052)
Amendment Effective January 24, 2002 Federal Register, November 19, 2001 (66 FR 57970)

NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES)

April 2002

Visit the OBA Web site at:
http://www4.od.nih.gov/oba
For current information on Guidelines, Protocols, Principal Investigators, Meetings, and information about upcoming Gene Therapy Policy Conferences

DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

These *NIH Guidelines* supersede all earlier versions and shall be in effect until further notice.

TABLE OF CONTENTS

A current version of the *NIH Guidelines* is available at:

http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

DLS LABORATORY CONSULTANTS

What type of research is covered by the *NIH Guidelines?*

- **The *NIH Guidelines* are applicable to all recombinant DNA research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH**
- **In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either:**
  - **(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or**
  - **(ii) molecules that result from the replication of those described in (i) above.**

DLS LABORATORY CONSULTANTS

Who must comply with the *NIH Guidelines?*

- **All institutions that receive National Institutes of Health (NIH) funding for recombinant DNA research must comply with the *NIH Guidelines*.**
- **Researchers at institutions that are subject to the *NIH Guidelines* must comply with the requirements even if their individual projects are not funded by NIH.**

DLS LABORATORY CONSULTANTS

Who must comply with the *NIH Guidelines?*

- **Even though they are called "guidelines," the *NIH Guidelines* are a term and condition of NIH funding for recombinant DNA research.**
- **If your institution is subject to the *NIH Guidelines* you must follow the requirements and adhere to the practices outlined in the document.**

DLS LABORATORY CONSULTANTS

Non compliance with the *NIH Guidelines*

Non compliance with the *Guidelines* may result in suspension or termination of NIH funds for recombinant DNA research, or the requirement to have all recombinant DNA projects at the institution receive prior NIH approval

Safety Considerations

Section II of the *NIH Guidelines* focuses on safety considerations for research with recombinant and synthetic nucleic acids.

Section II – Risk Groups

- **Appendix B of the *NIH Guidelines* lists biological agents known to infect humans as well as selected animal agents that have the potential to infect humans.**
- **Biological agents are assigned to one of four risk group based on the potential effect of the agent on a healthy human adult.**

Section II – Risk Groups

RG 1	RG 2	RG 3	RG 4
Agents that are not associated with disease in healthy adult humans	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

DLS LABORATORY CONSULTANTS

Containment

In proposing research, the PI must make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*.

The PI must also propose appropriate microbiological practices and laboratory techniques to be used for the research

Physical Containment

- **Four biosafety levels are described in Appendix G of the *NIH Guidelines*. These biosafety levels consist of a combination of lab practices and techniques, safety equipment, and lab facilities appropriate for the operations being performed.**
- **Biosafety level 4 provides the most stringent containment conditions, biosafety level 1 the least stringent.**

For more information see:

http://oba.od.nih.gov/oba/rac/guidelines_02/Appendix_G.htm

DLS LABORATORY CONSULTANTS

Physical Containment (continued)

APPENDIX P - PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING PLANTS

Appendix P of the *NIH Guidelines* specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals.

http://oba.od.nih.gov/oba/rac/guidelines_02/Appendix_P.htm

DLS LABORATORY CONSULTANTS

Physical Containment (continued)

APPENDIX Q - PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING ANIMALS

Appendix Q specifies containment and confinement practices for research involving whole animals, both transgenic animals and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals.

Appendix Q supersedes Appendix G when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. The animals covered in Appendix Q include but are not limited to cattle, swine, sheep, goats, horses, and poultry.

http://oba.od.nih.gov/oba/rac/guidelines_02/Appendix_Q.htm

DLS LABORATORY CONSULTANTS

Biological Containment

- **Biological containment is the application of highly specific biological barriers. Such barriers limit either the infectivity of a vector for specific hosts, or its dissemination and survival in the environment.**
- **Vectors can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the lab.**

For more information see:
http://oba.od.nih.gov/oba/rac/guidelines_02/Appendix_I.htm

DLS LABORATORY CONSULTANTS

What is an Institutional Biosafety Committee?

- **Institutional Biosafety Committees (IBCs) provide local review and oversight of nearly all forms of research utilizing recombinant DNA, they ensure that recombinant DNA research conducted at or sponsored by the institution is in compliance with the *NIH Guidelines*.**

Registration of Research with the IBC

- **A requirement of the *NIH Guidelines* is that an IBC must review and approve all research subject to the *NIH Guidelines*.**
- **Principal Investigator (PIs) are responsible for determining if their work is requires IBC review and approval because it falls under Section III-A, III-B, III-C, III-D or III-E of *the NIH Guidelines.***

Registration of Research with the IBC

- **PIs must submit a research proposal for IBC review and obtain IBC approval if the work is subject to Section III-A, III-B, III-C, III-D or III-E of the *NIH Guidelines*.**
- **IBC approval must be obtained <u>before</u> initiating research subject to Section III-A, III-B, III-C or III-D of the *NIH Guidelines*.**
- **PIs must determine the need for IBC review <u>before modifying</u> any recombinant DNA research already approved by the IBC.**

Section III - Levels of Review

- **Section III describes the levels of review necessary for certain types of recombinant DNA research.**
- **There are 6 categories of experiments under the *NIH Guidelines*. These categories reflect the risk of the research, with more stringent review required for the higher risk experiments.**
- **Experiments that are not considered to pose a risk to human health or the environment are exempt from the *NIH Guidelines* and do not require review.**

DLS LABORATORY CONSULTANTS

Summary of *NIH Guidelines* Levels of Review

Section of the *NIH Guidelines*	Level of review
III-A	**IBC, Recombinant DNA Advisory Committee (RAC) review, and NIH Director review and approval**
III-B	**IBC approval and NIH Office of Biotechnology Activities (OBA) review for containment determinations**
III-C	**IBC and Institutional Review Board (IRB) approval and RAC review before research participant enrollment**
III-D	**IBC approval before initiation**
III-E	**IBC notice at initiation**
III-F	**Exempt from the *NIH Guidelines*. IBC registration not required if experiment not covered by Sections III-A, III-B, or III-C**

Section III-A

- **Section III-A covers experiments that require IBC approval, RAC review and NIH Director approval before they can begin.**
  - **These types of experiments are known as "Major Actions" and involve the deliberate transfer of a drug resistance trait to microorganisms, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.**

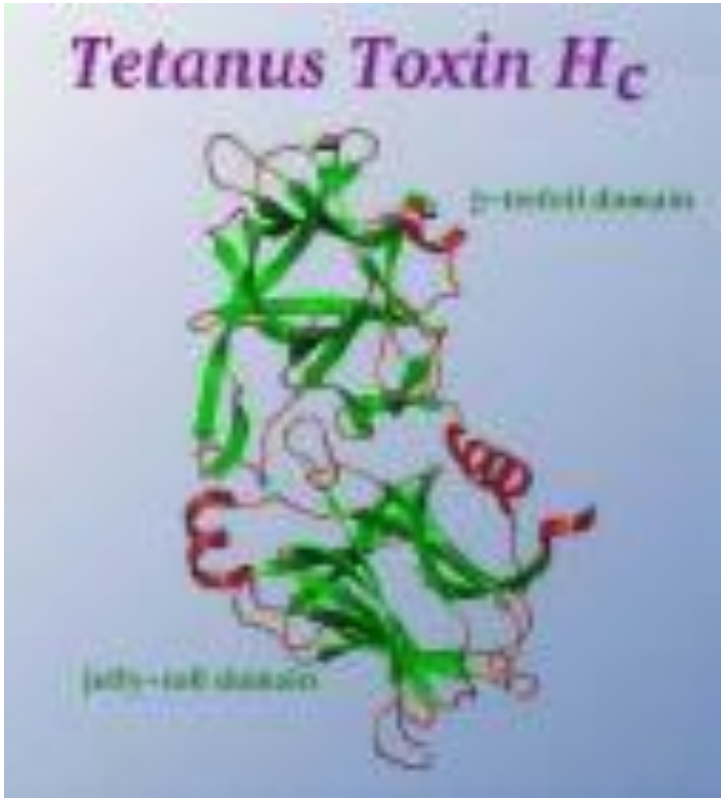
For more information on Major Actions see:

http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm#_Toc7261561

DLS LABORATORY CONSULTANTS

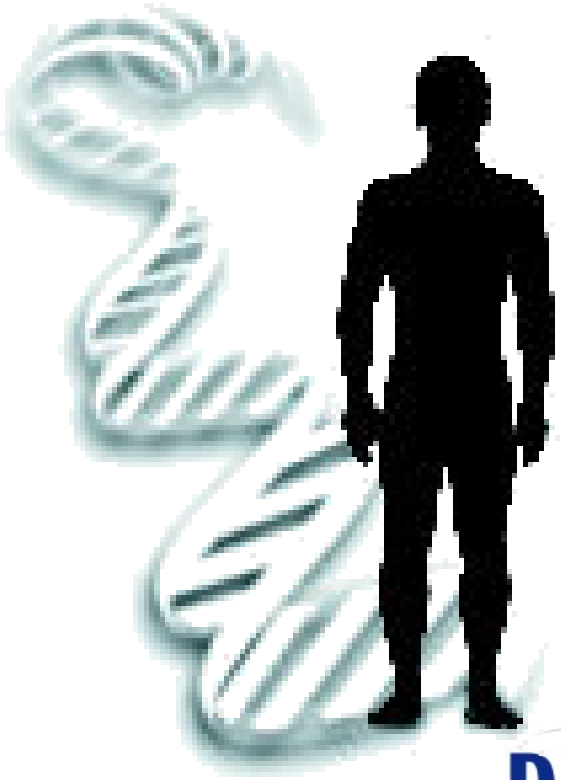
Section III-B

- **Section III-B covers experiments that require NIH/OBA review and IBC approval before initiation**
  - **Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight**



Section III-C

- **Section III-C experiments require RAC review, IBC approval and IRB approval <u>before</u> initiation**
  - **Deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants**



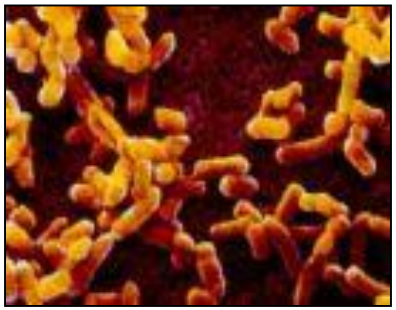
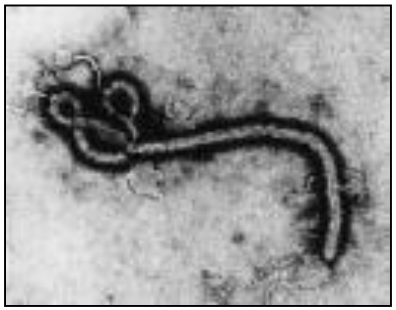
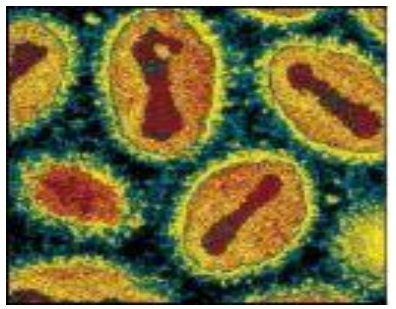
DLS LABORATORY CONSULTANTS

Section III-D

- **Section III-D covers experiments that require IBC approval <u>before</u> initiation**

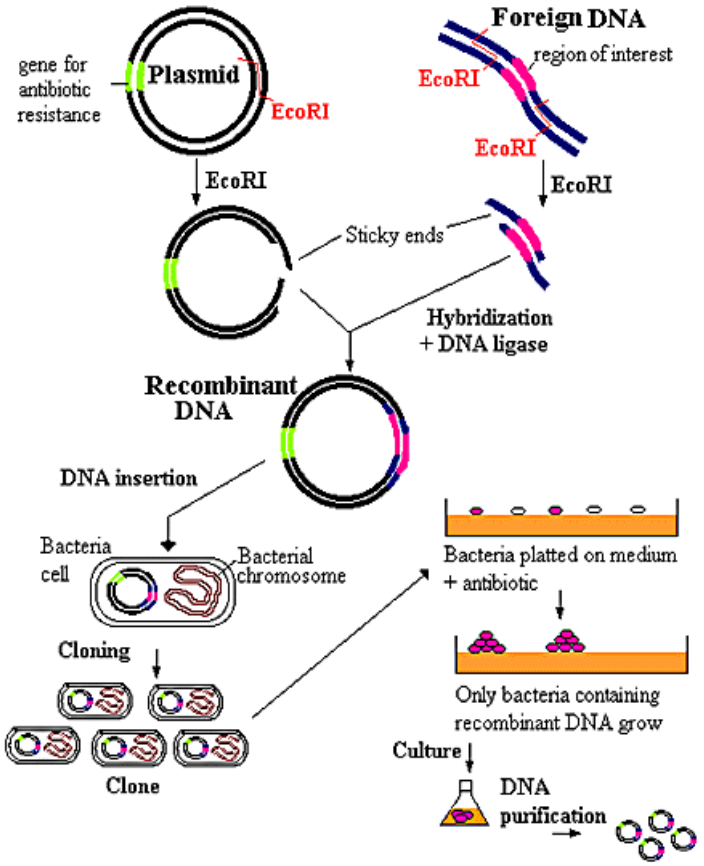
Section III-D-1

- **Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems**

Section III-D-2

- **Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems**



Cloning into a plasmid

Section III-D-3

- **Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.**

Section III-D-4

- **Experiments Involving Whole Animals**
  - **Includes experiments in which:**
    - **The animal's genome has been altered by stable introduction of recombinant DNA into germline (transgenic animals)**
    - **Viable recombinant DNA-modified microorganisms are tested on whole animals**
    - **BL2 or BL2-N or higher is required**

DLS LABORATORY CONSULTANTS

Section III-D-5

- **Experiments Involving Whole Plants**
  - **Includes experiments in which:**

- **Plants are genetically engineered by recombinant DNA methods**
- **Plants are used with recombinant DNA-modified insects**
- **Generally BL2-P through BL4-P, depending on risk.**

Section III-D-6

- **Experiments involving more than 10L of culture**

See Appendix K of the *NIH Guidelines*

http://oba.od.nih.gov/oba/rac/guidelines_02/Appendix_K.htm

Section III-E

- **Section III-E describes a class of experiments which require registration with the IBC at the time of initiation.**
- **All experiments not included in III-A through III-D or III-F fall under III-E.**
- **The IBC still reviews and approves these experiments but this review does not need to occur before the experiment commences.**

Section III-E (continued)

- **III-E-1: Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus.**

- **Such molecules may be propagated and maintained in tissue culture using BL1 containment. For such experiments it must be demonstrated that the cells lack helper virus for the specific Families of the defective viruses being used.**

Section III-E (continued)

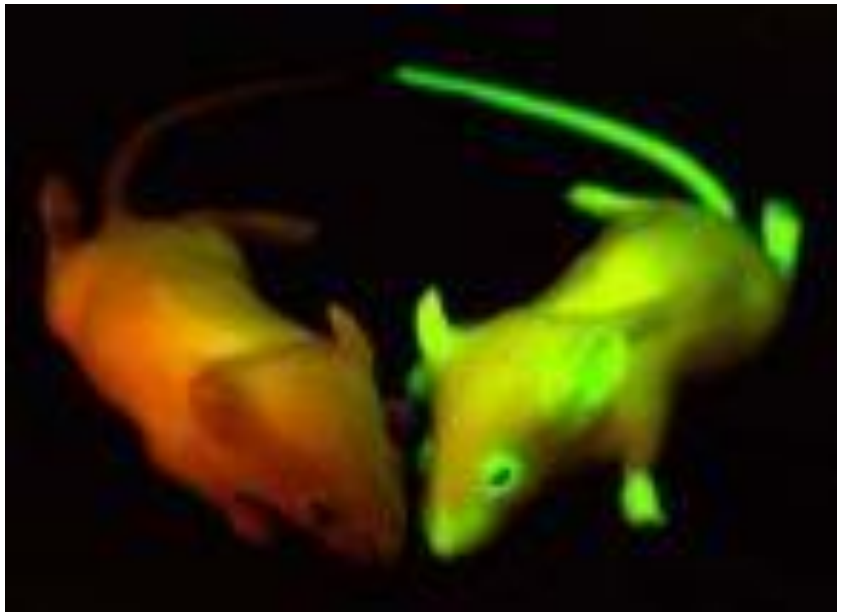
- **III-E-2: Covers experiments involving whole plants, and/or experiments involving recombinant DNA-modified organisms associated with plants, except those that fall under Section III-A, III-B, III-D or III-F.**

For further details see

http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm#_Toc72661575

Section III-E (continued)

- **Section III-E-3 covers experiments involving the generation of transgenic rodents**
  - **Rodent's genome has been altered by stable introduction of recombinant DNA into germline**
  - **BL1 containment is appropriate**



Section III-F

- **Section III-F describes experiments that are exempt from the *NIH Guidelines*. Registration with the IBC is not required (unless required by institutional policy)**

Section III-F (continued)

- **The following recombinant DNA molecules are exempt from the *NIH Guidelines*:**
  - **III-F-1 – Those that are not in organisms or viruses.**
  - **III-F-2 – Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.**

Section III-F (continued)

- **Exempt from the *NIH Guidelines*:**
  - **III-F-3 – Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host, or when transferred to another host by well established physiological means.**

Section III-F (continued)

- **Exempt from the *NIH Guidelines*:**
  - **III-F-4 – Those that consist entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).**
  - **III-F-5 – Those that consist entirely of DNA segments from different species that exchange DNA known physiological processes.**

DLS LABORATORY CONSULTANTS

Section III-F (continued)

- **Exempt from the *NIH Guidelines*:**
  - **III-F-6 – Those that do not present a significant risk to health or the environment, as determined by the NIH director, with the advice of the RAC, and following appropriate notice and opportunity for public comment – See Appendix C of the *NIH Guidelines* for other classes of experiments which are exempt from the *NIH Guidelines*.**

For further details see:

http://oba.od.nih.gov/oba/rac/guidelines_02/APPENDIX_C.htm

DLS LABORATORY CONSULTANTS

Appendix C-I

- **Recombinant DNA in Tissue Culture**
  - **Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome that are propagated and maintained in cells in tissue culture are exempt.**

Appendix C-II

- **Experiments which use *Escherichia coli* K-12 host-vector systems are exempt from the *NIH Guidelines* provided that:**
  - **the *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or**
  - **lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids**

Appendix C-III

- **Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems are exempt from the *NIH Guidelines*.**

Appendix C-IV

- **Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than 10^{-7} may be used for cloning DNA and is exempt from the *NIH Guidelines***

Appendix C-V

- **Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed, propagated and maintained in organisms listed are exempt from the *NIH Guidelines*.**

- **For list see**
  http://oba.od.nih.gov/oba/rac/guidelines_02/APPENDIX_C.htm

DLS LABORATORY CONSULTANTS

Appendix C-VI

- **The purchase or transfer of rodents for experiments that require BL-1 containment**
  - **Note: Further manipulations of these animals are not necessarily exempt from the *NIH Guidelines***

Section IV

- **Section IV of the *NIH Guidelines* outlines the roles and responsibilities of the:**
  - **Institution**
  - **Institutional Biosafety Committee (IBC)**
  - **Biological Safety Officer (BSO)**
  - **Principal Investigator (PI)**
  - **NIH**

Institutional Responsibilities under the *NIH Guidelines*

- **The Institution shall:**
  - **Establish and implement policies for the safe conduct of recombinant DNA research**
  - **Establish an Institutional Biosafety Committee**
  - **Assist and ensure compliance with the *NIH Guidelines* by investigators**
  - **Ensure appropriate training for IBC members and staff, PIs, laboratory staff**

DLS LABORATORY CONSULTANTS

Institutional Responsibilities under the *NIH Guidelines* (continued)

- **The Institution shall:**
  - **Determine necessity for health surveillance of personnel involved in recombinant DNA research**
  - **Report any significant accidents, incidents or violations of the *NIH Guidelines* to OBA within 30 days**

PI Responsibilities under the *NIH Guidelines*

PIs are responsible for full compliance with the *NIH Guidelines* during the conduct of recombinant DNA research.

PI Responsibilities under the *NIH Guidelines* (continued)

- **Investigators should:**
  - **Be adequately trained in good microbiological techniques**
  - **Provide laboratory research staff with protocols describing potential biohazards and necessary precautions**
  - **Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety and (ii) the procedures for dealing with accidents**

PI Responsibilities under the *NIH Guidelines* (continued)

- **Investigators should:**
  - **Inform the laboratory staff of the reasons and provisions for any precautionary medical practices (e.g. vaccinations)**
  - **Supervise laboratory staff to ensure that required safety practices and techniques are employed**
  - **Correct work errors and conditions that may result in the release of recombinant DNA material**

PI Responsibilities under the *NIH Guidelines* (continued)

- **Investigators should:**
  - **Comply with permit and shipping requirements for recombinant DNA molecules**

PI Responsibilities When Conducting Human Gene Transfer Research

- **PIs conducting research subject to section III-C of the *NIH Guidelines* (Human Gene Transfer) have a number of additional responsibilities that are outlined in Appendix M of the *NIH Guidelines:***
  - **Ensure all aspects of Appendix M have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OBA for review by the RAC**

DLS LABORATORY CONSULTANTS

PI Responsibilities When Conducting Human Gene Transfer Research (cont'd)

- Provide a letter signed by the PI(s) on institutional letterhead acknowledging the documentation being submitted to NIH OBA complies with the requirements set forth in Appendix M
- Not enroll research participants in a human gene transfer experiment until the RAC review process is completed; IBC approval has been obtained; IRB approval has been obtained; and all applicable regulatory authorizations have been obtained
- Comply with reporting requirements for human gene transfer experiments.

DLS LABORATORY CONSULTANTS

PI Responsibilities for Incident Reporting

PIs must report any significant problems pertaining to the operation of implementation of containment practices and procedures, violations of the NIH Guidelines, or any significant research related accidents and illnesses to the IBC, NIH OBA, and, as applicable, the Biological Safety Officer, Greenhouse or Animal Facilities Director, and other appropriate authorities.

DLS LABORATORY CONSULTANTS

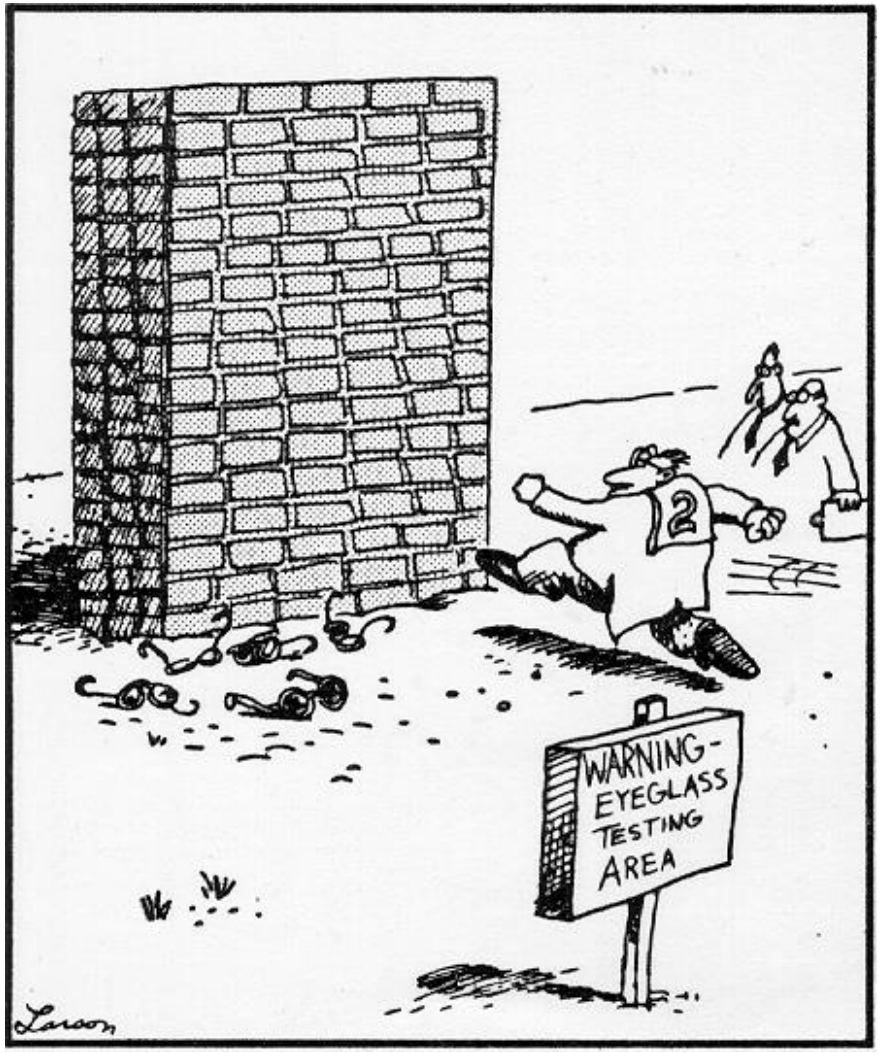
More Information

- **Further information about requirements under the *NIH Guidelines* can be obtained from the NIH Office of Biotechnology Activities**

http://oba.od.nih.gov

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

DLS LABORATORY CONSULTANTS

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**

DLS LABORATORY CONSULTANTS

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**

DLS LABORATORY CONSULTANTS

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

DLS LABORATORY CONSULTANTS

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

DLS LABORATORY CONSULTANTS

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**

DLS LABORATORY CONSULTANTS

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

DLS LABORATORY CONSULTANTS

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**

DLS LABORATORY CONSULTANTS

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

DLS LABORATORY CONSULTANTS

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?

DLS LABORATORY CONSULTANTS

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**

DLS LABORATORY CONSULTANTS

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**

DLS LABORATORY CONSULTANTS

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

Risk Assessment

Case Studies

Case #1

An investigator proposes to transduce human tumor samples with a lentiviral vector expressing GFP.

The lentiviral vector is an HIV-derived third generation vector that has been pseudotyped with VSV-G.

The transduced tumor cells will be injected into partially immune-compromised mice and tumor growth will be monitored by *in vivo* imaging.

Case #1

1. Under which section(s) of the *NIH Guidelines* does this work fall?

Transducing the tumor cells falls under Section III-D-3-b or III-E if no replication competent lentivirus can be confirmed.

Injecting the tumor cells into mice would fall under Section III-D-4-a ONLY IF using less than 2/3 of the viral genome AND it can be demonstrated that that no replication competent lentivirus is produced. Otherwise Section III-D-4-b

Case #1

2. Under what Biosafety Level should the animals be housed?

III-D-4-a = BSL1 at a minimum

III-D-4-b = Containment set by IBC (probably BSL2 at a minimum in this case)

NOTE: See lentiviral vector guidance issued by OBA.

Case #2

An investigator proposes to target a tumor suppressor that negatively regulates growth in cells by way of knock-down expression using RNAi.

Her first step will be to identify an siRNA sequence by developing a panel of synthetic RNA's and testing them in tissue culture.

Once she has identified a sequence, the PI will put it in a shRNA expression vector and move to lentiviral vector for animal delivery.

DLS LABORATORY CONSULTANTS

Case #2

1. What part, if any, of her research falls under the *NIH Guidelines*?

Developing the panel of synthetic RNA's is exempt.

Would testing the synthetic RNA's in tissue culture fall under Section III-D-3?

Introducing shRNA expression vector and moving to lentiviral vector for animal delivery falls under Section III-D-4.

DLS LABORATORY CONSULTANTS

Case #2

2. What special considerations should be given to the shRNA lentiviral vector use in animals?

The RNAi target negatively regulates growth in cells by way of knock-down expression.

While the lentiviral vector is replication incompetent, the RNAi will potentially transduce the cell disrupting growth regulation causing uncontrolled growth.

DLS LABORATORY CONSULTANTS

Case #3

An investigator proposes to conduct gene transfer in *Ruta graveolens* (a medicinal plant) using *Agrobacterium tumefaciens* in tissue culture.

The culture is manipulated via hormones to generate whole plants with genetic markers to study the furocoumarins biosythetic pathway.

Case #3

1. Under which section(s) of the *NIH Guidelines* does this work fall?

Since the experiment does not involve cloned genomes of exotic infectious agents or biosynthesis of toxin molecules . (i.e. Section III-D-5), it falls under Section III-E-2-a.

These experiments can be done using BL1-P containment.

Case #3

The whole plants are moved from the growth chambers and into the greenhouse.

2. What issues should be addressed?

The researcher should harvest before flowering or bag the flowers to prevent environmental release.

Case #4

An investigator proposes to use a series of mutants of *Yersinia pestis*.

Her research will involve introducing orthologs of *Y. pestis* genes from other organisms to analyze function and cross-complementation of those genes.

These genes will be introduced into *Y. pestis* using standard expression vectors that contain ampicillin and kanamycin resistance genes.

DLS LABORATORY CONSULTANTS

Case #4

1. **Under which section(s) of the *NIH Guidelines* does this work fall?**

Introducing the antibiotic-resistant genes into *Y. pestis* falls under Section III-D-1-b.

Case #4

2. What containment level should be used for these experiments?

Since the organism remains susceptible to the antibiotic treatment of choice, the experiments can be conducted at BL-3.

3. What other issues should be considered?

Y. pestis is a Select Agent under 42 CFR 73 and requires CDC registration.

Reducing Risk

- **The usual**
  - **Containment (engineering controls)**
  - **Work practices**
  - **PPE**
- **Lower risk agent**
  - **Is that vector necessary, or convenient?**
  - **Latest generation vectors**
- **Can the experiment be changed to be done more safely, yet still answer the question?**

DLS LABORATORY CONSULTANTS

What Should I Ask About?

- **Do you have the information you need?**
  - **Scale of work**
  - **Replication competent virus testing**
  - **Location of work**
  - **Knowledge/training/experience of personnel**
  - ***Details*** **of vector system (not 'adenovirus vector')**
  - ***Details*** **of the gene product and its action**
  - ***Details*** **of the work to be done**

DLS LABORATORY CONSULTANTS

Beware of Assumptions

The investigators at my institution are using:

A. The most advanced and safety-engineered generation of viral vector available

B. Something the post-doc down the hall gave me

C. The one that Invitrogen sells

D. What we've always used

E. All of the above

Thank You!

UNIVERSITY of WASHINGTON

Questions?