

NSABB: Synthetic Viruses

Risks and Benefits

Objectives

- Virus Biothreat Lists
- Virus Classification
 - Baltimore Scheme
 - ◆ Virus Reverse Genetic Strategies
- Reverse Genetics and Synthetic Genomics
- Technical Barriers to Synthetic Genome Reconstruction
- Chimeras and Synthetic Viruses
- Summary

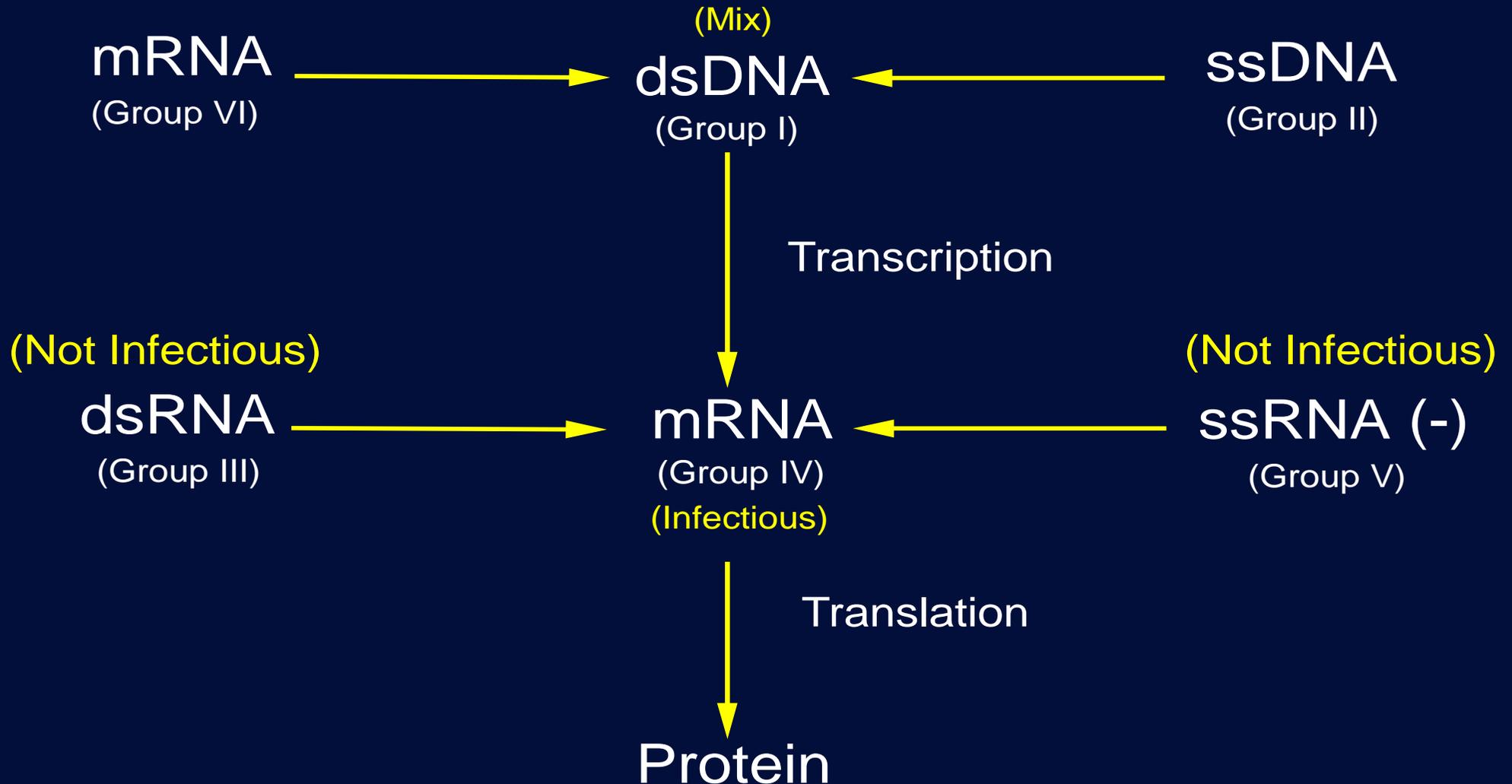
Goal: Provide a theoretical framework to initiate a broad discussion regarding the relative risks and benefits of synthetic genome technology

Biothreat Viruses

HHS/CDC, USDA, Dept Commerce, NIH Category A-C
(Lists of Biothreat Viruses)

- Very Heterogeneous group of viruses
 - HHS/CDC, USDA, Dept Commerce (Lists of Biothreat Viruses)
- Different genome organizations + replication strategies
 - different approaches are needed to develop infectious genomes
 - Genomes
 - ◆ dsDNA, ssRNA (+) polarity, ssRNA (-) polarity and dsRNA
- Simple classification scheme to understand virus reverse genetic strategies
 - All viruses must transcribe genome into mRNA \longrightarrow viral proteins.

Baltimore Classification Scheme



Genome Infectious?: Ability to induce mRNA expression and recover virus after injection of the genome into a cell

Figure 1. Baltimore Classification Scheme.

Baltimore Classification Scheme

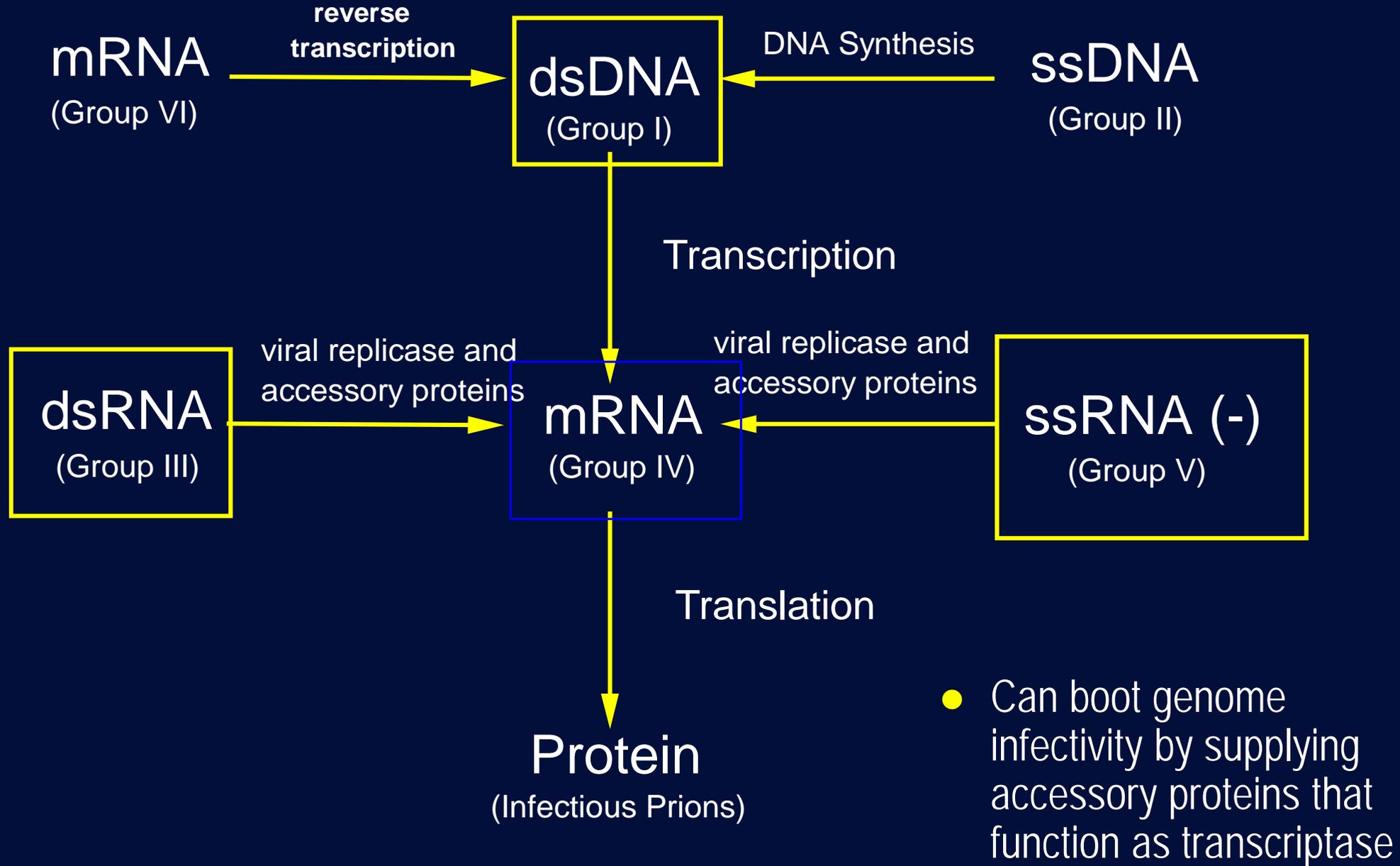


Figure 1. Baltimore Classification Scheme.

Virus Reverse Genetics

(Producing infectious virus from recombinant or synthetic DNA genomes)

- Group I (dsDNA Viruses)-Yes
 - Herpesviruses (e.g., HSV, HSV8, VZV)
 - Poxviruses (vaccinia virus)
 - ◆ Genome Size ~190 KB
 - ◆ Ends for covalently cloned hairpin loops
 - ◆ Genome is not infectious
 - Requires additional viral products to boot infectivity
- Group III (dsRNA Viruses)-No

Group IV (Positive Polarity ssRNA Viruses)-Yes

Picornavirus (FMDV, Swine Vesicular Disease Virus), Poliovirus (plum pox), Alphavirus (VEE, EEE)
Flavivirus (Central European TB, Far East TB encephallitis virus, others), Coronavirus (Yes)

Noroviruses-No

Group V (Negative Polarity ss RNA Viruses)-Yes

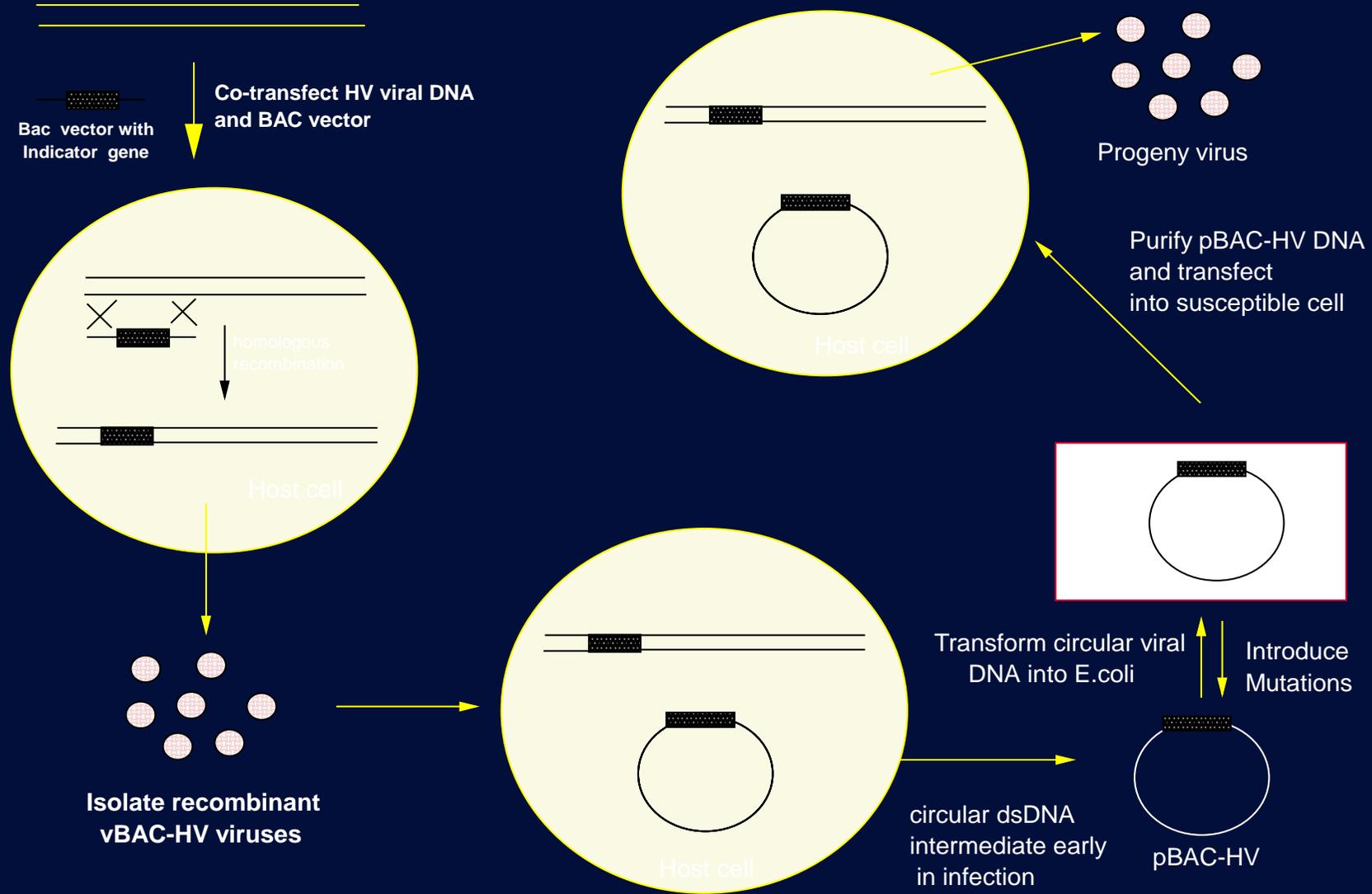
Myxoviruses (1918 Flu, H5N1), Paramyxoviruses (yes), Bunyaviruses (Yes-Rift Valley Fever),
Arenaviruses (yes-envelope exchange), Filoviruses (e.g., Ebola, Marburg), Rhabdoviruses (yes)

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Herpesvirus Molecular Clone Category I



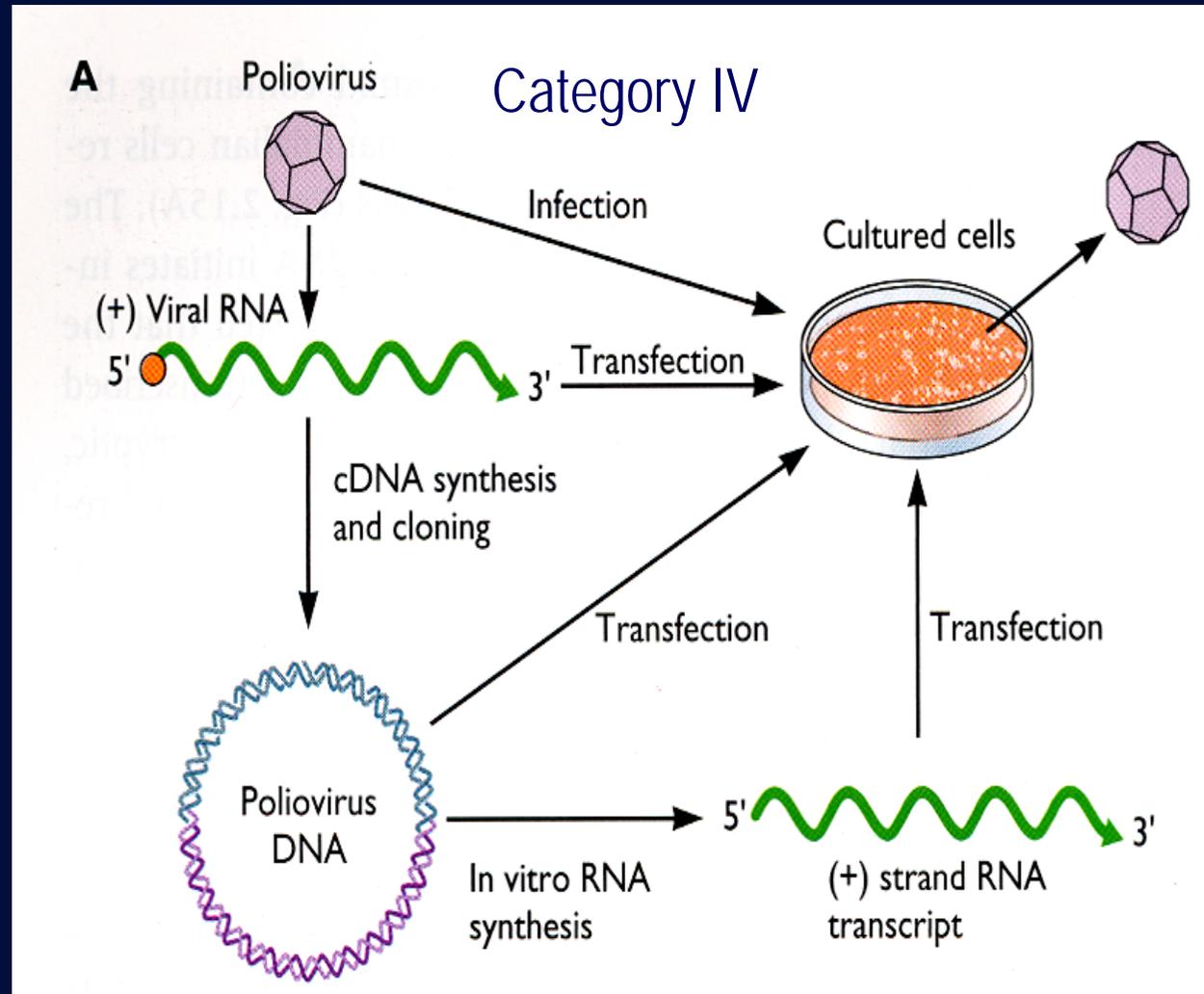
Virus Reverse Genetics Category IV

- Positive Strand RNA Viruses

- Picornaviruses
- Enteroviruses (e.g., PV, FMDV, HAV)
- Coronaviruses (e.g., SARS-CoV)
- Alphaviruses (e.g., VEE, WEE, EEE)
- Flaviviruses (e.g., Yellow fever, dengue, etc.)
- Noroviruses (not yet)

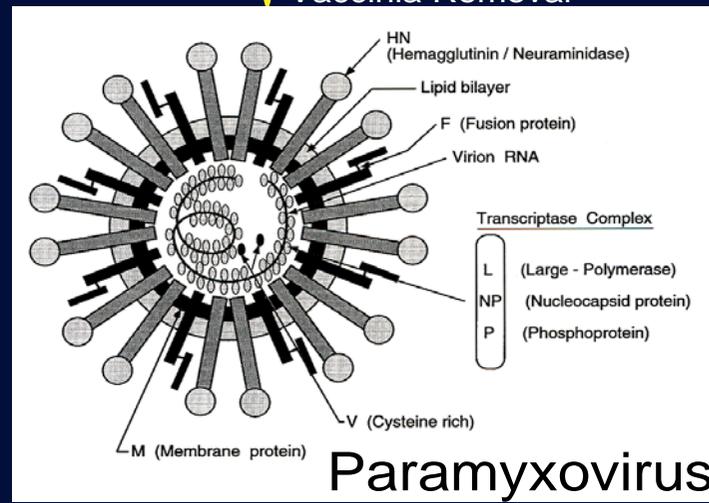
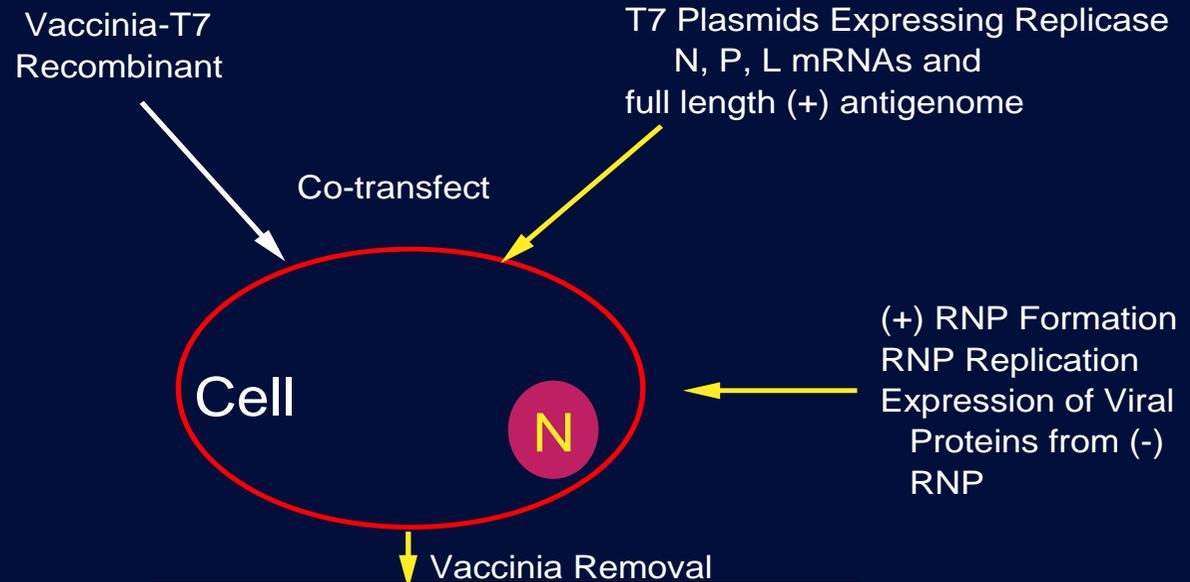
- Manipulate DNA and recover altered viruses

- Sequences readily available



Virus Reverse Genetics Category V

- Negative Strand RNA Viruses
- More complex (linear/**segmented**)
 - Paramyxoviruses (NDV, Hendra)
 - Rule of 6/size?
 - Filoviruses (e.g., Ebola, Marburg)
 - 19 Kb in length/stability
 - Rhabdoviruses (e.g., rabies)
 - Arenaviruses (LCM)
 - Bunyaviruses (LaCross Virus, Rift Valley Fever Virus)
 - Influenza Virus (e.g., 1918 Flu)



Other Methods of Virus Recovery

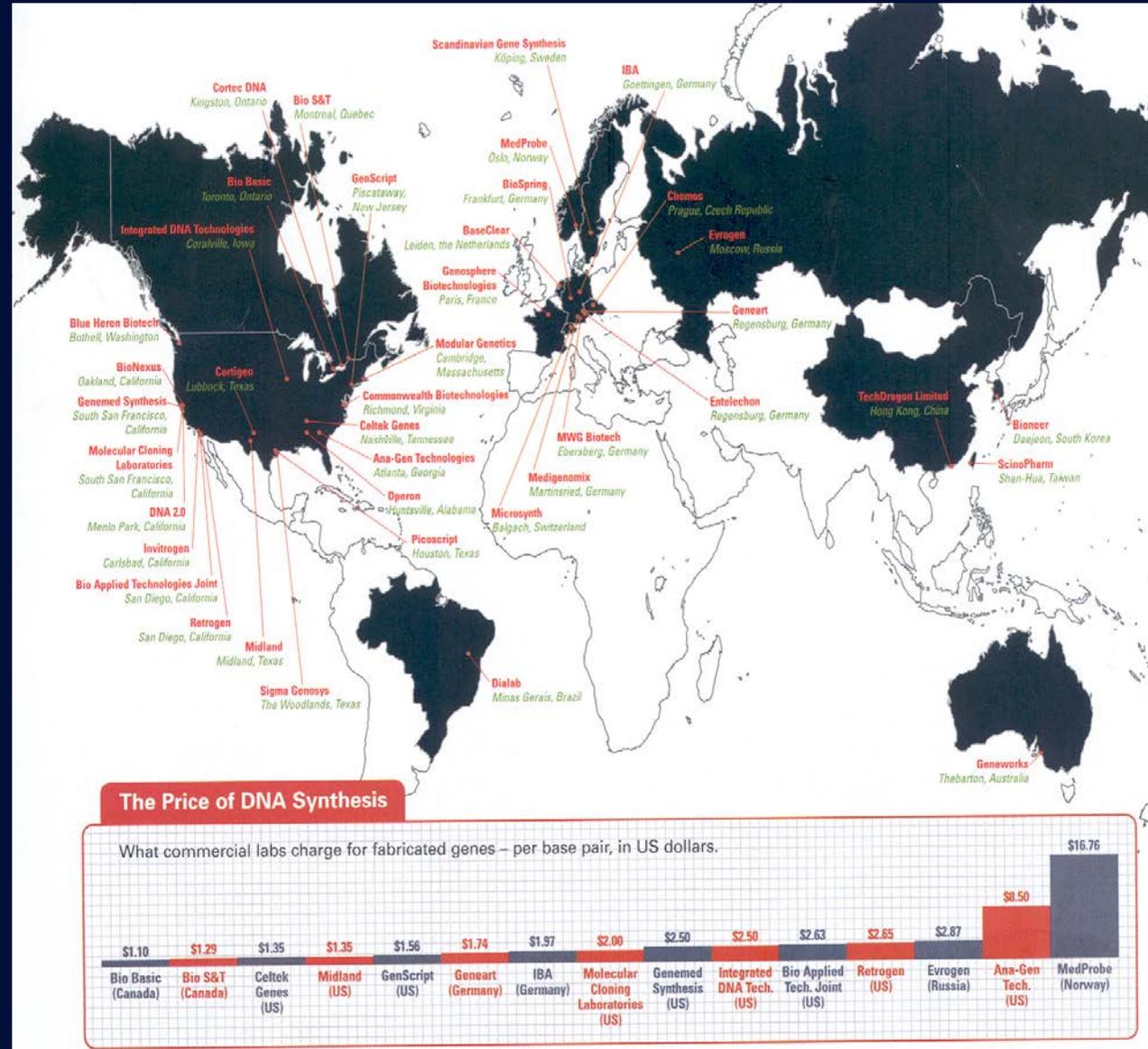
- Genome Infectious-Yes
 - dsDNA-Herpesviruses
 - ◆ Full length (>70%) HBV and poxvirus genomes are select agents
 - Positive polarity ssRNA viruses
 - ◆ DNA or RNA launch
 - Full length genomes of HHS/CDC (+) RNA viruses are select agents
- Genome Noninfectious
 - Boot Genome Infectivity
 - ◆ Strategies established for poxviruses
 - ◆ Strategies established for the negative polarity ssRNA viruses
 - Efficiency is lower

Synthetic Genomes, Molecular Clones and Reverse Genetics

- Synthetic DNA Applications.
 - Synthetic Genes Introduced into Molecular Clones
 - Full Length Genomes
 - Chimeric Viruses (Blends of genes from different viruses)
 - ◆ Designer Vaccines
 - ◆ Designer Pathogens
- Classic Recombinant DNA Approaches and Molecular Clones allow for Similar Constructs
 - Speed and Mutagenesis Capacity is Different

Infectious Genomes

- Constructed either Using Recombinant DNA Approaches or Synthetic Biology
 - ~50+ companies (de novo synthesis or PCA)
 - Synthetic DNAs (5-10 Kb)
- Infectious genomes can be synthesized for most viruses
 - Infectivity?
- Barriers



Barriers to Acquire Biodefense Pathogens

- Virus Availability:
 - Nature, Laboratory (Almost all available);
 - ◆ not necessarily easy (VEE-enzootic vs epidemic variants)
 - ◆ Cell culture attenuation
 - Extinct in wild (e.g., 1918 H1N1, H2N2, Smallpox, 2002-03 Epidemic SARS-CoV?, PV?)
 - Genome length sequences reported for most biodefense viruses

Accurate Sequence

Sequence Reported-doesn't make it infectious

Error rate Genbank: (1-500-1-10,000 bases)

Mistakes (1) in sequence can be lethal or attenuate pathogenesis

Smallpox (~190Kb), 1-10,000 error rate--20 mistakes--14 codon change

2.4×10^{19} possibilities to get correct genome (10^7 transfected cells make virus): (>7 mistakes/mutant pools fail)

Two full length sequences reported that differ in size by 525 bps, and contain ~1500 differences in sequence
(Both sequences right? Both sequences infectious?)

Size: Most synthetic DNA companies good for 1 to a few Kb in length

(PCA larger--more mistakes that must be fixed)

Virus genomes >10Kb become progressively harder to synthesize infectious genomes

Expertise

Smaller genome, easier to accomplish

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Steps

- Pick a Pathogen
- Obtain the sequence
 - Size considerations (larger is harder, +RNA easier than -RNA)
- Sequence validation
 - Infectious sequence reported (very helpful)
 - ◆ Is it pathogenic in animal models?
 - Phylogenetic Comparisons (bigger/better)
 - Choose (guess) a Candidate Sequence

Synthesize the Sequence

DNA vs RNA launch (DNA launch easier-problems-yes)

Accessory Factors to Boot Genome Infectivity?

Covert Operations?

One company/multiple companies: US vs global

Sequence Variation (~30-40%)-hide tracks/increase homology to benign strains

Gene fragments vs full length genomes (get around select agent DNA rules)

Designer pathogens (blend in virulence genes)

Assemble the Full Length Clone (<10 Kb)

Size Considerations: technical expertise

Recovery of Recombinant or Synthetic Virus from Cell Culture

Cell culture facilities, transfection techniques, trained staff, staff protection

FMDV/poliovirus, alpha/ flaviviruses very easy; purchase full length cDNAs with DNA launch capabilities

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Coronavirus Infectious Clone (30Kb)

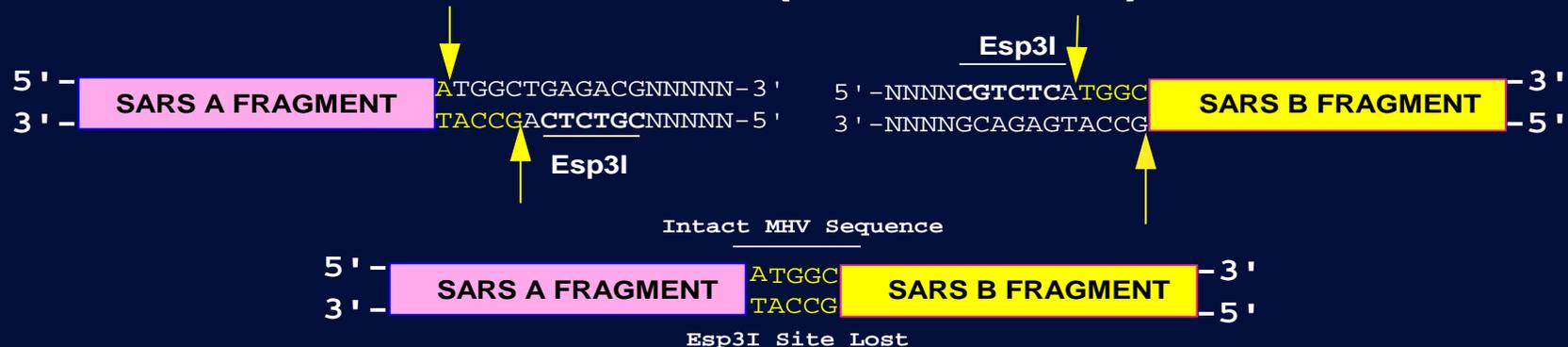
- Large Size of the Viral Genome
- Stable Cloning Vectors
- Regions of Chromosomal Toxicity
- Synthesizing Infectious Transcripts/Booting genome
- Ease of Manipulation
 - the availability of rare cutting restriction sites for reverse genetic applications
- Solutions: Systematic assembly from component clones

Class IIS Restriction Endonucleases (BsmB1/Esp3I)

Traditional



Seamless Junctions (No See'm)



Approach leaves no genetic signatures and allows assembly of DNAs greater than 1 million base pairs

Purify plasmid DNA containing SARS CoV fragments



Recombinant DNA
Guidelines (2/3rd
genome length)
Circumvented

Digest with Bgl2/Esp3I restriction endonuclease and purify



Ligate fragments



Finite source of non-replicating full length cDNA that is consumed in the reaction

Set of Contiguous ~5 Kb pieces

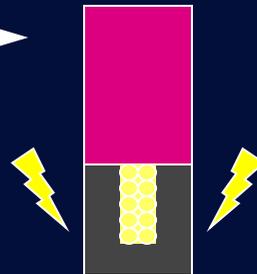
Transcribe genome length RNA



Boot Infectivity

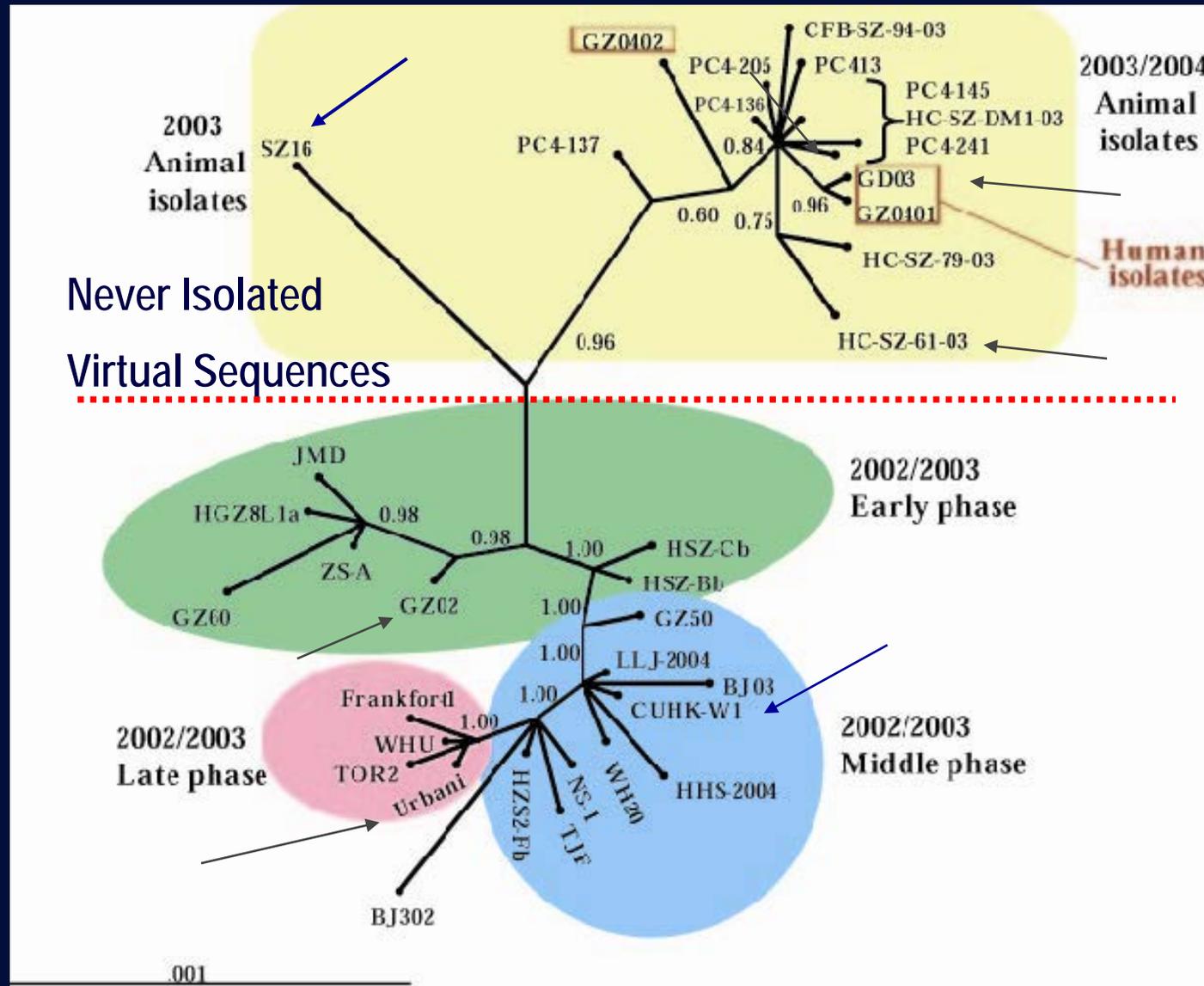
N transcripts (N protein)
“boots” infectivity by 10-
>1000 fold (enhances
transcription)

Transfect Vero E6 cells



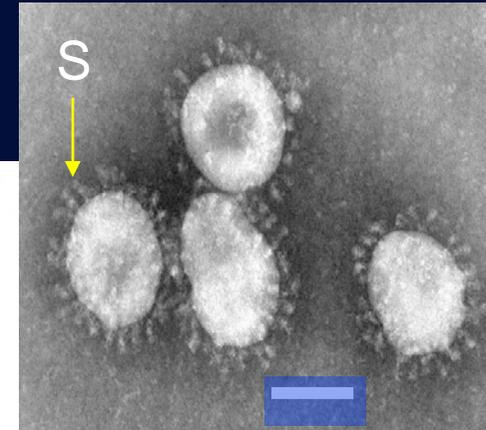
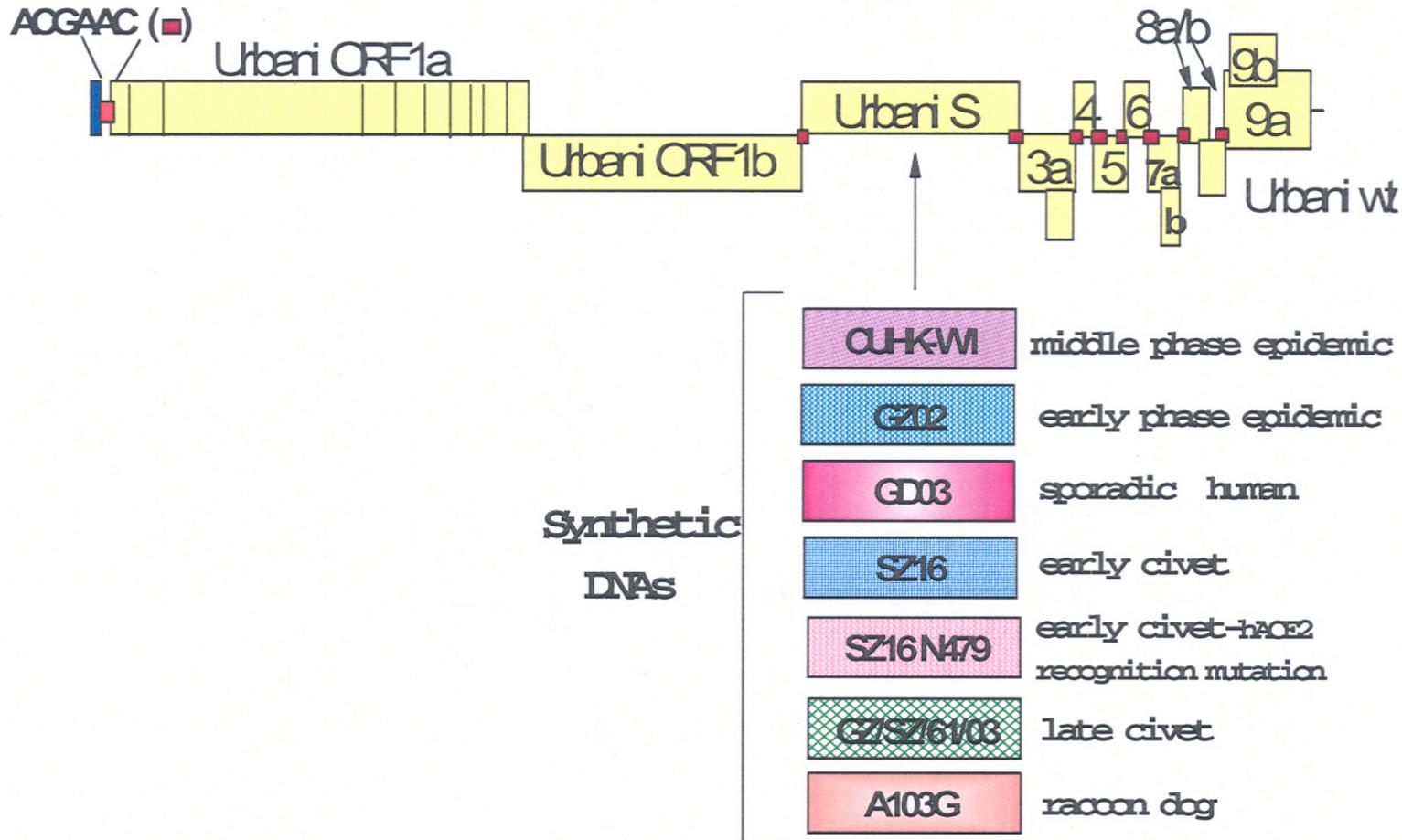
Virus recovered which replicated to wildtype levels

Molecular Resurrection of Early SARS-CoV Isolates from Sporadic Human Cases and Animals



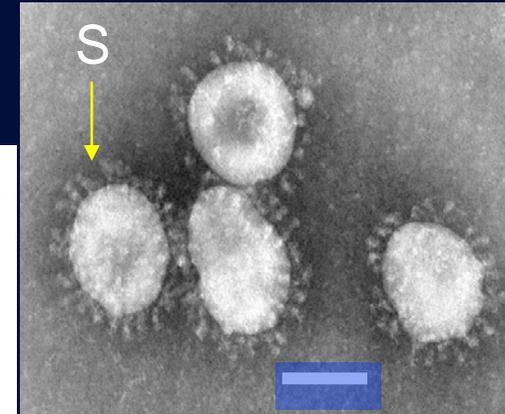
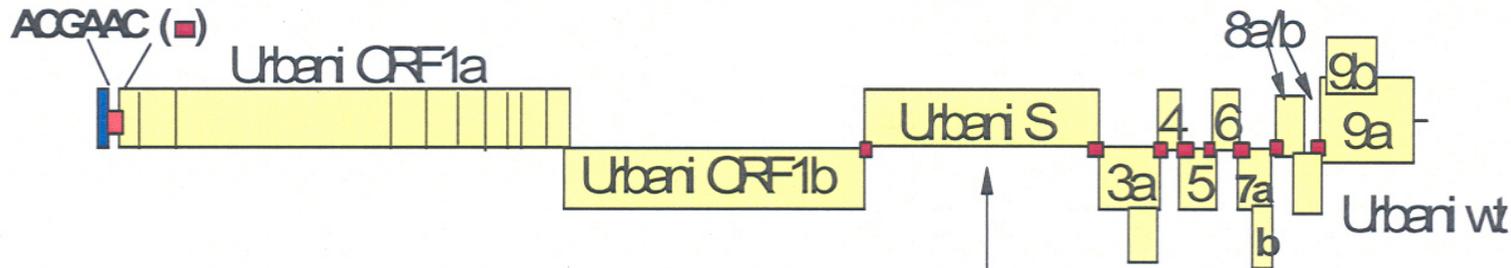
Synthetic Genomics

- Synthetic reconstruction of a viral genome/gene
 - Zoonotic SARS (bat, civet cat, racoon dog) only described in China
 - Why?

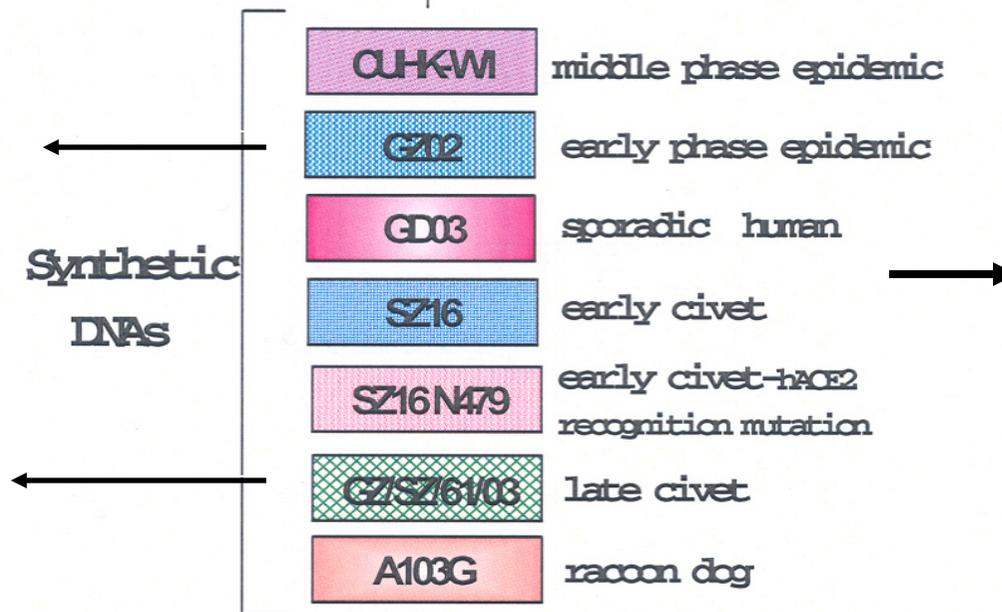


Synthetic Genomics

- Synthetic reconstruction of a viral genome/gene
 - Zoonotic SARS (bat, civet cat, racoon dog) only described in China
 - Why? Protection from zoonotic pools

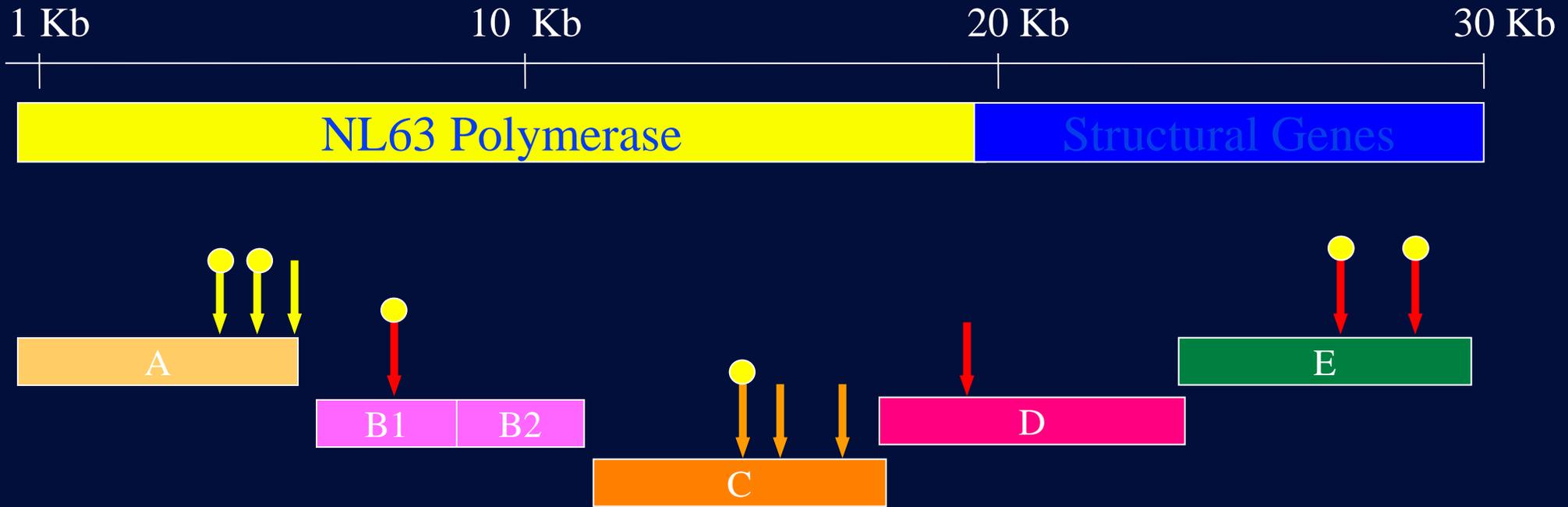


Viruses encoding the GZ02 and GZ/SZ/61/03 S genes kill senescent mice (pathogenic small animal model for SARS)(Rockx et al.)



Vaccines based on late phase isolates poorly protect against zoonotic S challenge: in senescent animals; Deming et al., submitted Plos Med

Synthetic HCoV NL63 Molecular Clone



- LRT Human Pathogen, major cause of croup in young children and infants
- Must have consensus sequence to rescue recombinant virus
 - Two reported differ at 64 positions
 - ◆ A deletion and insertion resulted in a set of 23 codon changes (identified by bioinformatic analysis)
- NL63 published sequence (two) were both incorrect; phylogenetic comparisons ~10 sites of concern
 - (~1/2 were predicted by bioinformatics); additional changes found

Risk: Designer Pathogens

Menu: Virus and Microbial Virulence Genes Grows Daily

- Cellular Signaling

- pro and anti-apoptotic activities
- Inhibiting host cell macromolecular expression
- MicroRNAs: targeting specific host cell processing pathways

- Antigen Processing/presentation

and HLA Expression (acquired immunity)

- Innate Immunity

- Interferon antagonists (e.g., Influenza NS1, Ebola VP35)
- Cytokine antagonists
- Immunomodulators

- Host genes that enhance virulence

- Chimerical Spikes

Blending genes into virulent pathogens is terribly complex; but synthetic and natural sources of these genes are readily available; part list increases monthly

Synthetic Genomes

Advantages

- Speed of synthesis
- Mutagenic superiority
- Ease of genome construction
- Low cost/rapid response

Disadvantages

- No outcome guarantee
- Design might be sophisticated
- Some Technical Expertise Required
- How Test?

Recombinant DNA Committee:

No real difference: a) origin of the DNA used in constructed a molecular clone: a) zoonotic genes viewed as likely reducing virulence; c) big problems with chimeric genomes that might modulate virulence (e.g., how to evaluate/safety recommendations).

SARS CoV-Related Research

NIH AI23946, AI059136, AI061819

- Baric Laboratory (UNC)
 - Boyd Yount
 - Will McRoy
 - Amy Sims
 - Lisa Lindesmith
 - Barry Rockx
 - Damon Deming
 - Eric Donaldson
 - Tim Sheahan
 - Rhonda Roberts
- Blue Heron Technology
 - John Mulligan