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 ⟶ viral proteins.

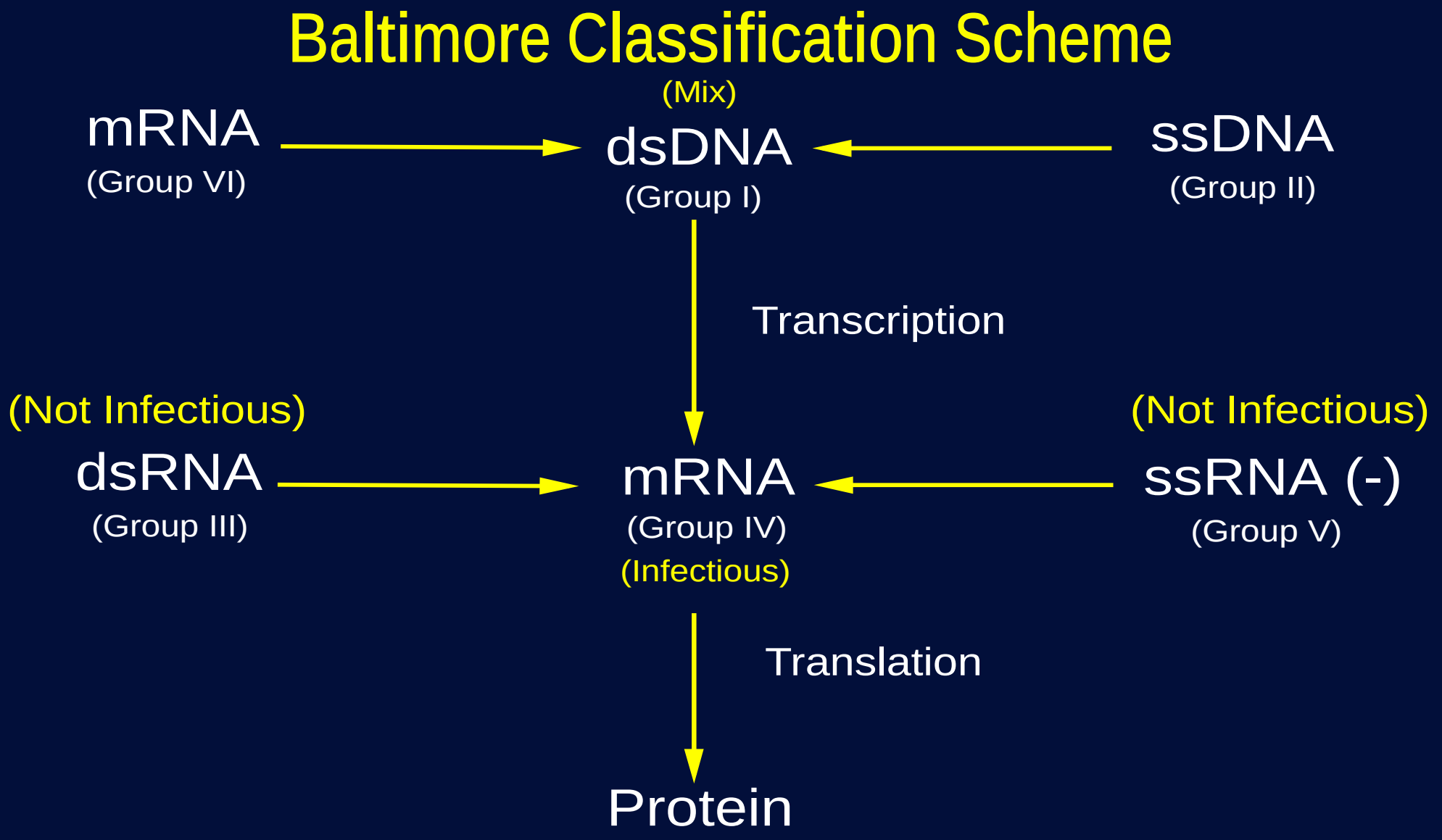


Figure 1. Baltimore Classification Scheme.

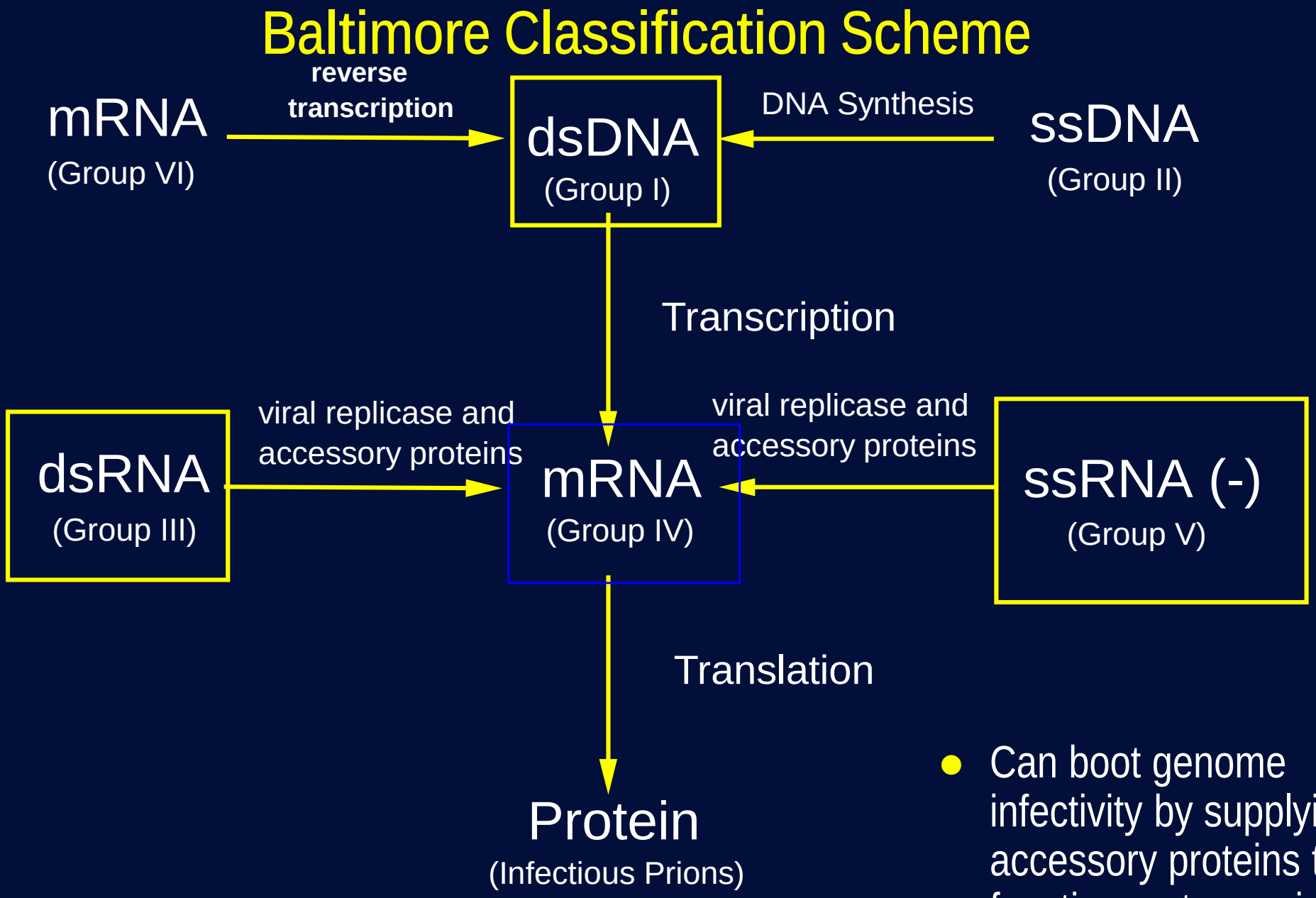


Figure 1. Baltimore Classification Scheme.

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), Potyvirus (plum pox), Alphavirus (VEE, EEE), Flavivirus (Central European TB, Far East TB encephalitis virus, others), Coronavirus (Yes)

Noroviruses-No

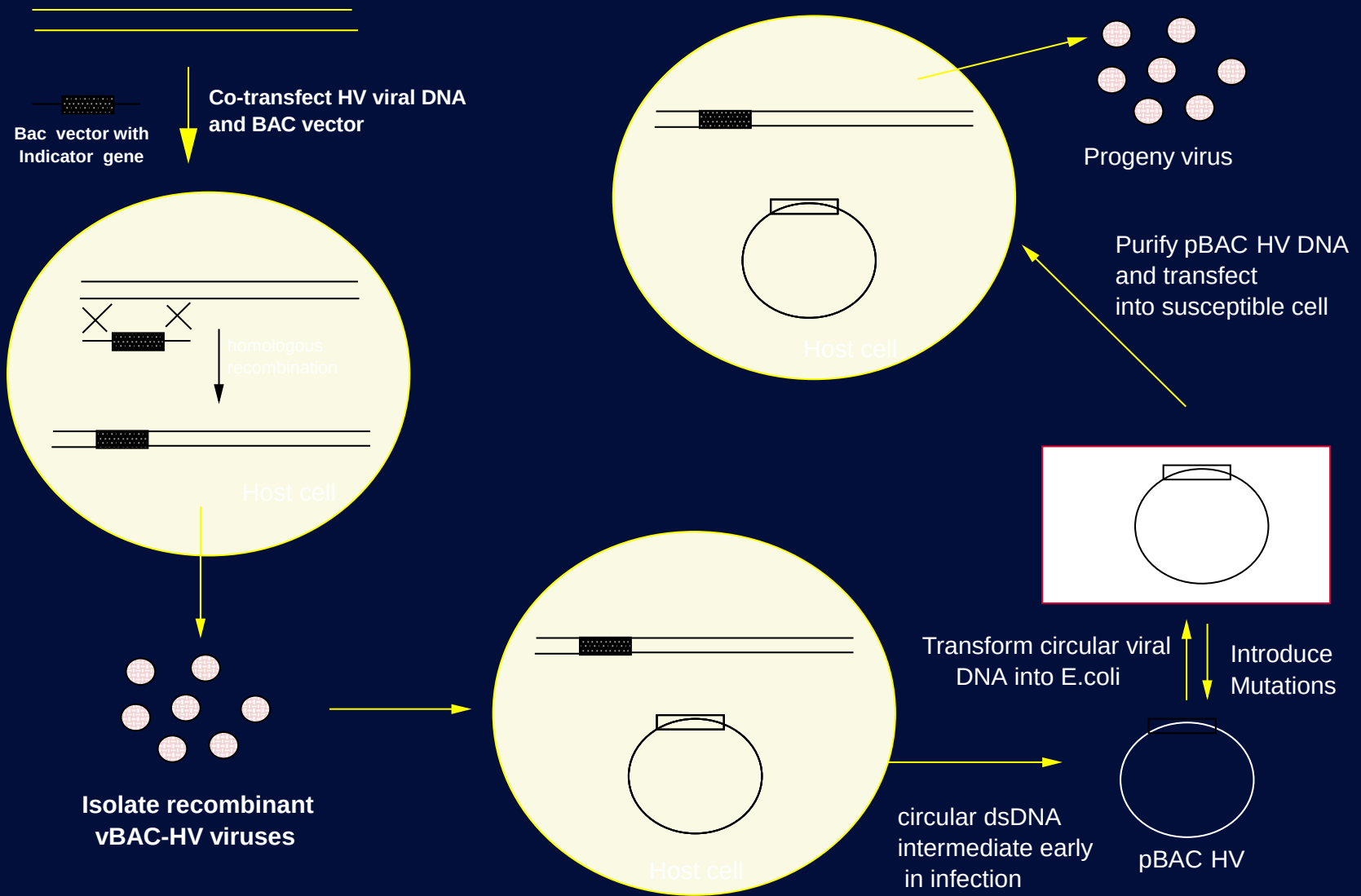
Group V (Negative Polarity ss RNA Viruses)-Yes

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

Virus Reverse Genetics
(Producing infectious virus from recombinant or synthetic DNA genomes)

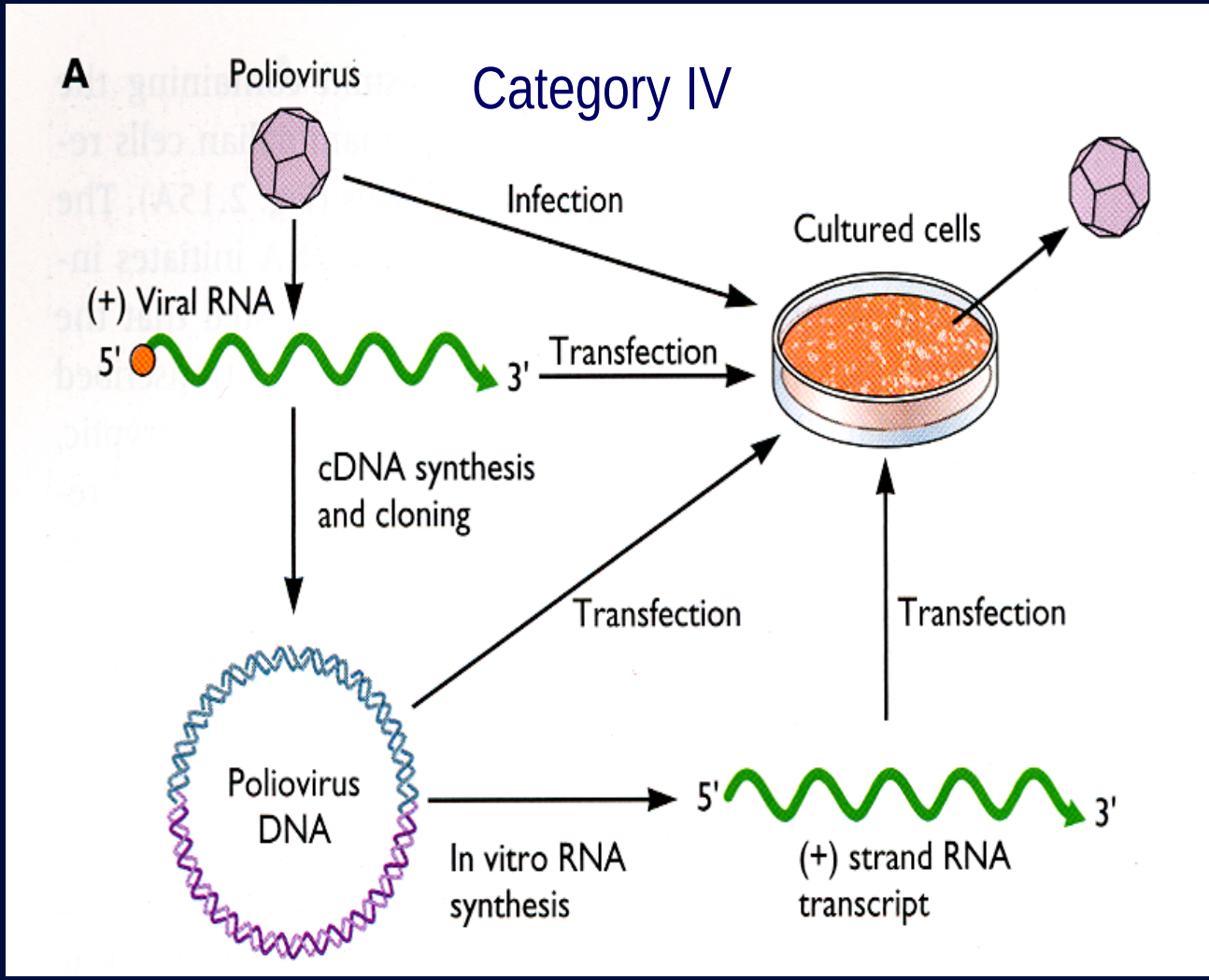
- Group I (dsDNA Viruses)-Yes
  - Herpesviruses (e.g., HSV, HSV8, VZV)
  - Poxviruses (vaccinia virus)
    - Genome Size ~190 KB
    - Ends for covalently cloned hairpin loops
    - Genome is not infectious
      - Requires additional viral products to boot infectivity
- Group III (dsRNA Viruses)-No
- Group IV (Positive Polarity ssRNA Viruses)-Yes
  - Picornavirus (FMDV, Swine Vesicular Disease Virus), Potyvirus (plum pox), Alphavirus (VEE, EEE), Flavivirus (Central European TB, Far East TB encephalitis 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)

Herpesvirus Molecular Clone
Category I



Virus Reverse Genetics
Category IV

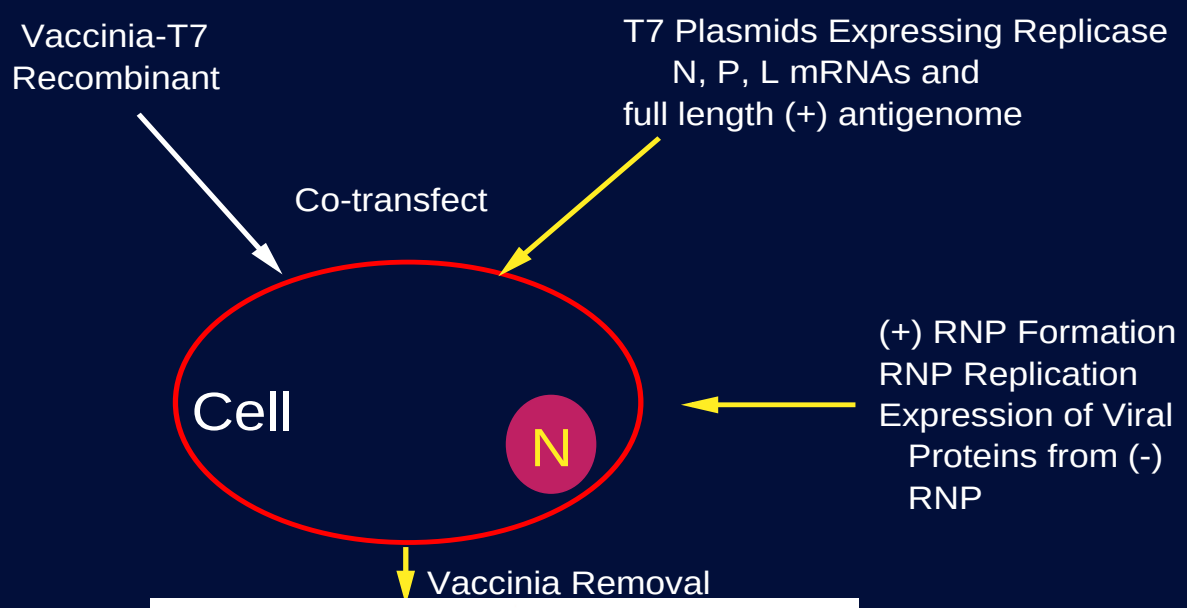
- Positive Strand RNA Viruses
  - Picornaviruses
  - Enteroviruses (e.g., PV, FMDV, HAV)
  - Coronaviruses (e.g., SARS-CoV)
  - Alphaviruses (e.g., VEE, WEE, EEE)
  - Flaviviruses (e.g., Yellow fever, dengue, etc.)
  - Noroviruses (not yet)

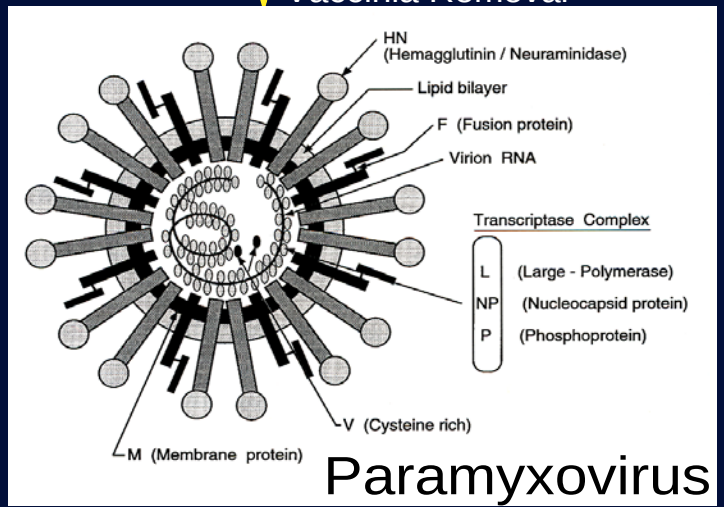


- Manipulate DNA and recover altered viruses
- Sequences readily available

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

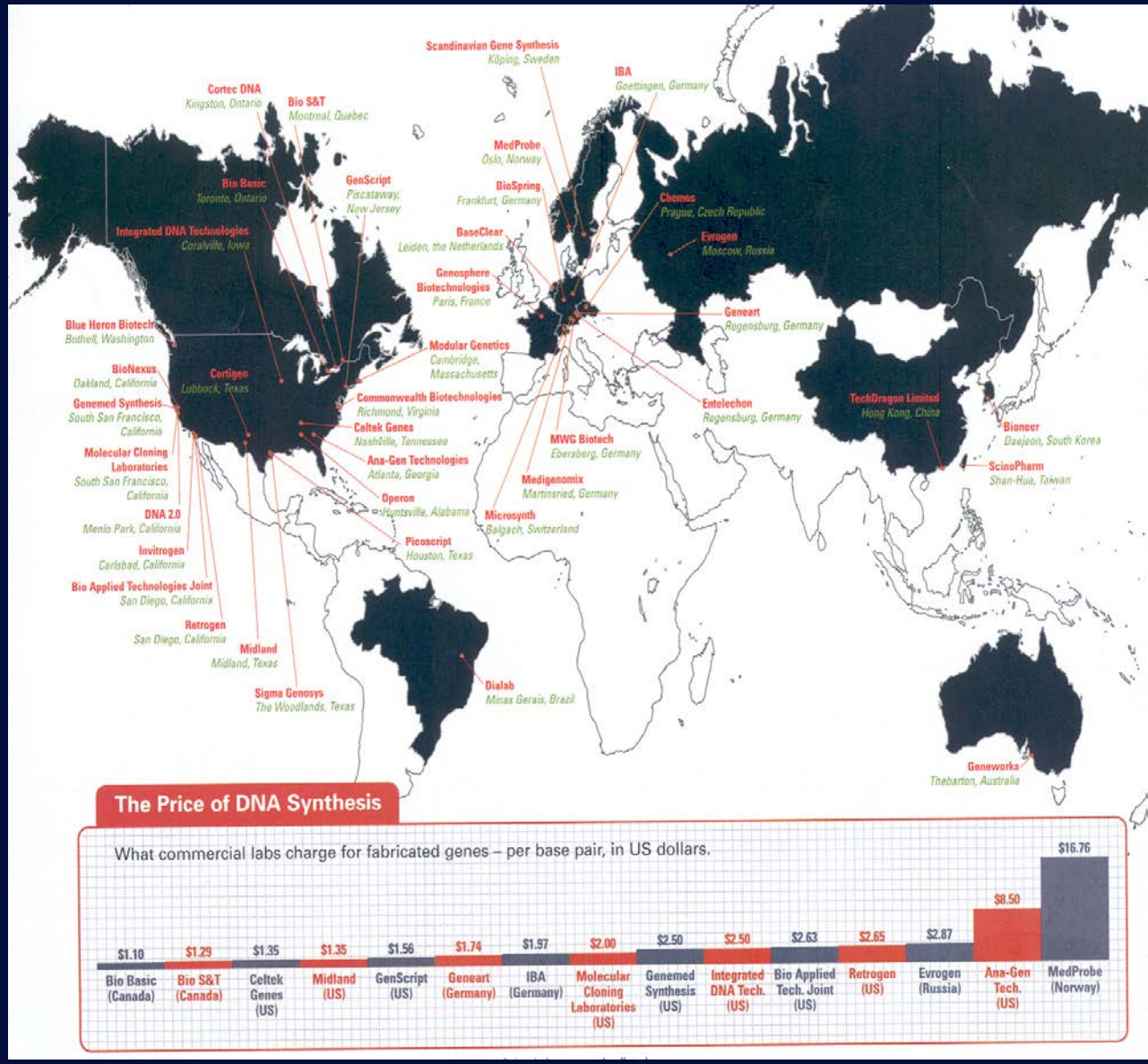
- Genome Infectious-Yes
  - dsDNA-Herpesviruses
    - Full length (>70%) HBV and poxvirus genomes are select agents
  - Positive polarity ssRNA viruses
    - DNA or RNA launch
      - Full length genomes of HHS/CDC (+) RNA viruses are select agents
- Genome Noninfectious
  - Boot Genome Infectivity
    - 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^{18} possibilities to get correct genome (10^4 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

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 Stability
  - 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 x 10^{18} possibilities to get correct genome (10^4 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

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 stability
  - 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^{18} possibilities to get correct genome (10^4 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

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

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/misdirection
    - 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

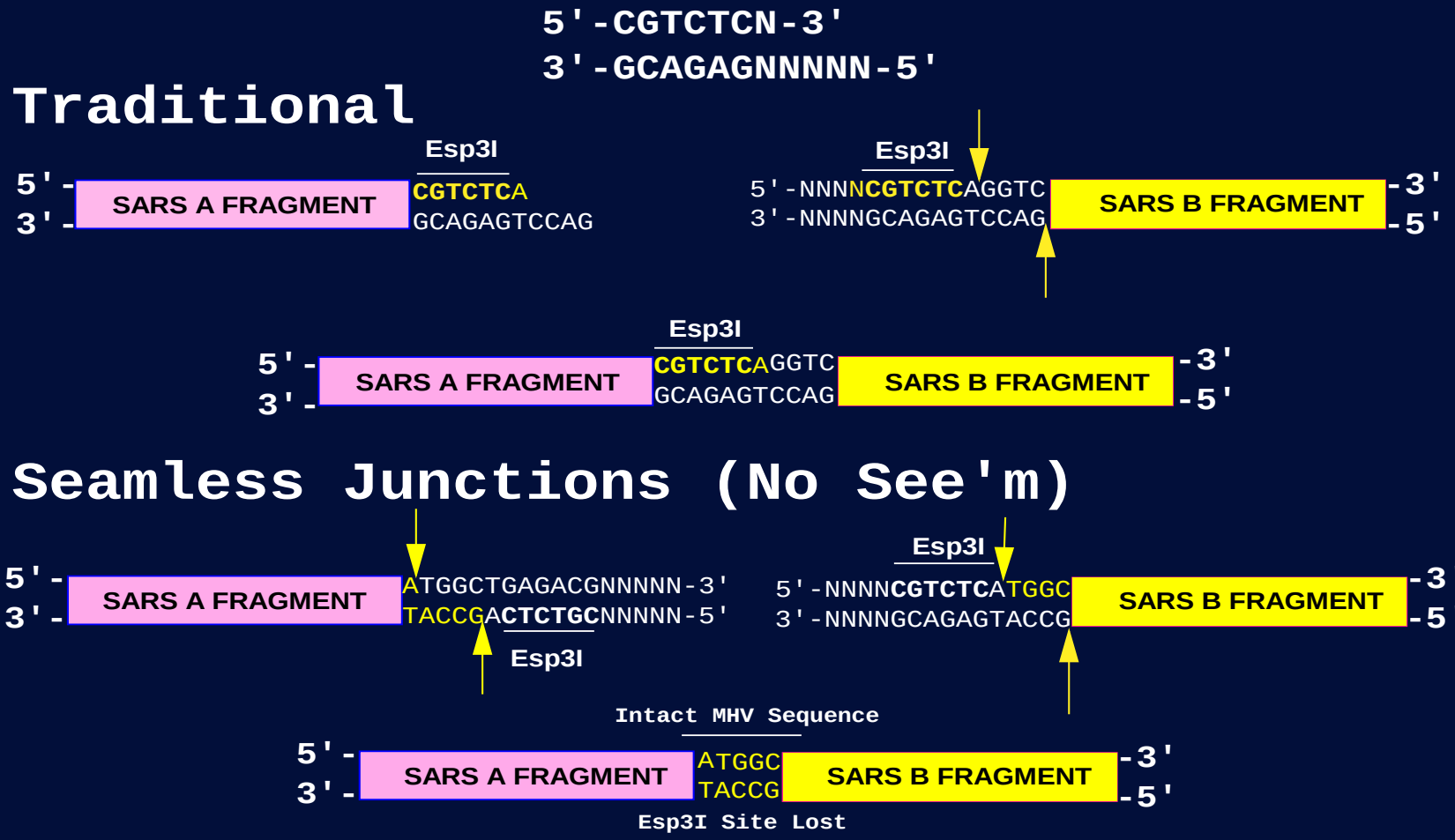
Steps

- 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 Comparison (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/misdirection
    - 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

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)



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

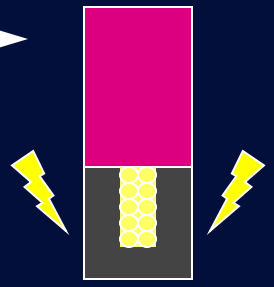
Finite source of non-replicating full length cDNA that is consumed in the reaction

Set of Contiguous ~5 Kb pieces

Transcribe genome length RNA

Boot Infectivity

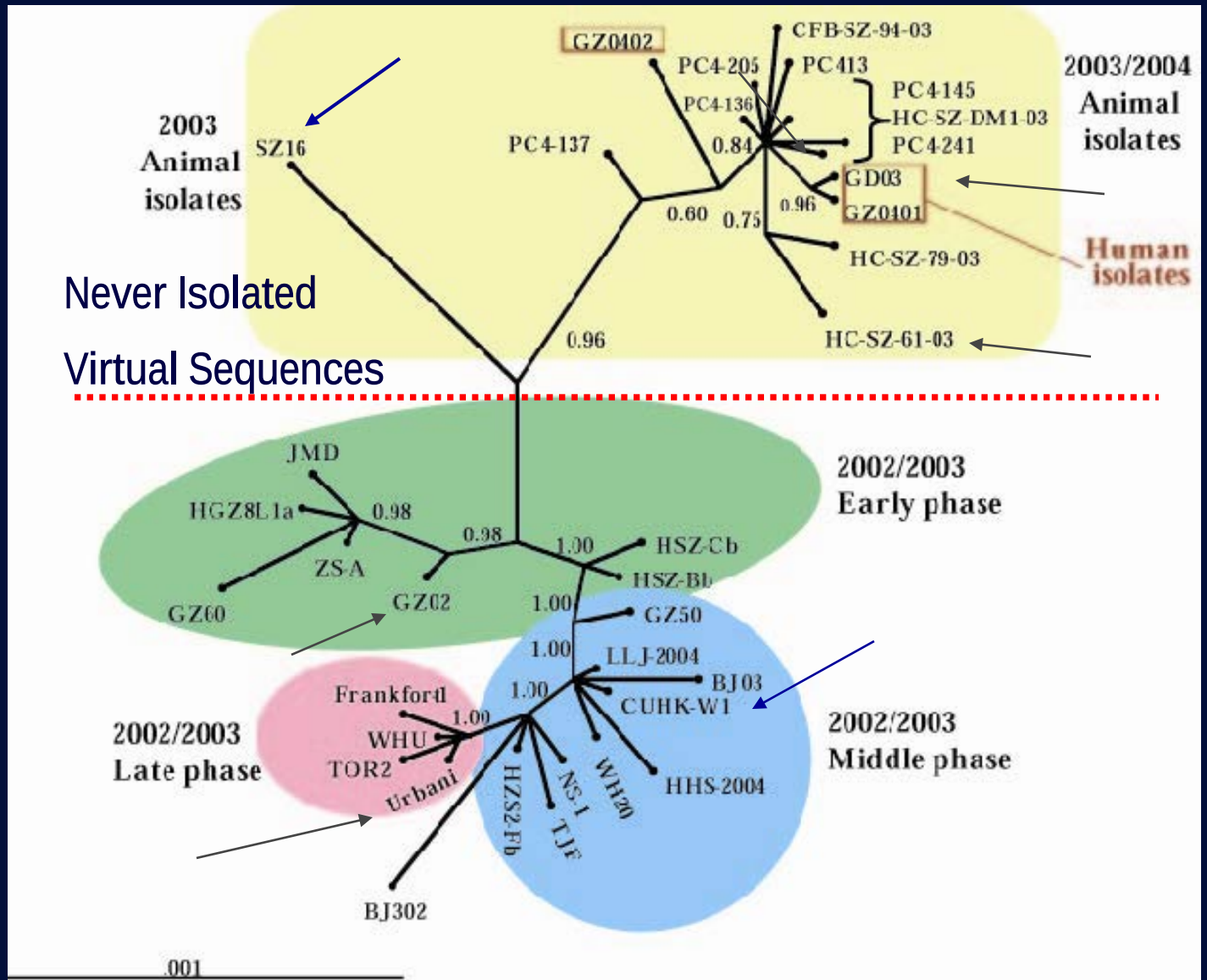
N transcripts (N protein) "boots" infectivity by 10->1000 fold (enhances transcription)



Transfect Vero E6 cells

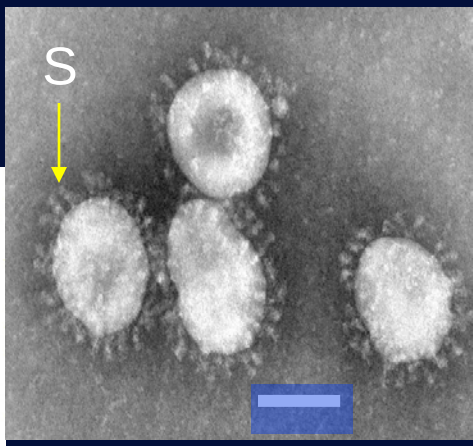
Virus recovered which replicated to wildtype levels

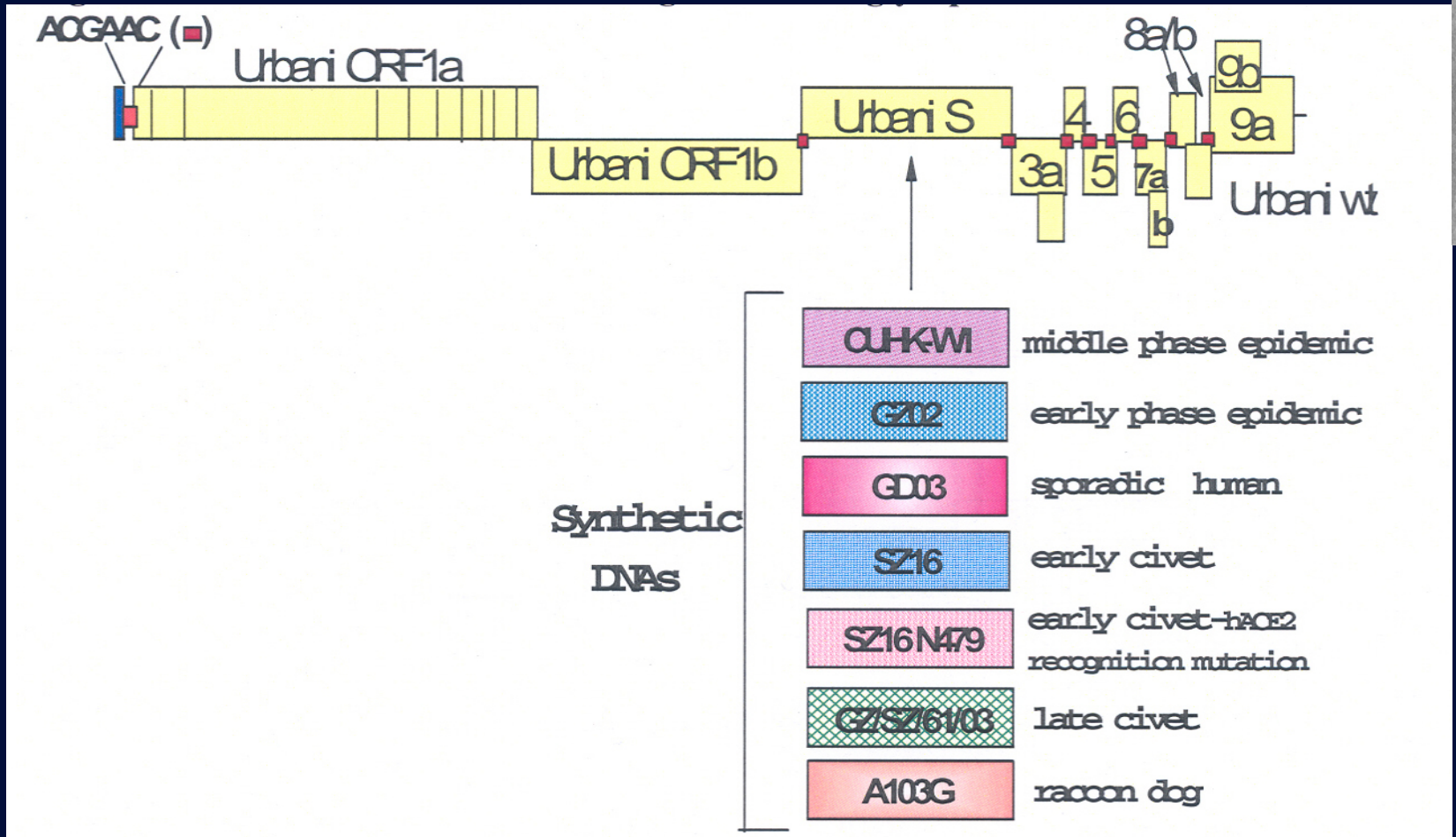
Molecular Resurrection of Early SARS-CoV Isolates from Sporadic Human Cases and Animals



Synthetic Genomics

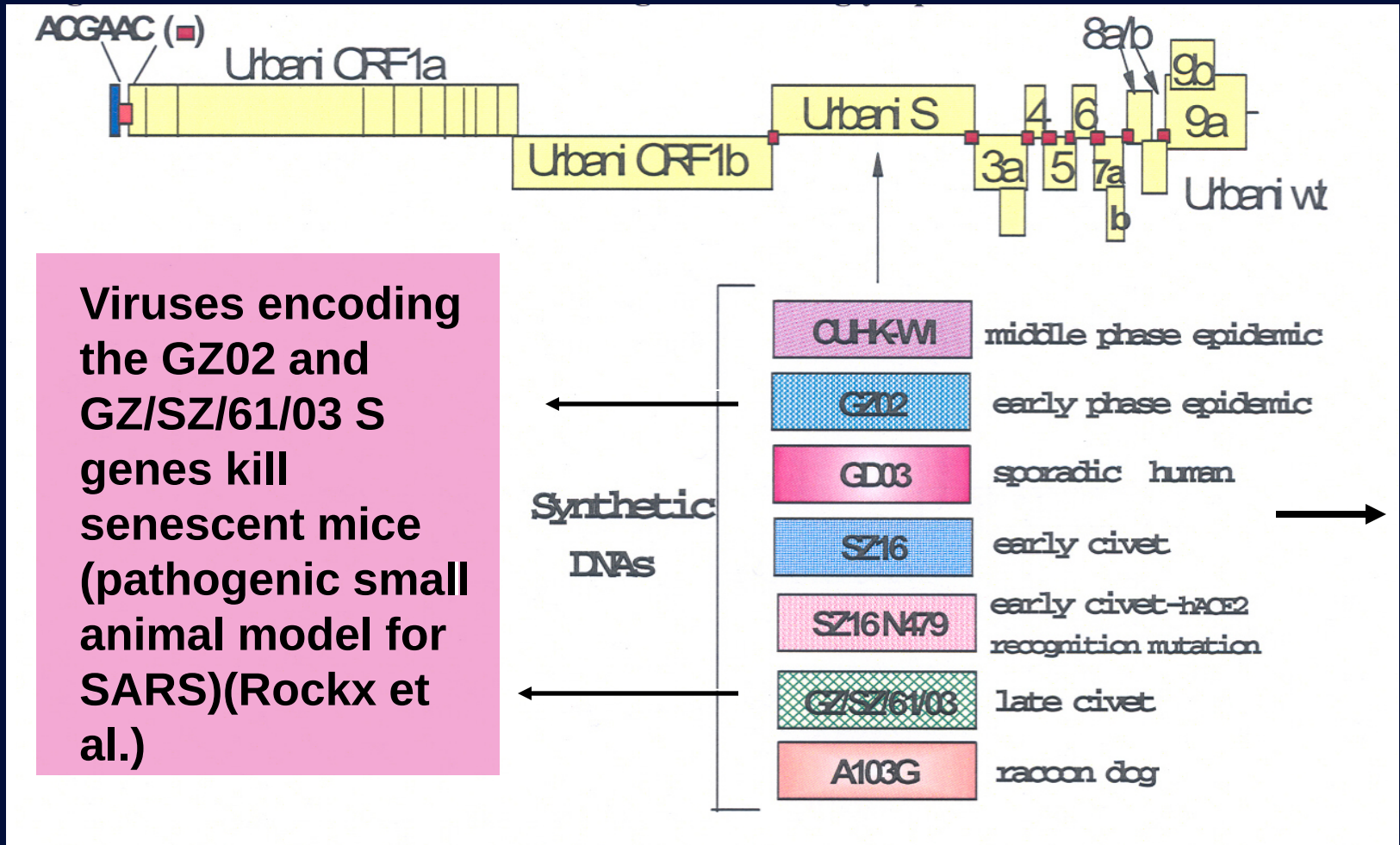
- Synthetic reconstruction of a viral genome/gene
  - Zoonotic SARS (bat, civet cat, racoon dog) only described in China
  - Why?



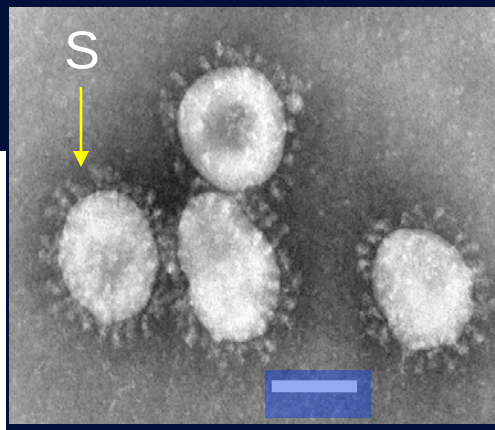


Synthetic Genomics

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

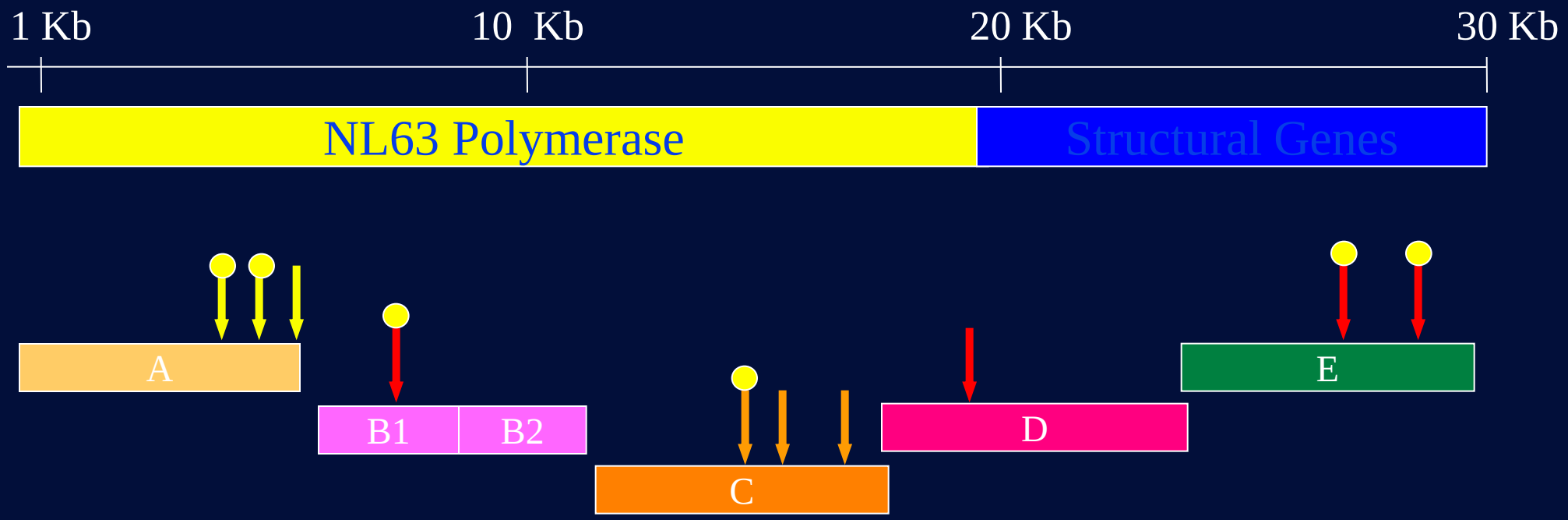


Viruses encoding the GZ02 and GZ/SZ/61/03 S genes kill senescent mice (pathogenic small animal model for SARS)(Rockx et al.)



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