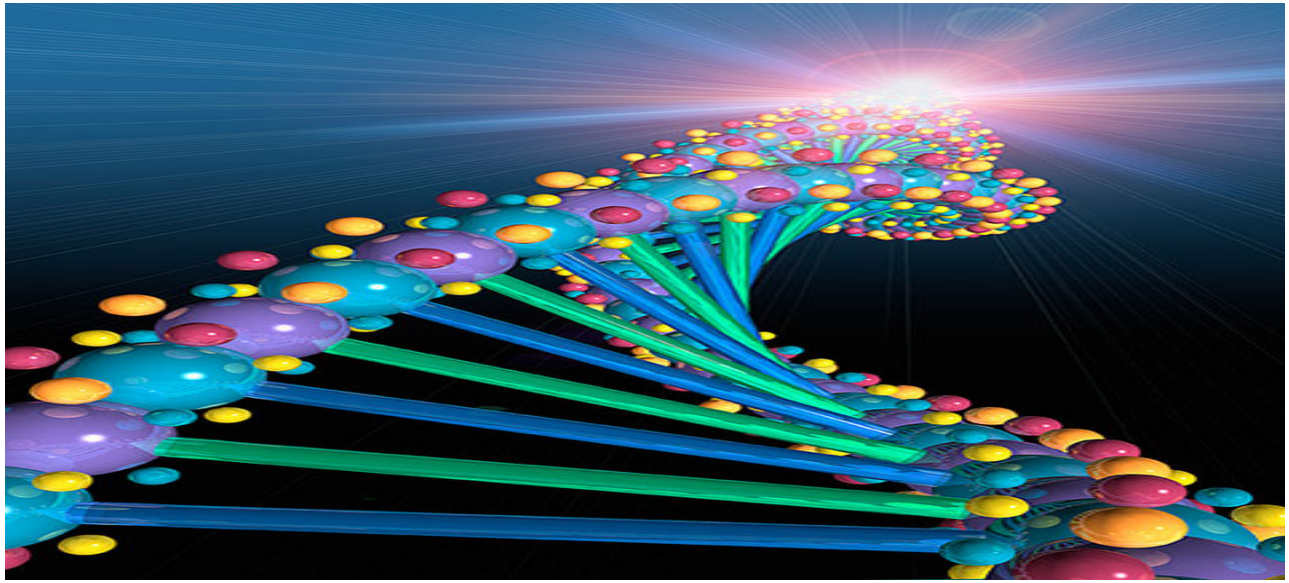
VirginiaTech
Invent the Future

Gene Amplification for cloning



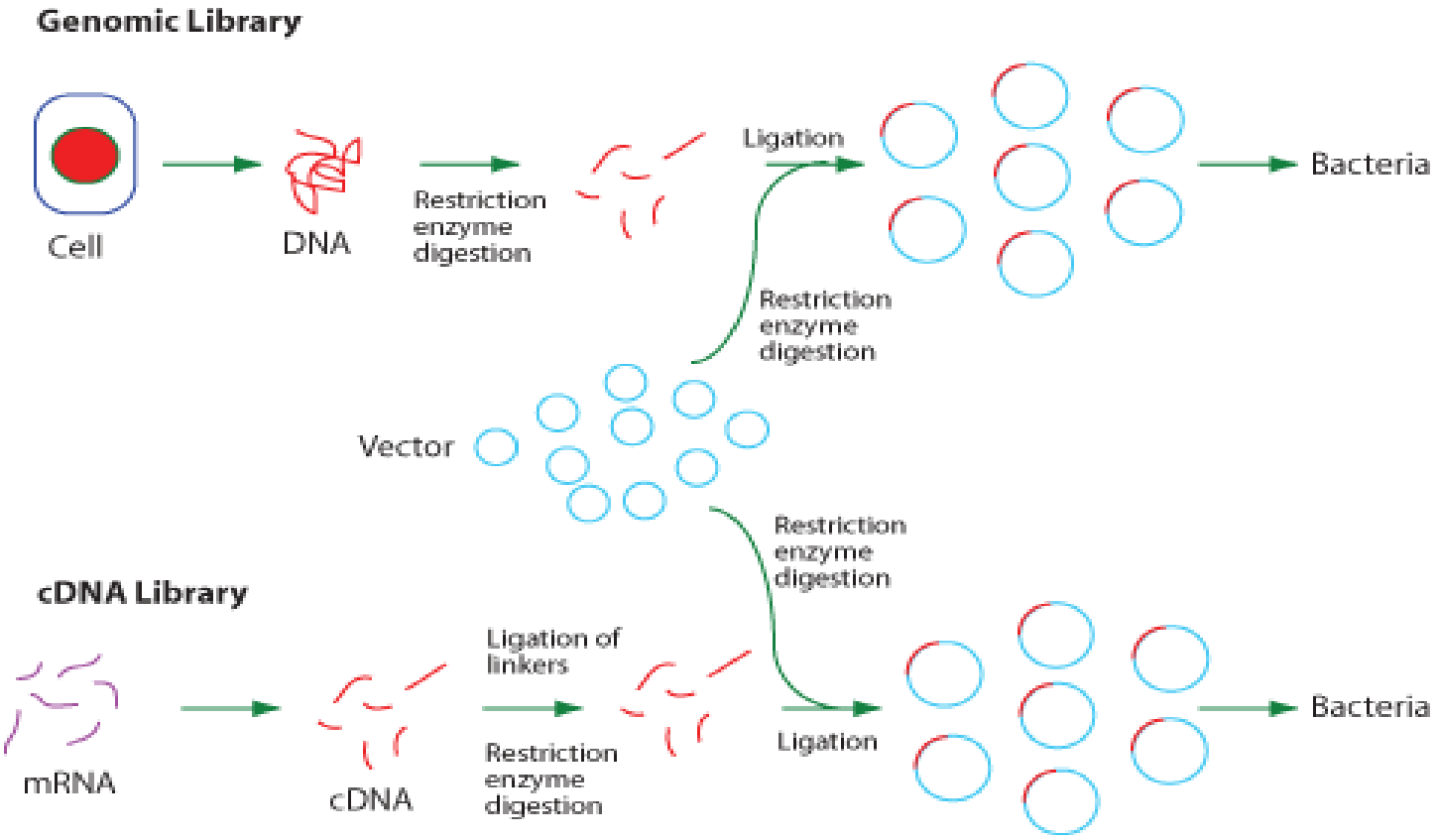
Mohamed N. Seleem

VirginiaTech
Invent the Future

How to pick up 1 out of thousands genes

VirginiaTech
Invent the Future

DNA library and screening

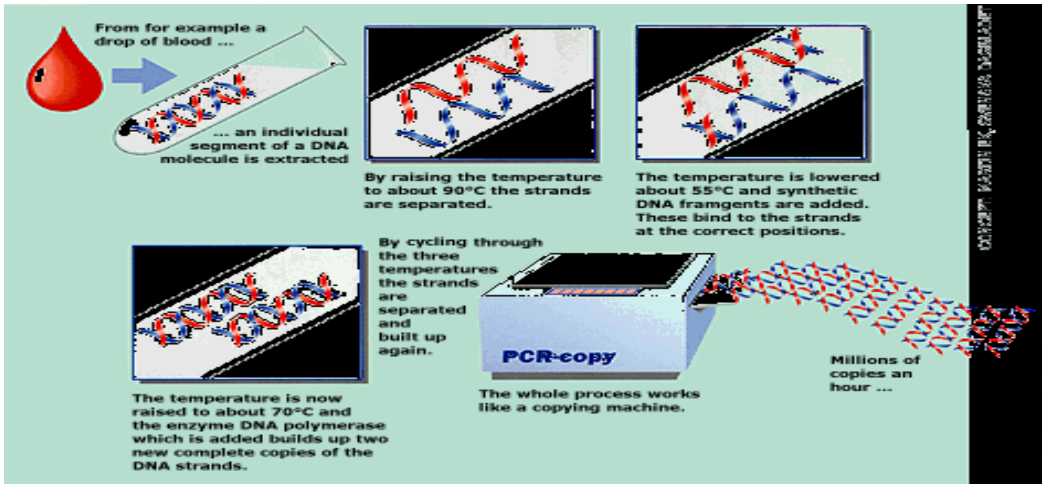


Polymerase Chain Reaction

- Selectively amplifying a particular segment of DNA.

e.g. a specific gene 1 copy can produce billions of copies

- It can be described as a molecular photocopier.



VirginiaTech
Invent the Future

Reagents in a tube

- Sample (DNA with gene of interest)
- Primers (forward and reverse)
- Enzyme (DNA polymerase, *Taq)*
- dNTPs (A, T, G, C)
- Buffer (50 mM KCl; 10 mM Tris-HCl; 1.5 mM MgCl2)

Master mix

VirginiaTech
Invent the Future

PCR Cycles

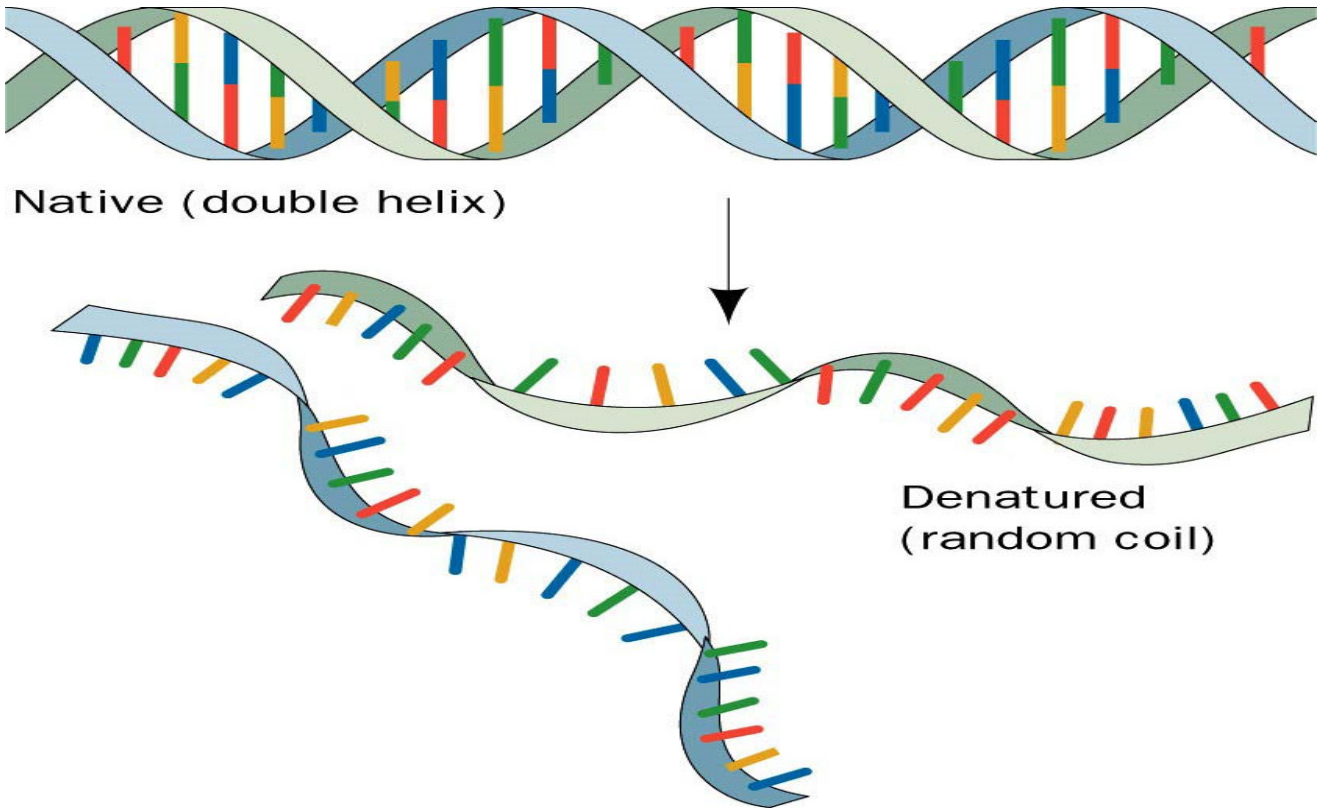
		Time and Temperature
•Initial denature		**94 °C for 2 minuets**
•denature	**25-40 cycles**	**94 °C for 30 sec**
•annealing		**55 °C for 30 sec**
•Extension		**68-72 °C for xxx sec**
•Final extension		**72 °C for 5 minuets**

VirginiaTech
Invent the Future

PCR Cycles

- **Denature**

94 °C for 30 sec



VirginiaTech
Invent the Future

PCR Cycles

- **Primer annealing**

55 °C for 30 sec

ACCATCGGACTGCATCAGTACCATCGG———ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGA

oligonucleotide=Primers

oligonucleotide=Primers

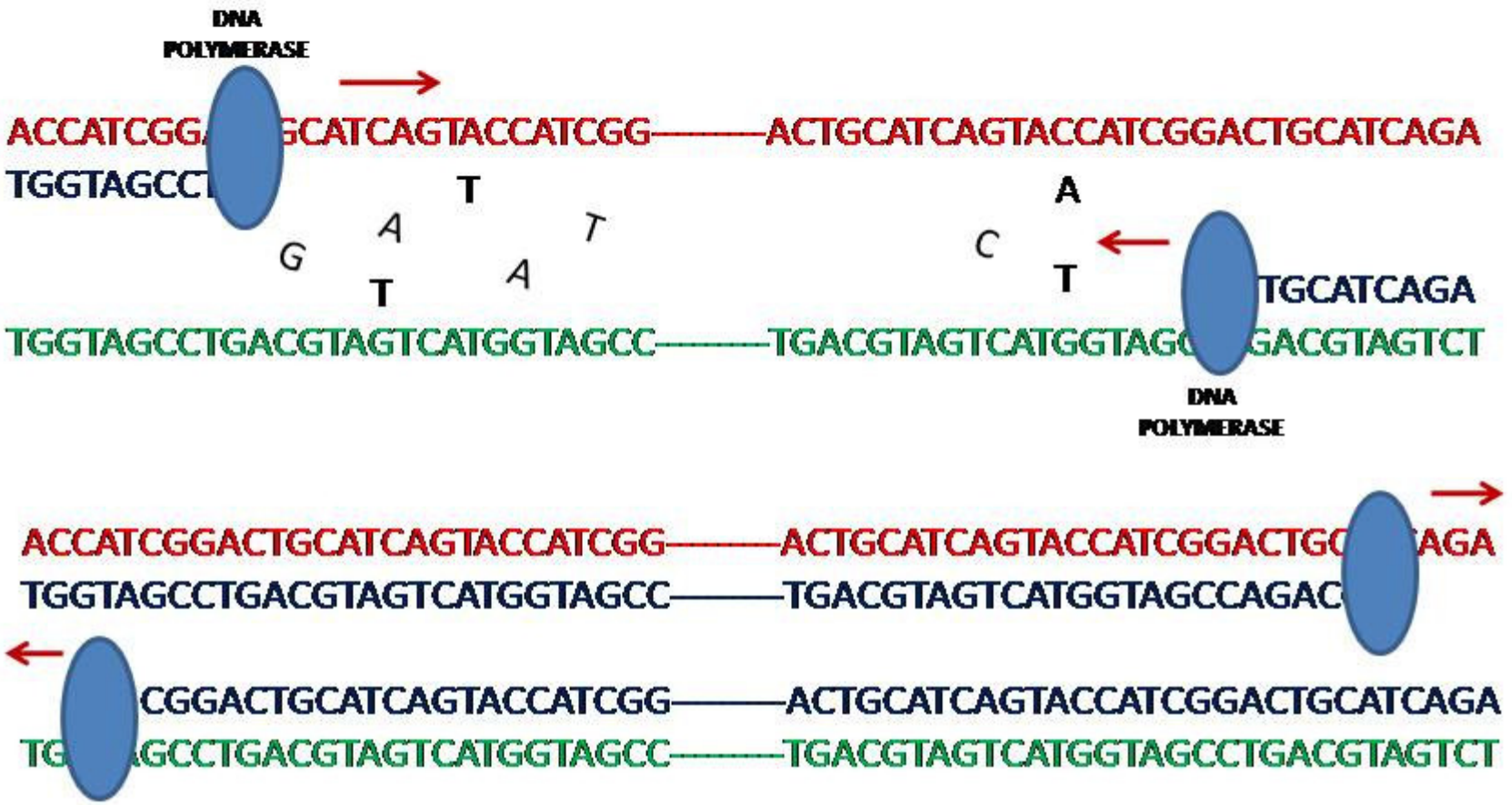
CTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC———TGACGTAGTCATGGTAGCCTGACGTAGTCT

VirginiaTech
Invent the Future

PCR Cycles

- **Extension** — 72 °C for XXX sec



2nd cycle

- **Denature**

94 °C for 30 sec

ACCATCGGACTGCATCAGTACCATCGG———ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC———TGACGTAGTCATGGTAGCCAGACGTAGTCT

ACCATCGGACTGCATCAGTACCATCGG———ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC———TGACGTAGTCATGGTAGCCTGACGTAGTCT

2nd cycle

- **Primer annealing**

55 °C for 30 sec

ACCATCGGACTGCATCAGTACCATCGG———ACTGCATCAGTACCATCGGACTGCATCAGA
TGGTAGCCTGA

CTGCATCAGA
TGGTAGCCTGACGTAGTCATGGTAGCC———TGACGTAGTCATGGTAGCCAGACGTAGTCT

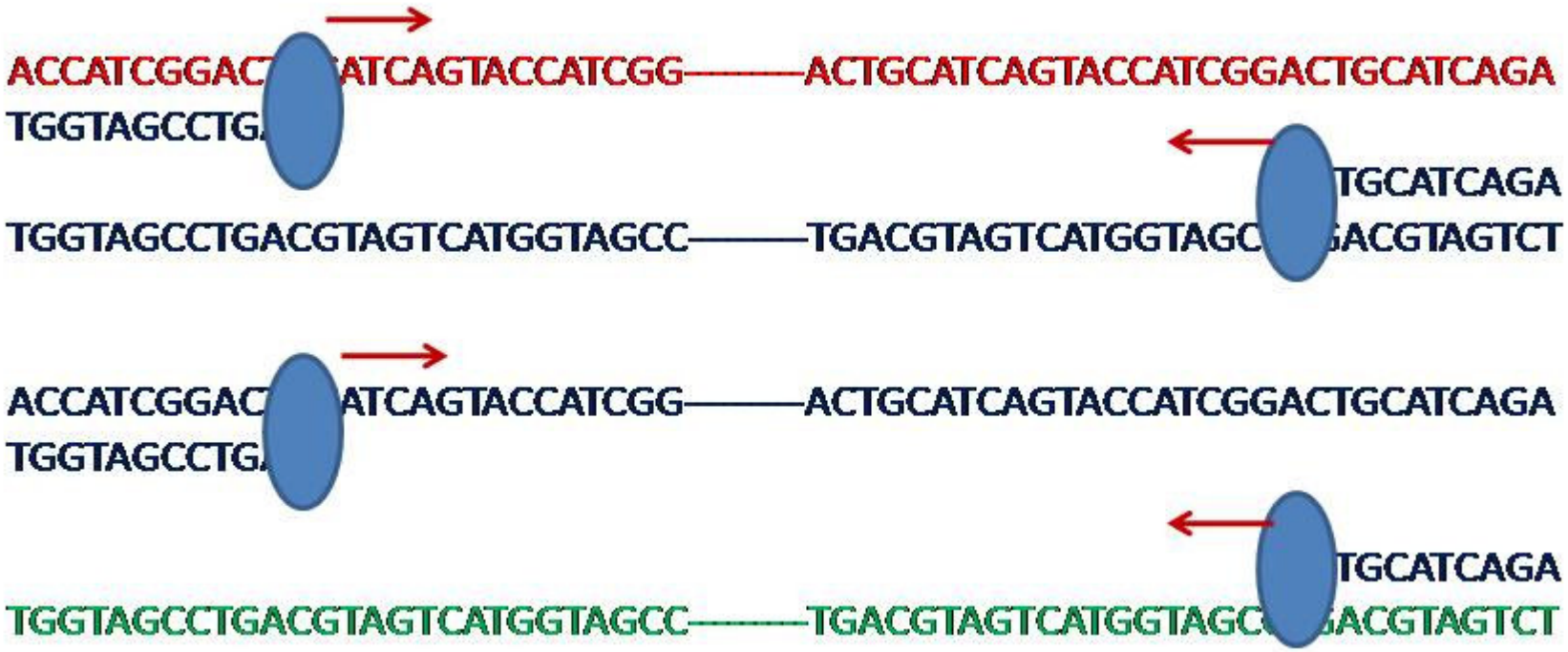
ACCATCGGACTGCATCAGTACCATCGG———ACTGCATCAGTACCATCGGACTGCATCAGA
TGGTAGCCTGA

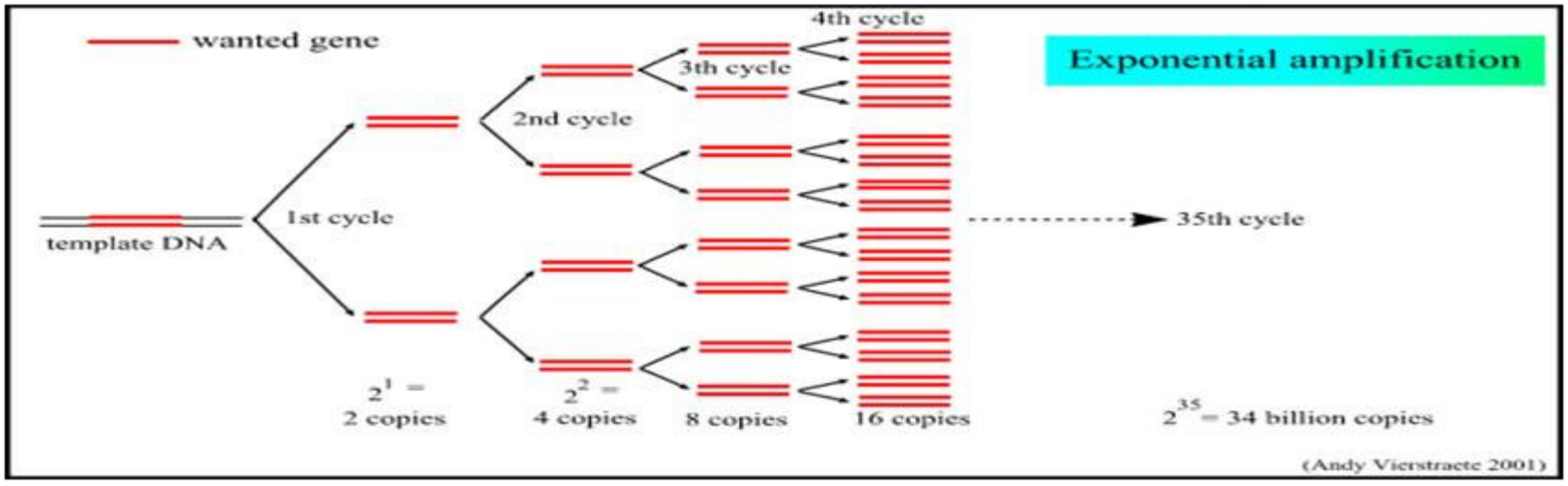
CTGCATCAGA
TGGTAGCCTGACGTAGTCATGGTAGCC———TGACGTAGTCATGGTAGCCTGACGTAGTCT

2nd cycle

- **Extension**

72 °C for XXX sec





VirginiaTech
Invent the Future

Set up PCR reaction

Reagents in a tube

1- 5 µl (200 ng)	•Sample (DNA)
1 µl F and 1 µl R 20 pmol	•Primers (forward and reverse)
25 µl – 50 µl	•Enzyme (DNA polymerase, *Taq)* •dNTPs (A, T, G, C) •Buffer (50 mM KCl; 10 mM Tris-HCl; 1.5 mM MgCl2)
If needed	•Water DNAase free water

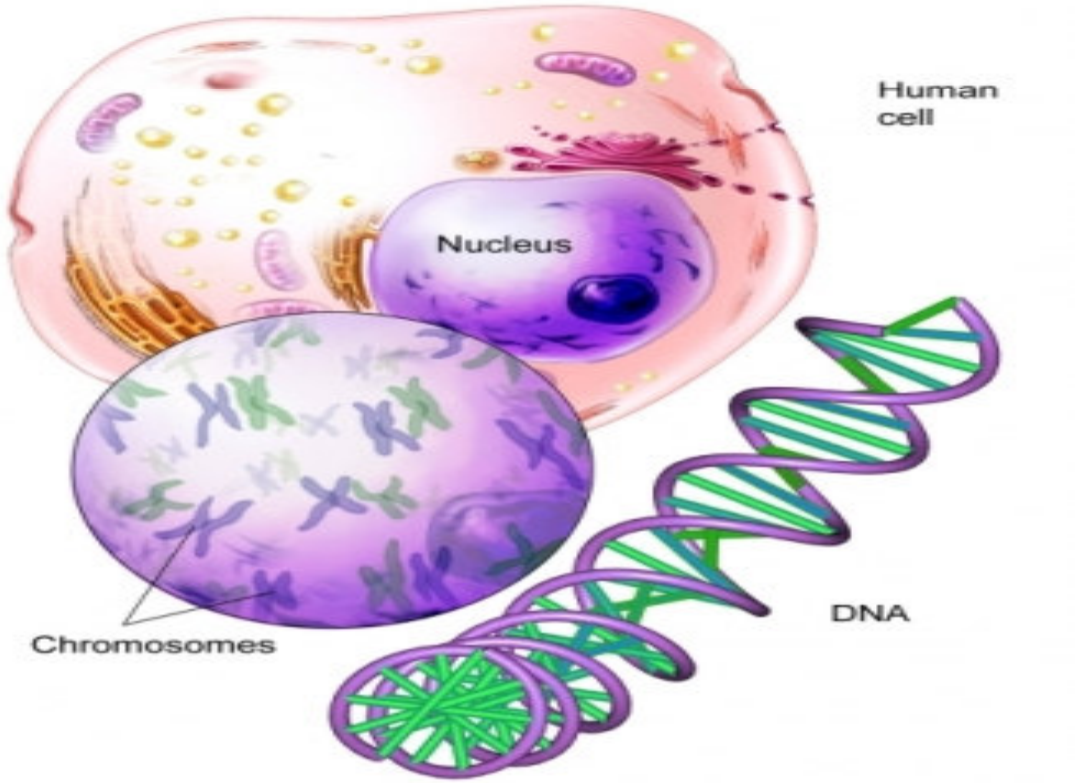
VirginiaTech
Invent the Future

Thermocycler

VirginiaTech
Invent the Future

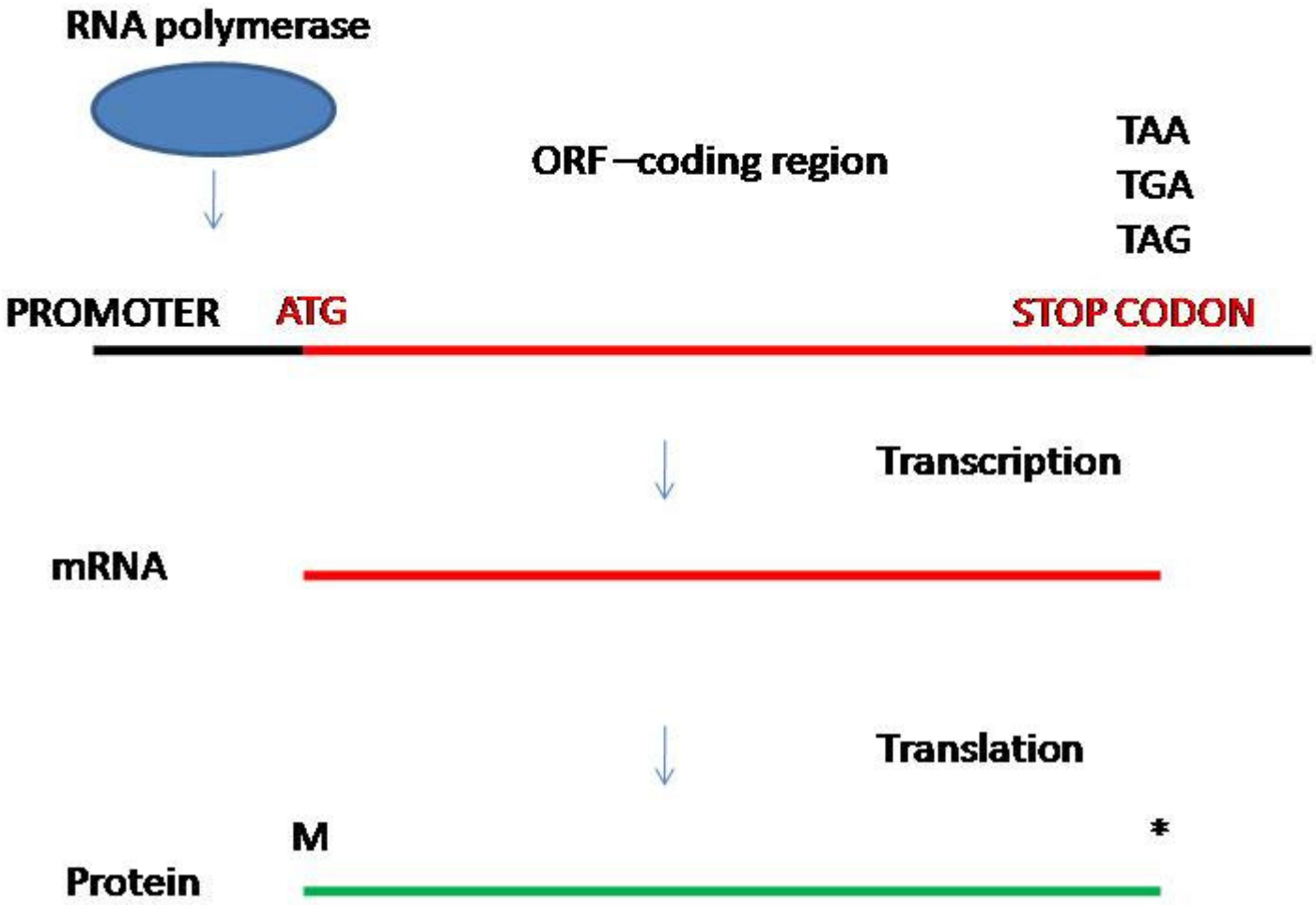
PCR for cloning

What is different ?



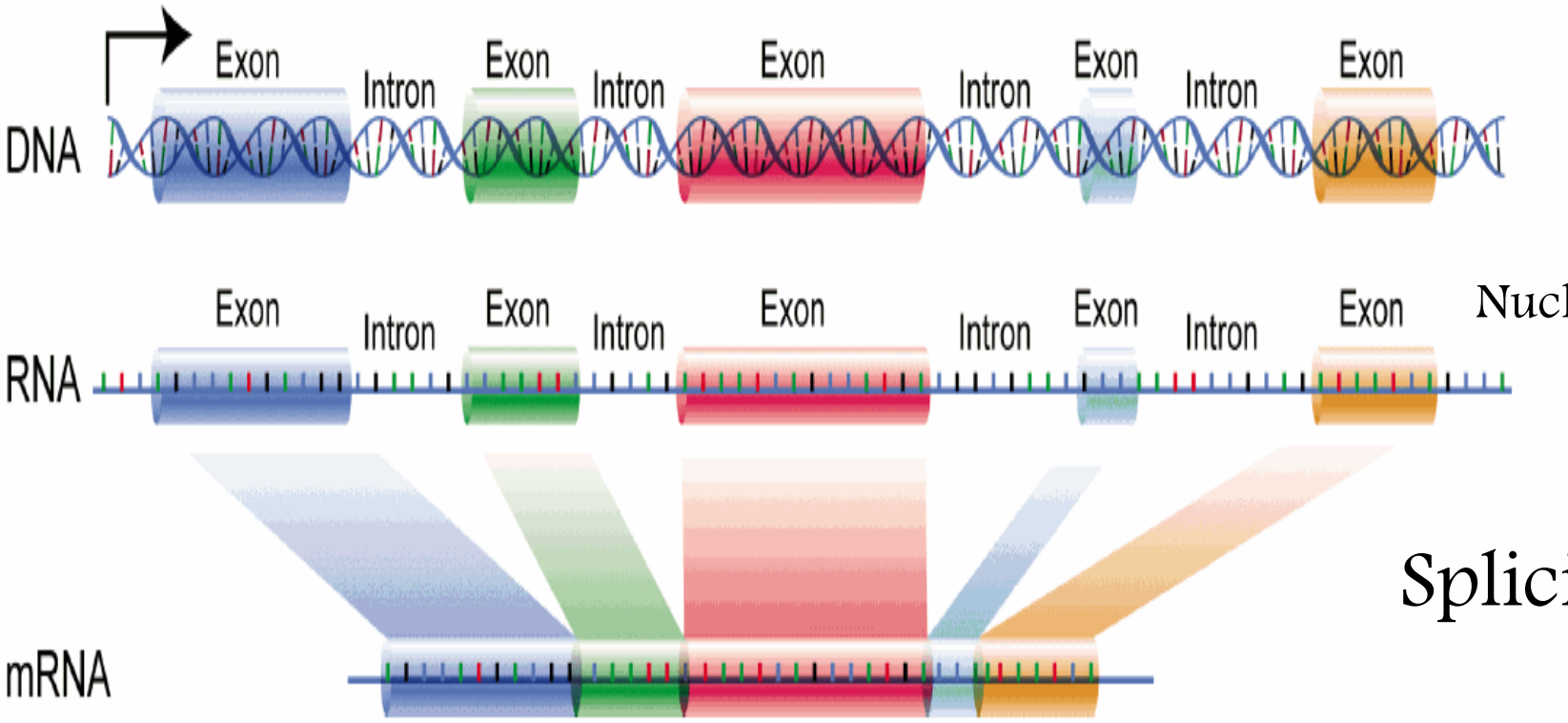
VirginiaTech
Invent the Future

Gene expression



VirginiaTech
Invent the Future

Eukaryotic gene expression

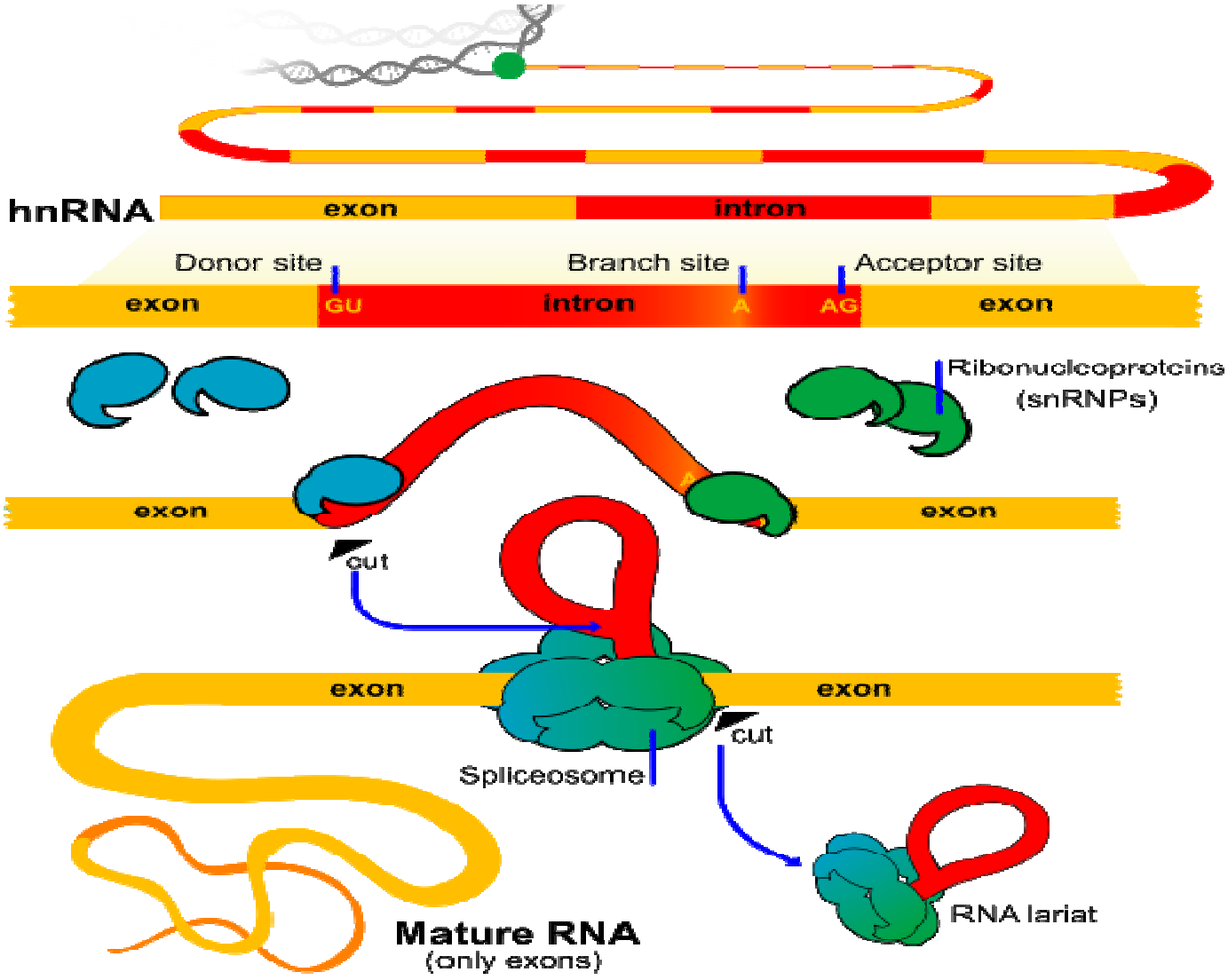


Nuclear RNA

Splicing

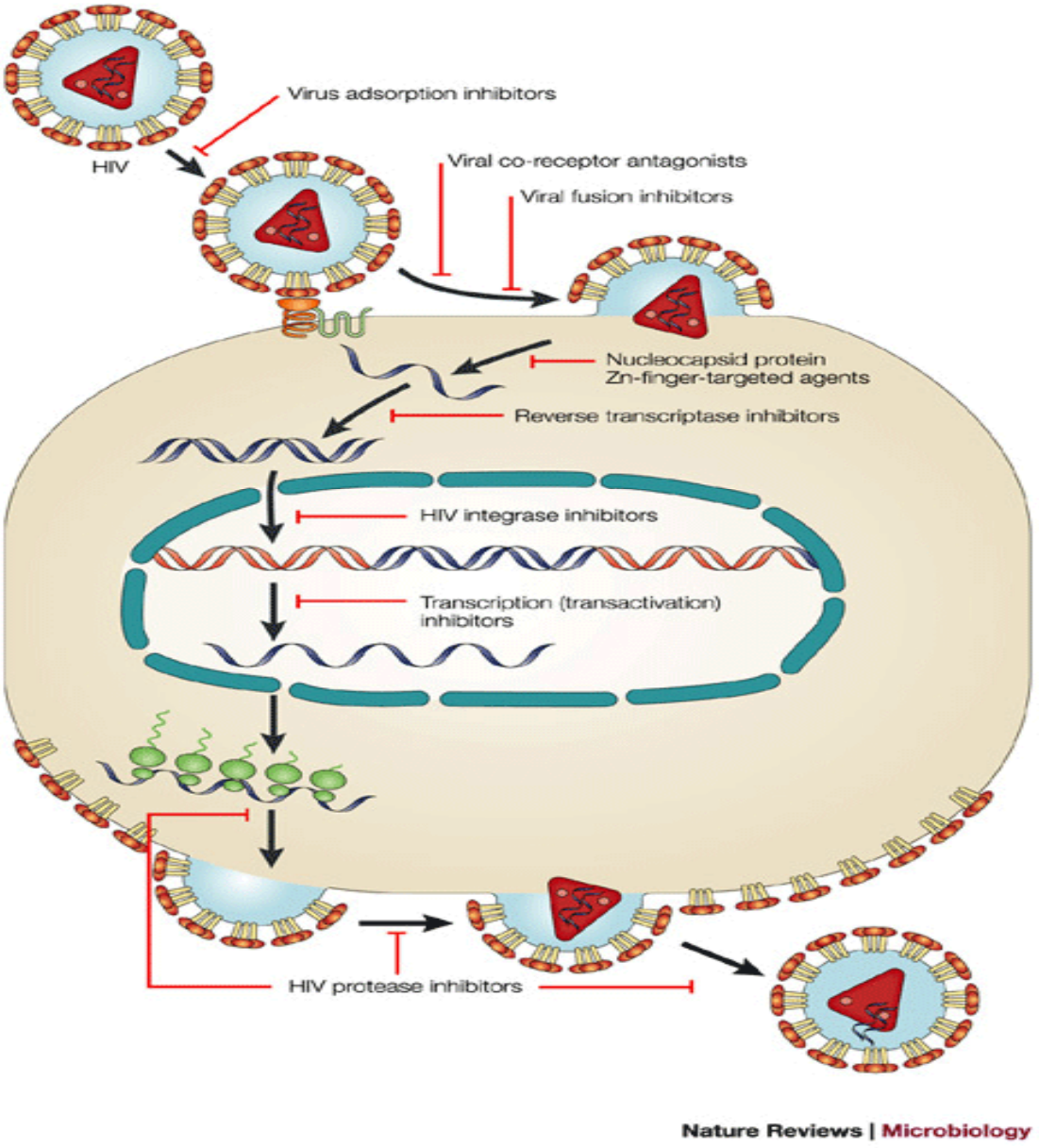
VirginiaTech
Invent the Future

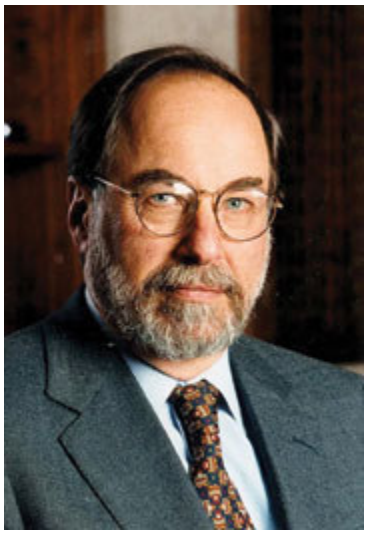
RNA Splicing



VirginiaTech
Invent the Future

HIV– Reverse transcriptase

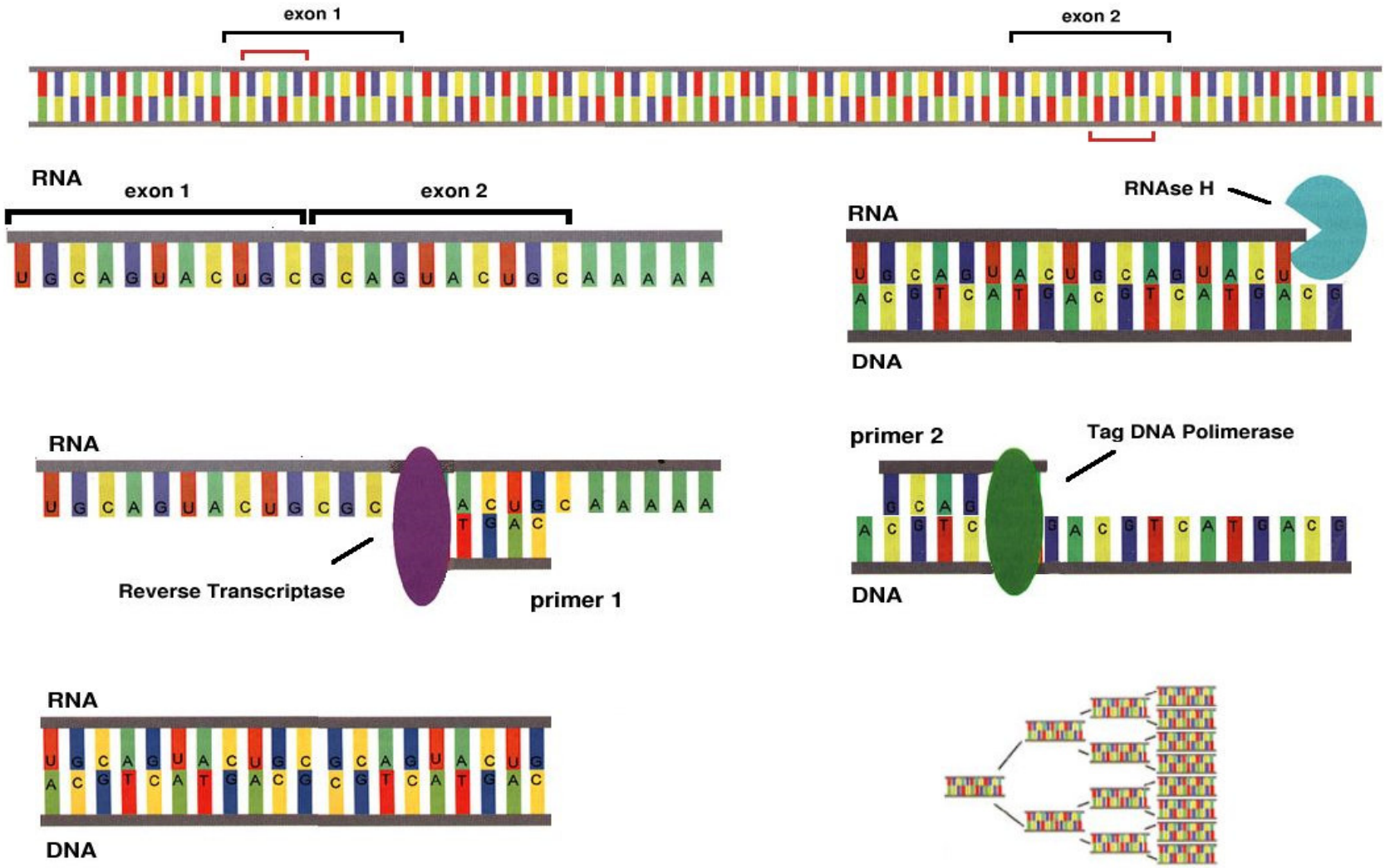




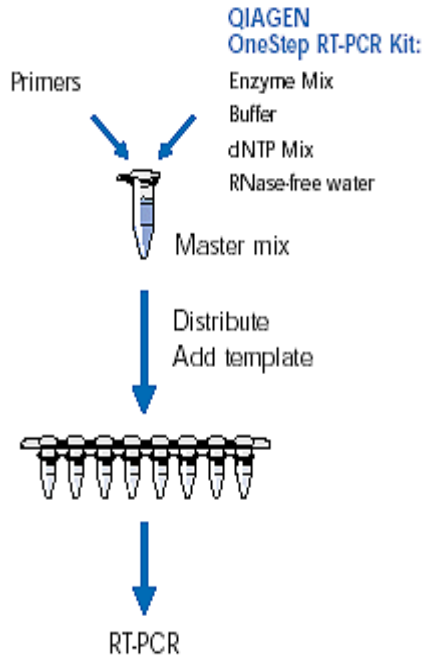
David Baltimore
1975 Nobel Prize
in Medicine

VirginiaTech
Invent the Future

RT–PCR= reverse transcriptase PCR



- One step RT–PCR
- Two steps RT and PCR

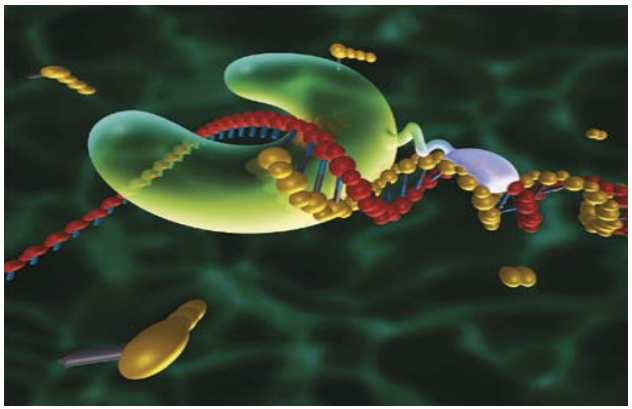


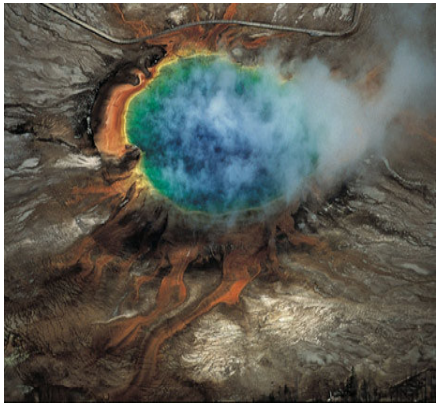
VirginiaTech
Invent the Future

DNA Polymerase

***T**hermus **aq**uaticus*
Taq enzyme
Heat stable
1988

Error 1:10,000

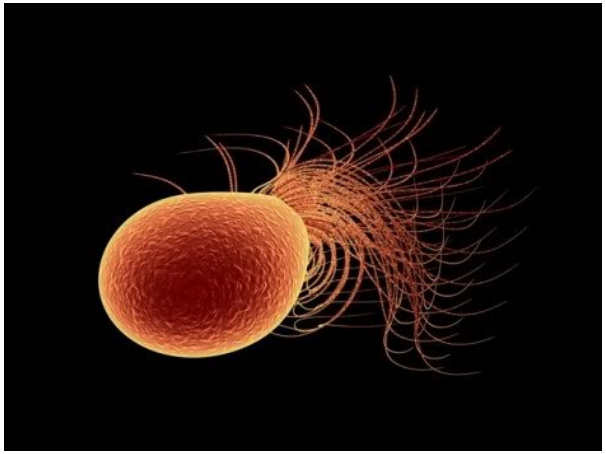




Hot spring

***P**yrococcus **fu**riosus*
Pfu enzyme
More stable
1991

Error 1: Million



3' to 5' exonuclease proofreading activity

VirginiaTech
Invent the Future

Things to avoid in Primer Design

A primer may be self-complementary and fold into a **hairpin**:

5-GTTGACTTGATATTATCAAG-3

5´-GTTGACTTGATA
|||||| T
3´-GAACTAT

A primer may form a **dimer** with itself or with the other primer.

5´-ACCGGTAGCCACGAATTCGT-3´

5´-ACCGGTAGCCACGAATTCGT-3´
||||||||||||
3´-TGCTTAAGCACCGATGGCCA-5´

VirginiaTech
Invent the Future

PCR Cycles and volume

More much
better than
little

Increase cycle to 40 cycles
Increase volume to 100 µl

VirginiaTech
Invent the Future

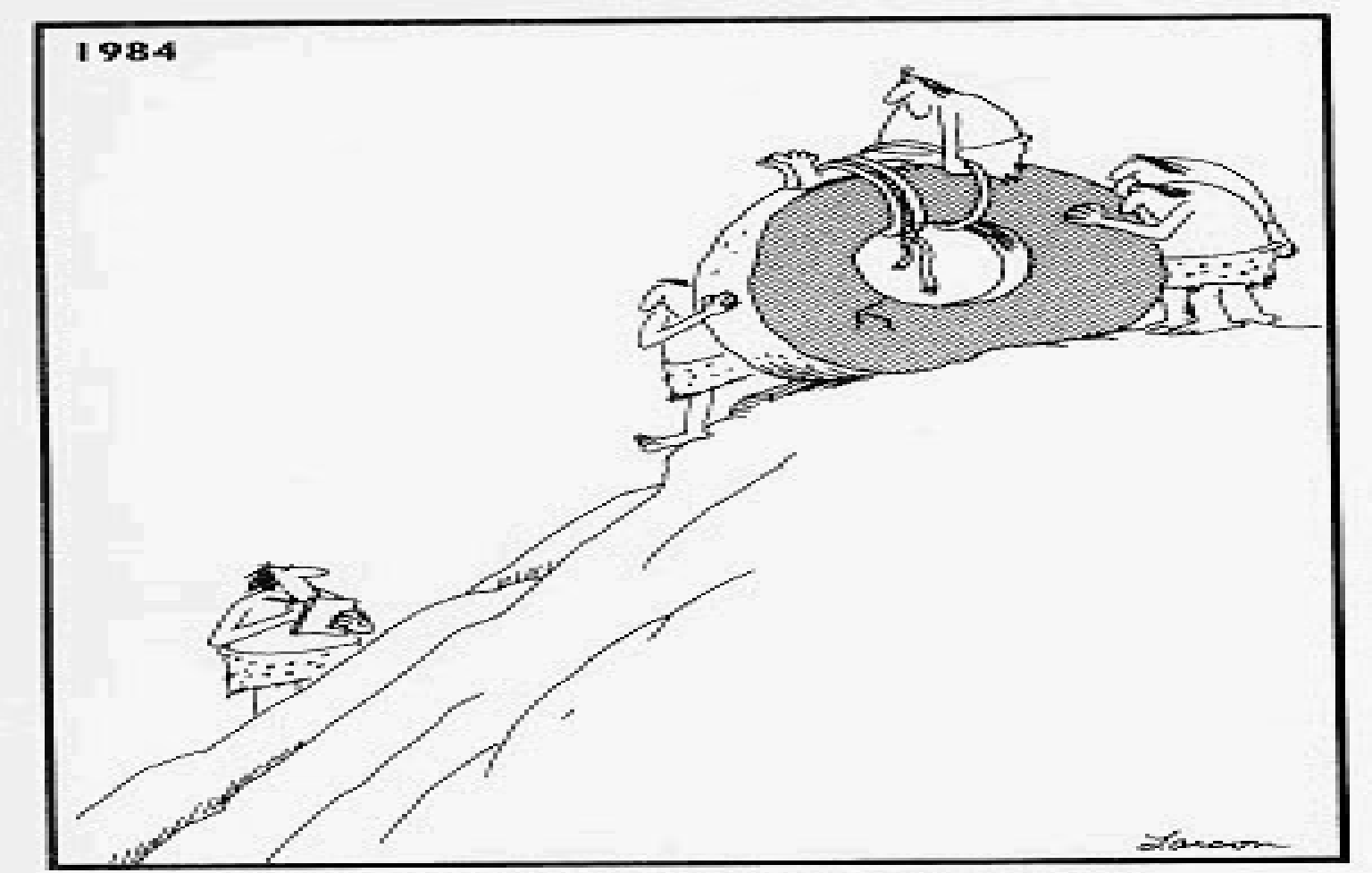
Overnight Thermocycler

Last step incubation

- 10 °C for gene cloning
- Room temperature for diagnosis

VirginiaTech
Invent the Future

Questions!!!



Early experiments in transportation