Molecular Biology Research Unit

Application of Real Time PCR

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Advantages of qRT-PCR

1. Rapid cycling times (1 hour).
2. High sample throughput (~200 samples/day).
3. Low contamination risk (sealed reactions).
4. Very sensitive (3pg or 1 genome eq of DNA).
5. Broad dynamic range (10 - 10^{10} copies).
6. Allows for quantitation of results.
7. Software driven operation.

Disadvantage

- **Current technology has limited capacity for multiplexing.**

  Simultaneous detection of 2 targets is the limit.

- **Development of protocols needs high level of technical skill and/or support.**

- **High capital equipment costs ($ 50,000 - 160,000).**

Application

The exponential amplification via reverse transcription polymerase chain reaction provides for a highly sensitive technique in which a very low copy number of RNA molecules can be detected.

There are different applications for quantitative polymerase chain reaction (RT-PCR).

Application

It is commonly used for:

1. Diagnostic uses.
2. Food safety and Industry include the quantification of microbial load in foods.
3. Gene expersion.
4. Basic research.
5. Agriculture.
6. GMOs (Genetically modified organisms).
7. Drug industry.
8. In forensic Medicine.

1. Diagnostic uses

qRT-PCR usually applied for rapid detection as well as quantification of nucleic acids of different pathogens.

A. Diagnosis of infectious diseases especially newly emerging diseases, such as new strains of flu.

B. Diagnosis of Virus

- **Viruses can be present in humans due to direct infection or co-infections which makes diagnosis difficult using classical techniques and can result in an incorrect prognosis and treatment.**
- **The use of qPCR allows both the quantification and genotyping (characterization of the strain).**
- **Detect anti-viral drug resistance ≠ Detect anti-viral drug efficacy.**

C. Cancer Detection

Diagnosis, prognosis and monitor response to therapy.

Circulating tumor cells produce unique mRNA transcripts depending on the type of cancer, this serve as the best biomarkers for a particular cancer cell type and then analyze its expression levels with RT-PCR.

2. Microbiological uses and food industry (Microbial load testing):

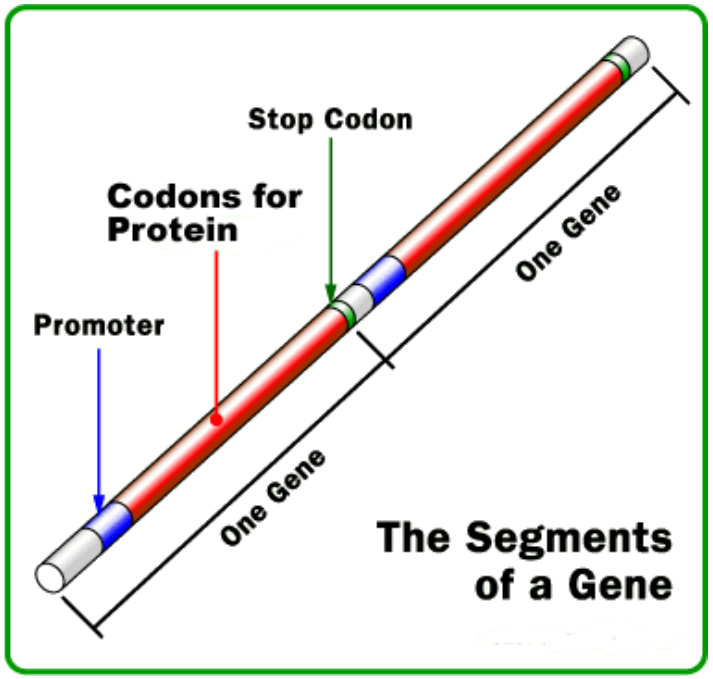
Microbial risk assessment:

- **Food safety, food spoilage and fermentation.**
- **Water quality for public health protection.**

For commensal organisms determine a "normal" microbial load.

- **Detect active infection by increasing load**

3. Uses in research (Gene expression)



- Upstream region of a gene, which regulates when/where the gene is to be transcribed.
- Essentially the promoter region acts as the "on switch" for the gene.

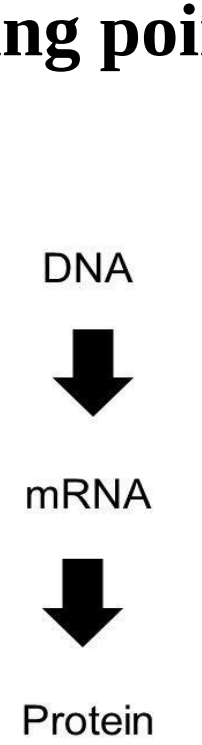
Gene expression

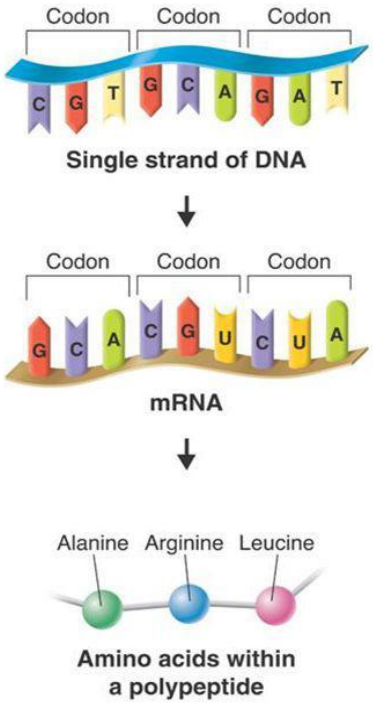
- **Reverse transcription (RT) followed by polymerase chain reaction (PCR) represents a powerful tool for the detection and quantification of mRNA.**
- **It is the most sensitive method for the detection and quantification of gene expression levels, in particular for low abundant transcripts in tissues with low RNA concentrations, from limited tissue sample.**

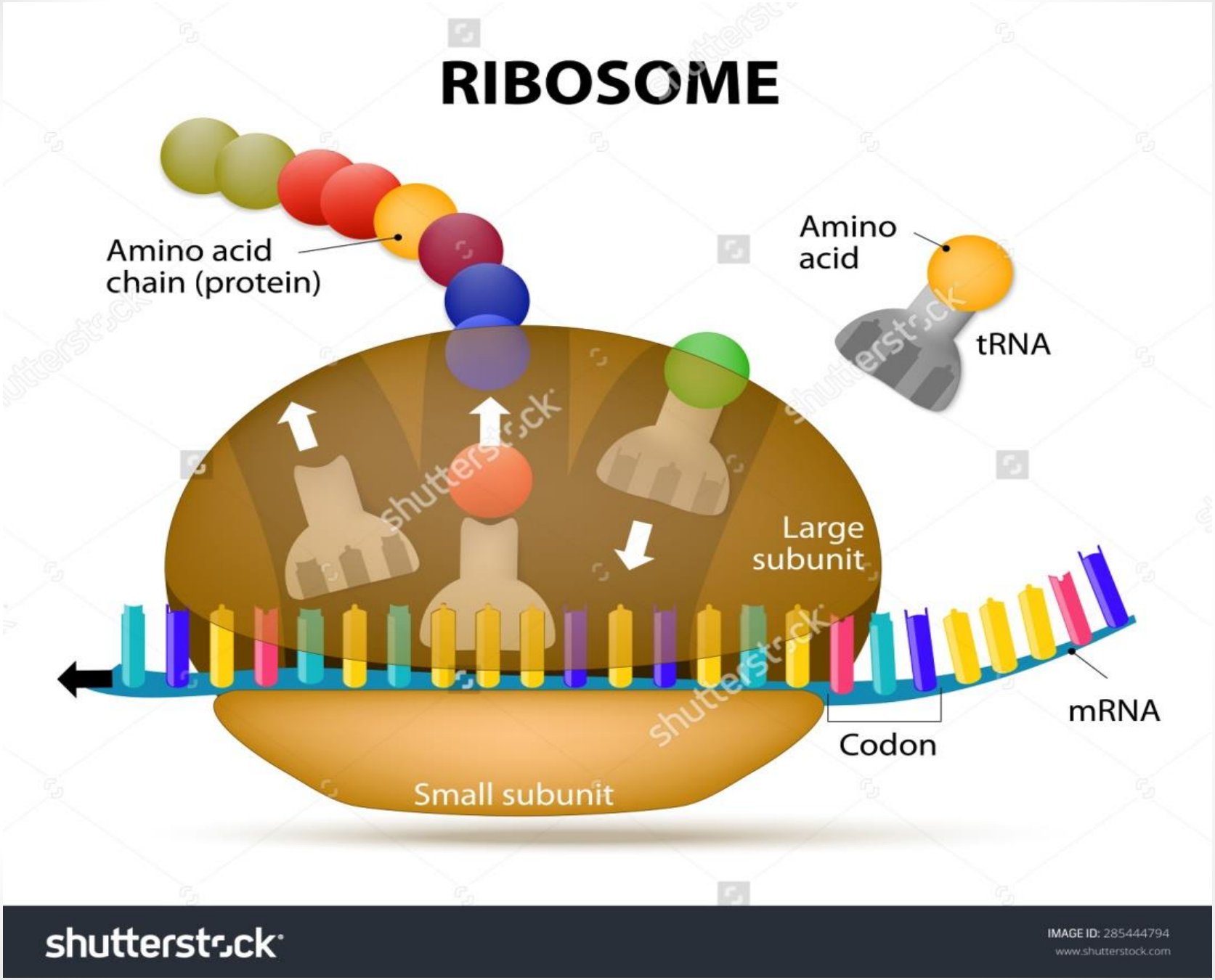
Gene expression studies by quantification of messenger RNA:

Presence of mRNA is:

- **Starting point for determining expression.**






RIBOSOME
Amino acid chain (protein)
Amino acid
tRNA
Large subunit
mRNA
Codon
Small subunit
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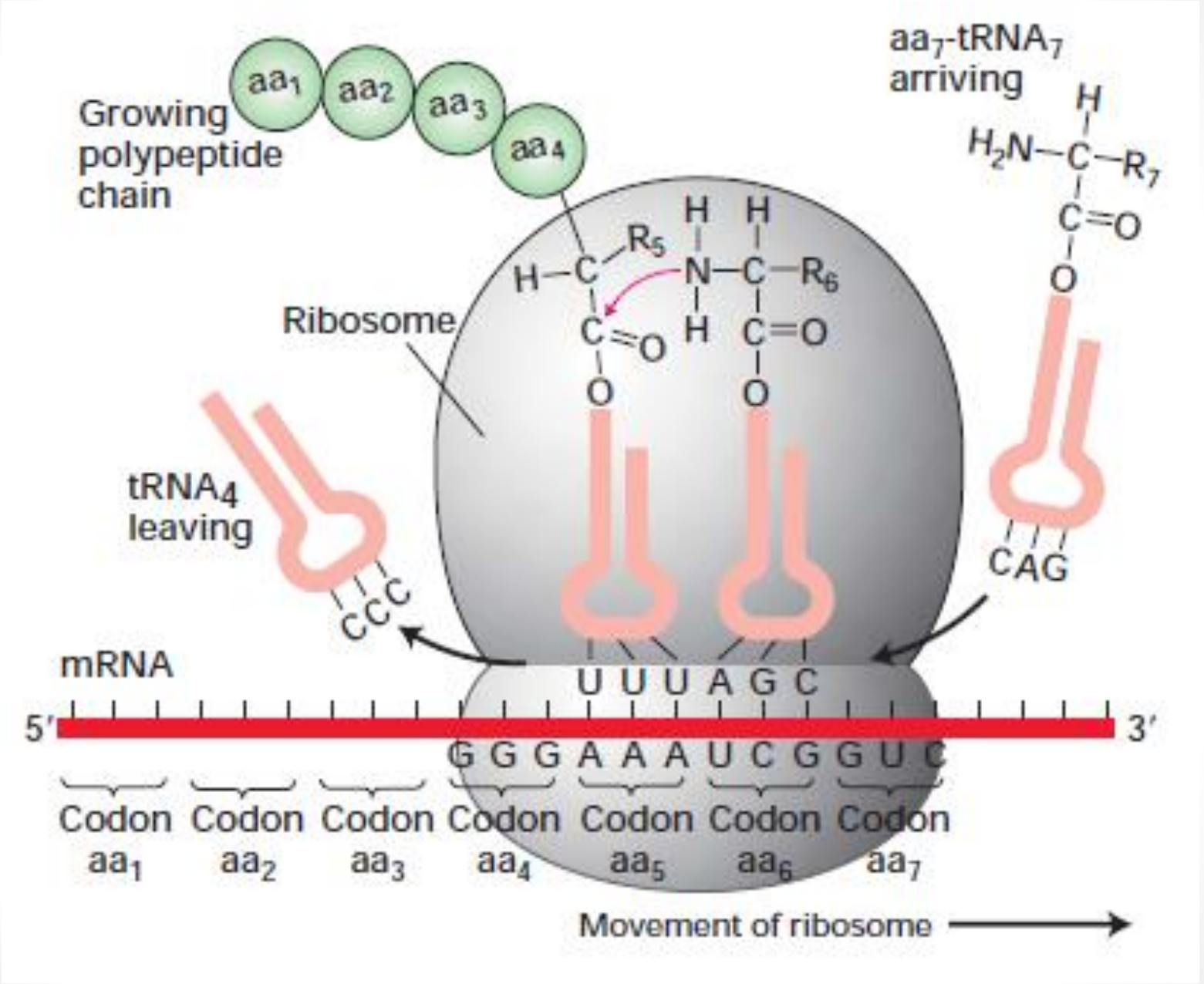
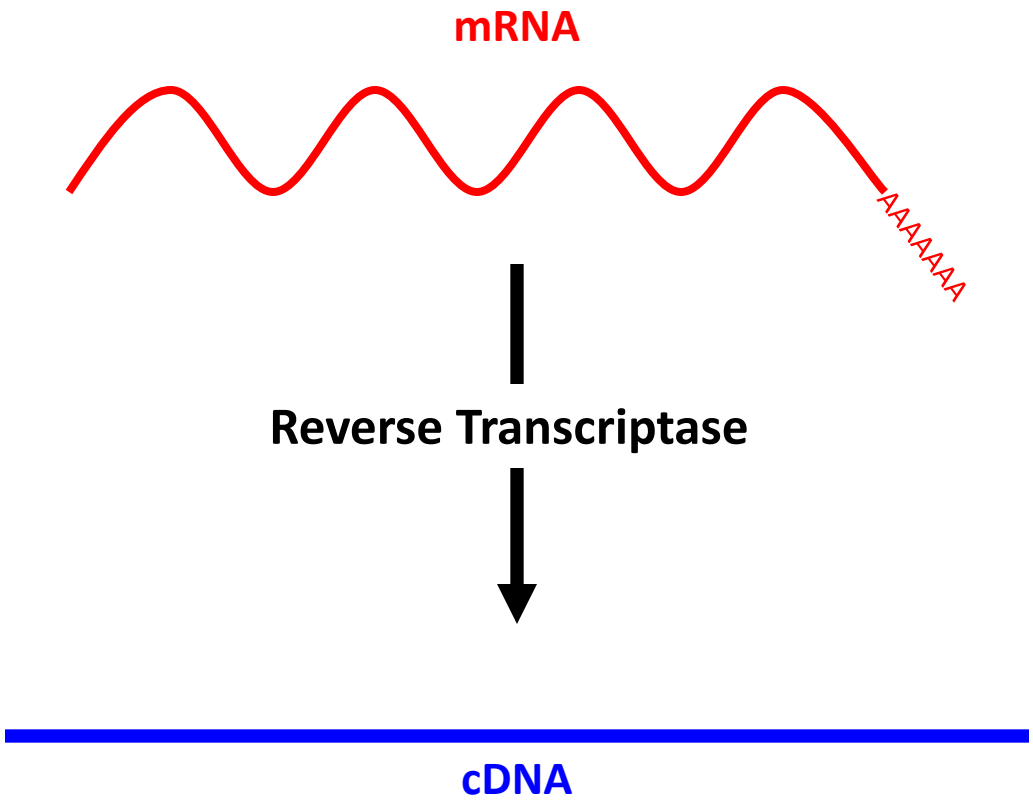
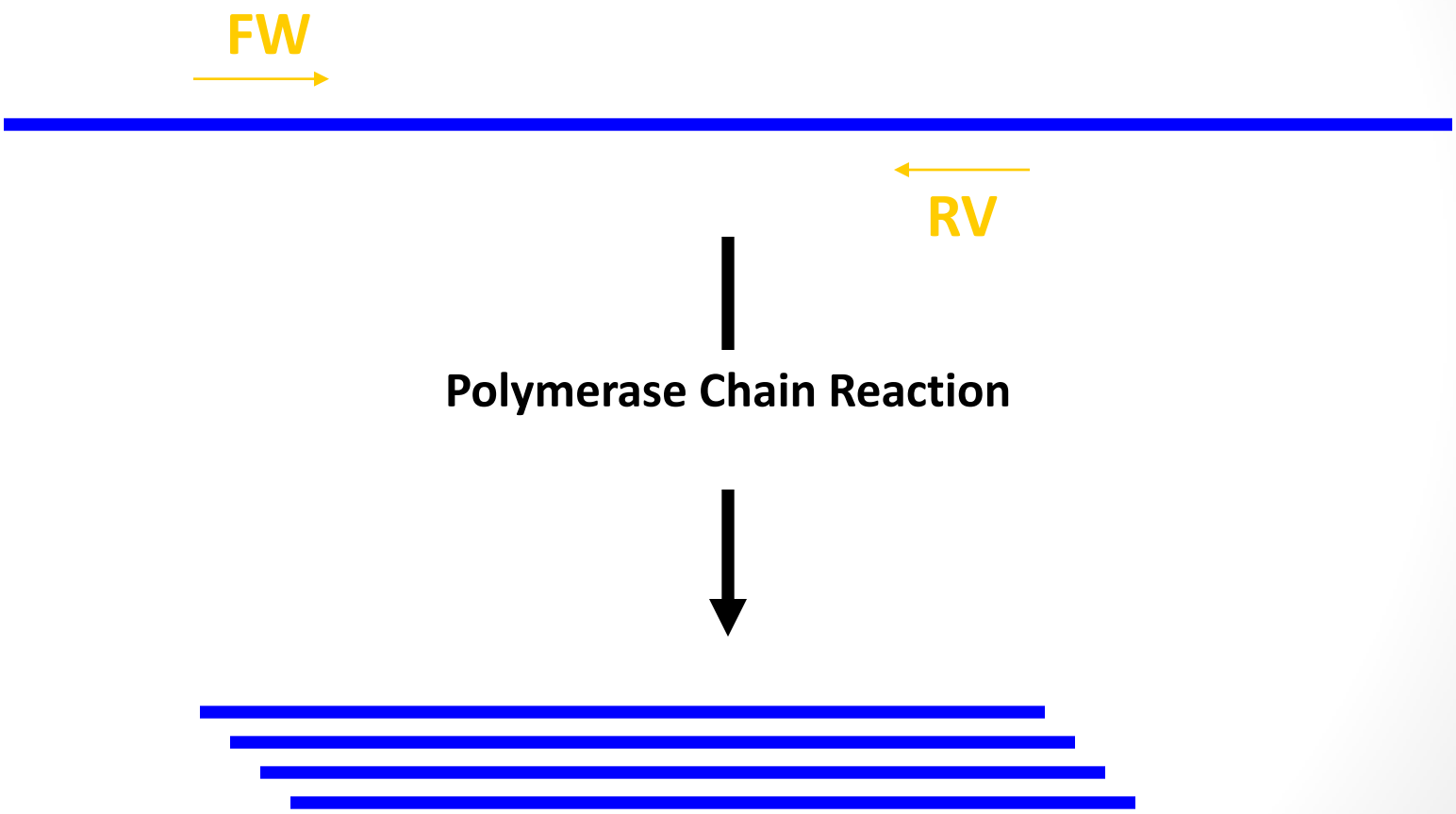

Growing polypeptide chain
aa1
aa2
aa3
aa4
aa7-tRNA7 arriving
Ribosome
tRNA4 leaving
CCC
CAG
mRNA
5′
3′
U U U A G C
G G G A A A U C G G U C
Codon aa1
Codon aa2
Codon aa3
Codon aa4
Codon aa5
Codon aa6
Codon aa7
Movement of ribosome

Synthesize cDNA Using RT



Amplify cDNA Using Primers



4. Uses in research

Gene transcription/Expression.

A. Determining how the gene expression and gene changes over time, such as in:

1. The response of tissue and cell cultures to an administration of a pharmacological agent,
2. Progression of cell differentiation,
3. Response to changes in environmental conditions.

Uses in research

B. It is also used for the determination of zygosity (Zygosity is the degree of similarity of the alleles for a trait in an organism) of transgenic animals used in research.

C. Gene Insertion

RT-PCR can also be very useful in the insertion of eukaryotic genes into prokaryotes.

4. Agriculture

A. Detection of phytopathogens

The agricultural industry is constantly striving to produce plant propagules or seedlings that are free of pathogens in order to prevent economic losses and safeguard health.

- **Discrimination between the DNA of the pathogen and the plant is based on the amplification of specific sequences in ribosomal RNA gene's coding area, which are characteristic for each taxon.**

Genetically modified organisms (GMO)

- **A genetically modified organism (GMO) is a living organism, e.g. a plant, whose genetic composition has been altered by means of gene technology.**
- **The genetic modification usually involves insertion of a piece of DNA (the insert), a synthetic combination of several smaller pieces of DNA, into the genome of the organism to be modified.**
- **These smaller pieces of DNA are usually taken from other naturally occurring organisms.**

Genetically modified organisms (GMO) :

- **A typical insert (gene construct) in a GMO is composed of three elements:**

1) **The promoter element functions as an on/off switch for reading of the inserted/altered gene.**
2) **The gene that has been inserted.**
3) **The terminator element functions as a stop signal for reading of the inserted/altered gene.**

5. Detection of genetically modified organisms (GMOs):

qPCR using reverse transcription (RT-qPCR) can be used to detect GMOs given its sensitivity and dynamic range in detecting DNA.

5. Detection of genetically modified organisms (GMOs):

- **Specific primers are used that amplify the promoter, terminator or even intermediate sequences used during the process of engineering the vector.**
- **This is often carried out by relative quantification using a control gene from the treated species that is only present as a single copy**

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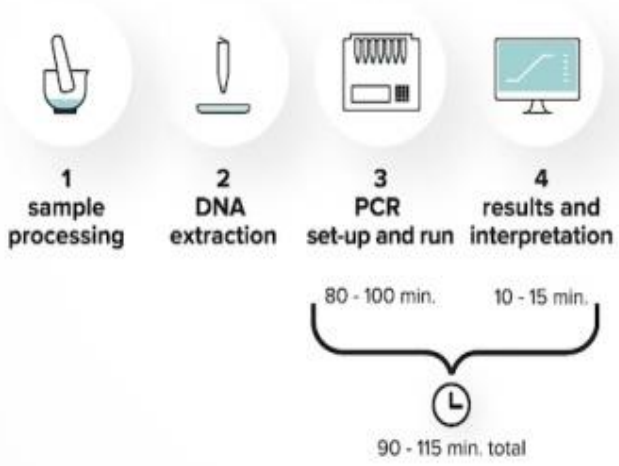
FOOD SAFE LINE

4 STEPS FOOD SAFE WORKFLOW



[NEW !]

GMO DETECTION KITS

BIOPFS-0016 • Food safe P35S and T-NOS detection kit

BIOPFS-0017 • Food safe P35S, T-NOS and P-FMV detection kit

BIOPFS-0018 • Food safe P-FMV detection kit

Biopremier food safe GMO detection kits are based on 5' nuclease real-time PCR reations to amplify unique genomic sequences and enable the simple and reliable detection of Genetic Modified Organisms in food. Also suitable to be used with animal feedstuff and other samples in which a rapid screening for GMO occurrence is needed.

- ✓ **Compatible with instruments working in FAM and ROX channels**
- ✓ **Method validated as recommended by CRL-GMFF**
- ✓ **Detects low amounts of target DNA**
- ✓ **Ready to use format**
- ✓ **100% specificity**

For development pipeline contact tech.support@biopremier.com

6-pharmatheutical uses: Detection of Anti-inflammatory effects of peptides

- **The epithelium lining the airways is the first tissue to encounter pathogens and their products; it is therefore, critical to the innate immune system and is the front line of the host defense.**
- **Some peptides which are classified in an antimicrobial group , can have an effect on immune system, for example Intercellular adhesion molecule-1(ICAM-1) is expressed at a low level in a subpopulation of haematopoietic cells, vascular endothelium, fibroblasts and epithelial cells.**

6-pharmatheutical uses:
Detection of Anti-inflammatory effects of peptides

- **However, its expression is dramatically increased at sites of inflammation.**
- **Increase in Expression of 1(ICAM-1) will be lowered due to the effect of antimicrobial peptide & this can be detected by Real time PCR using SYBR Green.**

7. Real time PCR in forensic medicine

- **The main task in The Forensic Medicine is to investigate deaths from unnatural causes.**
- **Forensic science has embraced the use of DNA molecular biology tools for diagnostic purposes.**
- **The process of routine forensic human identification involves sensitive PCR and can be performed successfully on most evidence materials found at a crime scene.**
- **This real-time technology monitors the accumulation of PCR product with each cycle and allows assessment of each sample individually during the exponential growth phase. quantification assay has proven to be highly sensitive, specific, rapid, cost-effective and flexible assay for analysis of forensic casework samples.**

7. Real time PCR in forensic medicine

DNA tests performed by a U.S. laboratory have proved that bone fragments exhumed in the Ural Mountains in 2007 belong to two children of Russia's last czar.

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