EVALUATION OF PCR AND REAL-TIME PCR METHODS FOR RAPID DETECTION OF FOODBORNE PATHOGENS

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Polymerase Chain Reaction (PCR)

- accepted as reliable for both rapidly determining the presence or absence and rapid identification of specific pathogens

PCR in Food Microbiology Research

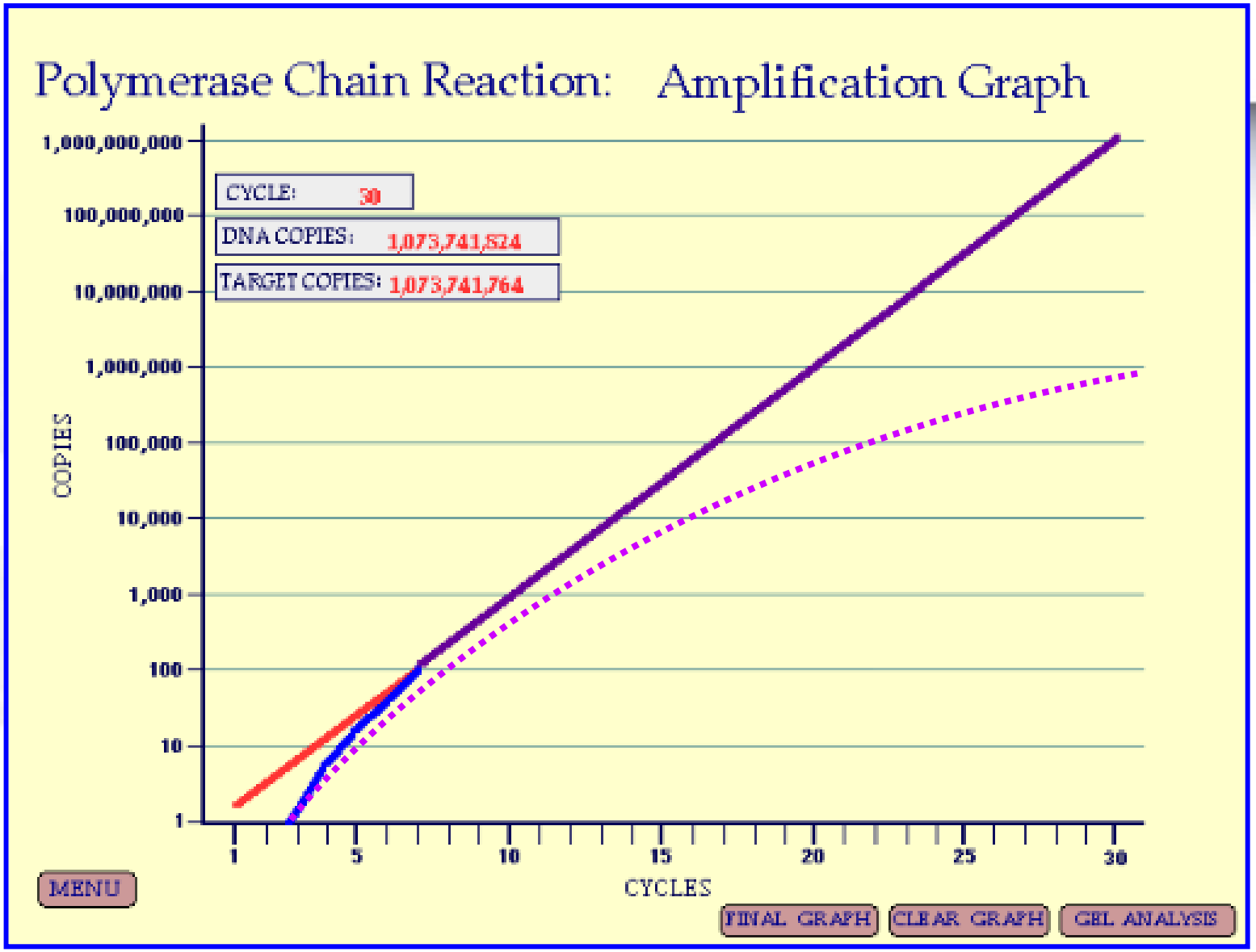
- rapidly detects low numbers of all known foodborne pathogens in food samples without the traditional, more taxing methods of cultivation and phenotypic characterization

What is PCR?

- rapidly copying and amplifying a selected template sequence from a pool of DNA *in vitro*
- involves a polymerase
- the products synthesized in each cycle can serve as templates in the next so the number of DNA copies approximately doubles at every cycle to create a chain reaction

The principle of PCR

- involves a repetitive series of cycles each of which consists of template denaturation, primer annealing and extension of the annealed primers by a DNA polymerase


Polymerase Chain Reaction: Amplification Graph
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
COPIES
CYCLE: 30
DNA COPIES: 1,073,741,824
TARGET COPIES: 1,073,741,764
1
5
10
15
20
25
30
CYCLES
MENU
FINAL GRAPH
CLEAR GRAPH
GEL ANALYSIS

Post-PCR Processing

the use of agarose gel electrophoresis for the visualization of the amplified target DNA fragments and further verification by sequencing or by hybridization of the amplified product with a DNA probe specific for the target fragment

Classical PCR Analysis of A Food

isolation of DNA from the food

amplification of the target sequences by PCR

agarose gel electrophoresis

verification of the PCR results

by specific cleavage
of the amplification products
by restriction endonuclease

by transfer of separated
amplification products onto
membranes (Southern Blot)
+ hybridization with a DNA probe
specific for the target sequence

by direct sequencing

by second nested or semi-nested PCR

Multiplex PCR

- adding of several primer pairs specific to the same or different pathogens but with similar annealing requirements to a PCR mixture to simultaneously detect several target sequences

Advantages of Multiplex PCR

- to save time
- to minimize the expense on detection of foodborne pathogens

In Food Microbiology

Advantages of PCR

- accurate
- reliable
- rapid
- specific

Disadvantages of PCR

- unable to differentiate viable and non-viable microorganisms in foods
- false-negative
- false-positive results

What is real-time PCR?

uses fluorescence to detect the presence or absence of a target amplified gene in real time

Advantages of real-time PCR

- accurate quantification
- viewing the increase in the amount of template as it is amplified
- a lower risk of contamination
- no need to use mutagenic staining dyes
- eliminate the need for time-consuming post-PCR processing
- high sensitivity
- high specificity

Disadvantages of real-time PCR

- require more expensive laboratory equipment