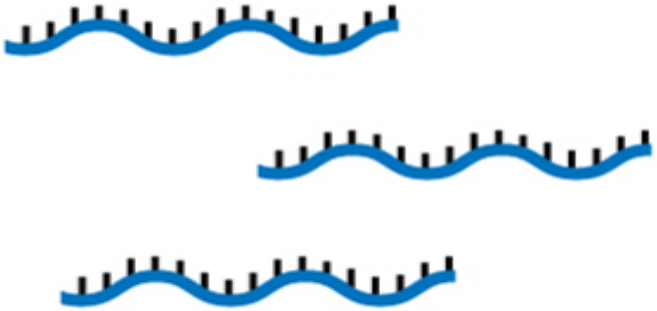
KGD lab workshop in 2025

Wan-Rung Chen

Quantitative Real-time PCR

Immunofluorescence



Quantitative Real-time PCR

- **Quantitative polymerase chain reaction (qPCR)** is a laboratory technique of molecular biology based on the PCR.
- It is a highly **sensitive** and **reliable** method for the detection and quantification of nucleic acids (DNA, RNA and cDNA).
- There are two common methods for the detection of products in real-time PCR.

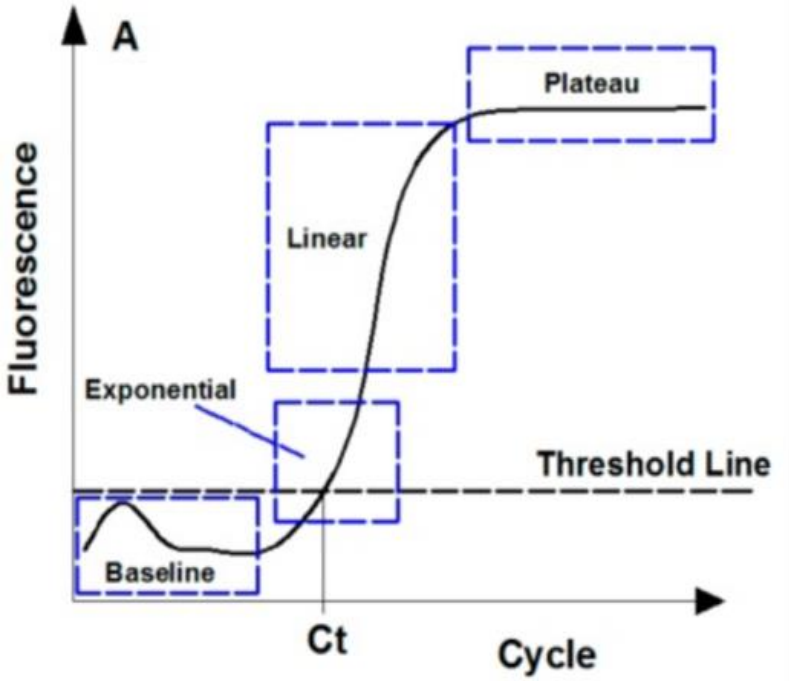
- **SYBR Green Dye**
- **TaqMan Probe**

SYBR Green

TaqMan

Three Phases of qPCR

- Exponential
  - Exact doubling of product
  - Reaction is very precise and specific
- Linear
  - The reaction components are becoming limited
  - The reaction efficiency is dropping
- Plateau
  - The reaction has stopped
  - No more products are being made



Yiqi Cao *et al*, *molecules*. 2020 Feb 6;25(3):706.

Ct value and Threshold in qPCR

- Threshold : The horizontal line in the amplification plot's y-axis marks the detection level, where fluorescence surpasses background levels.
- Ct : The number of reaction cycles it takes to reach that threshold is called Ct value.

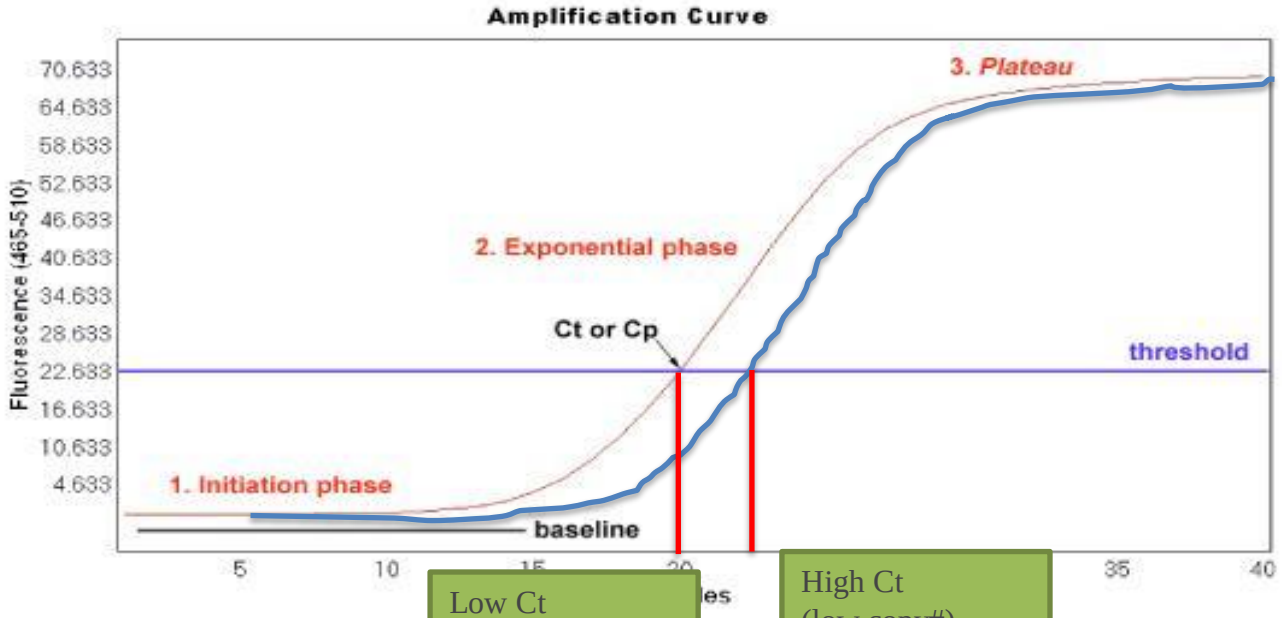
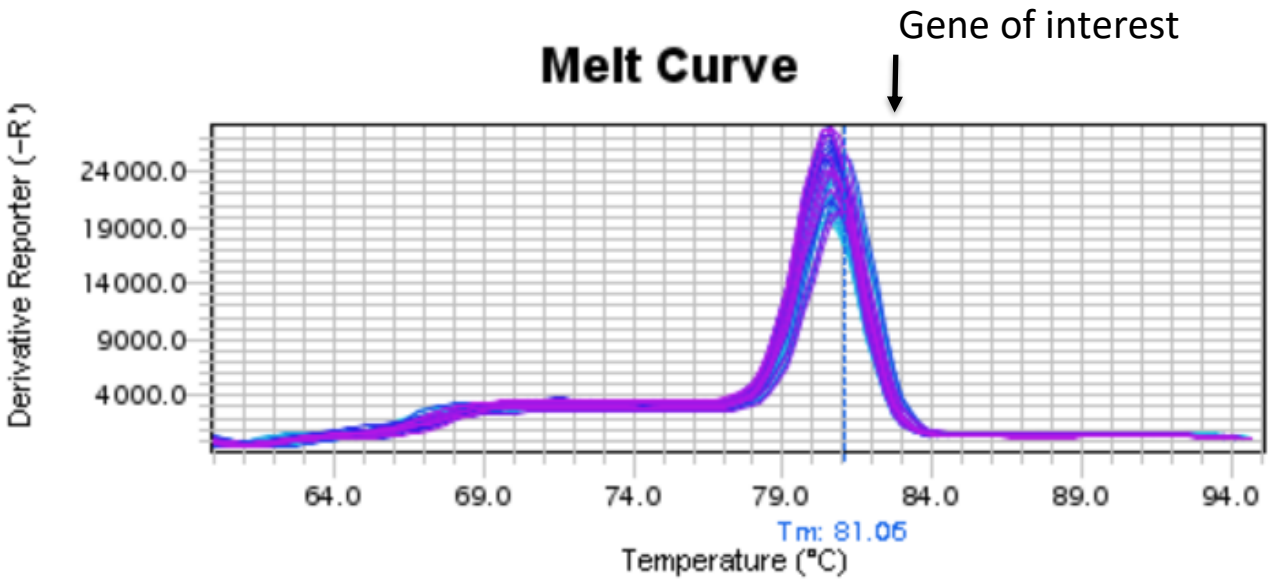


Figure 11. Phases of a PCR amplifica cation e le. Red: negative control.

Melt curve in qPCR

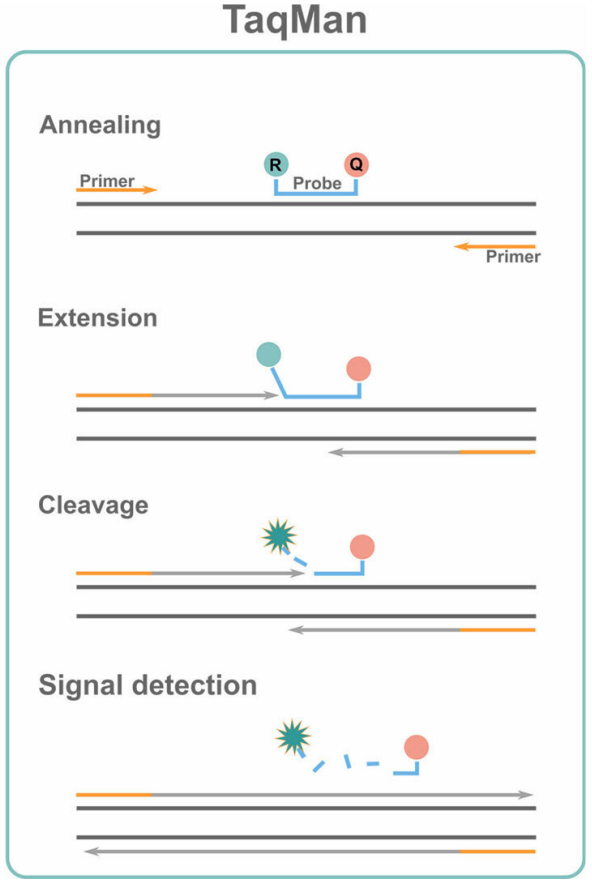
- It is used to assess the dissociation characteristics of double-stranded DNA during heating.
- Using it to determine the specificity of the qPCR assay.
- A single distinct peak in the plot indicates that the amplified double-stranded DNA products are a single discrete species.



Types of qPCR Techniques

TaqMan Probe

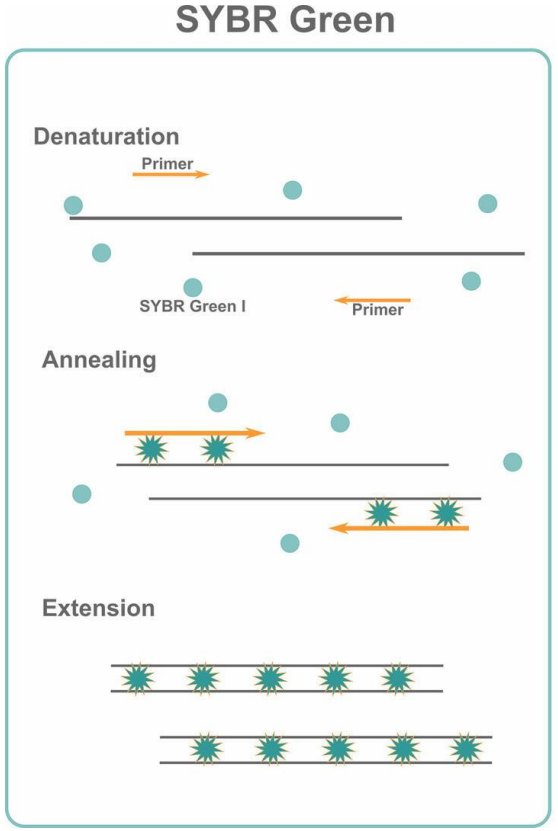
- Fluorophores is linked to oligonucleotides
- Uses specific probes to detect target genes
- Advantages: High specificity, High accuracy.
- Disadvantages: Higher cost



Types of qPCR Techniques

SYBR Green Dye

- Fluorescent dye binds to the minor groove of double-stranded DNA
- Advantages: Lower cost, simple operation
- Disadvantages: Lower specificity, potential for non-specific qPCR products (ex: primer dimers).

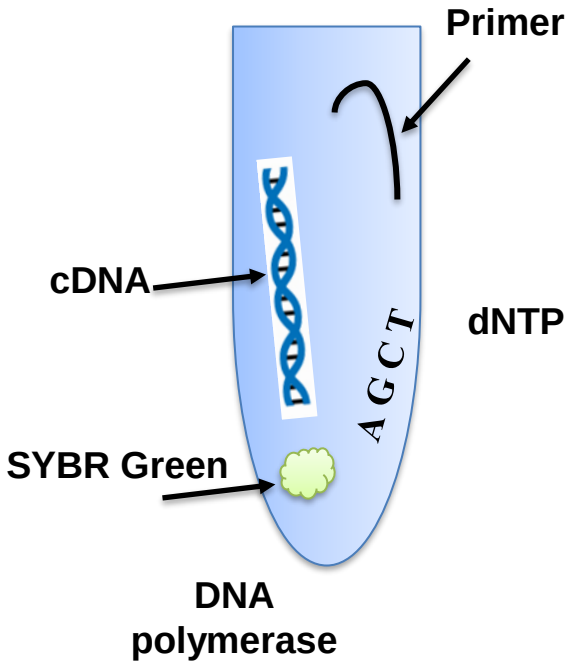


Components of qPCR

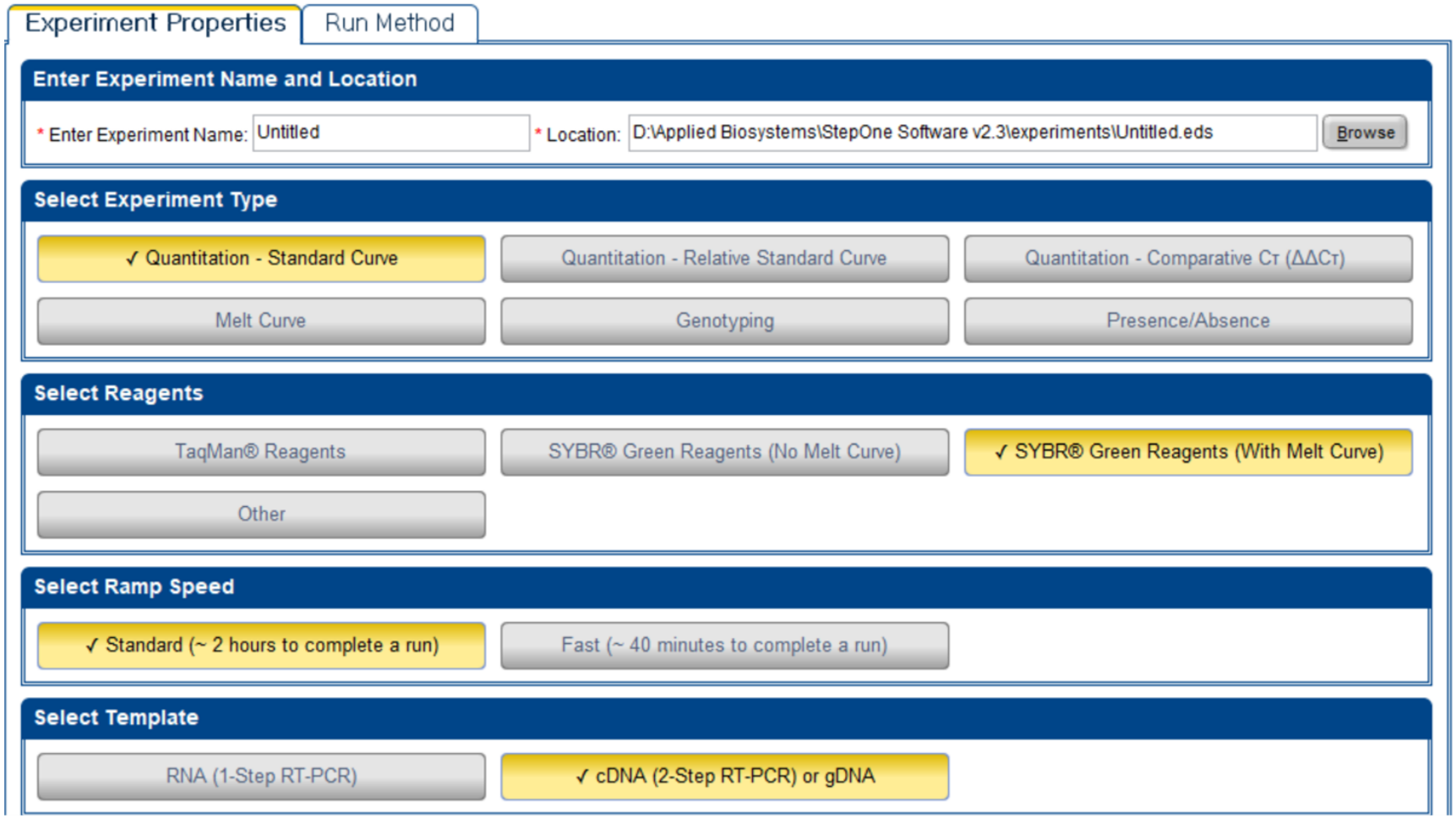
SYBR Green

2x SYBR Master Mix	1x	12.5 ul
F Primer	optimized	NA
R Primer	optimized	NA
Water		NA
cDNA	10-100 ng	5-10 ul
		25 ul

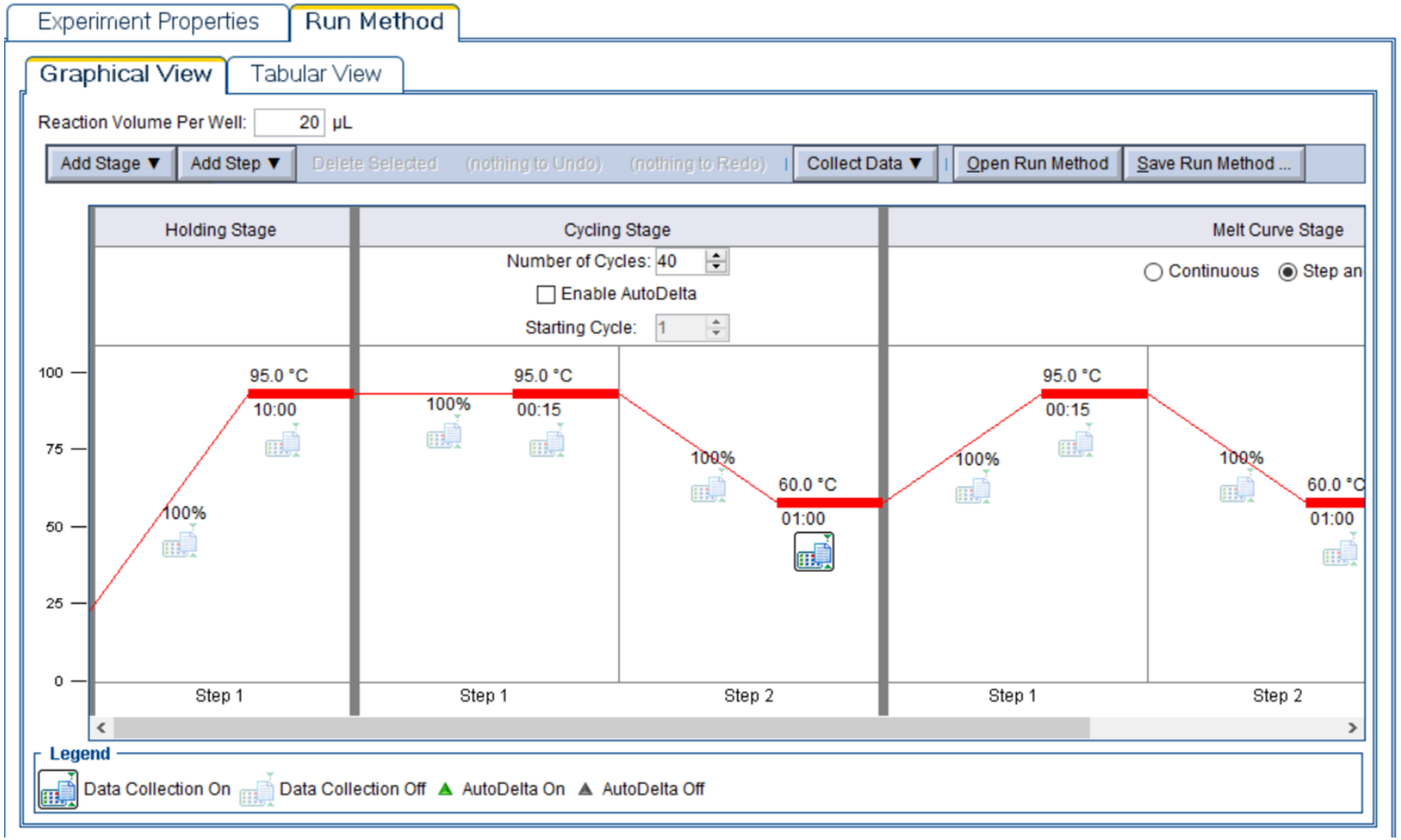
Components



StepOne™ Real-Time PCR System



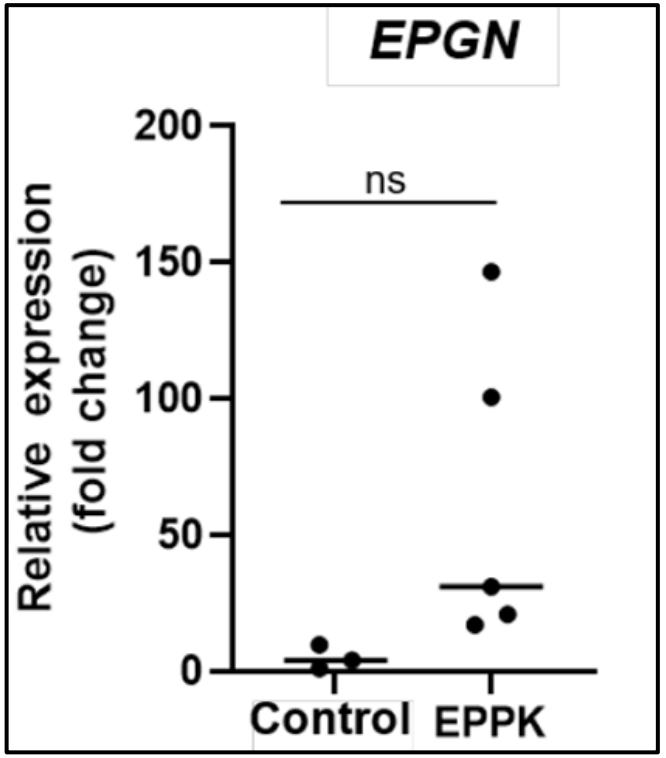
StepOne™ Real-Time PCR System



qPCR data analyze

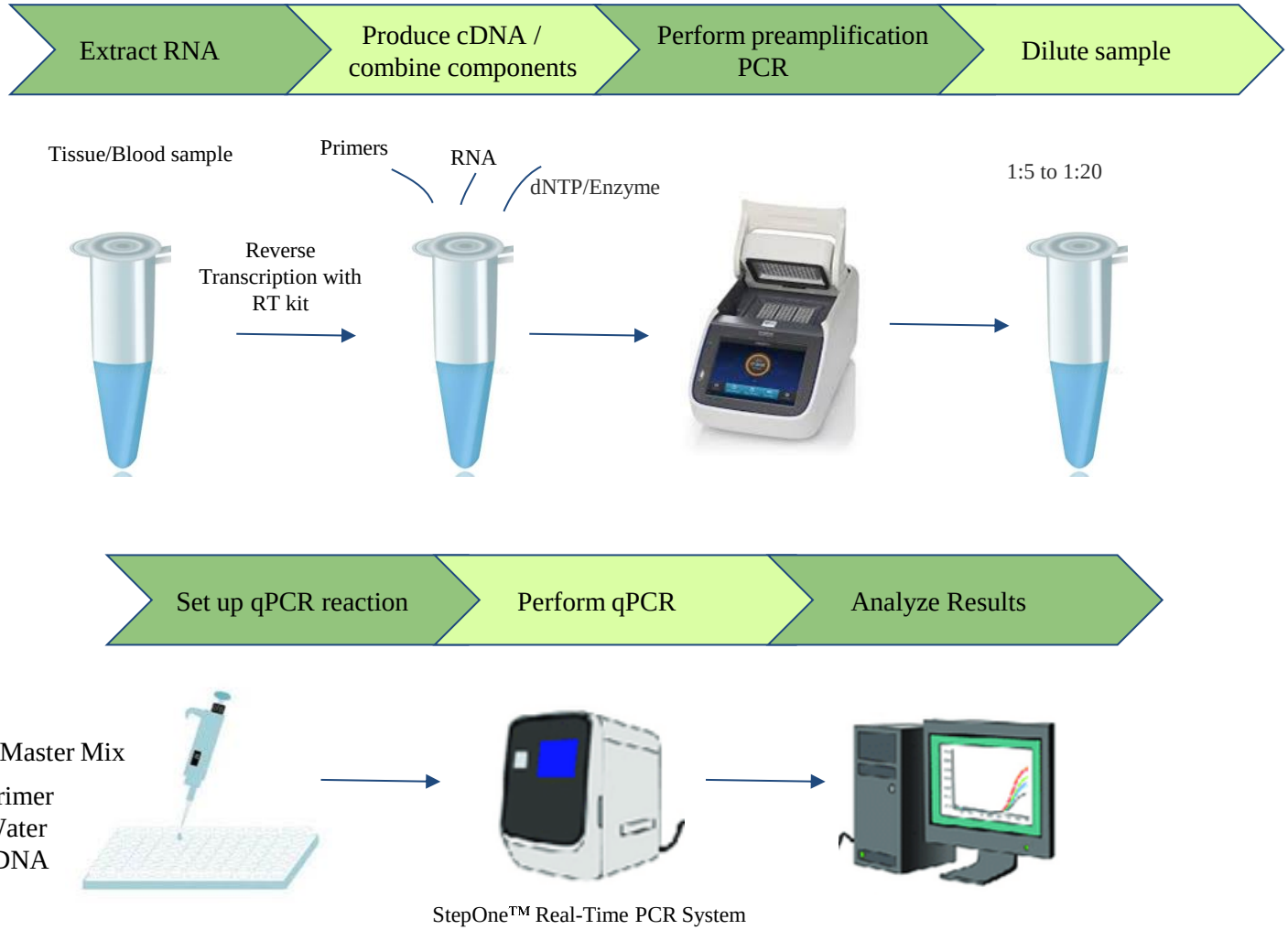
Calculation Formula

- **ΔCt**=
  Ct (Target gene)−Ct (reference gene)
- **ΔΔCt**=
  ΔCt (experimental group)−ΔCt (control group)
- **RQ (Fold change)** =2^−ΔΔCt



GraphPad Prism software

Workflow of qPCR



Immunofluorescence (IF)

Principle of Immunofluorescence

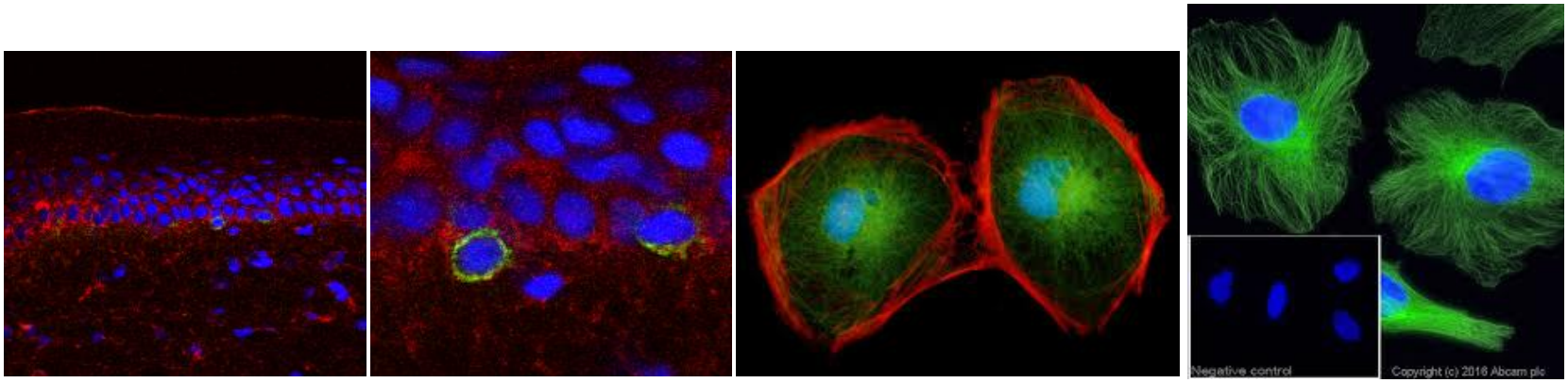
Direct and Indirect Immunofluorescence

Step by step procedure of IF

Immunofluorescence (IF)

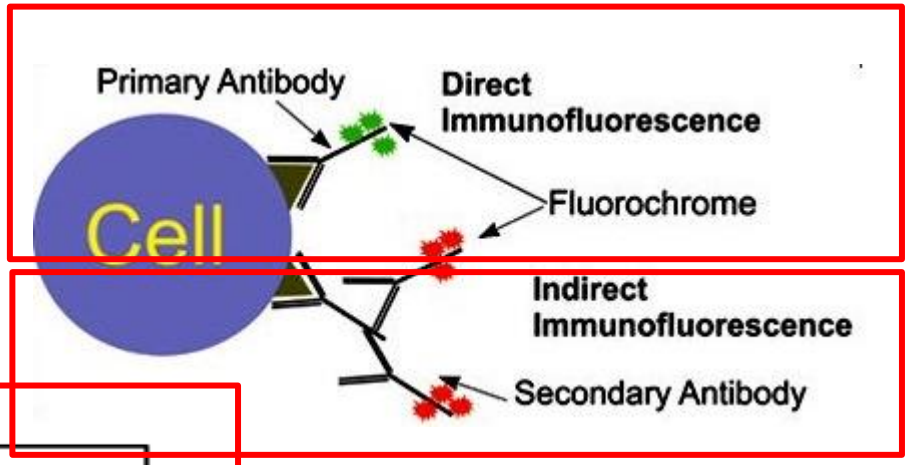
- Immunofluorescence is a powerful technique that use fluorescent-labeled antibodies to detect specific target antigens.

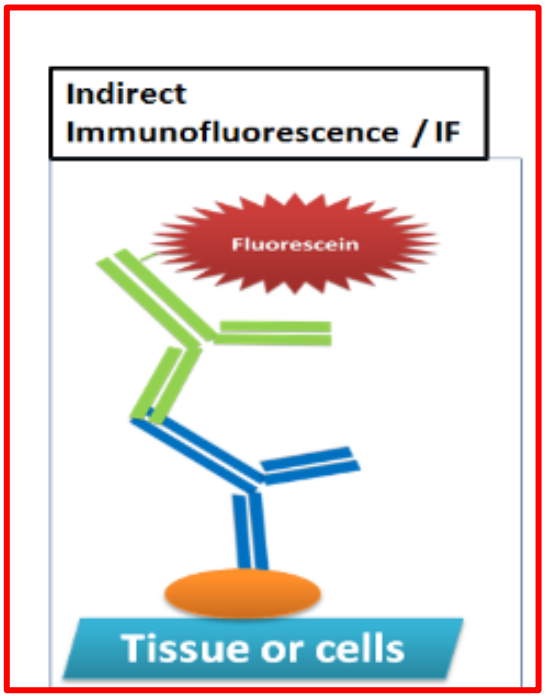
- Direct immunofluorescence
- Indirect immunofluorescence

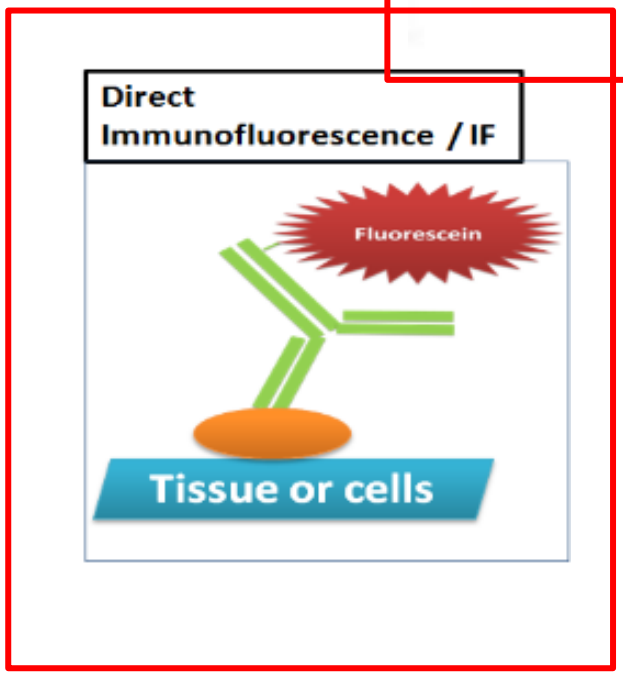


Types of Immunofluorescence

- Direct immunofluorescence
- Indirect immunofluorescence

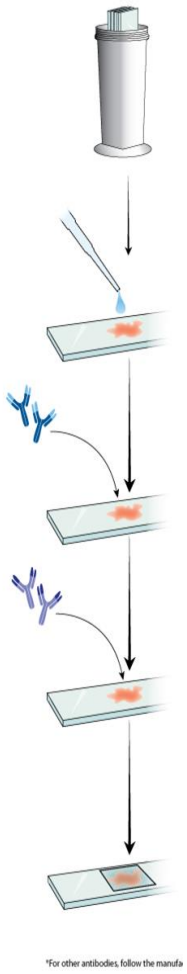






http://www.sinobiological.com/what-is-immunofluorescence-if.html

Procedure of Immunofluorescence



冷凍包埋/切片

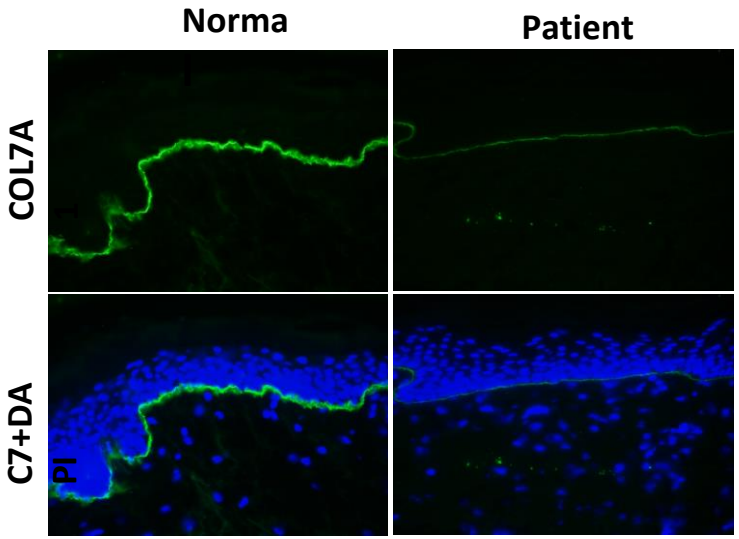
Tissue Fixation:
Acetone:Methanol=1:1, -20℃ for 10-15 min
Wash: 3 times for 5 min

Blocking: with Blocking buffer for 30 min at Room temperature
Wash: 3 times with washing buffer for 5 min

Primary antibody: Incubate at 4 ℃ overnight or 4 hr at 37 ℃
Wash: 3 times with washing buffer for 5 min

Secondary antibody/DAPI:
Incubate at 37 ℃ for 1 hr
Wash: 3 times with washing buffer for 5 min

Mount Sample:
Mounting media
Image:
microscopy



Thanks for your attention