In situ PCR

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Definition

- ***in Situ* Polymerase Chain Reaction (*In Situ* PCR)** is a technique where **PCR** is carried out in a section of **tissue** within a cell.

- It is a powerful method that detects minute quantities of rare or single-copy number nucleic acid sequences in *frozen* or *paraffin-embedded* cells or tissue sections for the **localization** of those sequences within the cells.

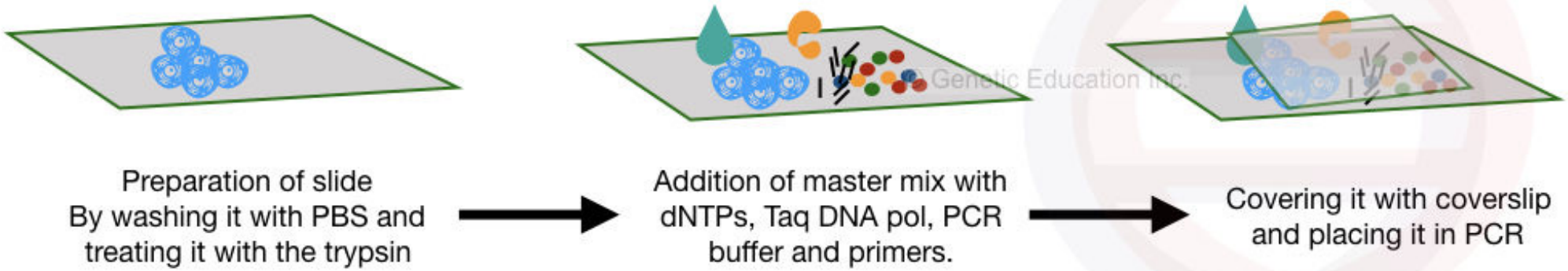
- If our sample is a layer of cells, the biological reaction taken place **on the surface** of the cell layer, is ***in situ.***

In-situ means "in the original place". In the biology context, this means in the cell, body or tissue.

PCR  ISH

In situ PCR is a **histological** technique that combines the extreme **sensitivity** and power of detection (DNA or mRNA) of **PCR** with the cellular **localization** (in cell organelles, intact cells, or tissue sections) provided by ***in situ* hybridization (ISH),** through the amplification of specific gene sequences within intact cells or tissue sections and increasing copy numbers to levels detectable by **ISH** or **immunohistochemistry**.

The three Steps



1. The tissue is **fixed** on the slide. After the tissue is **cleaned**, The **proteolytic enzymes** (Pepsin, trypsin and proteinase K) digest the protein portion of the cell.
2. The specialized **master mix** is applied to the surface of the cell along with the **primers** and other **PCR additives.**
3. After covering it, the slide is directly placed in the **thermocycler** for the **amplification**.

The Master mix

- The tissue sections of interest are attached on a microscopic **slide** and incubated with all necessary **reagents for PCR**.

- The **master mix** is directly applied on the surface of the slide, and the entire amplification process occurs on this slide. However, the master mix used into the *in situ* PCR is specially prepared.

    1. **Protease** step is generally needed.
    2. Higher **MgCl2** concentration.
    3. Higher **Taq** polymerase concentration.
    4. Addition of **BSA**.

The Primer

The primers used in in-situ PCR are coupled with "**marker**" molecules (e.g. biotin or digoxigenin molecule).

After PCR, the amplicons can be identified using antibodies against these markers. The antibodies can be made visible by attaching a ***fluorescent*** or **colorimetric** ***enzyme*** to it.

Duration: An average of **6 hours** is required to carry out the technique.

Application

- ✓ By using the *in situ* PCR even a single copy of the DNA present into the sample can be measured or amplified.
- ✓ Localizing and visualizing the amplicon within the cell is possible by the *in situ* PCR.
- ✓ The low copy number of DNA can be detected with high sensitivity by this method.
- ✓ It is widely used in the study of organogenesis and embryogenesis.
- ✓ It is used in infectious disease diagnosis such as HIV.
- ✓ allows the determination of various aspects of normal versus pathological conditions,
- ✓ Quantification of DNA is also possible in real-time *in situ* PCR.
- ✓ Gene expression can also be measured using the reverse transcription *in situ* PCR

References

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[3] https://handling-solutions.eppendorf.com/sample-handling/amplification/detailview-principles/news/in-situ-pcr-pcr-directly-inside-a-cell/

[4] Bagasra, O. Protocols for the *in situ* PCR-amplification and detection of mRNA and DNA sequences. *Nat Protoc* **2,** 2782–2795 (2007). https://doi.org/10.1038/nprot.2007.395