Basic Molecular Biology: PCR and Real-Time
RT-PCR Fluorescent Probe-Based Detection

Detection of PCR products in real-time can be accomplished by using fluorescent dyes or probes. Fluorescently-labeled probes detect the amount of specific double-stranded DNA sequences while fluorescent dyes detect only the amount of double-stranded DNA.

Here, we are going to look at the principles behind fluorescent dye-based detection. The fluorescent dye-based method utilizes a dye that emits fluorescence when incorporating itself into double-stranded DNA. The fluorescence signal increases at each PCR cycle as more double-stranded DNA molecules are generated.

The most commonly used dye for fluorescent-dye based detection is SyBr Green. The SyBr Green dye functions as an intercalating agent that emits detectable fluorescence when bound to double-stranded DNA. As the PCR progresses and the quantity of double-stranded DNA increases, more dye binds to the PCR products and hence, signal intensity increases. Since the dye binds to all amplified PCR products indiscriminately, artefacts such as those resulting from primer dimers or nonspecific binding of the primers may also contribute to the overall fluorescence.

A post-PCR melt curve analysis enables the detection of nonspecific PCR products, which melt at different temperatures than the specific PCR product. The fluorescence observed is proportional to the amount of DNA present.

Link to video job aid [Basic Molecular Biology: PCR and Real-Time PCR – RT-PCR Fluorescent Probe-Based Detection | OneLab REACH (cdc.gov)]