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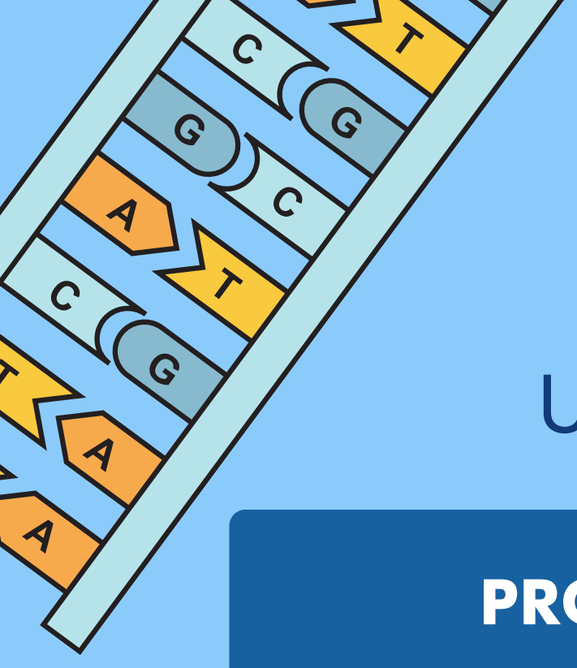
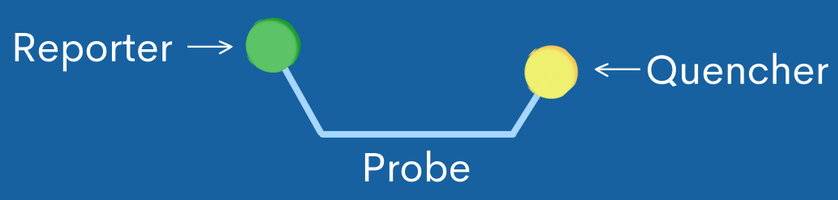


A LOOK AT qPCR PROBES

Understanding Probe and Dye Methods

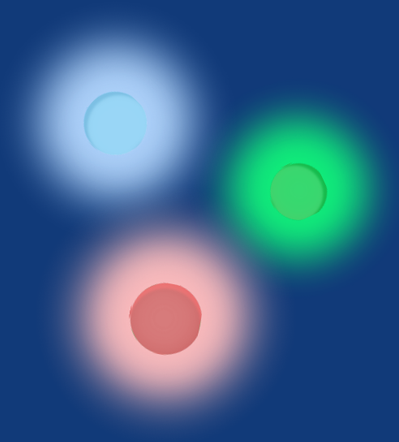
PROBE-BASED



High-Fidelity Tagging

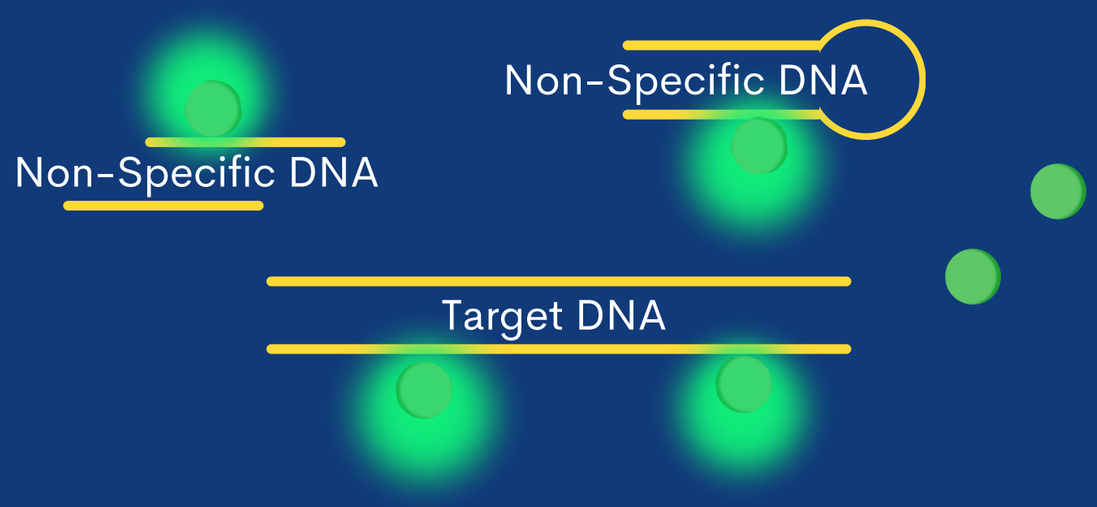
Probes ensure high specificity by only emitting a fluorescent signal upon binding to the exact target sequence and releasing the *quencher*. This allows detection of even minor variations like single nucleotide polymorphisms (SNPs).

Multiplexing with Probe



With probe-based qPCR, it is possible to detect several sequences in a single reaction by using probes that carry different reporter dyes.

DYE-BASED



Specificity Challenges

Dye molecules bind to any double-stranded DNA produced in the reaction, including off-target amplification products and primer-dimers, resulting in inaccurate quantification. Additional steps are often needed.

Single Target Limit

Only one target can be measured at a time in a dye-based qPCR run.

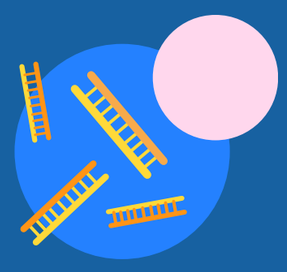
QUICK qPCR OVERVIEW

1
Denaturation: Prepping DNA for binding

Heating allows the reaction mix to separate the double-stranded DNA into single strands, allowing primers to bind. Both probes and dye can bind to single or double-stranded DNA.

2
Annealing: Finding a Match

Lowering the temperature enables primers to bind to complementary sequences on DNA. Probes need a perfectly matched sequence for binding whereas dye binds any double-stranded DNA.



3
Extension: Building the Target

DNA polymerase replicates the sequence for sensitive detection. Probe-based qPCR utilizes cleavable probes that release signals during extension, while dye simply detects all double-stranded DNA as it amplifies.