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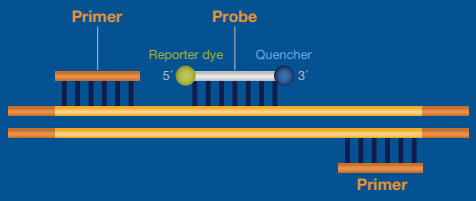
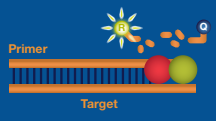
# Comparing SYBR Green I dye and TaqMan Assays for real-time PCR analysis

Real-time PCR instruments detect fluorescent signals generated during target amplification. Thermo Fisher Scientific offers products for two distinct detection chemistries that are widely used in research for fluorescence-based analysis of nucleic acids by real-time PCR.

## Reaction chemistry

| SYBR Green I dye | | TaqMan Assays |
|---|---|---|
| Dye-based detection: Invitrogen™ SYBR™ Green I dye binds nonspecifically to double-stranded DNA, then fluoresces upon excitation.  | **Detection method** | Probe-based detection: Sequence-specific Applied Biosystems™ TaqMan™ Assay probes are labeled with a fluorescent reporter dye and a quencher.   |
| SYBR Green dye–based assays include a forward primer and a reverse primer designed to amplify a specific target sequence. The dye is added to the reaction separately, usually as a component of a master mix.   | **Assay components** | TaqMan Assays for gene expression analysis include two PCR primers and a gene-specific TaqMan probe.   |
| SYBR Green I dye produces a fluorescent signal whether bound to the amplified target sequence, nonspecific amplification products, or primer-dimers.   | **Signal generation** | During polymerization, the 5´ nuclease activity of *Taq* DNA polymerase cleaves the bound probe. Once separated from the quencher, the reporter dye emits a permanent fluorescent signal.   |
| Suitable for applications with **low specificity** demands:<br>Mycoplasma infection in cell culture<br>Next-generation sequencing (NGS) library quantification<br>Telomere length<br>Chromatin immunoprecipitation (ChIP) | **When to use** | Ideal for applications with **high specificity** demands:<br>Gene expression analysis<br>miRNA analysis<br>Pathogen detection or quantification<br>Copy number variation, SNP genotyping<br>Clinical research |

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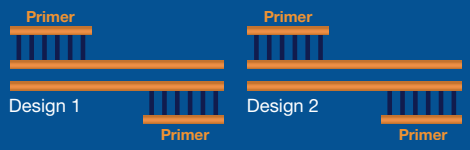
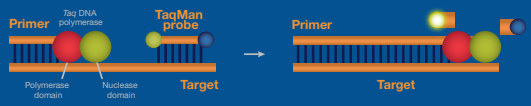
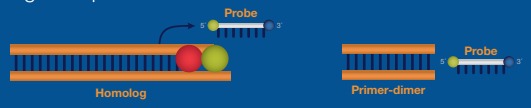
# Assay specificity

The specificity of an assay is its ability to detect targets and exclude nontargets in samples. The use of 3 oligonucleotides in TaqMan Assays helps to ensure **higher specificity** than the 2 oligonucleotides used in SYBR Green dye–based assays.

| SYBR Green I dye | | TaqMan Assays |
|---|---|---|
| Target detection relies on 2 primers, which must be optimized for specificity.<br>  | **Ability to detect target** | Target detection relies on 2 primers **and** cleavage of the bound sequence-specific probe, helping ensure higher specificity.<br>  |
| Signals from nontargets cannot be excluded. Post-run specificity monitoring is recommended, but because of inherent limitations it does not substitute for specificity verification.<br>Melt curve analysis<br>Gel electrophoresis<br>Sequencing | **Ability to exclude nontargets** | TaqMan probes help ensure nontargets are excluded. Mismatched probes are displaced from homologs without being cleaved, so no signal is produced.<br>TaqMan probes cannot bind to nonspecific PCR products (e.g., primer-dimers) and become cleaved, so no signal is produced.<br>  |

# Workflow design and optimization

| SYBR Green I dye | | TaqMan Assays |
|---|---|---|
| Predesigned assays are not available for gene expression analysis using SYBR Green I dye. Developing an assay typically requires user design and experimental optimization. | **Assay design and optimization** | Thermo Fisher Scientific offers over 2.8 million verified, ready-to-use TaqMan Gene Expression Assays for research, with guaranteed* performance.<br>* Terms and conditions apply. To see full details of the guarantee, go to **thermofisher.com/taqmanguarantee**. |
| No | **Multiplexing** | Yes |
| Applied Biosystems™ PowerTrack™ SYBR™ Green Master Mix | **Recommended reagents** | Applied Biosystems™ TaqMan™ Fast Advanced Master Mix or TaqMan™ Fast Virus 1-Step Master Mix |

Learn more about real-time PCR products and applications at **thermofisher.com/qpcr**

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