

## Protocol: Realtime qPCR with SYBR Green I

**SYBR Green I** is a very sensitive dye for the detection of double stranded DNA (dsDNA). The dye is used for non-specific detection of amplification in realtime qPCR experiments.

1. Calculate the volumes of reagents required for the reaction.

Reagent	Final concentration in the mixture
dNTP	10 mM each
DMSO	10% vol.
Taq polymerase buffer	1x
Primer 1	0.1-1 uM
Primer 2	0.1-1 uM
Taq DNA polymerase	1.25 u per reaction
MgCl <sub>2</sub>	2 mM
SYBR Green I	1x

2. On ice, prepare a 1x master mix containing no DNA, by mixing the components in the following order: water, DMSO, Taq polymerase buffer, dNTPs, MgCl<sub>2</sub>, SYBR Green I, Taq polymerase, and primers.
3. Transfer master mix to tubes or plates. Add DNA (50 ng per reaction).
4. Proceed with amplification according to your instrument manufacturer.

**Notes:**

Always use positive and negative controls when doing qPCR experiments.

The temperature program for the qPCR amplification does not differ from standard PCR program for the given template and primers.

For the detection, FAM or FAM/SYBR channel should be used.