Complementary information for qPCR assays :

Takyon® SYBR® assays

Primer design guidelines

- GC content should be between 30 % and 80 % (ideally 40-60%).
- Avoid runs of identical nucleotides, especially of 3 or more Gs or Cs at the 3' end.
- The Tm should be between 58 °C and 60 °C.

Custom assay design

The commonly used concentrations for primers are 100 nM. Optimal results may require titration of primers or adjustment of the ratio. The purpose of such a process is to determine the minimum amount of primers required to obtain the most sensitive results with your assay.

PRIMER TITRATION MATRIX

Titrate according to Table 1, perform a qPCR experiment and select the concentration which gives the lowest Cq value and clear No Template Controls (NTCs). By doing this type of titration it is also possible to compensate for differences up to 2°C in melt temperature of the primers.

Reverse	Forward		
	50 nM	100 nM	300 nM
50 nM	50 / 50	100 / 50	300 / 50
100 nM	50 / 100	100 / 100	300 / 100
300 nM	50 / 300	100 / 300	300 / 300

Table 1 : Primer titration matrix for SYBR® assays

$MGCL_2$ ADJUSTMENT MATRIX

Standard $MgCl_2$ concentration is 2.5 mM but optimal $MgCl_2$ concentration can vary between assays. If necessary adjust the $MgCl_2$ concentration with the provided 50 mM $MgCl_2$ tube. It is recommended to prioritize optimizing the primer concentrations before the $MgCl_2$ concentration. Adjust the amount of water if $MgCl_2$ is added to the reaction.

Complementary information for qPCR assays :

Takyon® Probe assays

Primer and probe design guidelines

PROBES:

- Avoid runs of identical nucleotides, especially of 4 or more Gs.
- The probe Tm should be 7 to 10 °C above primers Tm.
- Avoid 5'-end G as it quenches the fluorophore.
- For genotyping, the position of the polymorphism should be in the centre of the probes, and the probe length should be adjusted such that each probe has the same Tm

PRIMERS:

- GC content should be between 30 % and 80 % (ideally 40-60%).
- Avoid runs of identical nucleotides, especially of 3 or more Gs or Cs at the 3' end.
- The Tm should be between 58 °C and 60 °C.
- The primer should be placed as close as possible to the probe

Custom assay design

The commonly used concentrations for primers and for probes are 300 nM and 100 nM respectively. Optimal results may require titration of primers and probes or adjustement of the primer / probe ratio. The purpose of such a process is to determine the minimum amount of primers and probes required to obtain the most sensitive results with your assay.

PRIMER TITRATION MATRIX

Titrate according to Table 2, perform qPCR and select the concentration which gives the lowest Cq value. By doing this type of titration it is also possible to compensate for differences up to 2 °C in melt temperature of the primers.

Table 2: Primer titration matrix for probe assays

Reverse	Forward		
	50 nM	300 nM	900 nM
50 nM	50 / 50	300 / 50	900 / 50
300 nM	50 / 300	300 / 300	900 / 300
900 nM	50 / 900	300 / 900	900 / 900

PRIMER-PROBE RATIO MATRIX

Select optimal primer concentration as described in Table 2 and test with all probe concentrations described in Table 3. Select the concentration which gives the lowest Cq value.

Table 3: Primer ratio matrix for probe assays

	Probe		
Opt. primers conc	50 nM	100 nM	250 nM

$MGCL_2$ ADJUSTMENT MATRIX

Standard $MgCl_2$ concentration is 5.5 mM but optimal $MgCl_2$concentration can vary between assays. If necessary adjust the $MgCl_2$ concentration with the provided 50 mM $MgCl_2$ tube. It is recommended to prioritize optimizing the primer and probe concentrations before the $MgCl_2$ concentration. Adjust the amount of water if $MgCl_2$ is added to the reaction.

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