Sigma-Aldrich®
Lab & Production Materials

MERCK

OligoArchitect™ Online

Glossary of Parameters

SYBR® Green I

Template Structure Search Parameters

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers and template backbones.

The default value = 100.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 3.0 mM.

Folding Temperature: This is the temperature at which the algorithm searches for secondary structures along the template. Ideally, the temperature should be set to that used for the extension phase of the amplification reaction.

The default value = 55.0 °C.

Search Range: This is the length of the template that is searched for secondary structures, which might form during the extension phase of the amplification reaction. The minimum searchable length is 50 bases.

Maximum Range = 1200 bases.

Oligo Search Range

Sense Primer Search Range: Provides control as to where the sense primer is designed along the template, e.g. if the template is 1500 bases and the sense primer search range is set to 1 – 200, the sense primer will be designed in that range. The antisense primer will be designed elsewhere along the template, avoiding the first 200 bases.

Anti-Sense Primer Search Range: Provides control as to where the antisense primer is designed along the template, e.g. if the template is 1500 bases and the antisense primer search range is set to 1300 – 1500, the antisense primer will be designed in that range. The sense primer will be designed elsewhere along the template, avoiding the last 200 bases.

Amplicon Search Range: Provides control as to which part of the template undergoes amplification. The 5' ends of each primer are the ends of the amplicon.

Primer Parameters

Tm: The melting temperature (T_m) is the temperature at which 50% of the primer/template dissociates to become single-stranded and therefore is a measure of duplex stability. Primers with a T_m in the range of 50 – 60 °C typically produce the best results.

The default value = 55.0 ± 5.0 °C. OligoArchitect Online will identify primers for all sequences as close as possible to the specified value of the T_m. When a value for T_m is manually selected, the option to adjust the default annealing temperature (T_a) is eliminated. T_m values are calculated using the nearest neighbor formula with SantaLucia values (PNAS, Vol. 95, pp. 1460 – 1465, February 1998).

Ta: The annealing temperature (T_a) chosen for a particular PCR protocol depends on the length and GC content of the primers. As a rule of thumb, the T_a is 5 °C below the lowest T_m of the pair of primers. If the T_a is too low, secondary annealing will cause mismatches, leading to nonspecific amplification. If the T_a is too high, reduced annealing will lead to low amplification. The formula is as follows:

$T_a = [0.3 \times T_m(\text{primer})] + [0.7 \times T_m(\text{product})] - 14.9$

$T_m(\text{primer}) = T_m$ of the less stable primer-template pair

$T_m(\text{product}) = T_m$ of the PCR amplicon

The default value = 55.0 ± 5.0 °C. The tool will identify primers for all sequences as close as possible to the specified template T_a. The option to adjust the primer melting temperature (T_m) is not available when the default T_a remains selected.

Length: Since there are primarily four bases (A, C, G, T) in any natural DNA template sequence, an 18-base sequence will statistically be present only once in every 4^{18} bases or approximately 69 billion. Therefore, a primer of 18 bases should be complex enough to minimize secondary

annealing. Likewise, primers longer than approximately 24 bases generally do not show higher specificity.

The default value = 18 – 24 bp.

Amplicon Length: Typically, chosen lengths to be amplified are > 500 bases for traditional PCR and 100 – 200 bases for qPCR. The formula is as follows:

Amplicon Length = (position of antisense primer – position of sense primer) + 1

The default value = 75 - 200 bp.

Alternate Primer Pairs: While the best primers are displayed on the main results screen, up to 50 alternative designs are available.

The default value = 5 primer pairs.

Advanced Primer Parameters

Hairpin Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin that is acceptable at the 3′ end. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -3 kcal mol^{-1} is usually well tolerated.

The default value = -3.0 kcal mol^{-1}.

Hairpin Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin in the internal region of a primer. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -5 kcal mol^{-1} is usually well tolerated.

The default value = -5.0 kcal mol^{-1}.

3′ End Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable duplex between the last 5 bases from the 3′ end and its exact complement in the template. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for false priming as the 3′ end can initiate polymerization even if the remainder of the primer does not bind well. Primers with a ΔG more negative than the specified value are not considered. Primers with a ΔG between -4 and the specified value are rated lower.

The default value = -12.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -5 kcal mol^{-1} is usually well tolerated.

The default value = -5.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Run/Repeat (dinucleotide) maximum length: Primers with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to mispriming. Runs/repeats of 4 bases are usually well tolerated.

The default value = 4 bp.

GC clamp – Consecutive G/C's at 3′ end: The presence of consecutive G and C bases within the last 5 bases of the 3′ end help promote specific binding due to the greater number of hydrogen bonds present. For example, the sequence:

GCTTGCTGCGTTCACACTGC

contains 1 GC clamp. More than 3 G's or C's should be avoided, as it will make the 3′ end maximum ΔG more negative and therefore unfavorable. Primers with less than the specified number of G's or C′ s are rated lower. If the specified value is set to 0, the tool will attempt to design such primers. However, if no such primers can be designed, primers with GC clamps will be reported and rated lower.

The default value = 1.

GC%: It is an empirical observation that the optimal range for SYBR Green I primers is 30.0 – 80.0%.

The default value = 30.0 – 80.0%.

Advanced Primer Pair Parameters

Maximum Primer Pair T_m Mismatch: This is the maximum difference between the T_m values of the two primers. A difference of ≥ 5 °C can prevent amplification.

The default value = 4.0 °C.

Cross Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of the sense and antisense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the sense and antisense primers to hybridize to each other rather than to the template. ΔG = ≥ -5 kcal mol^{-1} is usually well tolerated.

The default value = -5.0 kcal mol^{-1}.

Cross Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of the sense and antisense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the

sense and antisense primers to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Reactions Conditions

Primer Concentration: The default value = 100.0 nM.

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers and template backbones.

The default value = 100.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 3.0 mM.

Total Na[+] Equivalent: The sodium equivalent is calculated as follows:

$[Na^+]$ = Monovalent ion concentration + 4 × (Free $Mg^{2+})^{1/2}$ (all in molarity)

Temperature for Free Energy Calculation: This is used to calculate the Gibbs free energy (ΔG) in the formula: ΔG = ΔH - T ΔS.

The default value = 25.0 °C (298K).

Dual-Labeled Probe

Template Structure Search Parameters

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers/probe and template backbones.

The default value = 100.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 5.0 mM.

Folding Temperature: This is the temperature at which the algorithm searches for secondary structures along the template. Ideally, the temperature should be set to that used for the extension phase of the amplification reaction.

The default value = 55.0 °C.

Search Range: This is the length of the template that is searched for secondary structures, which might form during the extension phase of the amplification reaction. The minimum searchable length is 50 bases.

Maximum Range = 1200 bases.

Oligo Search Range

Sense Primer Search Range: Provides control as to where the sense primer is designed along the template, e.g. if the template is 1500 bases and the sense primer search range is set to 1 – 200, the sense primer will be designed in that range. The antisense primer will be designed elsewhere along the template, avoiding the first 200 bases.

Anti-Sense Primer Search Range: Provides control as to where the antisense primer is designed along the template, e.g. if the template is 1500 bases and the antisense primer search range is set to 1300 – 1500, the antisense primer will be designed in that range. The sense primer will be designed elsewhere along the template, avoiding the last 200 bases.

Dual-Labeled Probe Search Range: Provides control as to where the probe is designed along the template, e.g. if the template is 1500 bases and the probe search range is set to 650 – 850, the probe will be designed in that range. The sense and antisense primers will be designed upstream and downstream, respectively, elsewhere along the template, avoiding the middle 200 bases.

Amplicon Search Range: Provides control as to which part of the template undergoes amplification. The 5′ ends of each primer are the ends of the amplicon.

Primer Parameters

Length: Since there are primarily four bases (A, C, G, T) in any natural DNA sequence, an 18-base sequence will statistically be present only once in every 4^{18} bases or approximately 69 billion. Therefore, a primer of 18 bases should be complex enough to minimize secondary annealing. Likewise, primers longer than approximately 25 bases generally do not show higher specificity.

The default value = 18 – 25 bp.

Tm: The melting temperature (T_m) is the temperature at which 50% of the primer / template dissociates to become single-stranded and therefore is a measure of duplex stability. Primers with a T_m in the range of 54 – 64 °C typically produce the best results. Primers with T_m > 65 °C often undergo secondary annealing.

The default value = 59.0 ± 5.0 °C. The tool will find primers for all sequences as close as possible to the specified value of the T_m. The values are calculated using the nearest neighbor formula with SantaLucia values (PNAS, Vol. 95, pp. 1460 – 1465, February 1998).

Amplicon Length: Typically, chosen lengths to be amplified are 100 – 150 bases for qPCR. The formula is as follows:

Amplicon Length = (position of antisense primer – position of sense primer) + 1

The default value = 70 - 200 bp.

Probe Parameters

Length: Non-modified probes shorter than 15 bases often lack specificity. Probes longer than 30 bases (canonical B-DNA has a rise—bp length along the helical axis—of 3.4 Å) may produce a poor signal to

noise ratio since FRET between a reporter and quencher typically occurs at a distance of no greater than 100 Å. Ideal probe lengths are typically 15 – 40 bases.

The default value = 20 – 27 bp.

Tm: The melting temperature (T_m) is the temperature at which 50% of the probe / template dissociate to become single-stranded and therefore is a measure of duplex stability. The probe T_m should be approximately 10 °C higher than the T_m of the primers to ensure that the probe is fully hybridized during primer extension.

The default value = Primer T_m + 10.0 °C ± 5.0 °C.

Output Options

Design Sense Probe / Design Anti-sense Probe: This option defines the sense of the probe and is only available when the sequence has a SNP.

The default value = Design Sense Probe.

Alternate Assays: While the best primer / probe set is displayed on the main results screen, up to 50 alternative assays are available.

The default value = 5 assays.

Advanced Design Parameters

Hairpin Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin in the internal region of a probe. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Self Dimer Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of two probes. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical probes to hybridize to each other rather than to the template. ΔG = ≥ -10 kcal mol^{-1} is usually well tolerated.

The default value = -10.0 kcal mol^{-1}.

Run/Repeat (Dinucleotide) Maximum Length: Probes with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to misbinding. Runs/repeats of 4 bases are usually well tolerated.

The default value = 4 bp.

Maximum Bases Between Primer & Probe: This is the maximum acceptable distance between primer and probe. The probe should be designed to within 10 – 20 bases of the primer that anneals to the same strand as the probe, without any overlaps.

The default value = 20 bp.

GC%: The ideal range for Dual-Labeled Probes in the 5′ nuclease assay is 30.0 – 80.0%.

The default value = 30.0 – 80.0%.

Probe must not have more G than C bases: It is an empirical observation that probes with more G than C bases will often produce reduced normalized fluorescence values.

The default value = checked.

Avoid G base at 5′end: Guanine is an effective quencher of fluorescence and should not be adjacent to the reporter dye.

The default value = checked.

Advanced Primer Parameters

3′ End Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable duplex between the last 5 bases from the 3′ end and its exact complement in the template. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for false priming as the 3′ end can initiate polymerization even if the remainder of the primer does not bind well. Primers with a ΔG more negative than the specified value are not considered. Primers with a ΔG between -4 and the specified value are rated lower.

The default value = -12.0 kcal mol^{-1}.

G/C Clamp – Consecutive G/C's at 3′ End: The presence of consecutive G and C bases within the last 5 bases of the 3′ end helps promote specific binding due to the greater number of hydrogen bonds present. For example, the sequence:

GCTTGCTGCGTTCACACTGC

contains 1 GC clamp. More than 3 G's or C's should be avoided, as it would make the 3′ end maximum ΔG more negative and therefore unfavorable. Primers with less than the specified number of G's or C′ s are rated lower. If the specified value is set to 0, the program will attempt to design such primers. However, if no such primers can be designed, primers with GC clamps will be reported and rated lower.

The default value = 1.

GC count in first 5 bases at 5′end >: This means that probes with more than two G's and C's in the first five bases at the 5′ end are considered.

The default value = 2.

Avoid T base at 3′ end: It is an empirical observation that thymidine tends to misprime more readily than other bases and therefore should be avoided.

The default value = checked.

Advanced Primer Pair Parameters

Maximum Primer Pair Tm Mismatch: This is the maximum difference between the T_m values of the two primers. A difference of ≥ 5 °C can prevent amplification.

The default value = 3.0 °C.

Cross Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of the sense and antisense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the sense and antisense

primers to hybridize to each other rather than to the template. ΔG = ≥ -9 kcal mol^{-1} is usually well tolerated.

The default value = -9.0 kcal mol^{-1}.

Reaction Conditions

Primer Concentration: The default value = 250.0 nM.

Probe Concentration: The default value = 100.0 nM.

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+ (potassium ion), present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers/probe and template backbones.

The default value = 100.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 5.0 mM.

Total Na[+] Equivalent: The sodium equivalent is calculated as follows:

$[Na^+]$ = Monovalent ion concentration + 4 × (Free $Mg^{2+})^{1/2}$ (all in molarity)

Temperature for Free Energy Calculation: This is used to calculate the Gibbs free energy (ΔG) in the formula: ΔG = ΔH - T ΔS.

The default value = 25.0 °C (298K).

Molecular Beacon

Step 1 – Primers Design
Template Structure Search Parameters

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers/probe and template backbones.

The default value = 100.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 3.0 mM.

Folding Temperature: This is the temperature at which the algorithm searches for secondary structures along the template. Ideally, the temperature should be set to that used for the extension phase of the amplification reaction.

The default value = 55.0 °C.

Search Range: This is the length of the template that is searched for secondary structures, which might form during the extension phase of the amplification reaction. The minimum searchable length is 50 bases.

Maximum Range = 1200 bases.

Oligo Search Range

Sense Primer Search Range: Provides control as to where the sense primer is designed along the template, e.g. if the template is 1500 bases and the sense primer search range is set to 1 – 200, the sense primer will be designed in that range. The antisense primer will be designed elsewhere along the template, avoiding the first 200 bases.

Anti-Sense Primer Search Range: Provides control as to where the antisense primer is designed along the template, e.g. if the template is 1500 bases and the antisense primer search range is set to 1300 –1500, the antisense primer will be designed in that range. The sense primer will be designed elsewhere along the template, avoiding the last 200 bases.

Amplicon Search Range: Provides control as to which part of the template undergoes amplification. The 5′ ends of each primer are the ends of the amplicon.

Primer Parameters

Tm: The melting temperature (T_m) is the temperature at which 50% of the primer/template dissociates to become single-stranded and therefore is a measure of duplex stability. Primers with a T_m in the range of 50 – 60 °C typically produce the best results.

The default value = 55.0 ± 5.0 °C. OligoArchitect Online will identify primers for all sequences as close as possible to the specified value of the T_m. When a value for T_m is manually selected, the option to adjust the default annealing temperature (T_a) is eliminated. T_m values are calculated using the nearest neighbor formula with SantaLucia values (PNAS, Vol. 95, pp. 1460 – 1465, February 1998).

Ta: The annealing temperature (T_a) chosen for a particular PCR protocol depends on the length and GC content of the primers. As a rule of thumb, the T_a is 5 °C below the lowest T_m of the pair of primers. If the T_a is too low, secondary annealing will cause mismatches, leading to nonspecific amplification. If the T_a is too high, reduced annealing will lead to low amplification. The formula is as follows:

$T_a = [0.3 \times T_m(\text{primer})] + [0.7 \times T_m(\text{product})] - 14.9$

$T_m(\text{primer}) = T_m$ of the less stable primer-template pair

$T_m(\text{product}) = T_m$ of the PCR amplicon

The default value = 55.0 ± 5.0 °C. The tool will identify primers for all sequences as close as possible to the specified template T_a. The option to adjust the primer melting temperature (T_m) is not available when the default T_a remains selected.

Length: Since there are primarily four bases (A, C, G, T) in any natural DNA template sequence, an 18-base sequence will statistically be present only once in every 4^{18} bases or approximately 69 billion. Therefore, a primer of 18 bases should be complex enough to minimize secondary annealing. Likewise, primers longer than approximately 25 bases generally do not show higher specificity.

The default value = 18 – 25 bp.

Amplicon Length: Typically, chosen lengths to be amplified are > 500 bases for traditional PCR and 100 – 200 bases for qPCR. The formula is as follows:

Amplicon Length = (position of antisense primer – position of sense primer) + 1

The default value = 100 - 200 bp.

Advanced Primer Parameters

Hairpin Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin that is acceptable at the 3′ end. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -3 kcal mol^{-1} is usually well tolerated.

The default value = -3.0 kcal mol^{-1}.

Hairpin Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin in the internal region of a primer. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -5 kcal mol^{-1} is usually well tolerated.

The default value = -5.0 kcal mol^{-1}.

3′ End Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable duplex between the last 5 bases from the 3′ end and its exact complement in the template. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for false priming as the 3′ end can initiate polymerization even if the remainder of the primer does not bind well. Primers with a ΔG more negative than the specified value are not considered. Primers with a ΔG between -4 and the specified value are rated lower.

The default value = -12.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -8 kcal mol^{-1} is usually well tolerated.

The default value = -8.0 kcal mol^{-1}.

Run/Repeat (Dinucleotide) Maximum Length: Primers with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to mispriming. Runs/repeats of 5 bases are usually well tolerated.

The default value = 5 bp.

GC clamp – Consecutive G/C's at 3′ end: The presence of consecutive G and C bases within the last 5 bases of the 3′ end help promote specific binding due to the greater number of hydrogen bonds present. For example, the sequence:

GCTTGCTGCGTTCACACTGC

contains 1 GC clamp. More than 3 G's or C's should be avoided, as it will make the 3′ end maximum ΔG more negative and therefore unfavorable. Primers with less than the specified number of G's or C's are rated lower. If the specified value is set to 0, the tool will attempt to design such primers. However, if no such primers can be designed, primers with GC clamps will be reported and rated lower.

The default value = 1.

Advanced Primer Pair Parameters

Maximum Ambiguous Bases in Amplicon: This is the number of ambiguous bases tolerated in the amplicon.

The default value = 0.

Maximum Primer Pair Tm Mismatch: This is the maximum difference between the T_m values of the two primers. A difference of ≥ 4 °C can prevent amplification.

The default value = 4.0 °C.

Cross Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of the sense and antisense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the sense and antisense primers to hybridize to each other rather than to the template. ΔG = ≥ -7 kcal mol^{-1} is usually well tolerated.

The default value = -7.0 kcal mol^{-1}.

Cross Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of the sense and antisense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the sense and antisense primers to hybridize to each other rather than to the template. ΔG = ≥ -8 kcal mol^{-1} is usually well tolerated.

The default value = -8.0 kcal mol^{-1}.

Reactions Conditions

Nucleic Acid Concentration: This is the concentration of the primers.

The default value = 250.0 nM.

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers/probe and template backbones.

The default value = 100.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 3.0 mM.

Total Na[+] Equivalent: The sodium equivalent is calculated as follows:

$[Na^+]$ = Monovalent ion concentration + 4 × (Free $Mg^{2+})^{1/2}$ (all in molarity)

Temperature for Free Energy Calculation: This is used to calculate the Gibbs free energy (ΔG) for the primers in the formula: ΔG = ΔH - TΔS.

The default value = 25.0 °C (298K).

Temperature for Beacon Free Energy Calculation: This is used to calculate the Gibbs free energy (ΔG) for the stem region of the Molecular Beacon—designed in step 2—in the formula: ΔG = ΔH - TΔS.

The default value = 55.0 °C (328K).

Step 2 – Probe Design

Oligo Search Range

Probe Search Range: Provides control as to where the probe is designed along the template within the confines of the already designed sense and antisense primer positions, e.g. if the sense primer begins at position 200, and the antisense primer begins at position 300 (assume a 20mer, so, the sense primer ends at 220 and the antisense primer ends at 280), the probe location can be changed along those 60 bases (from position 220 to 280).

Probe Parameters

Length: Non-modified probes shorter than 15 bases often lack specificity. Probes longer than 30 bases may not add any additional specificity. Therefore, typical probe lengths are 15 – 30 bases.

The default value = 18 – 30 bp.

Probe Tm: The melting temperature (T_m) is the temperature at which 50% of the loop region of the probe/template dissociates to become single-stranded and therefore is a measure of duplex stability. The probe T_m should be approximately 9 °C higher than the T_m of the primers to ensure that the probe is fully hybridized during primer extension.

The default value = T_a Opt of the primers + 9.0 ± 6.0 °C.

Beacon Tm: The melting temperature (T_m) is the temperature at which 50% of the stem region of the probe/template dissociates to become single-stranded and therefore is a measure of duplex stability. The probe T_m should be approximately 9 °C higher than the T_m of the primers to ensure that the probe is fully hybridized during primer extension.

The default value = T_a Opt of the primers + 9.0 ± 6.0 °C.

Stem Sequence: This is the sequence of the stem, the appropriate length of which is automatically selected by the algorithm.

The default values = CGCGA for 5 bases, CGCGAT for 6 bases, and CGCGATC for 7 bases.

Advanced Probe Parameters

Hairpin Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable alternative hairpin in the loop region. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -4 kcal mol^{-1} is usually well tolerated.

The default value = -4.0 kcal mol^{-1}.

Self Dimer Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of two probes. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical probes to hybridize to each other rather than to the template. ΔG = ≥ -7 kcal mol^{-1} is usually well tolerated.

The default value = -7.0 kcal mol^{-1}.

Run/Repeat (Dinucleotide) Maximum Length: Probes with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to misbinding. Runs/repeats of 4 bases are usually well tolerated.

The default value = 4 bp.

Cross Dimer Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between two different probes in a multiplex reaction. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the two probes to hybridize to each other rather than to the template. ΔG = ≥ -7 kcal mol^{-1} is usually well tolerated.

The default value = -7.0 kcal mol^{-1}.

Multiplexing Not Currently Available

Output Options

Design Sense Beacon / Design Anti-sense Beacon: This option defines the sense of the probe and is only available when the sequence has a SNP.

The default value = Design Sense Beacon.

LightCycler® Probe

Template Structure Search Parameters

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers/probes and template backbones.

The default value = 50.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 3.0 mM.

Folding Temperature: This is the temperature at which the algorithm searches for secondary structures along the template. Ideally, the temperature should be set to that used for the extension phase of the amplification reaction.

The default value = 55.0 °C.

Search Range: This is the length of the template that is searched for secondary structures, which might form during the extension phase of the amplification reaction. The minimum searchable length is 50 bases.

Maximum Range = 1200 bases.

Oligo Search Range

Sense Primer Search Range: Provides control as to where the sense primer is designed along the template, e.g. if the template is 1500 bases and the sense primer search range is set to 1 – 200, the sense primer will be designed in that range. The anti-sense primer will be designed elsewhere along the template, avoiding the first 200 bases.

Anti-Sense Primer Search Range: Provides control as to where the Anti-sense primer is designed along the template, e.g. if the template is 1500 bases and the anti-sense primer search range is set to 1300 – 1500, the anti-sense primer will be designed in that range. The sense primer will be designed elsewhere along the template, avoiding the last 200 bases.

Oligo Probe1 Search Range: Provides control as to where Probe 1 is designed along the template, e.g. if the template is 1500 bases and Probe 1 search range is set to 650 – 850, the probe will be designed in that range. Probe 2 and the primers will be designed elsewhere along the template.

Oligo Probe2 Search Range: Provides control as to where Probe 2 is designed along the template, e.g. if the template is 1500 bases and Probe 2 search range is set to 850 – 1050, the probe will be designed in that range. Probe 1 and the primers will be designed elsewhere along the template.

Amplicon Search Range: Provides control as to which part of the template undergoes amplification. The 5′ ends of each primer are the ends of the amplicon.

Primer Parameters

Tm: The melting temperature (T_m) is the temperature at which 50% of the primer/template dissociates to become single-stranded and therefore is a measure of duplex stability. Primers with a T_m in the range of 50 – 60 °C typically produce the best results.

The default value = 55.0 ± 5.0 °C. OligoArchitect Online will identify primers for all sequences as close as possible to the specified value of the T_m. When a value for T_m is manually selected, the option to adjust the default annealing temperature (T_a) is eliminated. T_m values are calculated using the nearest neighbor formula with SantaLucia values (PNAS, Vol. 95, pp. 1460 – 1465, February 1998).

Length: Since there are primarily four bases (A, C, G, T) in any natural DNA template sequence, an 18-base sequence will statistically be present only once in every 4^{18} bases or approximately 69 billion.Therefore, a primer of 18 bases should be complex enough to minimize secondary annealing. Likewise, primers longer than approximately 22 bases generally do not show higher specificity.

The default value = 18 – 22 bp.

Amplicon Length: Typically, chosen lengths to be amplified are > 500 bases for traditional PCR and 100 – 200 bases for qPCR. The formula is as follows:

Amplicon Length = (position of antisense primer – position of sense primer) + 1

The default value = 100 - 200 bp.

Advanced Primer Parameters

Hairpin Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin that is acceptable at the 3′ end. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -3 kcal mol^{-1} is usually well tolerated.

The default value = -3.0 kcal mol^{-1}.

Hairpin Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin in the internal region of a primer. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -5 kcal mol^{-1} is usually well tolerated.

The default value = -5.0 kcal mol^{-1}.

3′ End Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable duplex between the last 5 bases from the 3′ end and its exact complement in the template. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for false priming as the 3′ end can initiate polymerization even if the remainder of the primer does not bind well. Primers with a ΔG more negative than the specified value are not considered. Primers with a ΔG between -4 and the specified value are rated lower.

The default value = -12.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -8 kcal mol^{-1} is usually well tolerated.

The default value = -8.0 kcal mol^{-1}.

Run/Repeat (Dinucleotide) Maximum Length: Primers with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to mispriming. Runs/repeats of 5 bases are usually well tolerated.

The default value = 5 bp.

GC clamp – Consecutive G/C's at 3' end: The presence of consecutive G and C bases within the last 5 bases of the 3' end help promote specific binding due to the greater number of hydrogen bonds present. For example, the sequence:

GCTTGCTGCGTTCACACTGC

contains 1 GC clamp. More than 3 G's or C's should be avoided, as it will make the 3' end maximum ΔG more negative and therefore unfavorable. Primers with less than the specified number of G's or C' s are rated lower. If the specified value is set to 0, the tool will attempt to design such primers. However, if no such primers can be designed, primers with GC clamps will be reported and rated lower.

The default value = 2.

Advanced Primer Pair Parameters

Maximum Ambiguous Bases in Amplicon: This is the number of ambiguous bases tolerated in the amplicon.

The default value = 0.

Maximum Primer Pair Tm Mismatch: This is the maximum difference between the T_m values of the two primers. A difference of ≥ 4 °C can prevent amplification.

The default value = 4.0 °C.

Cross Dimer Maximum ΔG (3' End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3' ends of the sense and antisense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the sense and antisense primers to hybridize to each other rather than to the template. ΔG = ≥ -7 kcal mol^{-1} is usually well tolerated.

The default value = -7.0 kcal mol^{-1}.

Cross Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of the sense and antisense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the sense and antisense primers to hybridize to each other rather than to the template. ΔG = ≥ -8 kcal mol^{-1} is usually well tolerated.

The default value = -8.0 kcal mol^{-1}.

Probe Parameters

Length: Non-modified probes shorter than 25 bases often lack specificity. Probes longer than 35 bases may not add any additional specificity. Therefore, typical probe lengths are 25 – 35 bases.

The default value = 25 – 35 bp.

Probe Tm: The melting temperature (T_m) is the temperature at which 50% of the probe/template dissociates to become single-stranded and therefore is a measure of duplex stability. The probe T_m should be approximately 10 °C higher than the Tm of the primers to ensure that the probe is fully hybridized during primer extension.

The default value = Primer T_m + 8.0 °C ± 3.0 °C.

Tm difference between the probes: This is the difference between the T_m of the Probe 1 and Probe 2.

The default value = 5.0 to 8.0 °C.

Advanced Probe Parameters

Hairpin Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin in the internal region of a probe. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -3 kcal mol^{-1} is usually well tolerated.

The default value = -3.0 kcal mol^{-1}.

Self Dimer Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between two probes. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical probes to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Run/Repeat (Dinucleotide) Maximum Length: Probes with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to misbinding. Runs/repeats of 4 bases are usually well tolerated.

The default value = 4 bp.

Multiplex Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between two different probes in a multiplex reaction. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the two probes to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Multiplexing Not Currently Available

Avoid G base toward the fluorophore end: Guanine is an effective quencher of fluorescence and should not be adjacent to the reporter dye.

The default value = checked.

Output Options

Design Sense Probe / Design Anti-sense Probe: This option defines the sense of the probe.

The default value = Design Sense Probe.

Reactions Conditions

Nucleic Acid Concentration: This is the concentration of the Primers and probes.

The default value = 250 nM.

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primers/probes and template backbones.

The default value = 50.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 3.0 mM.

Total Na[+] Equivalent: The sodium equivalent is calculated as follows:

$[Na^+]$ = Monovalent ion concentration + 4 × (Free $Mg^{2+})^{1/2}$ (all in molarity)

Temperature for Free Energy Calculation: This is used to calculate the overall Gibbs free energy (ΔG) in the formula: $\Delta G = \Delta H - T\Delta S$.

The default value = 25.0 °C (298K).

Scorpions™ Probe

Technical Note: this module is for the uni-molecular Scorpions detection chemistry; it does not support bi-molecular designs.

Terminology Note: since the Scorpions Probe consists of two moieties with different functions that are conjugated to each other 1) the stem-loop region with the reporter and quencher dyes is referred to as 'Beacon' and 2) the primer region is referred to as 'SC Primer.' Also, in addition to the Scorpions Probe, this particular detection chemistry makes use of a standard PCR primer, which is referred to as 'Standard Primer.

Template Structure Search Parameters

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primer/probe:primer and template backbones.

The default value = 50.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 2.5 mM.

Folding Temperature: This is the temperature at which the algorithm searches for secondary structures along the template. Ideally, the temperature should be set to that used for the extension phase of the amplification reaction.

The default value = 55.0 °C.

Search Range: This is the length of the template that is searched for secondary structures, which might form during the extension phase of the amplification reaction. The minimum searchable length is 50 bases.

Maximum Range = 1200 bases.

Oligo Search Range

Scorpions Primer Search Range: Provides control as to where the SC Primer is designed along the template, e.g. if the template is 1500 bases and the SC Primer search range is set to 1 – 200, the SC Primer will be designed in that range. The Standard Primer will be designed elsewhere along the template, avoiding the first 200 bases.

Standard Primer Search Range: Provides control as to where the Standard Primer is designed along the template, e.g. if the template is 1500 bases and the Standard Primer search range is set to 1300 – 1500, the Standard Primer will be designed in that range. The SC Primer will be designed elsewhere along the template, avoiding the last 200 bases.

Amplicon Search Range: Provides control as to which part of the template undergoes amplification. The 5' ends of each primer are the ends of the amplicon.

Scorpions Probe Search Range: Provides control as to where the Beacon is designed along the template, e.g. if the template is 1500 bases and the Beacon search range is set to 650 – 850, the Beacon will be designed in that range. The SC Primer is attached and will therefore bind in the same region. The Standard Primer will be elsewhere along the template.

Primer Parameters

Tm: The melting temperature (T_m) is the temperature at which 50% of the primer/template dissociates to become single-stranded and therefore is a measure of duplex stability. Primers with a T_m in the range of 50 – 70 °C typically produce the best results.

The default value = 65.0 ± 5.0 °C. OligoArchitect Online will identify primers for all sequences as close as possible to the specified value of the T_m. When a value for T_m is manually selected, the option to adjust the default annealing temperature (T_a) is eliminated. T_m values are calculated using the nearest neighbor formula with SantaLucia values (PNAS, Vol. 95, pp. 1460 – 1465, February 1998).

Ta: The annealing temperature (T_a) chosen for a particular PCR protocol depends on the length and GC content of the primers. As a rule of thumb, the T_a is 5 °C below the lowest T_m of the pair of primers. If the T_a is too low, secondary annealing will cause mismatches, leading to nonspecific amplification. If the T_a is too high, reduced annealing will lead to low amplification. The formula is as follows:

$T_a = [0.3 \times T_m(\text{primer})] + [0.7 \times T_m(\text{product})] - 14.9$

$T_m(\text{primer}) = T_m$ of the less stable primer-template pair

$T_m(\text{product}) = T_m$ of the PCR amplicon

The default value = 58.0 ± 10.0 °C. The tool will identify primers for all sequences as close as possible to the specified template T_a. The option to adjust the primer melting temperature (T_m) is not available when the default T_a remains selected.

Length: Since there are primarily four bases (A, C, G, T) in any natural DNA template sequence, an 18-base sequence will statistically be present only once in every 4^{18} bases or approximately 69 billion. Therefore, a primer of 18 bases should be complex enough to minimize secondary annealing. Likewise, primers longer than approximately 28 bases generally do not show higher specificity.

The default value = 16 – 28 bp.

Amplicon Length: Typically, chosen lengths to be amplified are > 500 bases for traditional PCR and 50 – 200 bases for qPCR. The formula is as follows:

Amplicon Length = (position of antisense primer – position of sense primer) + 1

The default value = 50 - 150 bp.

Advanced Primer Parameters

Hairpin Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin that is acceptable at the 3′ end. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -2 kcal mol^{-1} is usually well tolerated.

The default value = -2.0 kcal mol^{-1}.

Hairpin Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable hairpin in the internal region of a primer. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -3 kcal mol^{-1} is usually well tolerated.

The default value = -3.0 kcal mol^{-1}.

3′ End Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable duplex between the last 5 bases from the 3′ end and its exact complement in the template. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for false priming as the 3′ end can initiate polymerization even if the remainder of the primer does not bind well. Primers with a ΔG more negative than the specified value are not considered. Primers with a ΔG between -4 and the specified value are rated lower.

The default value = -10.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -5 kcal mol^{-1} is usually well tolerated.

The default value = -5.0 kcal mol^{-1}.

Self Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of two same-sense primers. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical primers to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Run/Repeat (Dinucleotide) Maximum Length: Primers with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to mispriming. Runs/repeats of 4 bases are usually well tolerated.

The default value = 4 bp.

GC clamp – Consecutive G/C's at 3′ end: The presence of consecutive G and C bases within the last 5 bases of the 3′ end help promote specific binding due to the greater number of hydrogen bonds present. For example, the sequence:

GCTTGCTGCGTTCACACTGC

contains 1 GC clamp. More than 3 G's or C's should be avoided, as it will make the 3′ end maximum ΔG more negative and therefore unfavorable. Primers with less than the specified number of G's or C's are rated lower. If the specified value is set to 0, the tool will attempt to design such primers. However, if no such primers can be designed, primers with GC clamps will be reported and rated lower.

The default value = 2.

Advanced Primer Pair Parameters

Maximum Ambiguous Bases in Amplicon: This is the number of ambiguous bases tolerated in the amplicon.

The default value = 0.

Maximum Primer Pair T_m Mismatch: This is the maximum difference between the T_m values of the two primers. A difference of ≥ 3 °C can prevent amplification.

The default value = 3.0 °C.

Cross Dimer Maximum ΔG (3′ End): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the 3′ ends of the SC Primer and Standard Primer. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the Scorpions and standard primers to hybridize to each other rather than to the template. ΔG = ≥ -5 kcal mol^{-1} is usually well tolerated.

The default value = -5.0 kcal mol^{-1}.

Cross Dimer Maximum ΔG (Internal): The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between the internal regions of the SC Primer and Standard Primer. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the Scorpions and standard primers to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Probe Parameters

Length: Non-modified probes shorter than 14 bases often lack specificity. Probes longer than 26 bases may not add any additional specificity. Therefore, typical probe lengths are 14 – 26 bases.

The default value = 14 – 26 bp.

Probe Tm: The melting temperature (T_m) is the temperature at which 50% of the loop region of the Scorpions Probe/template dissociates to become single-stranded and therefore is a measure of duplex stability. The Scorpions probe and primer are attached to each other and therefore should anneal at the same temperature as the standard primer.

The default value = 60.0 ± 10.0 °C.

Beacon Tm: The melting temperature (T_m) is the temperature at which 50% of the stem region of the Scorpions Probe/template dissociates to become single-stranded and therefore is a measure of duplex stability. This T_m should be approximately 10 °C higher than the Tm of the Scorpions Probe:Primer to ensure that the Scorpions probe is fully hybridized during primer extension.

The default value = Probe T_m + 5.0 ± 10.0 °C.

Stem Sequence: This is the sequence of the stem, the appropriate length of which is automatically selected by the algorithm.

The default values = CCGCGC and CGCACG for 6 bases, and CCCATGCC and GGTCACCG for 7 bases.

Advanced Probe Parameters

Hairpin Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of the most stable alternative hairpin in the Beacon. ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable hairpins that can adversely affect the reaction. ΔG = ≥ -2 kcal mol^{-1} is usually well tolerated.

The default value = -2.0 kcal mol^{-1}.

Self Dimer Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between two Beacons. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for identical probes to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Run/Repeat (Dinucleotide) Maximum Length: Probes with long runs of the same base or repeats of a consecutive dinucleotide should be avoided as they lead to misbinding. Runs/repeats of 4 bases are usually well tolerated.

The default value = 4 bp.

Multiplex Maximum ΔG: The Gibbs free energy change (ΔG) is a measure of the spontaneity of formation of a dimer between two different Beacons in a multiplex reaction. ΔG is the energy required to break the secondary structure, and larger negative values indicate a higher propensity for the two probes to hybridize to each other rather than to the template. ΔG = ≥ -6 kcal mol^{-1} is usually well tolerated.

The default value = -6.0 kcal mol^{-1}.

Multiplexing Not Currently Available

Distance between Scorpions Primer and Probe: This is the maximum acceptable distance between SC Primer and Beacon, which should be as close as possible to each other.

The default value = 7 ± 3 bp.

Output Options

Design Sense Probe / Design Anti-sense Probe: This option defines the sense of the probe.

The default value = Design Sense Probe.

Determine ΔG and Tm for Probe Target Hybrid:

The default value = Unchecked

ΔG for Probe Target Hybrid Range = -4.0 to -10.0 kcal mol^{-1}.

Reactions Conditions

Nucleic Acid Concentration: This is the concentration of the Scorpions Probe and Standard Primer.

The default value = 0.25 nM.

Monovalent Ion Concentration: This is the concentration of all monovalent ions, e.g. K^+, present in the reaction mixture. K^+ encourages annealing by reducing charge repulsion between the negatively charged primer/probe:primer and template backbones.

The default value = 50.0 mM.

Free Mg^{++} Ion Concentration: This is the concentration of Mg^{2+} (magnesium ion) in the reaction mixture. Mg^{2+} is a cofactor required by thermostable DNA polymerases.

The default value = 2.5 mM.

dNTP Concentration: The default value = 0.2 mM.

Total Na[+] Equivalent: The sodium equivalent is calculated as follows:

$[Na^+]$ = Monovalent ion concentration + 4 × (Free $Mg^{2+})^{1/2}$ (all in molarity)

Temperature for Free Energy Calculation: This is used to calculate the Gibbs free energy (ΔG) for the primers in the formula: ΔG = ΔH - TΔS.

The default value = 25.0 °C (298K).

Temperature for Scorpions™ Free Energy Calculation: This is used to calculate the Gibbs free energy (ΔG) for the stem region of the Scorpions Probe in the formula: ΔG = ΔH - TΔS.

The default value = 60.0 °C (333K).

Merck KGaA,
Frankfurter Strasse 250,
64293 Darmstadt, Germany

Lit. No. MK_BR3011EN Ver. 0.0 2018 - 16286 09/2018