

Taqman assay design rules

Overview

9/2/12 RTK.

This page holds design rules and parameters used for Taqman assay design internally. These represent a combination of "traditional" PCR design rules, along with various empirical insights gleaned from experience with ddPCR and with Taqman MGB probes from ABI. When an assay cannot be found that exhibits "ideal" characteristics, rules may be bent to some extent, with the expectation that the design has a higher risk of problems or failure. Note that many PCR design software programs (e.g. Primer3) have quite strict rules by default. This means that oligos designed by such software should conform to "good" rules right out of the box; However, when you get stuck (i.e. very challenging targets) it is still reasonable to relax some rules and see what you can find.

Note: Rules for Taqman assays using **LNA probes** are not listed. The PCR components (i.e. non-LNA primers) should be the same, but rules for LNA probes, particularly mutation-discriminating SNP probe pairs, are not yet known with any confidence.

Details of **T_m calculations** are here: [DNA Thermodynamics](#)

Rules and parameters

Primers

Parameter	Ideal	OK	Allowed
Target T _m	61-62°C	60-62° C	58-63 allowed, lower increasingly disfavored.
T _m differences	0°C	1-2°C	Possible more if <i>absolutely</i> needed
Length	17-22	16-25 bases	Shorter or longer increasingly disfavored.
%GC	40-60%	30-70%	Maybe 15-85% if forced.
GCs in 3'-most five	2	3	4 or even 5 (not G's or only Cs)

Rows of G	2	3	4 or more (if not 3' end and can be synthesized)
Rows of non-G	3	4-5 (C) or 6 (A or T)	Maybe more, increasingly disfavored
"Primer-dimer"	2	4 or less 3' match	If GC-poor, higher may be OK
"Hairpin" stem	2	3-4 (G/C), 3-5 (A/T)	Maybe more, increasingly disfavored
Self complement	8 or less	12 or less total matching bases	Maybe more, depending on bases

Probes

Parameter	Ideal	OK	Allowed
Target Tm	67°C	66-68' C	64-70+ allowed, lower increasingly disfavored.
Tm above primers	> 5°C	4-5°C	Not less than 2.5°C
Tm difference (SNPs)	< 1°C	1-2°C	Note more than 2.5°C
Length (MGB)	15	14-18 bases	Shorter or longer increasingly disfavored.
Length (non-MGB)	20-25	18-30	Shorter or longer increasingly disfavored.
%GC	40-60%	30-70%	Maybe 15-85% if forced.
G at 5' end	0	0	1 if you must; Expected to lower signal.
Rows of G	2	3	4 or more (if not 3' end and can be synthesized)
Rows of non-G	3	4-5 (C) or 6 (A or T)	Maybe more, increasingly disfavored

SNP placement (MGB)	4-7 from 3' end	9-3 from from 3' end	Not last two bases
SNP placement (non-MGB)	center	center 50%	Not last two bases
SNP probe pairing	identical	overlap, except at one end	Increasingly disfavored with non-overlap
"Hairpin" stem	2	3-4 (G/C), 3-5 (A/T)	Maybe more, increasingly disfavored
Self complement	8 or less	12 or less total matching bases	Maybe more, depending on base