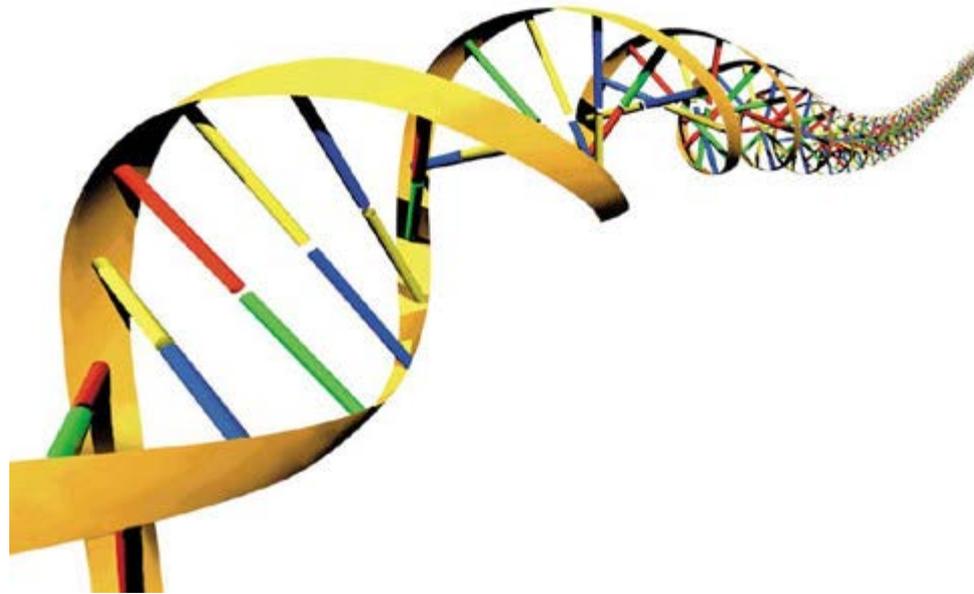


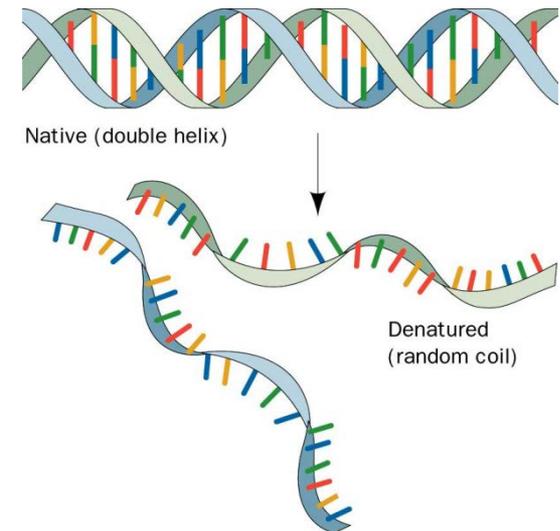
ÚVOD DO KVANTITATIVNÍ REAL-TIME PCR



V. Návrh primerů a sond

Hybridizace

- Úspěšný annealing sondy a primerů je kritický předpoklad úspěšné PCR
- Sekvence
- Koncentrace solí
- Tvorba heterodimerických stabilních struktur
- Párování bazí - nejen Watson a Crick
- Sekundární struktura
- Teplota tání DNA T_m





Melting temperature T_m

- jeden z nejdůležitějších parametrů, determinující annealingovou teplotu
- T_m – teplota, při které je 50% daného oligonukleotidu denaturováno
- „cooperativní melting“ – usnadněná denaturace po disociaci prvního páru bází
- Sekvence: $A=T < G \equiv C$

- Rychlost renaturace (a tedy i T_m) přímo úměrná délce řetězce a jeho koncentraci a nepřímo úměrná komplexitě molekuly (struktura)

- Elektrostatické interakce mezi fosfátovými molekulami
- kationty maskují + náboje fosfátů - vyšší iontová síla vede k vyšší T_m

Oligonukleotidy kratší než 20bp

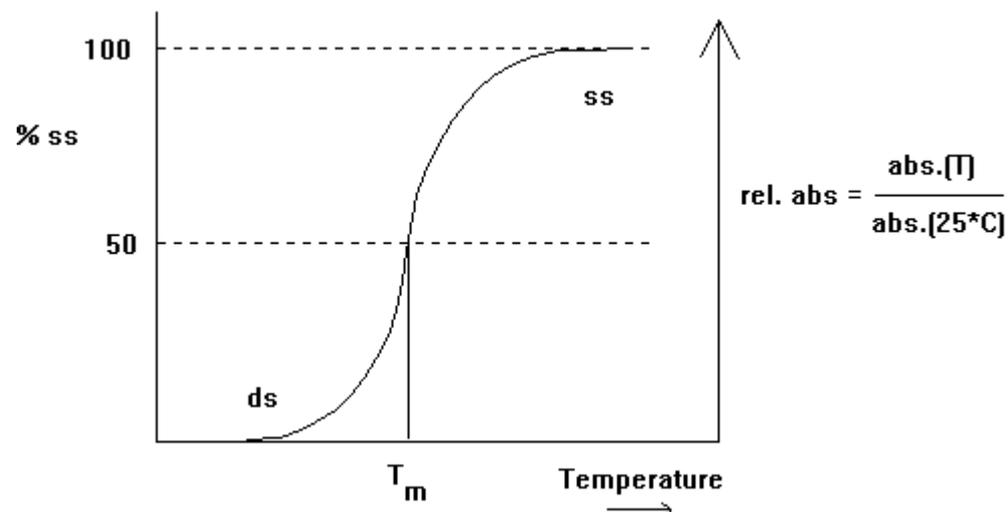
$$T_m = 2 \times (A+T) + 4 \times (G+C)$$

Iontová síla, %GC a délka řetězce (N)

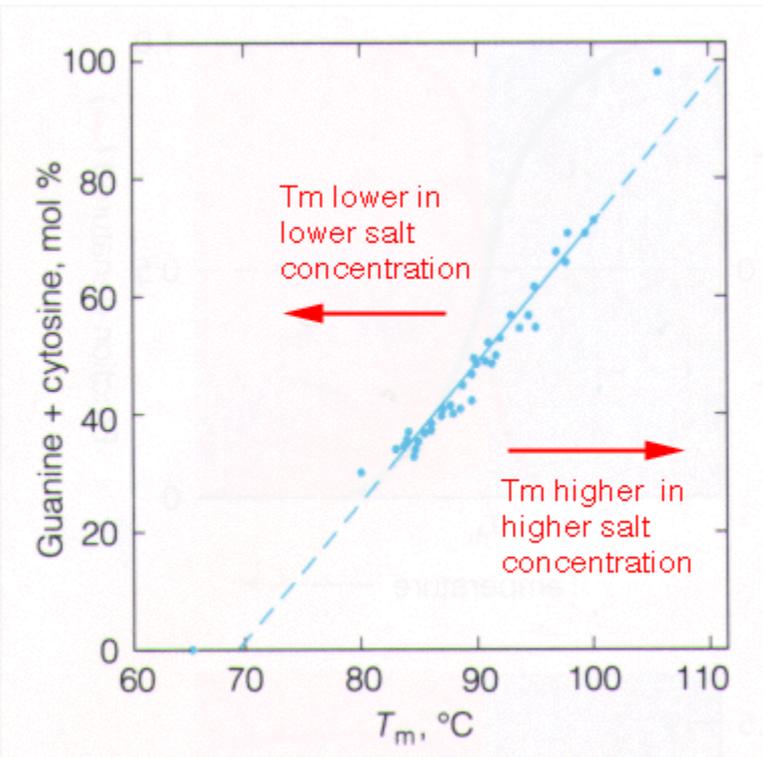
$$T_m = 81,5 + 16,6 (\log_{10}[\text{Na}^+] + 0,41(\%GC) - (625/N))$$

Web-based kalkulátory

<http://insilico.ehu.es/tm.php>



Melting temperature T_m



GCTATTCAACTGAAGAGGGGCACAGC

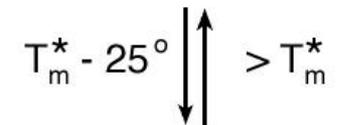
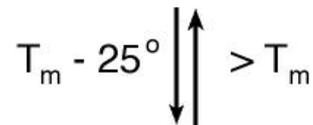
GCTATTCAACTG^GAGAGGGGCACAGC

+

+

CGATAAGTTGACTTCTCCCGTGTCG

CGATAAGTTGACTTCTCCCGTGTCG



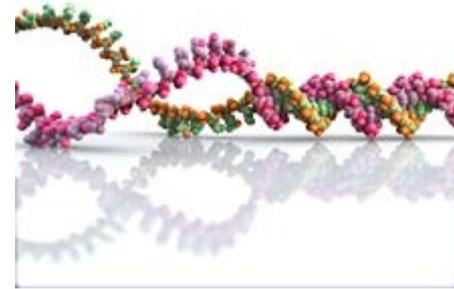
GCTATTCAACTGAAGAGGGGCACAGC
CGATAAGTTGACTTCTCCCGTGTCG

GCTATTCAACTG^GAGAGGGGCACAGC
CGATAAGTTGAC_TTCTCCCGTGTCG

note: T_m^* is 4° lower than T_m

(In general, there is a 1° drop for every 1% mismatch)

Gibbsova (volná) energie a její změna (ΔG , ΔG^0)



- Schopnost látek jít do reakce
- Sekundární struktura DNA
- ΔG závisí na změně vnitřní energie a entropie
- Změna volné energie ΔG^0 (množství energie uvolněné nebo absorbované během reakce za stejné teploty a tlaku) - spontánní reakce - $\Delta G < 0$
- Znalost termodynamického příspěvku párování bazí, mismatches, volných konců, vlásenkových struktur a smyček – predikce parametrů hybridizace
- Predice sekundární struktury – *nearest neighbor*
 - *helix initiation factor* (GC/AT)
 - *helix propagation* energie nutná pro vytvoření následujícího hybridizačního páru
 - symetrie sekvence (duplexu)
 - *Loop regions* – smyčky, vlásenky, výdutě atd.

Faktory ovlivňující stabilitu DNA DNA/RNA duplexu

1. Počet odpovídajících párů bází

- Kombinace vodíkových můstků a hydrofobních interakcí
- Pozice a typ neodpovídajícího páru (*mismatch*)

2. Sekvence – *nearest neighbor*

3. Sekundární struktura

- Charakter cílové sekvence
- Kompetice primeru nebo sondy s komplementárním řetězcem cílového duplexu

4. Volné konce

- Interakce mezi 5' a 3' konci hybridizovaného oligonukleotidu a nejbližší sousedící báze

Faktory ovlivňující stabilitu DNA DNA/RNA duplexu

5. Iontová síla

- Koncentrace iontů, zejména Mg^{II+}
- Kationty kompenzují negativní náboj fosfátových skupin a usnadňují formování duplexu
- Stabilita duplexu (T_m) je úměrná koncentraci iontů

6. Teplota

- Se stoupající T je udržení duplexu energicky náročnější, po překročení určité T je preferována ssDNA – vyšší entropie celého systému

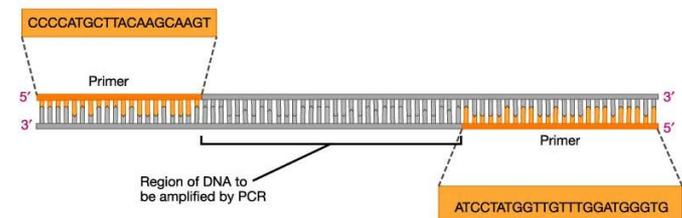
Není tedy nutná shodná T_m , ale shodná účinnost hybridizace obou primerů.

Primery se stejnou T_m , ale rozdílnou ΔG^0 , mohou vykazovat rozdílnou úspěšnost při tvorbě duplexu než primery s odpovídající ΔG^0 .

Design primerů

- Optimálně: primery jejichž 5'konce tvoří stabilní duplex, $\Delta G^0 < 10$ kcal/mol/37°C
- Plynulý přechod ΔG^0 směrem k 3'konci až k cca -6kcal/mol.
- Eliminace misprimingu (vzniklého hybridizací pouze 3'konce)
- Vyloučení repetitivních oblastí, které mohou tvořit sekundární struktury
- Komplementarita primerů – primer dimery
- Specifita – hybridizace k jedinečnému místu v genomu (BLASTn)

Vliv reakčního prostředí – i ideálně navržené primery mohou měnit své vlastnosti v závislosti na použitém PCR pufru a dalších parametrech PCR – vždy je nutná optimalizace jednotlivých PCR



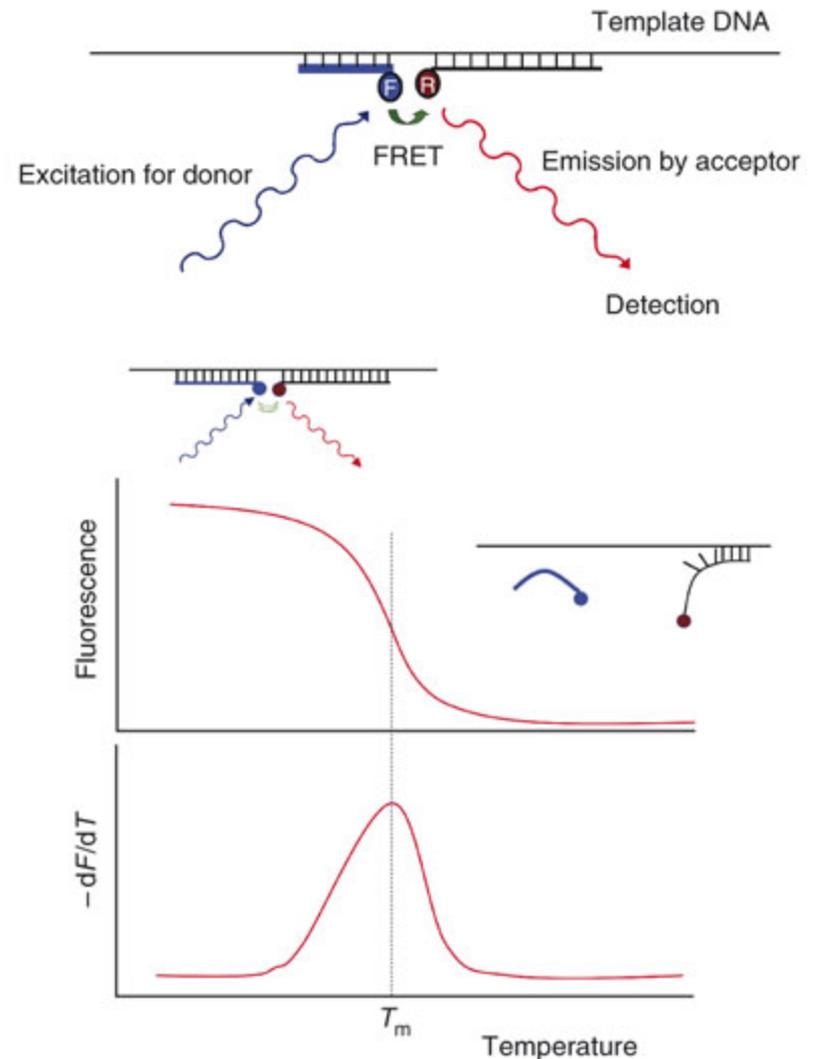
Design sond

- Různý design podle toho, zda je cílem kvantifikace DNA, mRNA nebo provedení alelické diskriminace nebo SNP
- Použitá chemie
- Detekce DNA, RNA nebo obou zároveň? Rozlišení HIV RNA od DNA začleněné do genomu
- Kombinace fluoroforu a zhášeče
- Modifikace sondy – LNA, PNA, MGB atd.
- Multiplex assay

Design hybridizačních sond

(Lightcycler probes)

- Sondy by měly být umístěny co nejdál od primeru 5' – odečet fluorescence v annealingové fázi
- GC 50%
- Každá sonda má délku 23-35bp
- Sondy o stejné T_m – musí se vázat současně ; T_m sond o 5-10°C vyšší než T_m primerů
- 3' konec akceptorové sondy fosforylován
- Donor FAM, akceptor Cy5 nebo Lightcycler Red 640/705
- Vzdálenost mezi sondami 1-5 bází (zajištění FRET)



Design molekulárních majáků

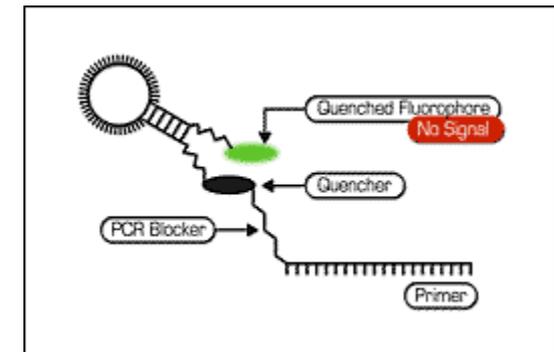
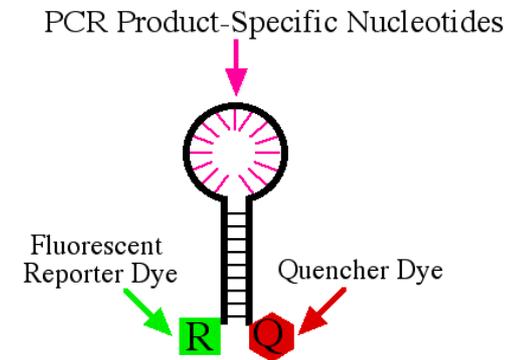
- Vazba majáku ideálně uprostřed ampliconu
- T_m komplementárních ramen o 7-10°C vyšší než T_m primerů
- Délka do 39 bp - omezení sekundárních struktur

Design scorpion primers

Sonda připojena k 5' konci primeru a je komplementární k nově syntetizovanému řetězci

- vlastní hybridizace sondy je intramolekulární událost
- 17-27bp; T_m sondy $< T_m$ primeru
- Cíl sondy – 0-20bp od 3' konce primeru
- Hairpin struktura
- výpočet ΔG pro uzavřenou i hybridizovanou formu

– MFold <http://www.bioinfo.rpi.edu/applications/mfold>



Design primerů

- Délka amplikonu, T_m , účinnost amplifikace i výtěžek
- Správná sekvence – BLASTn
- Sestřih – rozhraní exon/intron
- 3' konec – klíčový pro eventuální mispriming G/C
- Repetice (zejména GC)
- Sekundární struktura, intraprimer homology
- Obsah GC 35-65%
- Délka 15-25bp
- T_m 55-60°C
- ΔG do -10kcal/mol
- V případě převažujících AT – vhodné začlenění LNA
- Eventuální modifikace - na 5'konci

Design primerů a sond

Design primerů – web resources

Nový pár primerů

Nízká komplexita
sekvence (repetice)

Tm mimo rozsah

GC% mimo rozsah

Ne

Vysoká stabilita 3' konce

Ano

Vnitřní nebo vzájemná
komplementarita

Vysoké BLAST skóre

Primer – dimery

OK

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
Transcripts						
NM_005252.2	Homo sapiens v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), mRNA	40.1	40.1	100%	0.014	100%
XM_001718466.1	PREDICTED: Homo sapiens hypothetical protein LOC100128918 (LOC100128918), mRNA	32.2	32.2	80%	3.5	100%
XM_001717510.1	PREDICTED: Homo sapiens hypothetical protein LOC100128918 (LOC100128918), mRNA	32.2	32.2	80%	3.5	100%
XM_001716725.1	PREDICTED: Homo sapiens hypothetical protein LOC100128918 (LOC100128918), mRNA	32.2	32.2	80%	3.5	100%
NM_017780.2	Homo sapiens chromodomain helicase DNA binding protein 7 (CHD7), mRNA	30.2	30.2	75%	14	100%
NM_182923.3	Homo sapiens kinesin light chain 1 (KLC1), transcript variant 2, mRNA	30.2	30.2	75%	14	100%
NM_005552.4	Homo sapiens kinesin light chain 1 (KLC1), transcript variant 1, mRNA	30.2	30.2	75%	14	100%
XM_001726819.1	PREDICTED: Homo sapiens hypothetical protein LOC100131402 (LOC100131402), mRNA	28.2	28.2	70%	55	100%
XM_001725069.1	PREDICTED: Homo sapiens hypothetical protein LOC100131402 (LOC100131402), mRNA	28.2	28.2	70%	55	100%
Genomic sequences [show first]						
NW_001838113.2	Homo sapiens chromosome 14 genomic contig, alternate assembly (based on HuRef SCAF_11	40.1	901	100%	0.014	100%
NT_026437.11	Homo sapiens chromosome 14 genomic contig, reference assembly	40.1	3647	100%	0.014	100%
NW_001838847.2	Homo sapiens chromosome 2 genomic contig, alternate assembly (based on HuRef SCAF_110	34.2	258	100%	0.89	100%

Design primeru a sond

Design primeru – web resources

- Primer Bank

<http://pga.mgh.harvard.edu/primerbank/>

- RTPrimerDB

<http://medgen.ugent.be/rtpprimerdb/>

- Real Time PCR Primer Set

<http://www.realtimeprimers.org/>

- QPPD

<http://web.ncifcrf.gov/rtp/gel/primerdb/default.asp>

Primer Bank

PCR Primers for Gene Expression Detection and Quantification

Home/Search PCR Protocol Primer Statistics Comments Links Citation Policy Help/FAQ

Primer Search

Search for PCR Primers

Search where: GenBank Accession

Species: All Species

For text: Submit

You can blast your sequence against the primerbank sequence DB [here](#).

Order Oligos

You can have primers synthesized and PCR reaction products sequenced at:

DNA Core Facility

Center for Computational and Integrative Biology

Design primerů a sond

Design primerů a sond– web resources

- Primer 3

http://biotools.umassmed.edu/bioapps/primer3_www.cgi

- Primer Express

<http://www.appliedbiosystems.com>

- Premier Biosoft International

<http://www.premierbiosoft.com>

The image shows the 'Primer3: WWW primer tool' web interface. It features a search bar for a DNA sequence, with instructions to paste a sequence in FASTA format. Below the search bar are several checkboxes for selecting primer types (left, internal, right) and a 'Pick Primers' button. A 'General Primer Picking Conditions' section contains various sliders and input fields for parameters such as primer size, GC content, and GC skew. The interface is designed for users to input a DNA sequence and generate primers based on specific criteria.

The image shows the homepage of Premier Biosoft International. The header features the company logo and the tagline 'Software to Accelerate Research in Life Sciences'. Below the header is a navigation menu with links for HOME, COMPANY, PRODUCTS, SERVICES, DOWNLOAD, and ORDERING. The main content area lists several software products, each with a small icon and a brief description:

- AlleleID®** - Real Time PCR & Microarrays
- Array Designer** - Microarrays
- Beacon Designer™** - Real Time PCR Oligo Design
- PrimerPlex** - Oligo Design for xMAP® Based Multiplex Systems
- Primer Premier** - PCR Primer Design
- SimGlycan™** - MS/MS Data Analysis
- SimVector** - Draw Plasmid Maps & Plan Cloning Experiments
- TMA Foresight** - Tissue Microarray Data Analysis
- Xpression Primer** - Tagged Primer Design

Design primerů a sond

Návrh primerů a TaqMan sond – Primer Express

```
STS      /db_xref="UniSTS:47/415"  
1090..1143  
/gene="FOS"  
/gene_synonym="AP-1"  
/gene_synonym="C-FOS"  
/standard_name="BF250015A10G9"  
/db_xref="UniSTS:519218"  
  
ORIGIN  
1 atgatgttct cgggcttcaa cgcagactac gaggcgtcat cctcccgtg cagcagcgcg  
61 tcccggcgcg gggatagcct ctcttactac cactcaaccg cagactccct ctcacagatg  
121 ggctcgctg tcaacgcgca ggaactctgc acggaactag cctgtccag tgcacacttc  
181 attcccacgg tcactgcat ctgcaccagt cgggaactgc agtggctgt ggaagccgcg  
241 ctgctctct ctgtggccc atcgcagacc agagccctc acccttcgg agtcccgcg  
301 cctcccgtg gggcttactc cagggtggc gttgtgaaga ccatgacag aggcagcgcg  
361 cagagcattg gcaggagggg caagtgga aagtattctc cagaagaaga agagaaaagg  
421 agaactccga gggaaaggaa taagatggct gcagccaaat gccgcaacc gaggagggag  
481 ctgactgata cactccaagc ggagacagac caactagaag atgcaagtc tgccttgag  
541 accgagattg ccaactctgc gaaggagaag gaaaaactag agtctatct gccagctcac  
601 cgacctgctt gcaagatccc tgatgacctg ggcttcccag aagagatgc tgtggcttcc  
661 cttgatctga ctgggggccc gccagaggtt gccaccggg agtctgagga ggccttcaac  
721 ctgctctctc tcaatgccc tgagcccaag cctcagtggg aacctgcaa gagcatcagc  
781 agcatggagc tgaagaccga gccctttgat gacttctgt tcccagatc atccaggccc  
841 agtggctctg agacagcccg ctcccggcca gacatggacc tatctgggtc cttctatgca  
901 gcagactggg agcctctgca cagtggctcc ctggggatgg ggcctatggc cacagagctg  
961 gagccctctg gcaactccgtt ggtcactctg actcccagct gcaactgta cacgtcttcc  
1021 ttctgtctca cctaccgga ggcctactcc tcccagact gtgcagctgc ccaccgcaag  
1081 ggcagcagca gcaatgagcc ttctctgac tgcctcagct caccacagct gctggcctct  
1141 tga
```

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Applied Biosystems

Primer Express® Software for Real-Time PCR

Version 3.0

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Primer Express 3.0

TaqMan® MGB Quantification # 1

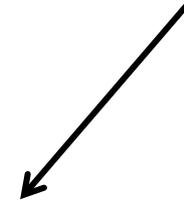
Sequence Parameters Primers / Probes Order

File Name

Length 1143 bp. Selection 1144 to 1144 Double Stranded

ATGATGTTCT	CGGGCTTCAA	CGCAGACTAC	GAGGCCTCAT	CCTCCCCTG	50
CAGCAGCGCG	TCCCCGGCCG	GGGATAGCCT	CTCTTACTAC	CACCTACCCG	100
CAGACTCTCT	CTCCAGCATG	GGCTCGCCTG	TCAACCGCCA	GGAATCTTGC	150
ACGGACTTGG	CGGTCTCCAG	TGCCAACTTC	ATTCCCACGG	TCACTGCCAT	200
CTCGACCACT	CGGACCTGCG	AGTGGCTGGT	GCAGCCCGCC	CTGCTCTCT	250
CTGTGGCCCC	ATCGCAGACC	AGAGCCCTTC	ACCCTTTCGG	AGTCCCCTGC	300
CCCTCGCTG	GGGCTTACTC	CAGGGCTGGC	GTGTGAAGA	CAATGACAGG	350
AGGCCGAGCG	CAGAGCATTG	GCAGGAGGGG	CAAGTGGAA	CAGTATCTC	400
CAGAAAAGA	AGAGAAAAGG	AGAATCCGAA	GGGAAAAGAA	TAAGATGGCT	450
GCAGCCAAAT	GCCCAACCGG	GAGGAGGGAG	CTGACTGATA	CACCTCAAGC	500
GGAGACAGAC	CAACTAGAAAG	ATGAGAAGTC	TGCTTTGCGA	ACCGAGATTG	550
CCAACCTGCT	GAAAGGAAAG	GAAAAACTAG	AGTTCATCCT	GCCAGCTCAC	600
CGACTTGCCT	GCAAGATCCC	TGATGACCTG	GGCTTCCCAG	AAGAGATGTC	650
TGTGGCTTCC	CTTGATCTGA	CTGGGGCCCT	GCCAGAGGTT	GCCACCCCGG	700
AGTGTGAGGA	GGCTTTCACC	CTGGCTTCTC	TCAATGACCC	TGAGCCCAAG	750
CCCTCAGTGG	AACCTTCAA	GAGCATCAGC	AGCATGGAGC	TGAAAGCCGA	800
GGCCTTTGAT	GACTTCTGT	TCCCAGATC	ATCCAGGCC	AGTGGCTCTG	850
AGACAGCCCC	CTCCGTGCCA	GACATGGACC	TATCTGGGTC	CTTCTATGCA	900
GCAGACTTGG	AGCCTTGTCA	CAGTGGCTCC	CTGGGGATGG	GGCCCATGGC	950

To find Primers & Probes, click the "Find Primers/Probes" button



Design primeru a sond

TaqMan® MGB Quantification # 1	
Sequence	Parameters / Primers / Probes / Order
Parameter	Value
<input type="checkbox"/> Primer Tm	
Min Primer Tm	58
Max Primer Tm	60
Max Difference in Tm of Two Primers	2
<input type="checkbox"/> Primer GC Content	
Min Primer %GC Content	30
Max Primer %GC Content	80
Max Primer 3' GC's	2
Primer 3' End Length	5
Primer 3' GC Clamp Residues	0
<input type="checkbox"/> Primer Length	
Min Primer Length	9
Max Primer Length	40
Optimal Primer Length	20
<input type="checkbox"/> Primer Composition	
Max Primer G Repeats	3
Max Num Ambig Residues in Primer	0
<input type="checkbox"/> Primer Secondary Structure	
Max Primer Consec Base Pair	4
Max Primer Total Base Pair	8
<input type="checkbox"/> Primer Site Uniqueness	
Max % Match in Primer	75
Max Consec Match in Primer	9
Max 3' Consec Match in Primer	7
<input type="checkbox"/> Probe Tm	
Min Probe Tm	68
Max Probe Tm	70
<input type="checkbox"/> Probe GC Content	
Min Probe %GC Content	30
Max Probe %GC Content	80
<input type="checkbox"/> Probe Length	
Min Probe Length	13
Max Probe Length	25
<input type="checkbox"/> Probe Composition	
Max Probe G Repeats	3
Max Num Ambig Residues in Probe	0
No G at 5' End in Probe	<input checked="" type="checkbox"/>
Select Probe with more C's than G's	<input type="checkbox"/>
<input type="checkbox"/> Probe Secondary Structure	
Max Probe Consec Base Pair	4
Max Probe Total Base Pair	8
<input type="checkbox"/> Amplicon	
Min Amplified Region Tm	0
Max Amplified Region Tm	85
Min Amplified Region Length	50
Max Amplified Region Length	150
<input type="checkbox"/> General	
Max Primers / Probes	50

Design primerů a sond

TaqMan® MGB Quantification # 1



Sequence Parameters Primers / Probes Order

Candidate Primers & Probes

#	Fwd Start	Fwd Stop	Fwd Len...	Fwd Tm	Fwd %GC	Fwd Seq	Rev Start	Rev Stop	Rev Len...	Rev Tm	Rev %GC	Rev Seq	Probe
1	162	181	20	58	55	CGTCTCCA...	217	199	19	58	63	GGTCCGGA...	183
2	161	180	20	59	60	CCGTCTCC...	217	199	19	58	63	GGTCCGGA...	182
3	161	180	20	59	60	CCGTCTCC...	217	199	19	58	63	GGTCCGGA...	183
4	745	762	18	58	61	CCCAAGCC...	809	790	20	59	55	TCAAAGGG...	765
5	745	762	18	58	61	CCCAAGCC...	809	790	20	59	55	TCAAAGGG...	765
6	745	762	18	58	61	CCCAAGCC...	809	790	20	59	55	TCAAAGGG...	765
7	800	822	23	60	48	AGCCCTTT...	864	847	18	59	67	GGAGCGGG...	827
8	800	822	23	60	48	AGCCCTTT...	864	847	18	59	67	GGAGCGGG...	828
9	800	822	23	60	48	AGCCCTTT...	864	847	18	59	67	GGAGCGGG...	829
10	745	762	18	58	61	CCCAAGCC...	810	791	20	58	50	ATCAAAGG...	765
11	745	762	18	58	61	CCCAAGCC...	810	791	20	58	50	ATCAAAGG...	765
12	745	762	18	58	61	CCCAAGCC...	810	791	20	58	50	ATCAAAGG...	765
13	745	762	18	58	61	CCCAAGCC...	810	790	21	59	52	ATCAAAGG...	765
14	745	762	18	58	61	CCCAAGCC...	810	790	21	59	52	ATCAAAGG...	765
15	745	762	18	58	61	CCCAAGCC...	810	790	21	59	52	ATCAAAGG...	765
16	799	821	23	60	48	GAGCCCTT...	864	847	18	59	67	GGAGCGGG...	827
17	799	821	23	60	48	GAGCCCTT...	864	847	18	59	67	GGAGCGGG...	828
18	799	821	23	60	48	GAGCCCTT...	864	847	18	59	67	GGAGCGGG...	829
19	745	762	18	58	61	CCCAAGCC...	811	792	20	58	55	CATCAAAG...	765
20	745	762	18	58	61	CCCAAGCC...	811	792	20	58	55	CATCAAAG...	765
21	745	762	18	58	61	CCCAAGCC...	811	792	20	58	55	CATCAAAG...	765
22	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	820
23	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	820
24	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	821
25	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	821
26	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	822
27	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	827
28	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	828
29	798	818	21	59	52	CGAGCCCT...	864	847	18	59	67	GGAGCGGG...	829
30	745	762	18	58	61	CCCAAGCC...	812	793	20	58	50	TCATCAA...	765
31	745	762	18	58	61	CCCAAGCC...	812	793	20	58	50	TCATCAA...	765

Click to show Locations

Click to show Secondary Structures

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Name	Value
<input type="checkbox"/> Forward Primers	
Total primers tested:	35792
GC test passed:	35149
Ambiguity test passed:	963
Clamp test passed:	963
Tm test passed:	963
Avoid Excluded regions test passed:	963
Repeat test passed:	900
Self compare test passed:	741
Limit GC test passed:	214
Sequence compare passed:	84
Reverse sequence compare passed:	83

<input type="checkbox"/> Reverse Primers	
Total primers tested:	35296
GC test passed:	34657
Ambiguity test passed:	946
Clamp test passed:	946
Tm test passed:	946
Avoid Excluded regions test passed:	946
Repeat test passed:	861
Self compare test passed:	703
Limit GC test passed:	205
Sequence compare passed:	95
Reverse sequence compare passed:	95
<input type="checkbox"/> Primer Pairs	
Total pairs tested:	7885
Amplicon Length test passed:	691
Avoid Excluded regions test passed:	691
Tm Difference test passed:	691
Amplicon Tm test passed:	630

<input type="checkbox"/> TaqMan Probes	
Total probes tested:	14450
GC test passed:	14128
Ambiguity test passed:	1178
Tm test passed:	1178
Avoid Excluded regions test passed:	1178
Repeat test passed:	1126
Self compare test passed:	1076
Sequence compare passed:	475
Reverse sequence compare passed:	458
Probe start test passed:	351

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#	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
	#	Fwd Start	Fwd Stop	Fwd Length	Fwd Tm	Fwd %GC	Fwd Seq	Rev Start	Rev Stop	Rev Length	Rev Tm	Rev %GC	Rev Seq	Probe Start	Probe Stop	Probe Length	Probe Tm	Probe %GC	Probe Seq	Amp Tm	Amp %GC	Amp Ta	Amp Len	Penalty	
2	1	162	181	20	58	55	CGTCTCCAGTGCCCAACTCA	217	199	19	58	63	GGTCCGGAGTGGTCCGAGAT	183	197	15	69	67	TCCCAAGGCTCACTGC	84	61	62	56	31	
3	2	161	180	20	59	60	CCGTCTCCAGTGCCCAACTC	217	199	19	58	63	GGTCCGGAGTGGTCCGAGAT	182	197	16	69	63	TTCCCAAGGCTCACTGC	85	61	62	57	36	
4	3	161	180	20	59	60	CCGTCTCCAGTGCCCAACTC	217	199	19	58	63	GGTCCGGAGTGGTCCGAGAT	183	197	15	69	67	TCCCAAGGCTCACTGC	85	61	62	57	36	
5	4	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	809	790	20	59	55	TCAAAGGGCTCGGTCTTCAG	765	780	16	68	50	TGTCAGAGCATCAGC	83	57	61	65	77	
6	5	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	809	790	20	59	55	TCAAAGGGCTCGGTCTTCAG	765	781	17	69	47	TGTCAGAGCATCAGCA	83	57	61	65	77	
7	6	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	809	790	20	59	55	TCAAAGGGCTCGGTCTTCAG	765	782	18	69	50	ATCATCCAGGCCAAGTGG	83	57	61	65	77	
8	7	800	822	23	60	48	AGCCCTTTGATGACTTCTGTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	827	844	18	70	61	CATCATCAGGCCAAGTGG	84	60	62	65	80	
9	8	800	822	23	60	48	AGCCCTTTGATGACTTCTGTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	828	845	18	70	61	ATCATCCAGGCCAAGTGG	84	60	62	65	80	
10	9	800	822	23	60	48	AGCCCTTTGATGACTTCTGTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	829	845	17	69	65	TCATCCAGGCCAAGTGG	84	60	62	65	80	
11	10	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	810	791	20	58	50	ATCAAAGGGCTCGGTCTTCA	765	780	16	68	50	TGTCAGAGCATCAGC	83	56	60	66	82	
12	11	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	810	791	20	58	50	ATCAAAGGGCTCGGTCTTCA	765	781	17	69	47	TGTCAGAGCATCAGCA	83	56	60	66	82	
13	12	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	810	791	20	58	50	ATCAAAGGGCTCGGTCTTCA	765	782	18	69	50	TGTCAGAGCATCAGCAG	83	56	60	66	82	
14	13	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	810	790	21	59	52	ATCAAAGGGCTCGGTCTTCAG	765	780	16	68	50	TGTCAGAGCATCAGC	83	56	60	66	83	
15	14	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	810	790	21	59	52	ATCAAAGGGCTCGGTCTTCAG	765	781	17	69	47	TGTCAGAGCATCAGCA	83	56	60	66	83	
16	15	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	810	790	21	59	52	ATCAAAGGGCTCGGTCTTCAG	765	782	18	69	50	TGTCAGAGCATCAGCAG	83	56	60	66	83	
17	16	799	821	23	60	48	GAGCCCTTTGATGACTTCTGTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	827	844	18	70	61	CATCATCAGGCCAAGTGG	84	61	62	66	85	
18	17	799	821	23	60	48	GAGCCCTTTGATGACTTCTGTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	828	845	18	70	61	ATCATCCAGGCCAAGTGG	84	61	62	66	85	
19	18	799	821	23	60	48	GAGCCCTTTGATGACTTCTGTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	829	845	17	69	65	TCATCCAGGCCAAGTGG	84	61	62	66	85	
20	19	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	811	792	20	58	55	CATCAAAGGGCTCGGTCTTC	765	780	16	68	50	TGTCAGAGCATCAGC	83	57	61	67	87	
21	20	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	811	792	20	58	55	CATCAAAGGGCTCGGTCTTC	765	781	17	69	47	TGTCAGAGCATCAGCA	83	57	61	67	87	
22	21	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	811	792	20	58	55	CATCAAAGGGCTCGGTCTTC	765	782	18	69	50	TGTCAGAGCATCAGCAG	83	57	61	67	87	
23	22	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	820	835	16	69	50	TTCCAGCATCATCCA	85	61	62	67	88	
24	23	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	820	836	17	69	53	TTCCAGCATCATCCA	85	61	62	67	88	
25	24	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	821	835	15	69	53	TCCAGCATCATCCA	85	61	62	67	88	
26	25	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	821	836	16	69	56	TCCAGCATCATCCA	85	61	62	67	88	
27	26	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	822	834	13	69	62	CCCAGCATCATCC	85	61	62	67	88	
28	27	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	827	844	18	70	61	CATCATCAGGCCAAGTGG	85	61	62	67	88	
29	28	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	828	845	18	70	61	ATCATCCAGGCCAAGTGG	85	61	62	67	88	
30	29	798	818	21	59	52	CGAGCCCTTTGATGACTTCT	864	847	18	59	67	GGAGCGGGTGTCTCAGA	829	845	17	69	65	TCATCCAGGCCAAGTGG	85	61	62	67	88	
31	30	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	812	793	20	58	50	TCATCAAAGGGCTCGGTCTT	765	780	16	68	50	TGTCAGAGCATCAGC	82	56	60	68	92	
32	31	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	812	793	20	58	50	TCATCAAAGGGCTCGGTCTT	765	781	17	69	47	TGTCAGAGCATCAGCA	82	56	60	68	92	
33	32	745	762	18	58	61	CCCAAGCCCTCAGTGGAA	812	793	20	58	50	TCATCAAAGGGCTCGGTCTT	765	782	18	69	50	TGTCAGAGCATCAGCAG	82	56	60	68	92	
34	33	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	820	835	16	69	50	TTCCAGCATCATCCA	85	62	62	68	92	
35	34	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	820	836	17	69	53	TTCCAGCATCATCCA	85	62	62	68	92	
36	35	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	821	835	15	69	53	TCCAGCATCATCCA	85	62	62	68	92	
37	36	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	821	836	16	69	56	TCCAGCATCATCCA	85	62	62	68	92	
38	37	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	822	834	13	69	62	CCCAGCATCATCC	85	62	62	68	92	
39	38	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	822	836	15	68	60	CCCAGCATCATCCA	85	62	62	68	92	
40	39	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	823	837	15	68	60	CCAGCATCATCAGG	85	62	62	68	92	
41	40	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	827	840	14	68	64	CATCATCAGGCCC	85	62	62	68	92	
42	41	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	827	842	16	68	63	CATCATCAGGCCAAGTGG	85	62	62	68	92	
43	42	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	827	844	18	70	61	CATCATCAGGCCAAGTGG	85	62	62	68	92	
44	43	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	828	845	18	70	61	ATCATCCAGGCCAAGTGG	85	62	62	68	92	
45	44	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	829	845	17	69	65	TCATCCAGGCCAAGTGG	85	62	62	68	92	
46	45	797	816	20	58	55	CCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	830	845	16	68	69	CATCCAGGCCAAGTGG	85	62	62	68	92	
47	46	800	822	23	60	48	AGCCCTTTGATGACTTCTGTTC	867	851	17	59	71	CACGAGCGGGTGTCT	827	844	18	70	71	CATCATCAGGCCAAGTGG	84	60	62	68	96	
48	47	800	822	23	60	48	AGCCCTTTGATGACTTCTGTTC	867	851	17	59	71	CACGAGCGGGTGTCT	828	845	18	70	61	ATCATCCAGGCCAAGTGG	84	60	62	68	96	
49	48	800	822	23	60	48	AGCCCTTTGATGACTTCTGTTC	867	851	17	59	71	CACGAGCGGGTGTCT	829	845	17	69	65	TCATCCAGGCCAAGTGG	84	60	62	68	96	
50	49	796	816	21	59	52	ACCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	820	835	16	69	50	TTCCAGCATCATCCA	85	61	62	69	98	
51	50	796	816	21	59	52	ACCGAGCCCTTTGATGACTTC	864	847	18	59	67	GGAGCGGGTGTCTCAGA	820	836	17	69	53	TTCCAGCATCATCCA	85	61	62	69	98	

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Choose Species

Filter by Amplicon Lengths

- H. sapiens
- R. norvegicus
- M. musculus
- M. mulatta (Rhesus)
- D. rerio (Zebrafish)
- G. gallus (Chicken)
- S. scrofa (Pig)
- A. thaliana
- D. melanogaster
- C. elegans
- C. familiaris (Canine)
- B. taurus (Cow)
- O. cuniculus (Rabbit)
- Amplicon length less than 70
- Amplicon length between 71 and 85
- Amplicon length between 86 and 100
- Amplicon length greater than or equal to 101

Choose Set Membership

- Search All Assays (excludes Gene Copy Number Assays)
- Search Gene Copy Number Assays
- Limit Assay Sets to:

TARGET CLASS	ASSAY ATTRIBUTE	MICROARRAY VALIDATION	COLLABORATOR SETS
<input type="checkbox"/> Apoptosis	<input type="checkbox"/> Ambion siRNA	<input type="checkbox"/> 1700	<input type="checkbox"/> Immune Tolerance Network
<input type="checkbox"/> Fusion Transcripts	<input type="checkbox"/> Endogenous Controls	<input type="checkbox"/> 3' Most	<input type="checkbox"/> Mammalian Gene Collection

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Assay ID	Availability	Gene Symbol	Gene Name	Alias	RefSeq	GenBank mRNA	Species	Amplicon Length
1. Assay ID Details: Hs00170630_m1 Alignment Map siRNAs & Related Products	Inventoried	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	AP-1 C-FOS	NM_005252.2	5 GenBank mRNAs	Homo sapiens	77
2. Assay ID Details: Hs99999140_m1	Inventoried	FOS	v-fos FBJ murine	AP-1 C-FOS	NM_005252.2	5 GenBank mRNAs	Homo sapiens	77

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Assay ID	Availability	Gene Symbol	Gene Name	Alias	RefSeq	GenBank mRNA	Species	Amplicon Length
1. Assay ID Details: Hs00170630_m1 Alignment Map siRNAs & Related Products	Inventoried	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	AP-1 C-FOS	NM_005252.2	5 GenBank mRNAs	Homo sapiens	78
							Homo sapiens	67

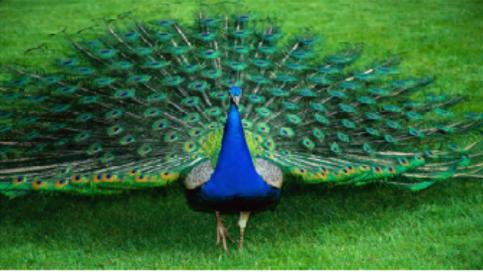
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Specify your target(s):

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By sequence ID, gene name or keyword

e.g. ENST00000331789, NM_001101 or X00351 or beta-actin

or

By sequence

e.g.
>part of X00351 Human mRNA for beta-actin
CACGGCATCGTCACCAACTGGGACGACATGGAGAAAATCTGGCACCACACCTTCTACAAT
GAGCTGCGTGTGGCTCCCGAGGAGCACCCCGTGTGCTGACCGAGGCCCCCTGAACCCC
AAGGCCAACCGGAGAAAGATGACCCAGATCATGTTTGAGACCTTCAACACCCGACCATG
TACGTTGCTATCCAGGCTGTGCTATCCCTGTACGCCCTTGCCCGTACCCTGGCATCGTG
ATGGACTCCGGTGACGGGGTACCCACACTGTGCCATCTACGAGGGGTATGCCCTCCC

Automatically select an intron spanning assay. Design multiplex PCR with reference gene.

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Please choose the sequence(s) you would like to continue with. You can select up to 10 sequences.

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	Name	Length	Description
<input type="checkbox"/>	ENST00000400991.1	2669	AL139130.28-201 Clone_based_ensembl_transcri Transcriptional activator of the c-fos promoter CROC4 (CROC-4). [Source:Uniprot/SPTREMBL;Acc:Q8N964]
<input type="checkbox"/>	ENST00000303562.2	2103	FOS-201 HGNC automatic transcript Proto-oncog fos) (G0/G1 [Source:Uni
<input type="checkbox"/>	ENST00000297904.2	2110	FIGF-001 HGNC automatic transcript endothelial g (c-fos-induc [Source:Uni
<input type="checkbox"/>	NM_003367.2	1732	Homo sapie c-fos interac
<input type="checkbox"/>	NM_207291.1	1531	Homo sapie c-fos interac
<input type="checkbox"/>	NM_003131.2	4343	Homo sapie response el (SRF), mRN
<input type="checkbox"/>	NM_004469.2	2128	Homo sapie (vascular en mRNA.
<input type="checkbox"/>	AB022275.1	300	Homo sapie partial cds.
<input type="checkbox"/>	AB022276.1	700	Homo sapie partial cds.
<input type="checkbox"/>	AB209128.1	5672	Homo sapie (c-fos serum transcription
<input type="checkbox"/>	AF126533.1	238	Homo sapie

ProbeFinder has designed the optimal real-time PCR assay for:

[NM_003367.2](#) Homo sapiens upstream transcription factor 2, c-fos interacting (USF2), transcript variant 1, mRNA.

Assay details:

Use Universal ProbeLibrary probe: #26, cat.no. 04687574001

Primer	Length	Position	Tm	%GC	Sequence
Left Primer	18	449 - 466	60	67	gtgaccacaggtgggtgtg
Right Primer	21	540 - 560	59	43	tgaagggttttgatcacag

Amplicon (112 nt)

```
gtgaccacaggtgggtgtggaagggaagccagcagccgggccccgcctctgtg
ccccaggtcctgcagcgccttcccgtggtgtgatccaaaatccctca
```

Transcript overview:



Detailed view:



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Po dnešní přednášce:

- Rozumíte vlastnostem primerů i základních typů sond a znáte faktory, které ovlivňují jejich hybridizaci a účinnost
- Umíte navrhnout optimální sekvenci primerů i hydrolyzační sondy pomocí dostupných programů a rozumíte parametrům designu

