

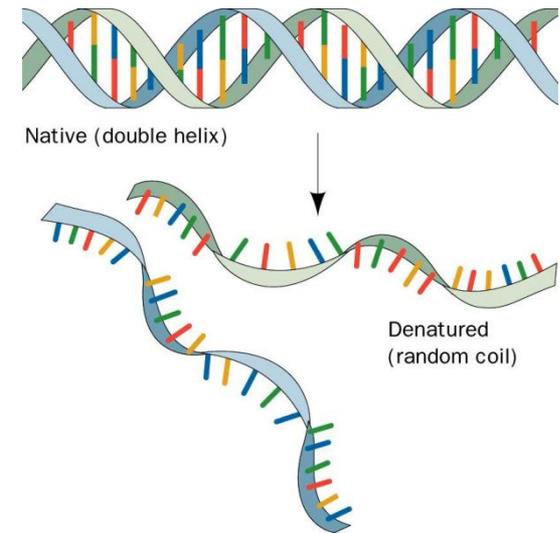
ÚVOD DO KVANTITATIVNÍ REAL-TIME PCR



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

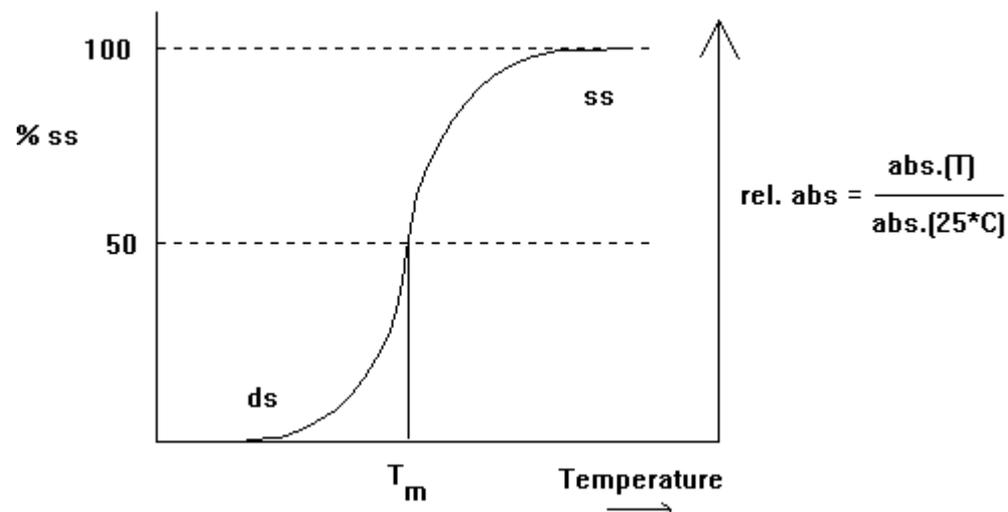
$$T_m = 2 \times (A+T) + 4 \times (G+C)$$

Iontová síla, %GC a délka řetězce (N)

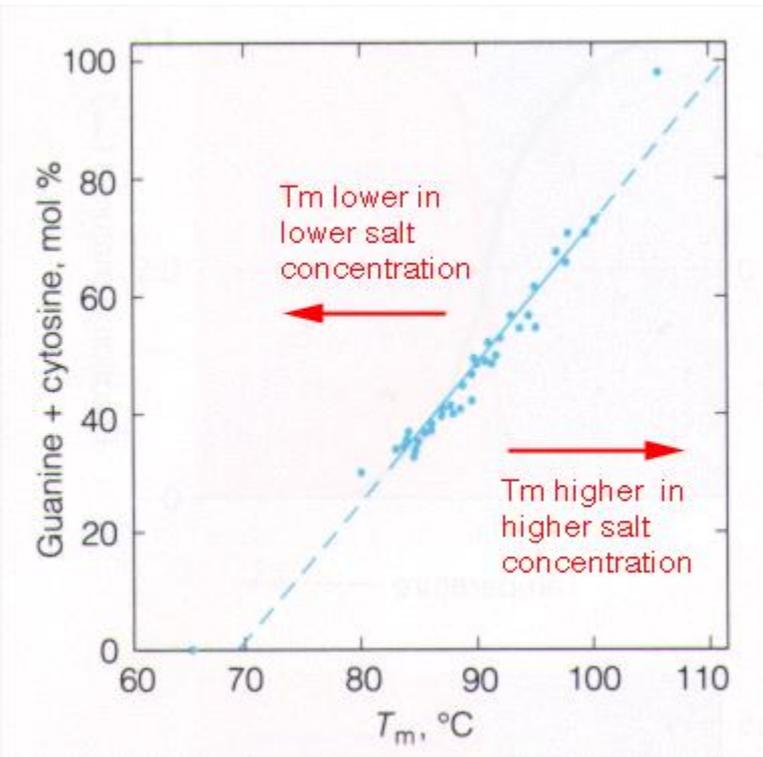
$$T_m = 81,5 + 16,6 (\log_{10}[\text{Na}^+] + 0,41(\%GC) - (625/N))$$

Web-based kalkulátory

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



Melting temperature T_m



GCTATTCAACTGAAGAGGGGCACAGC

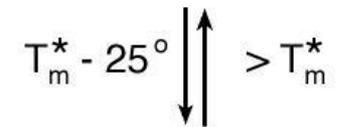
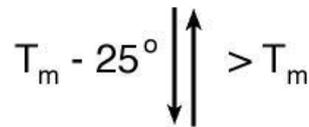
GCTATTCAACTG^GAGAGGGGCACAGC

+

+

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

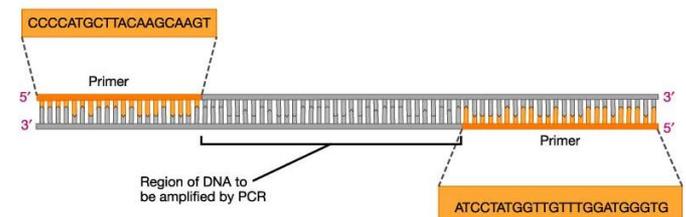
Není tedy nutná shodná T_m , ale shodná účinnost hybridizace obou primerů.

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

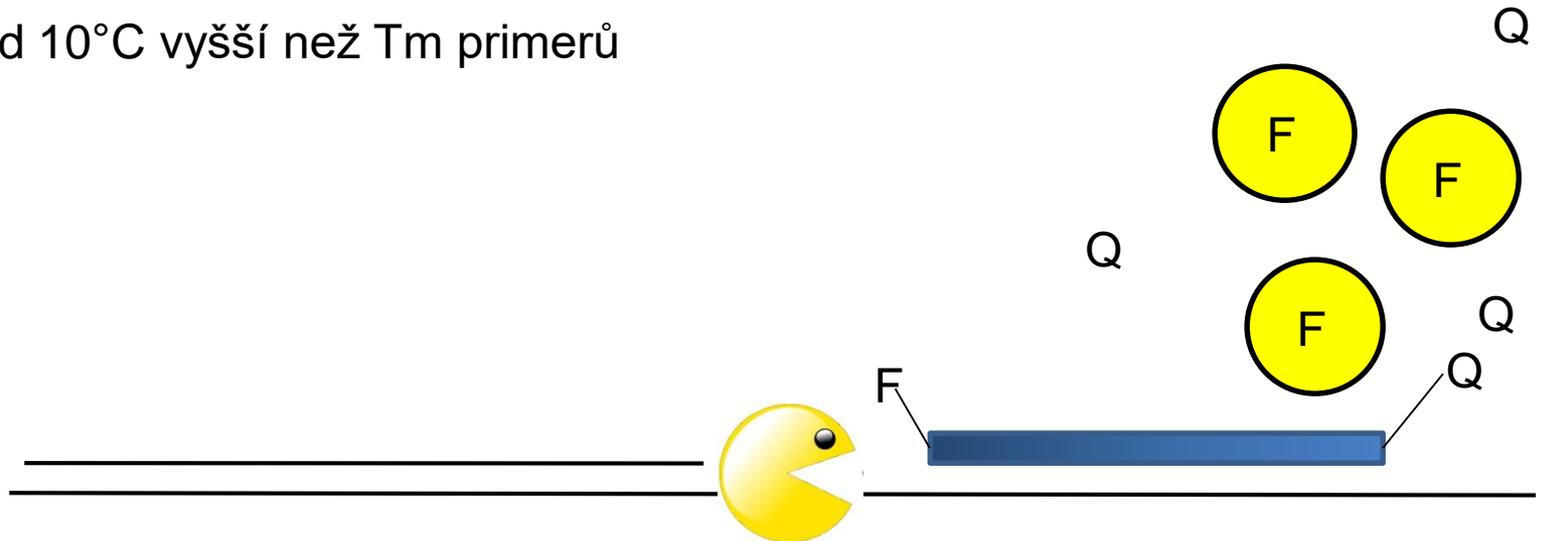


Design sond

- 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 hydrolyzačních sond

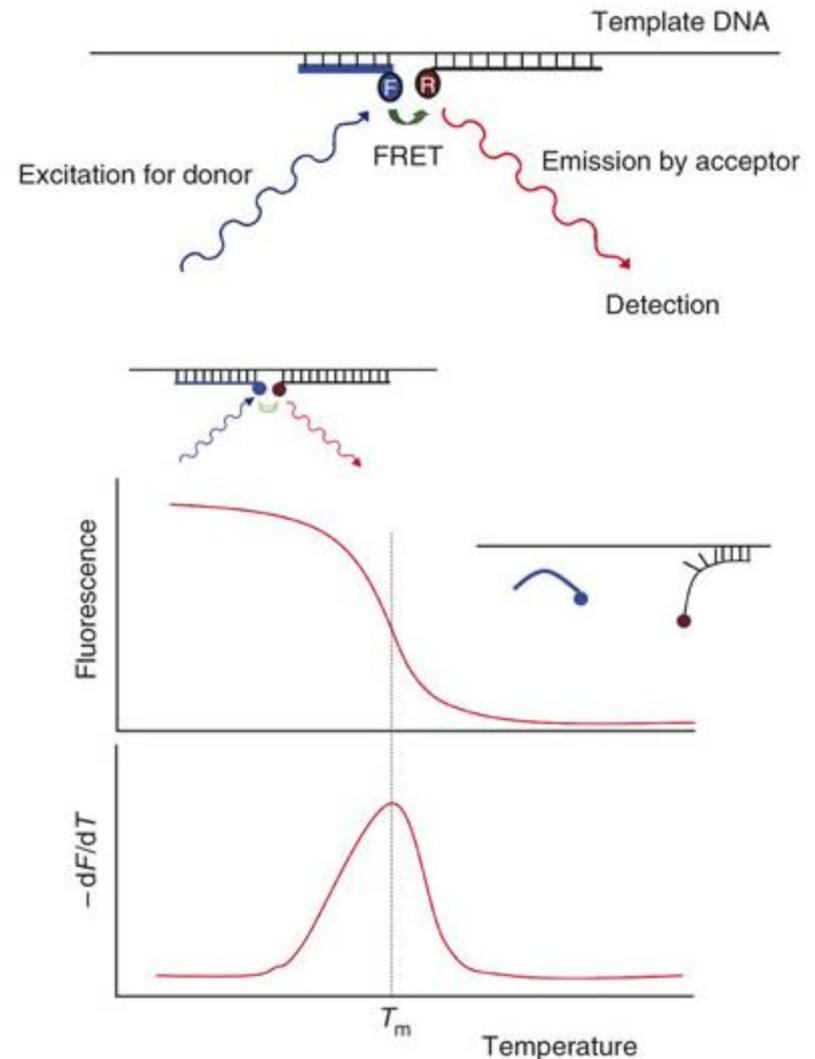
- qPCR TaqMan - dvoukrokový proces – denaturace a annealing/extension
- Co nejnižší Ct a nejvyšší ΔR (ΔR_n)
- Umístění 5' konce sondy v rámci stanovované sekvence co nejbliže 3' konci jednoho z primerů – účinné štěpení sondy
- Optimální délka do 30 nukleotidů, obsah GC do 30%
- AT bohaté sekvence – začlenění LNA, PNA nebo MGP
- G – účinný quencher
- Minimum repeticí, zejména GGGG, začlenění inosinu do repetice řeší tento problém
- T_m probe od 10°C vyšší než T_m primerů



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)



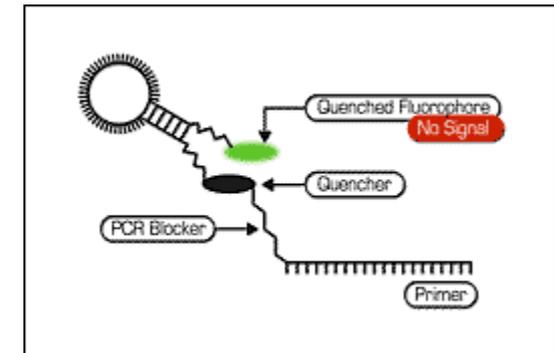
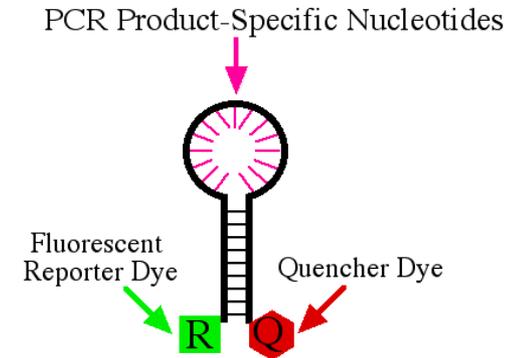
Design molekulárních majáků

- 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)

T_m 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 primerů a sond

Design primerů – 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

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Primer Search

Search for PCR Primers

Search where:

Species:

For text:

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

Quicksearch - Filter settings

RTPrimerDB ID: Gene: Species:

Home | Search | In silico evaluation | Log In

Introduction

RTPrimerDB is a public database for primer and probe sequences used in real-time PCR assays employing popular chemistries (SYBR Green I, TaqMan, Hydrolysis Probes, Molecular Beacons). It provides time-consuming primer design and experimental optimization, and to introduce a certain level of uniformity and standardization among different laboratories.

We strongly encourage researchers to submit their validated primer and probe sequences, so that other users can benefit from their expertise. The database can be [queried](#) using the official gene name or symbol, [Entrez](#) or [Ensembl](#) Gene identifier, [SRA](#) identifier, or oligonucleotide sequence.

Different options make it possible to restrict a query to a particular application (Gene Expression Quantification/Detection, DNA Copy Number Quantification/Detection, SNP Detection, Mutation Analysis, Fusion Gene Quantification/Detection, Chromatin Immunoprecipitation (ChIP), organome Human, Mouse, Rat, and others) or detection chemistry. Data submission is allowed after free registration whereby you obtain a login name and password.

Currently, 7755 real-time PCR assays for 5397 genes are available, submitted by 184 people.

Last submission link:

Publications

- PATTYN, F., SPELEMAN, F., DE PAEPE, A. & VANDESCHPELE, J. (2003). RTPrimerDB: the Real-Time PCR primer and probe database. *Nucleic Acid Research*, 31(1), 120-123. [\[PubMed\]](#)
- PATTYN, F., ROBERTSCHT, P., SPELEMAN, F., DE PAEPE, A. & VANDESCHPELE, J. (2005). RTPrimerDB: the Real-Time PCR primer and probe database - major update 2004. *Nucleic Acid Research*, 33(Database issue), D684-D688. [\[PubMed\]](#)
- LEFEBVER, S., VANDESCHPELE, J., SPELEMAN, F., PATTYN, F. (2006). RTPrimerDB: the portal for real-time PCR primers and probes. *Nucleic Acid Research*, Oct 23. [pub ahead of print] [\[PubMed\]](#)

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Quantitative PCR Primer Database

Q P P D

[QPPD Home](#) [Search Primer](#) [Submit Primer](#)

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>

Primer3: WWW primer tool

pick primers from a DNA sequence

fasta source sequence below (5'>3', string of ACGTNacgtm -- other letters treated as N -- numbers and blanks ignored; FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Microarray Library (Mount Shair).

NOTE: []

Pick left primer or use left primer below Pick hybridization probe (internal oligo) or use oligo below Pick right primer or use right primer below (5'>3' on opposite strand)

Sequence ID: [] A string to identify your output

Targets: [] E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and] e.g. ...ATCT|CCCC|TCAT... means that primers must flank the central CCCC

Included Regions: [] E.g. 401,7,68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and > e.g. ...ATCT<CCCC>TCAT... forbids primers in the central CCCC

Product Size: Max: 100 Opt: 200 Max: 1000

Number To Return: 5 Max 3' Stability: 9.0

Max Mismatches: 10.00 Max Min Mismatches: 24.00

General Primer Picking Conditions

Primer Size: Max: 18 Opt: 20 Max: 27

Primer Tm: Max: 57.0 Opt: 60.0 Max: 63.0 Max Tm Difference: 100.0

Product Tm: Max: [] Opt: [] Max: []

Primer GC%: Max: 20.0 Opt: [] Max: 80.0

Min Self Complementarity: 0.00 Max 3' Self Complementarity: 3.00

Max GC%: 0 Max Poly-S: 5

Inside Target Penalty: [] Outside Target Penalty: 0 Set Inside Target Penalty to allow primers inside a target

First Base Index: 1 CG Clamp: 0

Salt Concentration: 50.0 Annealing Oligo Concentration: 50.0 (Not the concentration of oligos in the reaction mix, but of those annealing to template)

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- 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 ctttactact cactcaaccg cagactccct ctcacagatg
121 ggctcgctg tcaacgcgca ggaactctgc acggaactag cctgtccag tgcacacttc
181 attcccacgg tcaactgcat ctcgaccagt cgggaactgc agtggctgt ggaagccgcg
241 ctgctctct ctgtggccc atcgcagacc agagccctc acccttcgg agtcccgcg
301 cctcccgtg gggcttactc cagggtggc gttgtgaaga ccatgacagg aggcagcgcg
361 cagagcattg gcaggagggg caaggtgaa cayttatct cagaagaaga agagaaaagg
421 agaactccga ggaagaggaa taagatggct gcagccaaat gccgcaaccg gagagggag
481 ctgactgata cactccaagc ggagacagac caactagaag atgcaagtc tgccttgag
541 accgagattg ccaactcgtc gaaggagaag gaaaaactag agtctatct gccagctcac
601 cgacctcct ccaagatccc tgatgacctg ggcttcccag aagagatgc tgtggcttcc
661 cttgatctga ctgggggccc gccagaggtt gccaccggg agtctgagga gccctcaacc
721 ctgctctctc tcaatgccc tgagcccaag cctcagtggg aacctgcaa gagcatcagc
781 agcatggagc tgaagaccga gcccttggat gacttctgt tcccagatc atccaggccc
841 agtggctctg agacagcccg ctcccggca gacatggacc tatctggctc cttctatgca
901 gcagactggg agcctctgca cagtggctcc ctggggatgg ggcccagtc cacagagctg
961 gagccccctg gcaactcggg ggtcacctgt actcccagct gcaactgta cacgtcttcc
1021 ttctgttcca cctaccgga ggtgactcc ttcccagct gtgcagctgc ccacgccaag
1081 ggacgacgca gcaatgagcc ttctctgac tgcctcagct caccacagct gctggcccct
1141 tga
```

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**Primer Express®
Software
for Real-Time PCR**

Version 3.0

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Primer Express 3.0

TaqMan® MGB Quantification # 1

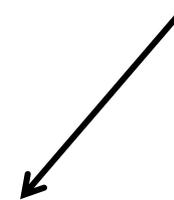
Sequence Parameters Primers / Probes Order

File Name

Length 1143 bp. Selection 1144 to 1144 Double Stranded

ATGATGTTCT	CGGGCTTCAA	CGCAGACTAC	GAGGCCTCAT	CCTCCCCTG	50
CAGCAGCGCG	TCCCCGGCCG	GGGATAGCCT	CTCTTACTAC	CACTCACCCG	100
CAGACTCCTT	CTCCAGCATG	GGCTCGCCTG	TCAACCGCCA	GGAATCTTGC	150
ACGGACTTGG	CGGTCTCCAG	TGCCAACTTC	ATTCCCACGG	TCACTGCCAT	200
CTCGACCACTG	CGGACCTGCG	AGTGGCTGGT	GCAGCCCGCC	CTGCTCTCCT	250
CTGTGGCCCC	ATCGCAGACC	AGAGCCCTTC	ACCCTTTCGG	AGTCCCCTGC	300
CCCTCCGCTG	GGGCTTACTC	CAGGGCTGGC	GTGTGAAGA	CAATGACAGG	350
AGGCCGAGCG	CAGACATTG	GCAGGAGGGG	CAAGTGGAA	CAGTATATCT	400
CAGAAGAAGA	AGAGAAAAGG	AGAATCCGAA	GGGAAAAGAA	TAAGATGGCT	450
GCAGCCAAAT	GCCCAACCGG	GAGGAGGGAG	CTGACTGATA	CACTCCAAGC	500
GGAGACAGAC	CAACTAGAAG	ATGAGAAGTC	TGCTTTGCA	ACCGAGATTG	550
CCAACCTGCT	GAAAGAGAA	GAAAAACTAG	AGTTCATCCT	GCCAGCTCAC	600
CGACTCGCCT	GCAAGATCCC	TGATGACCTG	GGCTTCCCAG	AAGAGATGTC	650
TGTGGCTTCC	CTTGATCTGA	CTGGGGCCCT	GCCAGAGGTT	GCCACCCCGG	700
AGTGTGAGGA	GGCCTTACC	CTGGCTTCTC	TCAATGACCC	TGAGCCCAAG	750
CCCTCAGTGG	AACCTTCAA	GAGCATCAGC	AGCATGGAGC	TGAGCCCAAG	800
GGCCTTTGAT	GACTTCTGT	TCCCAGATC	ATCCAGCCCG	AGTGGCTCTG	850
AGACAGCCCG	CTCCGTGCCA	GACATGGACC	TATCTGGGTC	CTTCTATGCA	900
GCAGACTGGG	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

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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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Ordering Information **Assay Search** Product Description Specifications Literature/Support Related Products

To begin, select a search method below

- Keyword:** Search by gene symbol, gene name, public accession number, biological process, or molecular function.
- Batch ID:** Search by uploading a file containing multiple assay IDs, RefSeq accession numbers, GenBank GI #s, LocusLink IDs, gene symbols, IMAGE Clone IDs, or species.

Search Help

Keyword Search | Batch ID Search

Search for in All Text

Disable wildcard search

[Advanced Keyword Search](#)

Search Reset

Choose Species

Filter by Amplicon Lengths

H. sapiens A. thaliana Amplicon length less than 70

R. norvegicus D. melanogaster Amplicon length between 71 and 85

M. musculus C. elegans Amplicon length between 86 and 100

M. mulatta (Rhesus) C. familiaris (Canine) Amplicon length greater than or equal to 101

D. rerio (Zebrafish) B. taurus (Cow)

G. gallus (Chicken) O. cuniculus (Rabbit)

S. scrofa (Pig)

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

Ordering Information **Assay Search** Product Description Specifications Literature/Support Related Products

Your search for **C-Fos** in **All Text** returned **27 results**. (Species: Homo sapiens Amplicon Length: ALL Set Membership: ALL) If you wish to refine your search results by product availability, click a radio button below, and then click Filter Results. To filter your results by other criteria, select from the categories list to the left of your results.

Search Again

Previous | 1 | 2 | Next

View Results by Category

All Results/

Panther Classification:

- Panther Function (26)
- Panther Process (26)

Filter Results by availability

Inventoried Assays Made to order Assays Inventoried and Made to order Assays [Filter Results](#)

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25 items/page

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

[Give us your Feedback](#)

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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 - LightCycler[®] 480 System
 - Universal ProbeLibrary System**
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 - Technology
 - Assay Design Center
 - User Statements and Application
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- ◆ Design real-time qPCR assays online in seconds.
- ◆ Rely on just 165 prevalidated probes for over five million qPCR assays for a large variety of organisms.
- ◆ Reduce the cost of gene expression analysis by performing multiplex qPCR assays with Universal ProbeLibrary Reference Gene Assays.

Universal ProbeLibrary for Human

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Specify your target(s):

[Advanced primer3 settings](#)

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
AAGGCCAACCGGAGAAAGATGACCCAGATCATGTTTGAGACCTTCAACACCCGAGCCATG
TACGTTGCTATCCAGGCTGTGCTATCCCTGTACGCCTCTGGCCGTACCACTGGCATCGTG
ATGGACTCCGGTGACGGGGTACCCACACTGTGCCATCTACGAGGGGTATGCCCTCCCC

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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 - ▶ LightCycler® 480 System
- ▼ Universal ProbeLibrary System
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- ▶ Assay List
- ▶ Performance
- ▶ Product List
- ▶ Support
- ▶ Literature and References
- ▶ Multimedia Presentations
- ▶ Product Information and Pack Inserts

	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 HG 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	tgaagggattttggatcacag

Amplicon (112 nt)

```
gtgaccacaggtgggtgtggaagggaagccagcagccgggccccgcctctgtg
ccccaggtcctgcagcgccttcccgtggctgtgatccaaaaaccctca
```

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

