

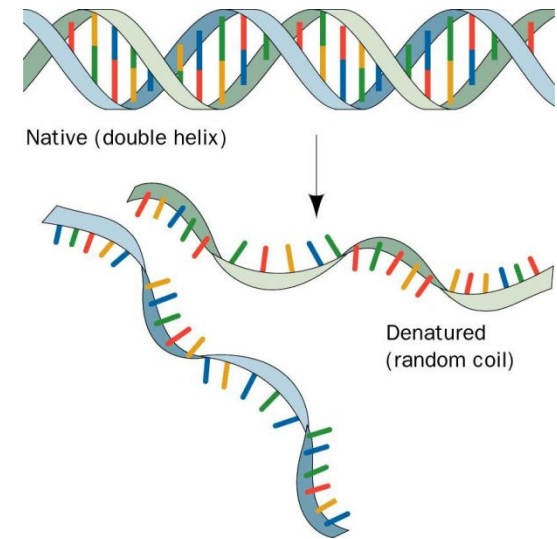
ÚVOD DO KVANTITATIVNÍ REAL-TIME PCR



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

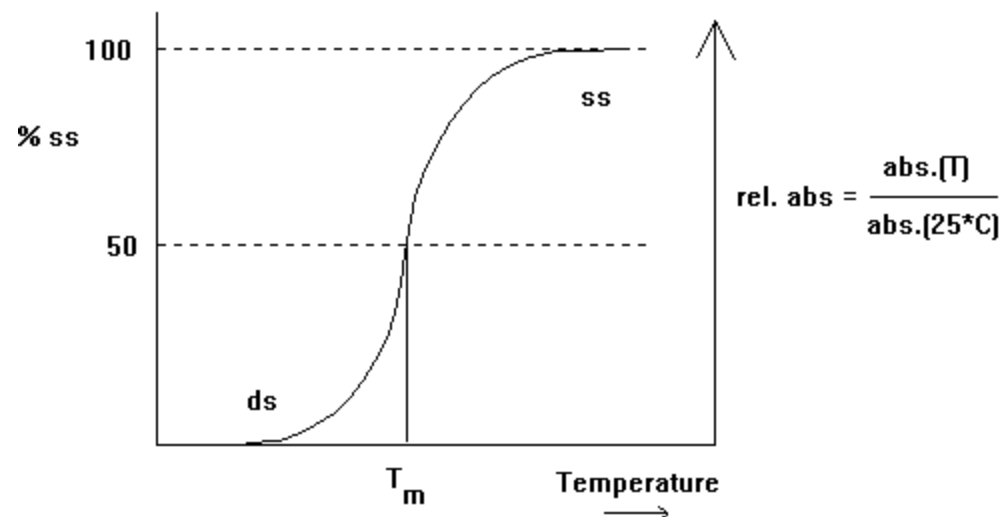
$$T_m = 2 \times (A+T) + 4 \times (G+C)$$

Iontová síla, %GC a délka řetězce (N)

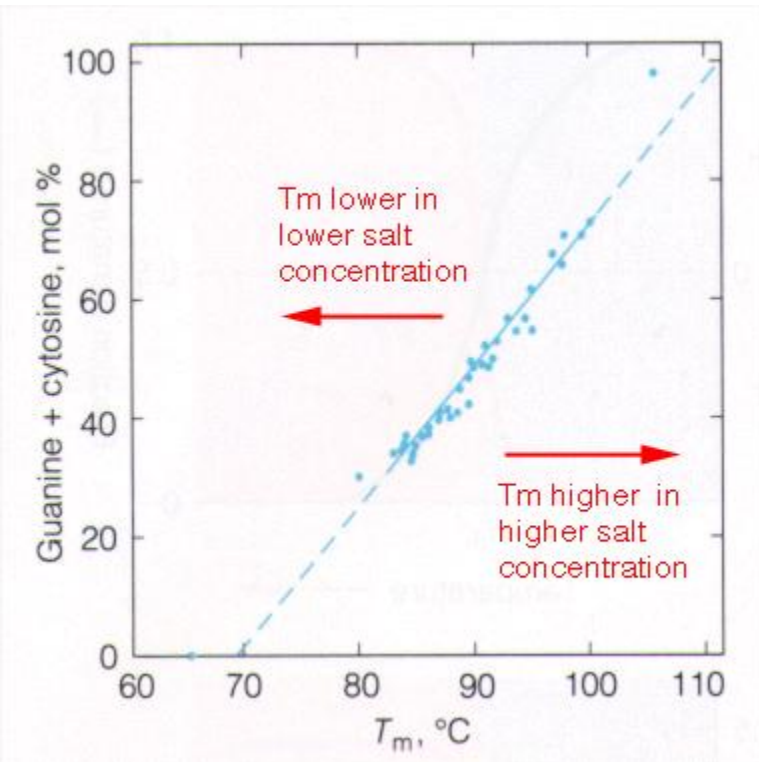
$$T_m = 81,5 + 16,6 (\log_{10}[\text{Na}^+] + 0,41(\%GC) - (625/N))$$

Web-based kalkulátory

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



Melting temperature T_m



GCTATTCAACTGAAGAGGGGCACAGC

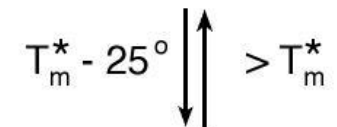
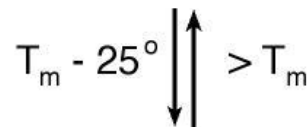
GCTATTCAACTG^GAGAGGGGCACAGC

+

+

CGATAAGTTGACTTCTCCCGTGTCTG

CGATAAGTTGACTTCTCCCGTGTCTG



GCTATTCAACTGAAGAGGGGCACAGC
CGATAAGTTGACTTCTCCCGTGTCTG

GCTATTCAACTG^GAGAGGGGCACAGC
CGATAAGTTGAC_TTCTCCCGTGTCTG

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

- Příklad:

$$\Delta G^0 (\text{GC}) -0,96 \text{ kcal/mol}$$

$$\Delta G^0 (\text{AT}) -0,50 \text{ kcal/mol}$$

$$\Delta G^0 \text{ W-C (TA/AT)} -0,58 \text{ kcal/mol}$$

$$\Delta G^0 \text{ W-C (GC/CG)} -2,24 \text{ kcal/mol}$$

GTAGACAATCTCCATCTCCTATCCTGATTAGAG

GTTAGAGGTAGAGGATAGGA

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

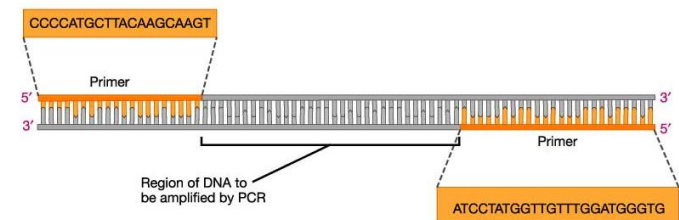
Není tedy nutná shodná T_m , ale shodná účinnost hybridizace obou primerů.

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

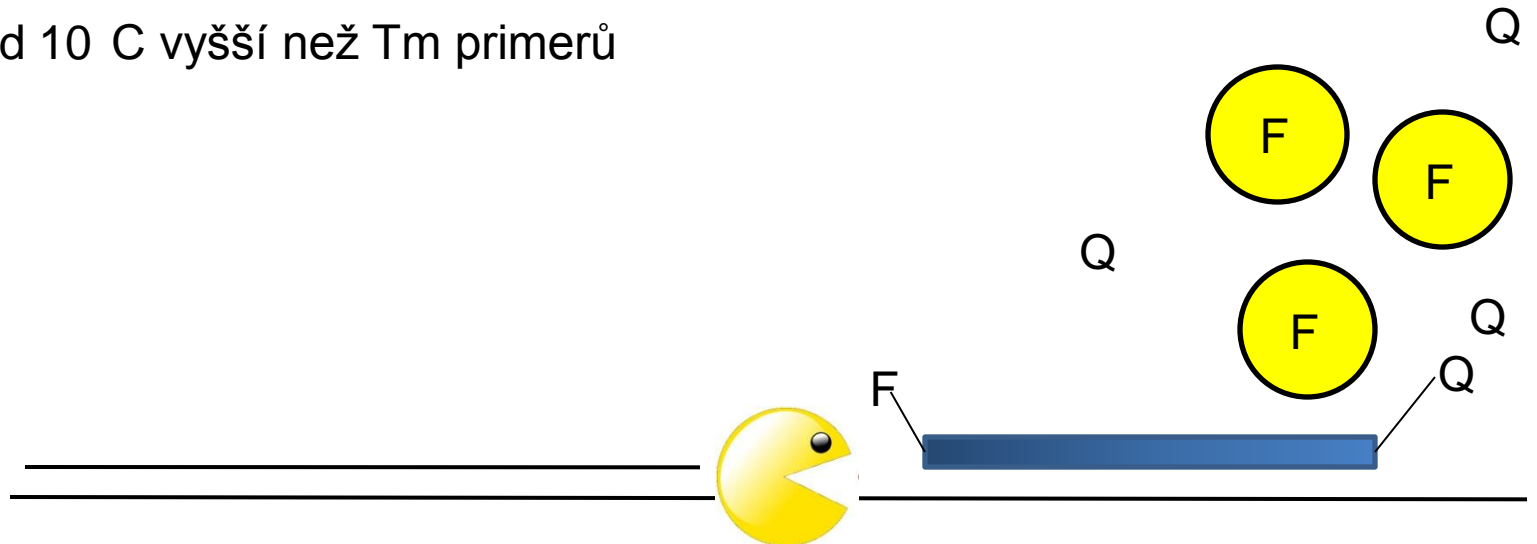


Design sond

- 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

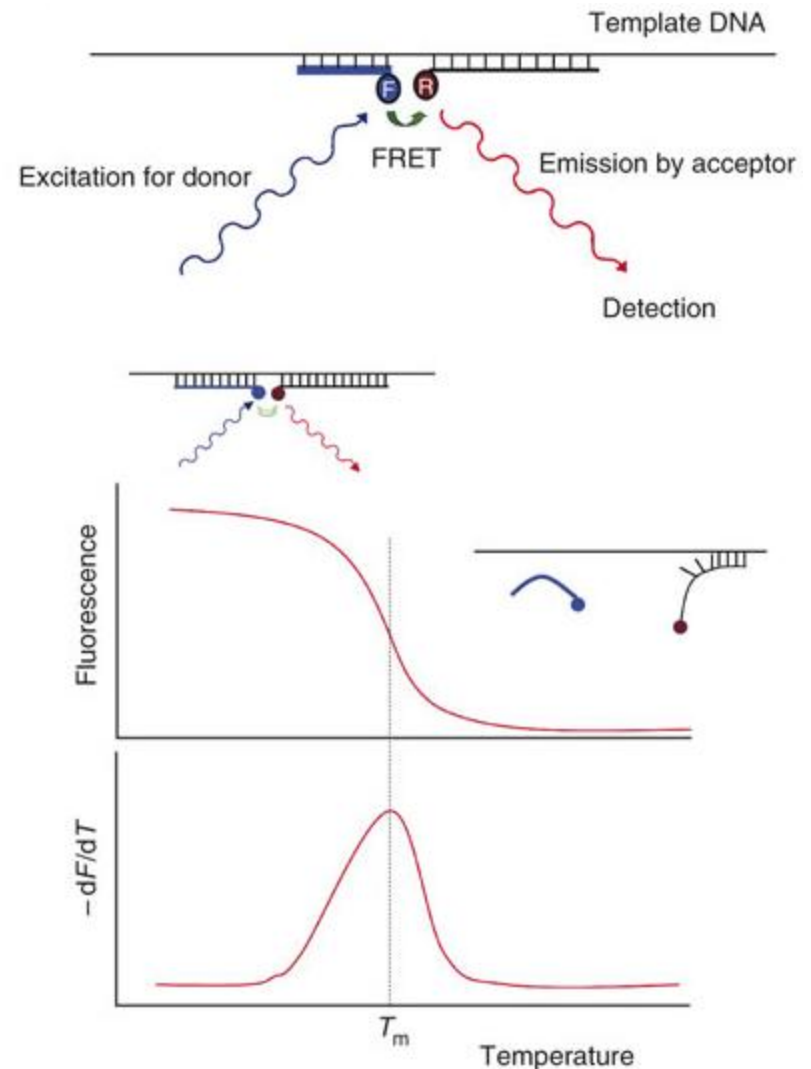
- qPCR TaqMan - dvoukrokový proces – denaturace a annealing/extension
- Co nejnižší Ct a nejvyšší ΔR (ΔR_n)
- Umístění 5' konce sondy v rámci stanovované sekvence co nejbliže 3' konci jednoho z primerů – účinné štěpení sondy
- Optimální délka do 30 nukleotidů, obsah GC do 30%
- AT bohaté sekvence – začlenění 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)



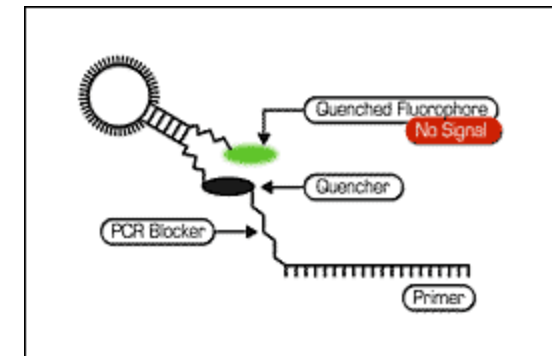
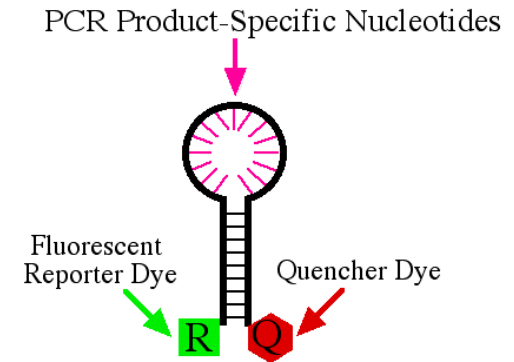
Design molekulárních majáků

- 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)

Tm mimo rozsah

GC% mimo rozsah

Vysoká stabilita 3' konce

Vnitřní nebo vzájemná
komplementarita

Vysoké BLAST skóre

Primer – dimery

Ne

Ano

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

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

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 Expression Database

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, Hybridization Probes, Molecular Beacons) to provide time-consuming primer design and experimental optimization, and to introduce a certain level of uniformity and standardisation among different laboratories.

We strongly encourage researchers to submit their validated primer and probe sequence, 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, [SNP](#) 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), expression Human, Mouse, Rat, and others) or detection chemistry. Data submission is allowed after free registration whereby you obtain a login name and password.

Currently, **7750 real-time PCR assays** for 5397 genes are available, submitted by 164 people.

Last submission: [link](#)

Publications

- PATTYN, F., SPELEMAN, F., DE PAEPE, A. & VANDESOMPELE, J. (2003). RTPrimerDB: the Real-Time PCR primer and probe database. Nucleic Acid Research, 31(1), 122-123. [\[PubMed\]](#)
- PATTYN, F., ROBERGHE, P., SPELEMAN, F., DE PAEPE, A. & VANDESOMPELE, J. (2005). RTPrimerDB: the Real-Time PCR primer and probe database, major update 2005. Nucleic Acid Research, Supplement 1, Database issue. [\[PubMed\]](#)
- LEFEVER, S., VANDESOMPELE, J., SPELEMAN, F., PATTYN, F. (2006). RTPrimerDB: the portal for real-time PCR primers and probes. Nucleic Acid Research, Oct 23. [\[PubMed\]](#)

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Ask by Google

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[Molecular Beacons](#) [Submit Primers/Probes](#) [Links](#)

Quantitative PCR Primer Database

QPPD

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

Paste source sequence below (5'>3', using of ACGTNacgts -- 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 \(report library\)](#).

NONE


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]CAT. means that primers must flank the central CCCC.
Excluded 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 7: Stability: 9.0
Max Mismatches: 12.00 Pair Max Mismatches: 24.00

General Primer Picking Conditions










Primer Size: Min: 18 Opt: 20 Max: 27
Primer Tm: Min: 57.0 Opt: 60.0 Max: 63.0 Max Tm Difference: 100.0
Primer Tm: Min: _____ Opt: _____ Max: _____
Primer GC%: Min: 20.0 Opt: _____ Max: 80.0
Min Self Complementarity: 0.00 Max 2 Self Complementarity: 3.00
Max dN's: 0 Max Pch: 3
Inside Target Penalty: _____ Outside Target Penalty: 0 Set Inside Target Penalty to allow primers inside a target.
First Base Index: 1 CG Change: 0
Salt Concentration: 50.0 Annealing Oligo Concentration: 50.0 (Do not the concentration of oligos in the reaction mix, but of those annealing to template.)
Library Size: 1 Show Detailed Info



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-  **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 gagggctcat cctcccgtg cagcagcgcg
61 tcccggcgcg gggatagcct ctttactact cactcaaccg cagactccct ctcceagatg
121 ggctcgctg tcaacggcga ggaactctgc acggactcag cctgtccag tgcgaacttc
181 attcccacgg tcactgcat ctcgaccagt cgggactcgc agtggctgt ggaagccgcg
241 ctgctctct ctgtggccc atcgcagacc agagccctc acccttcgg agtcccgcg
301 cctcccgtg gggcttact cagggtggc gttgtgaaga ccatgacag aggcgagcgc
361 cagagcattg gcaggaggg caaggtgaa cayttatct cagaagaaga agagaaaagg
421 agaactccga ggaagaggaa taagatggct gcagccaaat gccgcaacc gaggagggag
481 ctgactgata cactccaagc ggagacagac caactagaag atgcaagtc tgccttgag
541 accgagattg ccaactcgtc gaaggagaag gaaaaactag agtctatct gccagctcac
601 cgactcctc gcaagatccc tgatgacctg gcttcccag aagagatgc tgtggcttcc
661 cttgatctga ctgggggccc gccagaggtt gccaccggg agtctgagga gccctcaacc
721 ctgcctctcc tcaatgccc tgagcccaag cctcagtgag aacctgcaa gagcatcagc
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961 gagccctctg gactccgggt ggtcactctg actcccagct gcaactgta cacgtcttcc
1021 ttctgtctca cctaccgga ggcgactcc ttcccagct gtgcagctgc ccaccgcaag
1081 ggacgacga gcaatgagcc ttctctgac tgcctcagct caccacagct gctggcctg
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

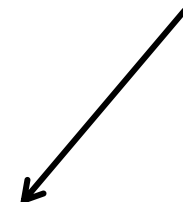
Sequence Parameters Primers / Probes Order

File Name

Length 1143 bp. Selection 1144 to 1144 Double Stranded

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CAGCAGCGCG TCCC CGCGCG GGGATAGCCT CTCTTACTAC CACTCACCG 100
CAGACTCCTT CTC CAGCATG GCTCGCCTG TCAACCGCCA GAACTTCTGC 150
ACGGACTTGG CGGTCTCCAG TGCCAATTC ATTCCCACGG TCACTGCCAT 200
CTCGACCACTG CCGGACTTGC AGTGGCTGGT GCAGCCCGCC CTGCTCTCT 250
CTGTGGCCCC ATCGCAGACC AGAGCCCTC ACCCTTTCGG AGTCCC CGCC 300
CCCTCGCTG GGGCTTACTC CAGGCTGGC GTTGTGAAGA CATTGACAGG 350
AGGCCGAGCG CAGAGCATTG GCAGGAGGG CAAGTGGAA CAGTTATCTC 400
CAGAAGAAGA AGAGAAAAG AGAATCCGAA GGGAAAAGAA TAAGATGGCT 450
GCAGCCAAAT GCCCAACCG GAGGAGGGAG CTGACTGATA CACTCCAAGC 500
GGAGACAGAC CAACTAGAAG ATGAGAAGTC TGCTTTGCG ACCGAGATTG 550
CCAACCTGCT GAAGGAGAA GAAAAACTAG AGTTCATCT GCCAGCTCAC 600
CGACTTGCCT GCAAGATCC TGATGACTG GCTTCCCAG AAGAGATGTC 650
TGTGGCTTCC CTTGATCTGA CTGGGGGCTT GCCAGAGGTT GCCACCCCG 700
AGTGTGAGGA GGCCTTACC CTGGCTTCT TCAATGACC TGAGCCCAAG 750
CCCTCAGTGG AACCTTCAA GAGCATGAG AGCATGGAG TGAAGCCGA 800
GCCCTTGGAT GACTTCTGT TCCCAGATC ATCCAGGCC AGTGGCTCTG 850
AGACAGCCCG CTCGGTGCCA GACATGGACC TATCTGGGTC CTCTTATGCA 900
GCAGACTTGG AGCTCTGCA CAGTGGCTCC CTGGGGATGG GGCCTATGG 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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- **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.

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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.

Previous | 1 | 2 | Next

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.

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



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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 |

| | 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):

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
AAGGCCAACCGGAGAGATGACCCAGATCATGTTTGAGACCTTCAACACCCCGCCATG
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.

- ▼ Real-Time PCR Systems
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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_automatc_transcript Proto-oncog fos) (G0/G1 [Source:Uniprot/SPTREMBL;Acc:Q8N964] |
| <input type="checkbox"/> | ENST00000297904.2 | 2110 | FIGF-001 HGNC_automatc_transcript endothelial g (c-fos-induc [Source:Uniprot/SPTREMBL;Acc:Q8N964] |
| <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
ccccaggtcctgcagcgccttcccgtggctgtgatccaaaatccctca
```

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Transcript overview:



Detailed view:



Design primerů a sond



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

