Primer and Probe Design

Primer

Good primer design is essential for a successful PCR reaction. There are many factors to take into account when designing the optimal primers for your gene of interest. Here are some tips to consider when designing primers.

1. In general, a length of 18–25 nucleotides for primers is good.

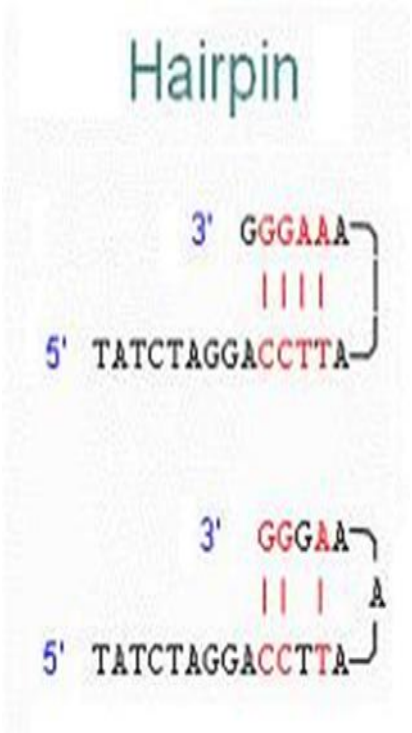
2. Try to make the melting temperature (Tm) of the primers between 52°C and 65°C.

3. If the Tm of your primer is very low, try to find a sequence with more GC content, or extend the length of the primer a little.

4. Aim for the GC content to be between 40 and 60%, with the 3' of a primer ending in C or G to promote binding.

5. Try to avoid regions of secondary structure, and have a balanced distribution of GC-rich and AT-rich domains.
6. Try to avoid runs of 4 or more of one base, or dinucleotide repeats (for example, ACCCC or ATATATAT).
7. Avoid intra-primer homology (more than 3 bases that complement within the primer) or inter-primer homology (forward and reverse primers having complementary sequences). These circumstances can lead to self-dimers or primer-dimers instead of annealing to the desired DNA sequences.

If primers can anneal to themselves, or anneal to each other rather than anneal to the template, the PCR efficiency will be decreased dramatically. They shall be avoided.



- The common Tm formulas for calculating the theoretical Tm of an oligo

- Tm = 4°C x (number of G's and C's in the primer) + 2°C x (number of A's and T's in the primer)
- Tm = 4°C(GC) + 2°C(AT)

- Ta = Tm - (2-5)

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National Center for Biotechnology Information

Gene FOXP2 Search

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See FOXP2 forkhead box P2
foxp2 in Homo sapiens Mus musculus Rattus norvegicus All 193 Gene records

Search results

Items: 1 to 20 of 241

<< First < Prev Page 1 of 13 Next > Last >>

See also 3 discontinued or replaced items.

Name/Gene ID	Description	Location	Aliases	MIM
ACOT13 ID: 55856	acyl-CoA thioesterase 13 [*Homo sapiens* (human)]	Chromosome 6, NC_000006.12 (24667035..24705069)	HT012, PNAS-27, THEM2	615652
AES ID: 166	amino-terminal enhancer of split [*Homo sapiens* (human)]	Chromosome 19, NC_000019.10 (3052910..3063107, complement)	AES-1-2, ESP1, GRG, GRG5, Grg-5, TLE5, AES	600188
Cadm1 ID: 54725	cell adhesion molecule 1 [*Mus musculus* (house mouse)]	Chromosome 9, NC_000075.6 (47530170..47862280)	2900073G06Rik, 3100001I08Rik, AI987920, Bl2, Igsf4, Igsf4a, Necl2, RA175, RA175A, RA175B, RA175C, RA175N, ST17, SglGSF, SynCam, Tslc1	
CCNC	cyclin C [*Homo sapiens*	Chromosome 6,	CycC	123838

Filters: Manage Filters

Results by taxon

Top Organisms [Tree]
Homo sapiens (34)
Mus musculus (7)
Rattus norvegicus (4)
Xenopus laevis (3)
Taeniopygia guttata (2)
All other taxa (191)
More...

Find related data

Database: Select

Find items

Search details

FOXP2[All Fields] AND alive[prop]

polyglutamine tract and is an evolutionarily conserved transcription factor, which may bind directly to approximately 300 to 400 gene promoters in the human genome to regulate the expression of a variety of genes. This gene is required for proper development of speech and language regions of the brain during embryogenesis, and may be involved in a variety of biological pathways and cascades that may ultimately influence language development. Mutations in this gene cause speech-language disorder 1 (SPCH1), also known as autosomal dominant speech and language disorder with orofacial dyspraxia. Multiple alternative transcripts encoding different isoforms have been identified in this gene.[provided by RefSeq, Feb 2010]

Orthologs mouse all

Genomic context

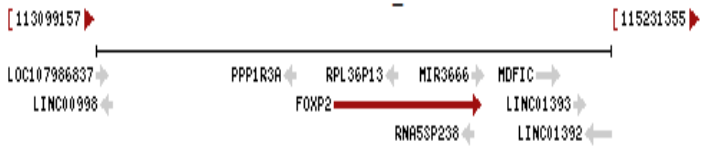
Location: 7q31.1

See FOXP2 in Genome Data Viewer Map Viewer

Exon count: 24

Annotation release	Status	Assembly	Chr	Location
108	current	GRCh38.p7 (GCF_000001405.33)	7	NC_000007.14 (114086310..114693772)
105	previous assembly	GRCh37.p13 (GCF_000001405.25)	7	NC_000007.13 (113726365..114333827)

Chromosome 7 - NC_000007.14



Genomic regions, transcripts, and products

Go to reference s

Genomic Sequence: NC_000007.14 Chromosome 7 Reference GRCh38.p7 Primary Assembly

Go to nucleotide: Graphics FA

NC_000007.14: 114M..115M (790Kbp) Find: Tools Tracks

Related sequences

Additional links

Locus-specific Databases

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Map Viewer

Variation Viewer (GRCh37.p13)

Variation Viewer (GRCh38)

1000 Genomes Browser (GRCh37.p13)

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Related information

BioAssays, RNAi Target, Tested

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Inspect Ctrl+Shift+I

FASTA Send:

Homo sapiens chromosome 7, GRCh38.p7 Primary Assembly

NCBI Reference Sequence: NC_000007.14

GenBank Graphics

>NC_000007.14:114086310-114693772 Homo sapiens chromosome 7, GRCh38.p7 Primary Assembly
AGACAGCGCGAGCCTCCGAGAAAGCGCGAGACACGCCGGCGCGTGCAGCTCCGCGGCCGCCGCTTCGCCC
TAGCTCTAGCCCCGCGCCACCCGCAGCCCGCCCGCGAACGCCCGCCCCGGTTATTTATGCGGCGGCCGCG
TCCGCTGGCTGCGGCTTCCTCGGCCCCCCCCCTCCCCGGGCGCGCCCCCCACTCGCGGCAGCAGCTGCCC
GGACTCGCGCGTGGGTGTGTTGTTTGGGGGCTTCTGCCTCGCCGCCGCGGGTGCCACCTCCCGGGACGCT
GCCCACGGCGTCCCCGGTCGCGGTAAGTTTCTTGGCCCTCACTCTGGCGCGCTACACCTCCGCACCCCAC
CCTGTCCCAGCCACCTCCACGCTGGGCCGAGCTGCGACTTTACTCTGCTCCGCGCTCCTCCCGCGGTGGC
GACAAAGTTTCGCCCCAAAGGCAGCGCCCTGCTTGCCGGGGGCGAGTGTGACATGTGTCAAATTTGGGCTC
GGCGTTGGGGTCGCATTCCGGACCCAGCATCACCACTTGTTGTTCTCTTTCATTTTGCTTTGTGATGGGG
GAAAAAGGGGTGGAGAGGGGAGATTTGCTGTTGGCTCACGGATGATTTTTAGTTTGGATAATGCAAAATG
TTGCTTCGTCCGGAGAGACCTCGGCTGGAGGAGAATGTGTGTCGAAACACCAGATGTGTGTTTGTTTACT
CTCTTCTTTTTAATTGTTTGTTGTCTTTTCCCCTTCCTCCCCGCACCCCCACCCCCAACCTCGGGAGGAG
AAACAACAGTAAAAACATCTGGCGGTTAGAAGCACACACTTTATTGATCCAACTGCTGACCTTTATTTAC
TCAGTTGGGCAAGTGCACGCTTCTCGCGTCTAAGTTGGGCACTTCAGCGTTCATCTCAGAAGTACTTCTC

Change region shown

Whole sequence

Selected region

from: 114086310 to: 114693772

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Primer-BLAST

A tool for finding specific primers

NCBI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST).

Reset page | Save search parameters | Retrieve recent results | Publication | Tips for finding specific primers

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

```
TACAATTTTGATTTATTTGTATCTATATTAAAAGAGTGGGTATAAAATTATATAAACTGTGATCGTTAGG
GTCATACAATTTCATATCAGTTTTAGAGCTTTTTTAACTTACAATTTTGATTTATTTGTATCTATATTAA
AAGAGTGGGTATAAAATTCTGTACACTGTGATCATTAGGGCCAAACAATTTCATATCAGCTTTAGAGCTT
TTTAAAGGCCCCTTTTCTCTTGCCTGCTTAACTTCTTTCCTTTTATTTTTATTGCCTAACTAGACTCATA
TTCAGTCGTCAGTCTAGTTAAATGCTTCCTTACGACACACACTATTTCATTGTTATCTAAAATTAAGTGA
```

Range

	From	To
Forward primer		
Reverse primer		

Clear

Or, upload FASTA file Choose File No file chosen

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	70	1000

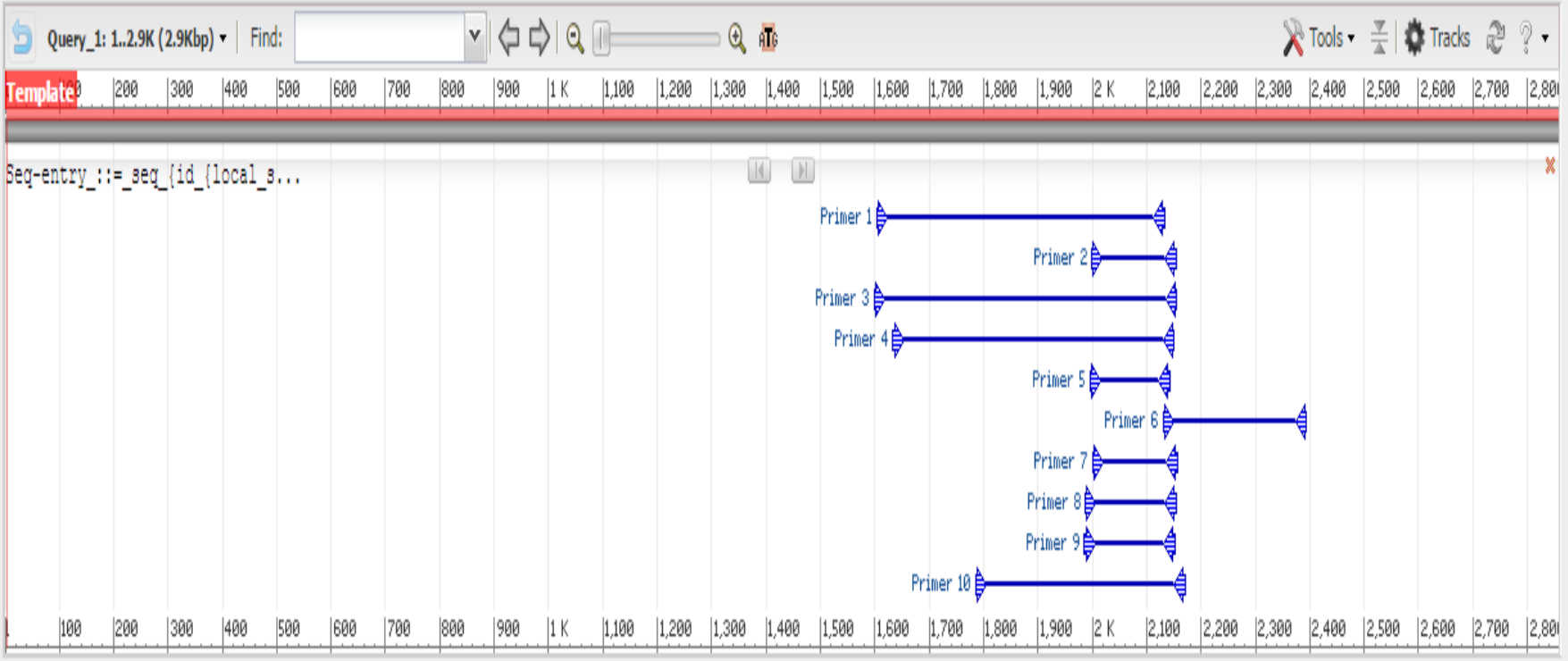
# of primers to return: 10

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

NCBI/ Primer-BLAST : results: Job id=urBlqNmz1BvzlcIkz0TmFrVf9ySYTOw5mQ more...

Input PCR template	
Range	1 - 2870
Specificity of primers	Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports	Search Summary

Graphical view of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGTCTGGGGATTGGAAAGCG	Plus	20	1604	1623	60.04	55.00	3.00	2.00
Reverse primer	CCCTTGGGCTTACCTGCTAC	Minus	20	2132	2113	60.11	60.00	4.00	1.00
Product length	529								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GAAGGACCTGTGGGAGTGTG	Plus	20	1999	2018	59.96	60.00	5.00	0.00
Reverse primer	TGACAACAGGGCGTCACTTT	Minus	20	2154	2135	60.11	50.00	4.00	2.00
Product length	156								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTGCAAGTCTGGGGATTGGA	Plus	20	1599	1618	59.96	55.00	4.00	2.00
Reverse primer	TTGACAACAGGGCGTCACTT	Minus	20	2155	2136	60.11	50.00	5.00	0.00
Product length	557								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCGTTTTGCACAGGGATGAG	Plus	20	1634	1653	60.11	55.00	4.00	0.00
Reverse primer	AACAGGGCGTCACTTTCTCC	Minus	20	2150	2131	60.25	55.00	3.00	1.00
Product length	517								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TACGAAGGACCTGTGGGAGT	Plus	20	1996	2015	59.89	55.00	5.00	1.00

Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

BLASTN programs search nucleotide databases using a nucleotide query. more...

Reset page | Bookmark

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

Clear

AGTCTGGGGATTGGAAAGCG

Query subrange

From

To

Or, upload file

Choose File No file chosen

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database

Human genomic + transcript | Mouse genomic + transcript | Others (nr etc.):

Nucleotide collection (nr/nt)

Organism
Optional

Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude
Optional

Models (XM/XP) Uncultured/environmental sample sequences

Limit to

Sequences from type material

BLAST Results

Your search parameters were adjusted to search for a short input sequence.

Edit and Resubmit | Save Search Strategies | Formatting options | Download | How to read this page | Blast report description

Nucleotide Sequence (20 letters)

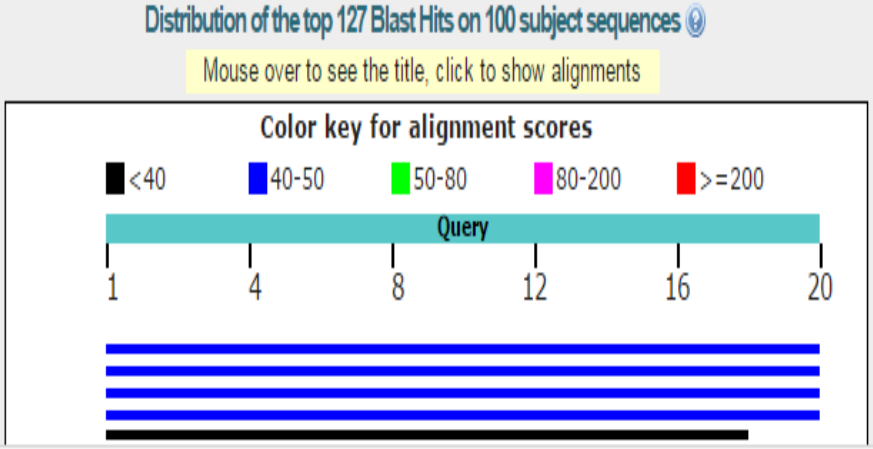
RID 6ZX9J7R9014 (Expires on 01-08 02:51 am)

Query ID lcl|Query_176353
Description None
Molecule type nucleic acid
Query Length 20

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.6.0+ Citation

Other reports: Search Summary [Taxonomy reports] [Distance tree of results]

Graphic Summary



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PREDICTED: Homo sapiens forkhead box P2 (FOXP2), transcript variant X1, mRNA

Sequence ID: XM_017012801.1 Length: 7035 Number of Matches: 1

Related Information

Range 1: 219 to 238 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
40.1 bits(20)	0.22	20/20(100%)	0/20(0%)	Plus/Plus

```
Query  1    AGTCTGGGGATTGGAAAGCG  20
            ||||||||||||||||||||
Sbjct  219  AGTCTGGGGATTGGAAAGCG  238
```

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Homo sapiens forkhead box P2 (FOXP2), RefSeqGene on chromosome 7

Sequence ID: NG_007491.2 Length: 614463 Number of Matches: 1

Related Information

Map Viewer - aligned genomic context

Range 1: 6604 to 6623 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
40.1 bits(20)	0.22	20/20(100%)	0/20(0%)	Plus/Plus

```
Query  1     AGTCTGGGGATTGGAAAGCG  20
             ||||||||||||||||||||
Sbjct  6604  AGTCTGGGGATTGGAAAGCG  6623
```

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Pan troglodytes BAC clone RP43-39F11 from chromosome 7, complete sequence

Sequence ID: AC145868.2 Length: 209787 Number of Matches: 1

Related Information

Map Viewer - aligned genomic context

Range 1: 184048 to 184067 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
40.1 bits(20)	0.22	20/20(100%)	0/20(0%)	Plus/Minus

```
Query  1       AGTCTGGGGATTGGAAAGCG  20
               ||||||||||||||||||||
Sbjct  184067  AGTCTGGGGATTGGAAAGCG  184048
```

Primer3web version 4.0.0 - Pick primers from a DNA sequence.

disclaimer | code | cautions

Select the Task for primer selection generic

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library) NONE

```
TCAGTATATTCCCAGTGTACTGGAATTTTTAAATGTAGTGTTTGTTTAATGTTGTTAAACACAATTAATT
TACAATTTTGATTTATTTGTATCTATATTAAAAGAGTGGGTATAAAATTATATAAACTGTGATCGTTAGG
GTCATACAATTTCATATCAGTTTTAGAGCTTTTTTAACTTACAATTTTGATTTATTTGTATCTATATTAA
AAGAGTGGGTATAAAATTCTGTACACTGTGATCATTAGGGCCAAACAATTTCATATCAGCTTTAGAGCTT
TTTAAAGGCCCCTTTTCTCTTGCCTGCTTAACTTCTTTCCTTTTATTTTTATTGCCTAACTAGACTCATA
TTCAGTCGTCAGTCTAGTTAAATGCTTCCTTACGACACACACTATTTCATTGTTATCTAAAATTAAGTGA
```

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' to 3' on opposite strand)

Pick Primers | Download Settings | Reset Form

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.
Overlap Junction List		E.g. 27 requires one primer to overlap the junction between positions 27 and 28. Or mark the source sequence with -: e.g. ...ATCTAC-TGTCAT.. means that primers must overlap the junction between the C and T.
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.
Pair OK Region List		See manual for help.
Included Region		E.g. 20,400: only pick primers in the 400 base region starting at position 20. Or use { and } in the source sequence to mark the beginning and end of the included region: e.g. in ATC{TTC...TCT}AT the included region is TTC...TCT.

Primer3 Output

```
No mispriming library specified
No internal oligo mishyb library specified
Using 1-based sequence positions
```

OLIGO	start	len	tm	gc%	any_th	3'_th	hairpin	seq
LEFT PRIMER	956	20	59.01	55.00	5.24	0.00	0.00	AAGAAACTCCTGGGCCCTAC
RIGHT PRIMER	1143	20	59.03	55.00	0.00	0.00	0.00	GATTTCACCCCAAACCCACC
INTERNAL OLIGO	988	20	60.02	50.00	19.67	7.48	39.99	ATAACCGTGCACAGGGATGA

```
SEQUENCE SIZE: 2870
INCLUDED REGION SIZE: 2870

PRODUCT SIZE: 188, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

    1 AGACAGCGCGAGCCTCCGAGAAAGCGCGAGACACGCCGGCGCGTGCAGCTCCGCGGCCGC

   61 CGCTTCGCCCTAGCTCTAGCCCCGCGCCACCCGCAGCCCGCCCGCGAACGCCCGCCCCGG

  121 TTATTTATGCGGCGGCCGCGTCCGCTGGCTGCGGCTTCCTCGGCCCCCCCCCTCCCCGGG

  181 CGCGCCCCCCACTCGCGGCAGCAGCTGCCCGGACTCGCGCGTGGGTGTGTTGTTTGGGGG

  241 CTTCTGCCTCGCCGCCGCGGGTGCCACCTCCCGGGACGCTGCCCACGGCGTCCCCGGTCG

  301 CGGTAAGTTTCTTGGCCCTCACTCTGGCGCGCTACACCTCCGCACCCCACCCTGTCCCAG

  361 CCACCTCCACGCTGGGCCGAGCTGCGACTTTACTCTGCTCCGCGCTCCTCCCGCGGTGGC
```

```
 841 TCAGTTGGGCAAGTGCACGCTTCTCGCGTCTAAGTTGGGCACTTCAGCGTTCATCTCAGA

 901 AGTACTTCTCCAGGAAGGAGAGAGATGGAAAGGGGACACTCCTGTTTCTGGAGTCAAGAA
                                                              >>>>>

 961 ACTCCTGGGCCCTACTGACGCTTCGGGATAACCGTGCACAGGGATGACTGCTGCCTTGAT
     >>>>>>>>>>>>>>>                ^^^^^^^^^^^^^^^^^^^^

1021 CGCGTTCCTTTCCCTGTCCACGCGTTAGCACGACCGGCTTCCCCCGGTGTCTGGCCTGGT

1081 TTTACTTTTATTCCGCTTTAGAGAGGTCGCCTGGCTGTTTGTGGGTGGGTTTGGGGTGAA
                                                  <<<<<<<<<<<<<<<<<

1141 ATCGCACCTCGCGGCACTTGGTGAGGGGACGTGGGAGGAGCGCAGACACCTTTCGGTGAT
     <<<

1201 AGGGAGGGCTTCTCACTTGGCTGTTACCTGGAAGTCCACAGTGGCCCCGGCGGGCAGGCG

1261 GGCGGGCAGAGCGCGGGTCCGAACGCCCTTCGCGCTCGCGGCGGCGCACGTGCGGCGGCG
```

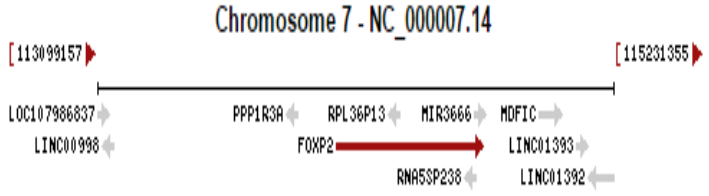
Genomic context

Location: 7q31.1

See FOXP2 in Genome Data Viewer Map Viewer

Exon count: 24

Annotation release	Status	Assembly	Chr	Location
108	current	GRCh38.p7 (GCF_000001405.33)	7	NC_000007.14 (114086310..114693772)
105	previous assembly	GRCh37.p13 (GCF_000001405.25)	7	NC_000007.13 (113726365..114333827)

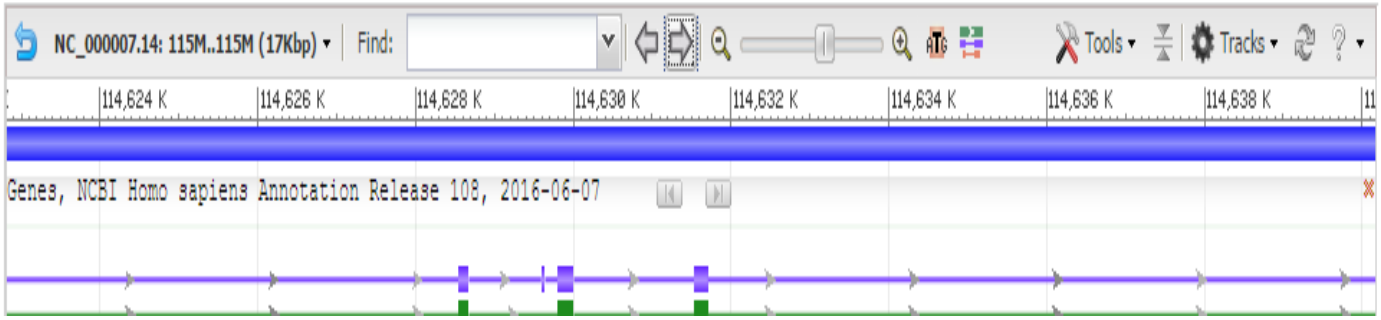


Genomic regions, transcripts, and products

Go to reference sequence details

Genomic Sequence: NC_000007.14 Chromosome 7 Reference GRCh38.p7 Primary Assembly

Go to nucleotide: Graphics FASTA GenBank



Map Viewer

Variation Viewer (GRCh37.p13)

Variation Viewer (GRCh38)

1000 Genomes Browser (GRCh37.p13)

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UCSC

Related information

Order cDNA clone

3D structures

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BioAssay by Target (Summary)

BioAssay, by Gene target

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BioAssays, RNAi Target, Tested

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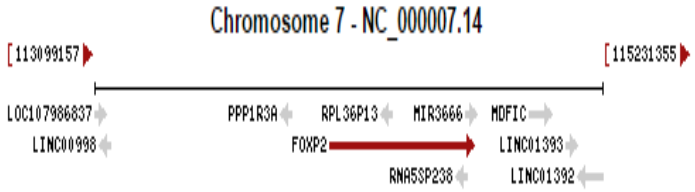
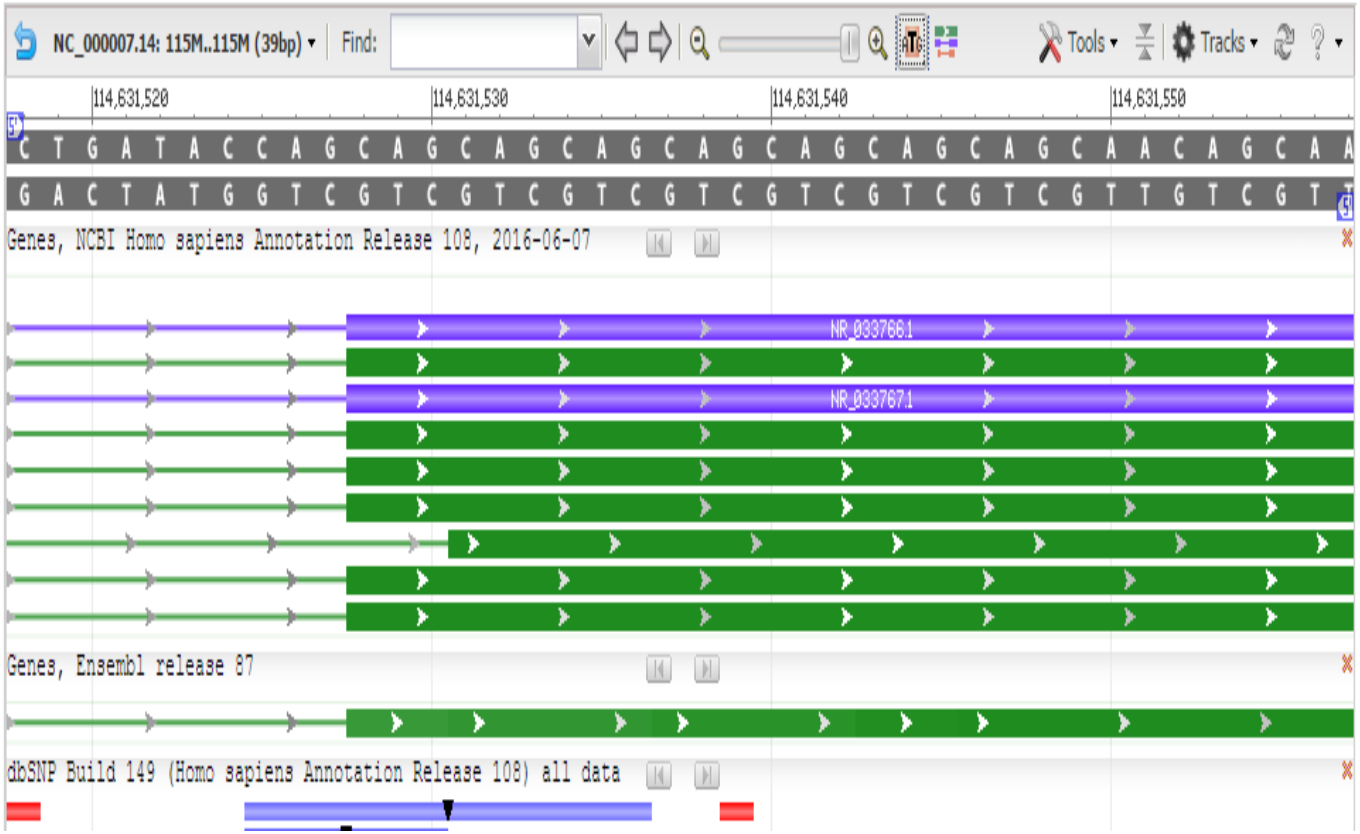


Genomic regions, transcripts, and products

Go to reference sequence details

Genomic Sequence: NC_000007.14 Chromosome 7 Reference GRCh38.p7 Primary Assembly

Go to nucleotide: Graphics FASTA GenBank



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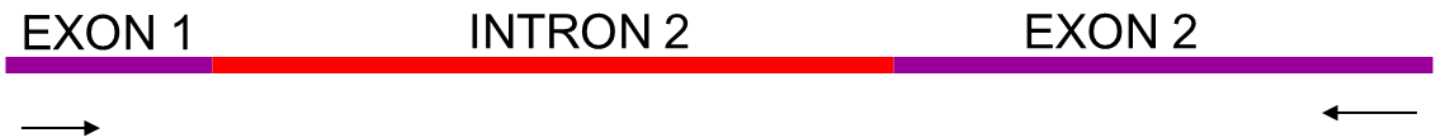
- BioAssay by Target (Summary)
- BioAssay, by Gene target
- BioAssays, RNAi Target, Active
- BioAssays, RNAi Target, Tested
- BioProjects
- BioSystems
- Books
- CCDS
- ClinVar
- Conserved Domains
- dbVar
- EST
- Full text in PMC
- Full text in PMC_nucleotide
- Gene neighbors



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Full text in PMC_nucleotide
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Genes with a similar H3K4me3 profile
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GTR
HomoloGene
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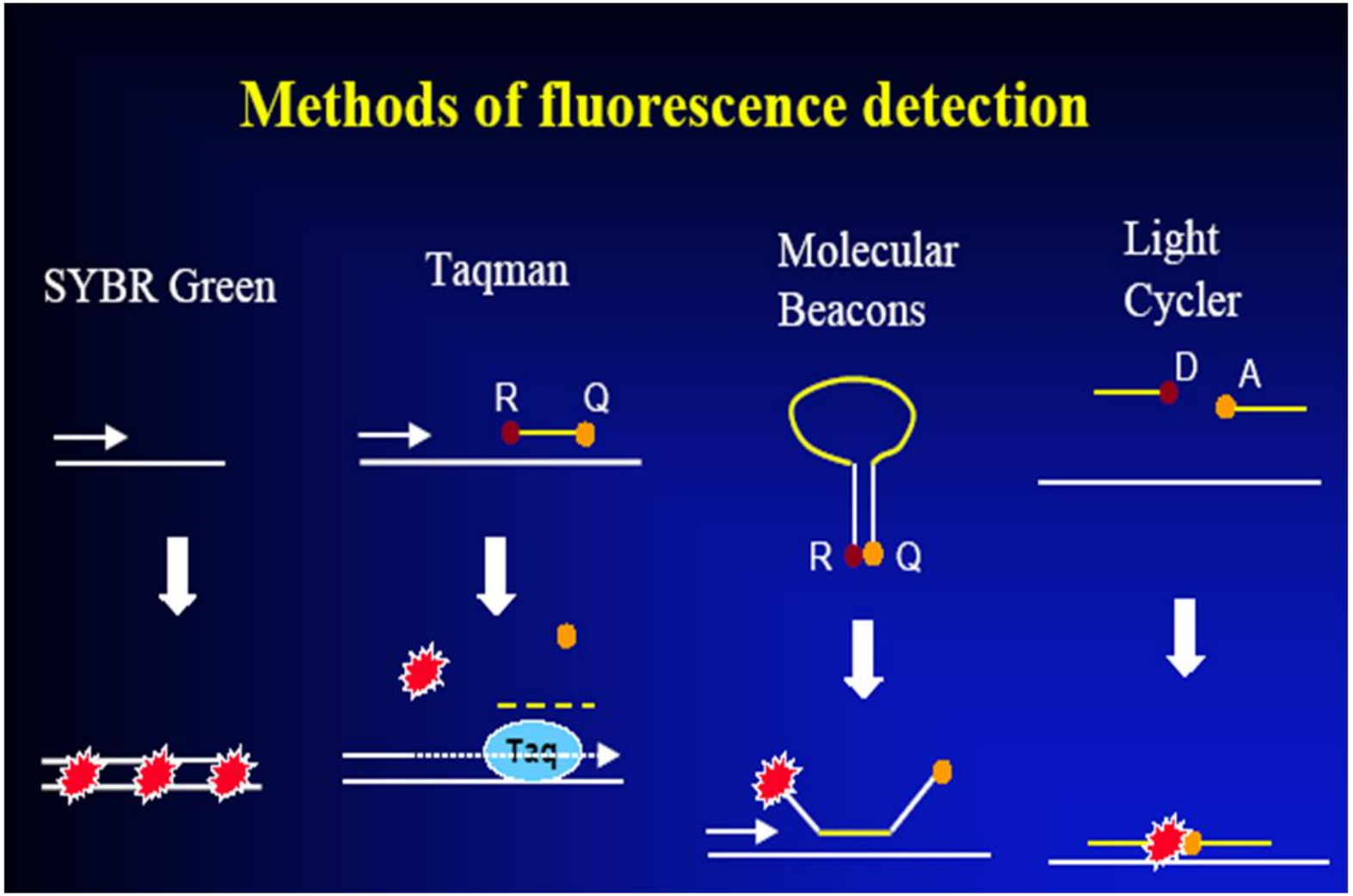
MedGen
Nucleotide
OMIM

AACTCCAGCAGCAGCAGCATCTGCT

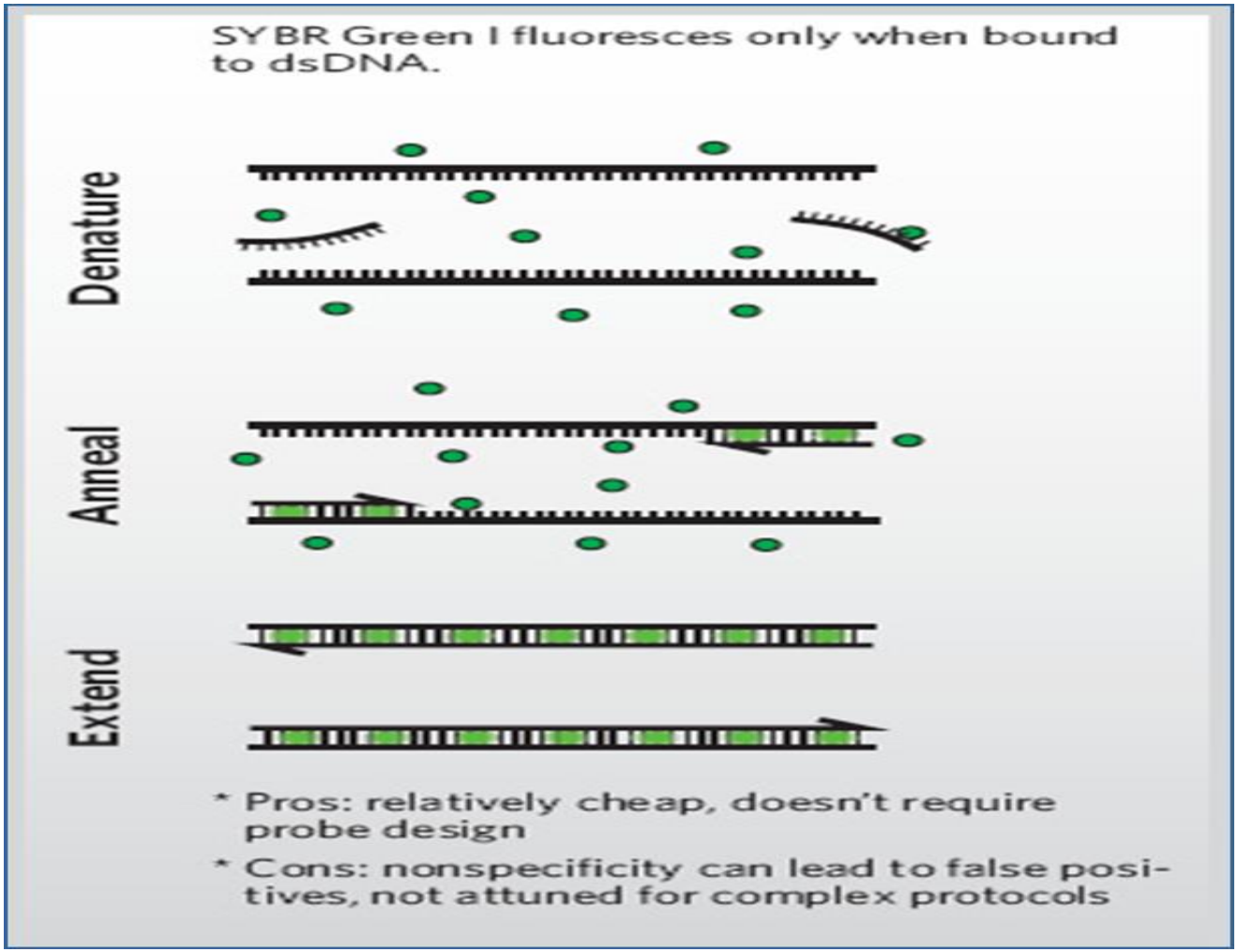
Y(C/T)

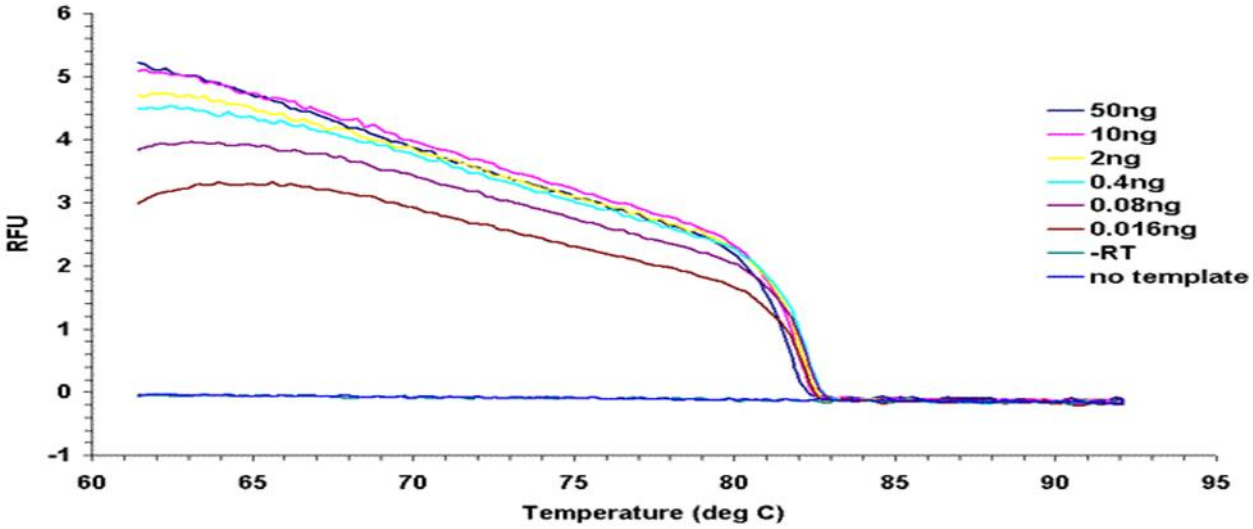
AGCCTTCAGC GTCAGGGACTCATCT

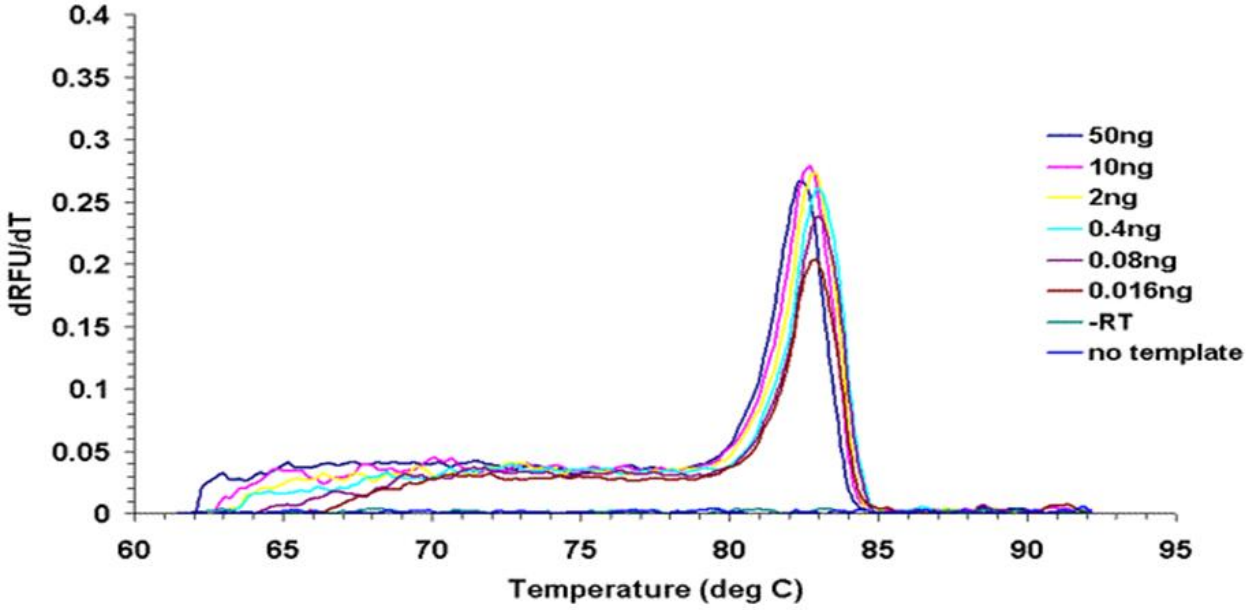
Detection in real time PCR

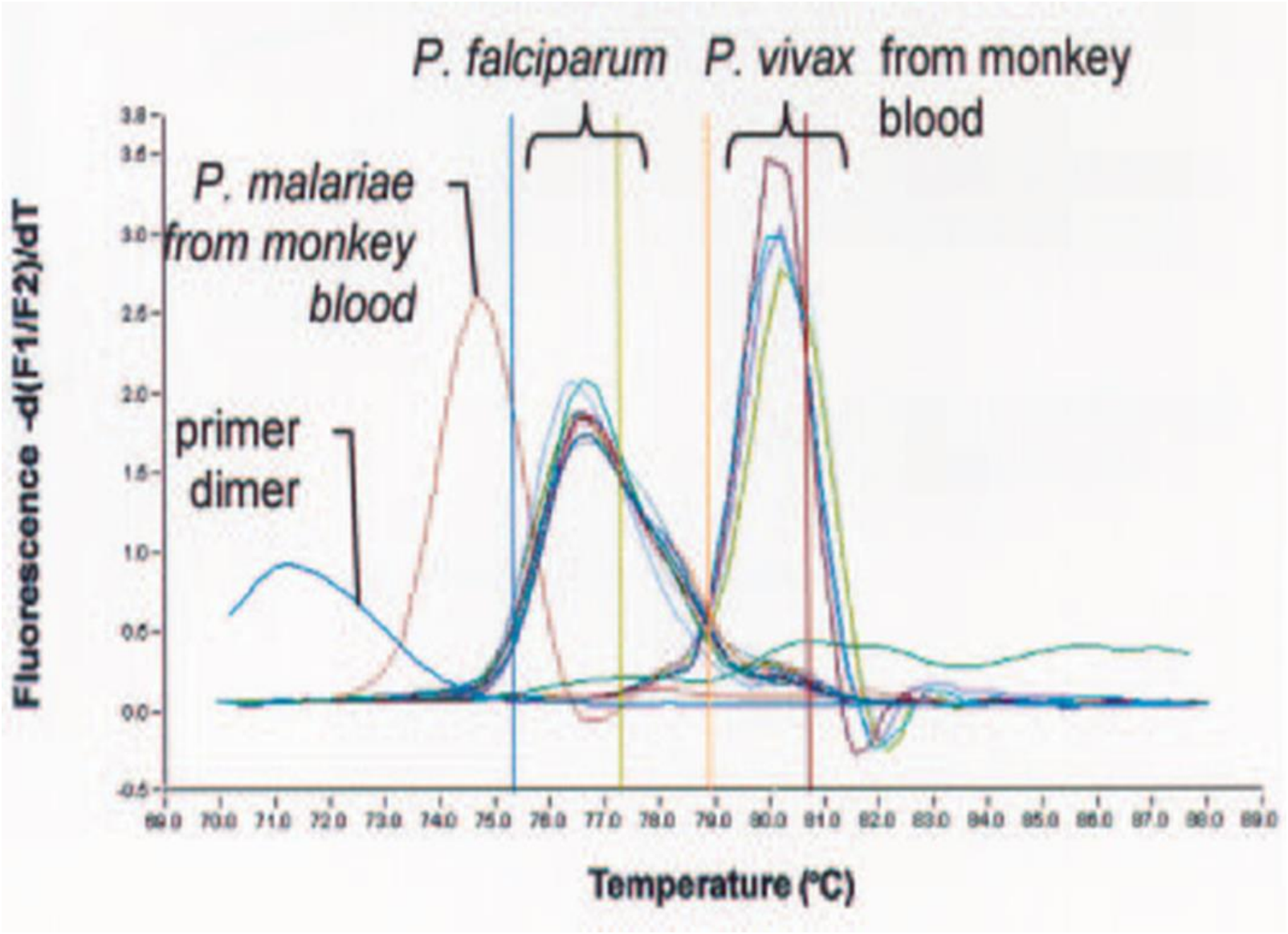


SYBR® green

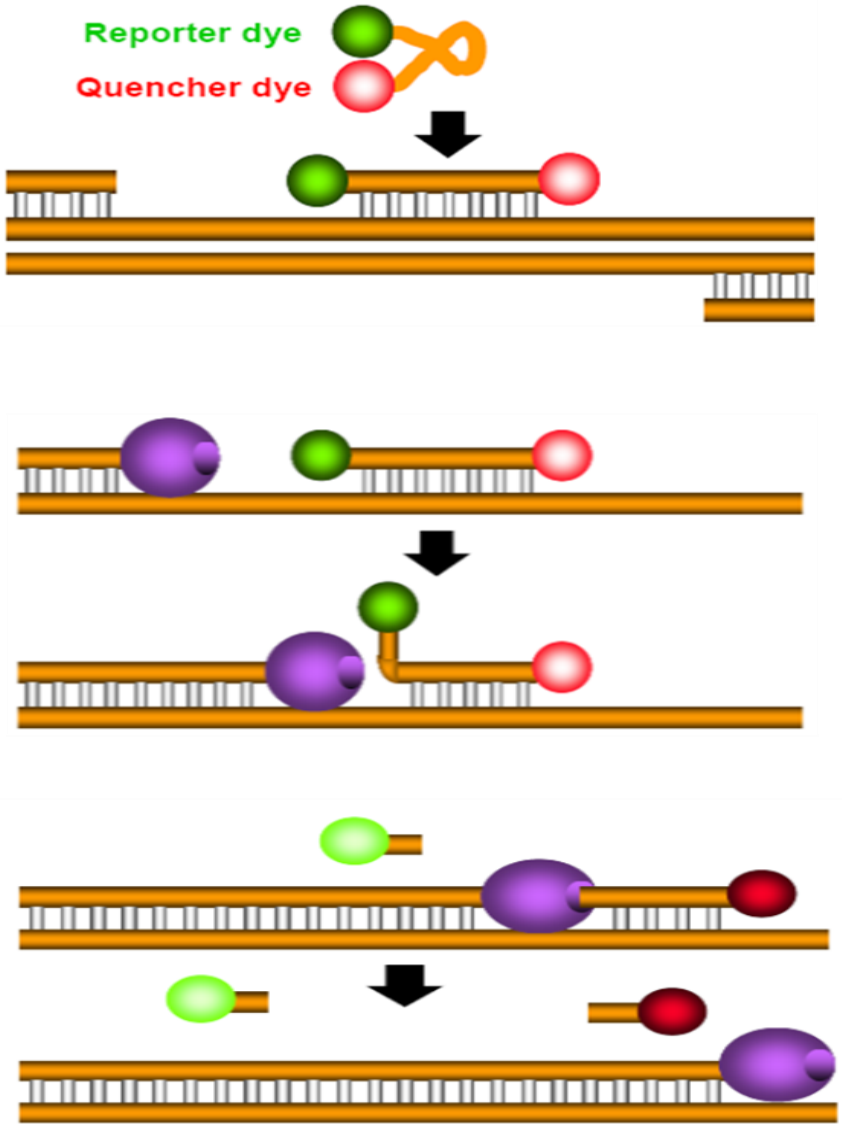

SYBR Green I fluoresces only when bound to dsDNA.
Denature
Anneal
Extend
* Pros: relatively cheap, doesn't require probe design
* Cons: nonspecificity can lead to false positives, not attuned for complex protocols


RFU
Temperature (deg C)
50ng
10ng
2ng
0.4ng
0.08ng
0.016ng
-RT
no template

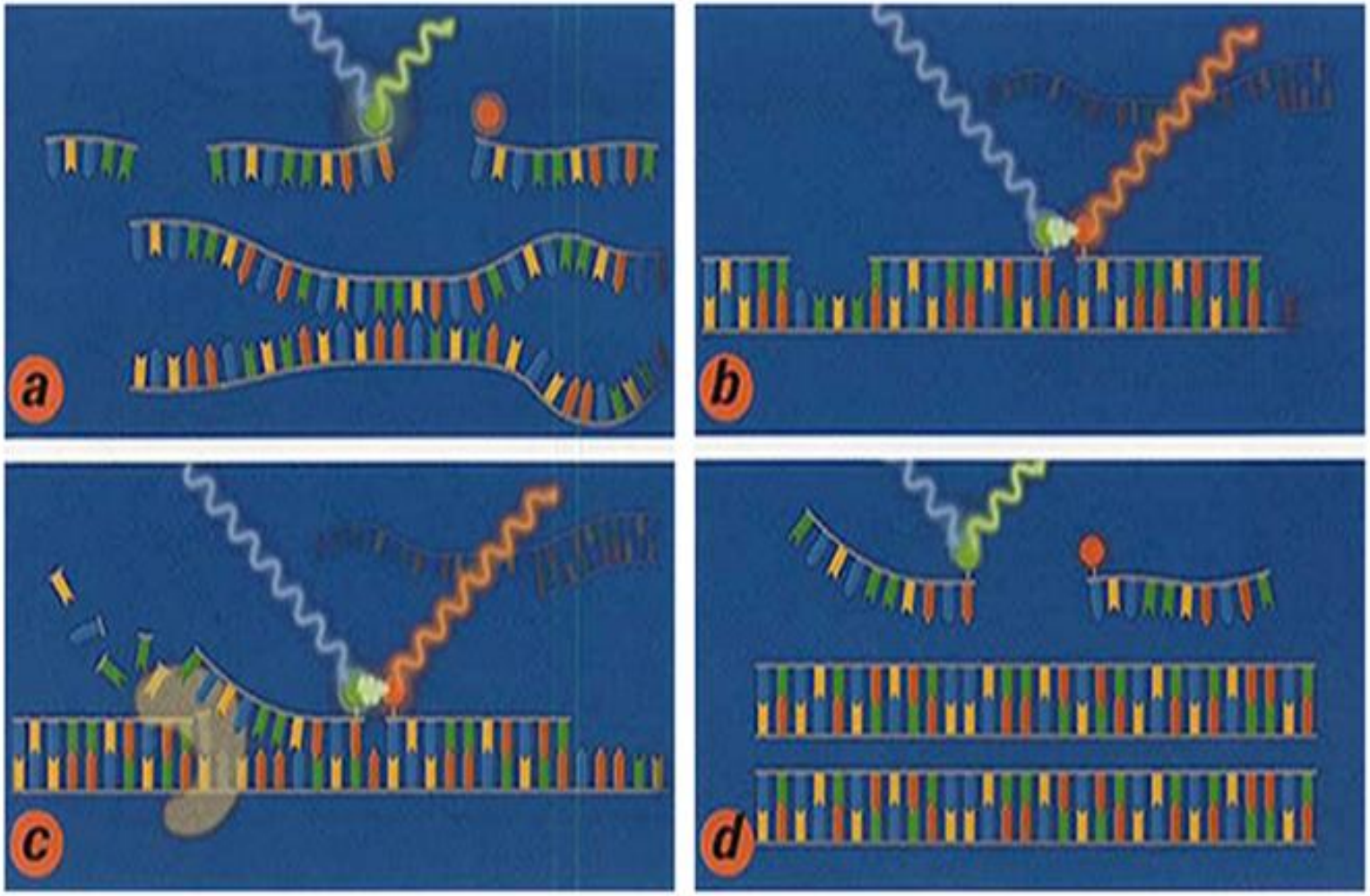

dRFU/dT
Temperature (deg C)
50ng
10ng
2ng
0.4ng
0.08ng
0.016ng
-RT
no template


P. falciparum
P. vivax from monkey blood
P. malariae from monkey blood
primer dimer
Fluorescence -d(F1/F2)/dT
Temperature (°C)

Taqman



Hybridization probes technique




Thank You!