DESIGNING PRIMERS

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Primer features:

- single stranded DNA molecules.

- Types of primers
  - Random (short 10 – 18 b)
  - Specific (long 17 – 30 b)

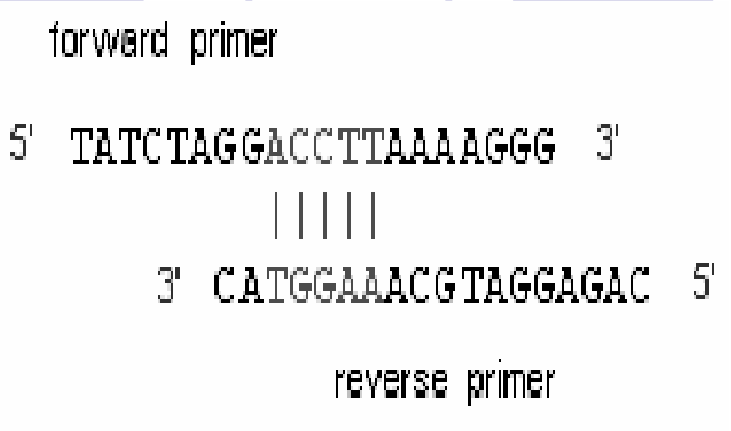
*as the primer increases in size, the chances of matching the target size increase.

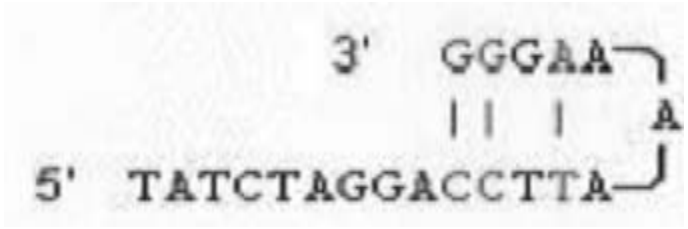
*Longer the primer, the higher the annealing temperature.

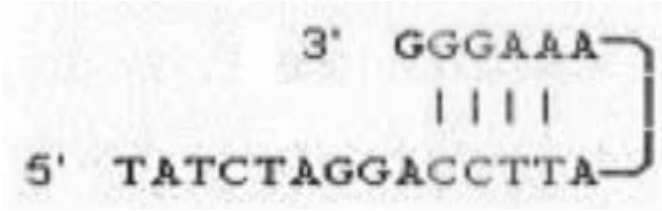
- The higher the annealing temperature, the greater the specificity

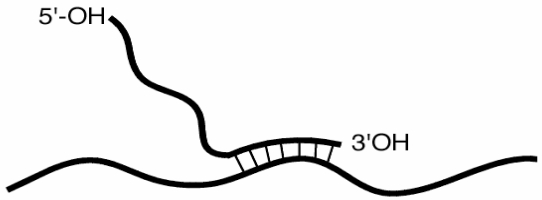
Primer features:

- **Tm of forward primer = Tm of reverse primer (~ 2 to 4 °C).**
- **The G/C content of the primers should be 40 - 60%, and having G or C at 3′ end.**
- **Avoid :**
  - **primer dimmers.**
  - **Hairpins**
  - **The GC clamps cause false priming**





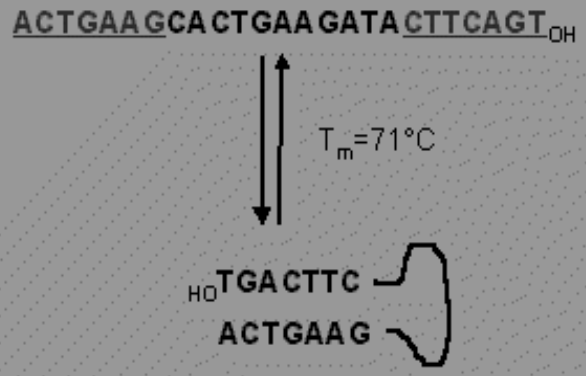




hairpin

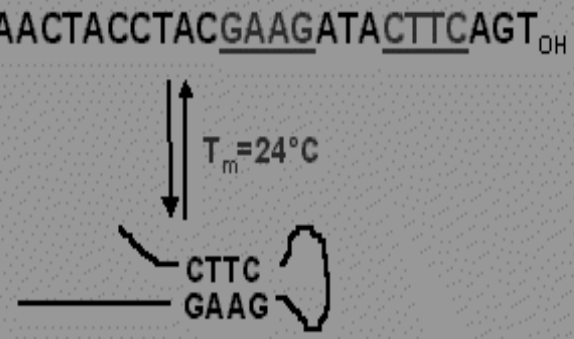
Self Complementarity

- hairpin formation

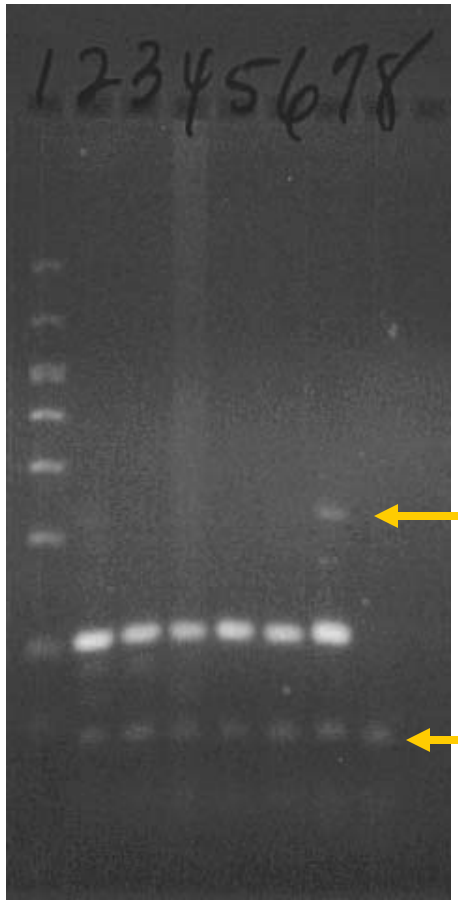


Self Complementarity

- "harmless" hairpin formation



Primer features:



Molecular weight markers

Reagent blank

Misprime

PCR product

Primer dimers

How I can design primers???

- by hand

- Using Comp. Prog (Blast search)

  Blast search primers – avoid repetitive DNA

designing primers by hand

- **Determine the start and end sites of the DNA fragment you want to clone.**

3`GACTAAGGACAGTTGCACAGTTACC..CTCAGGACTTCAGGAACGTAGCAC5`
5`CTGATTCCTGTCAACGTGTCAATGG..GAGTCCTGAAGTCCTTGCATCGTG3`

- **Choose the first 17 -24 bases in the 5` ends as forward and reverse primers.**

3`GACTAAGGACAGTTGCACAGTTACC..CTCAGGACTTCAGGAACGTAGCAC5`
5`CTGATTCCTGTCAACGT3`----→
Forward primer

Reverse primer
←-----3`CTTCAGGAACGTAGCAC5`
5`CTGATTCCTGTCAACGTGTCAATGG..GAGTCCTGAAGTCCTTGCATCGTG3`

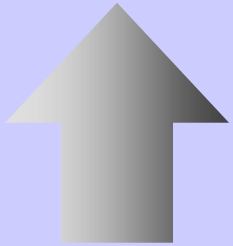
Calculating Annealing Temperature

3`**CTTCAGGAACGTAGCAC**5`→ *Reverse primer*
5`**CTGATTCCTGTCAACGT**3`→ *Forward primer*

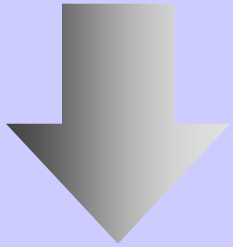
- (#As+#Ts)x2 + (#Cs+#Gs)x4 = Tm
- Tm = melting temperature
- Ta = Annealing temperature
- Ta = Tm - 4ºC

Annealing Temperature

$$T_{anneal} = T_m - (2 - 4\ ^\circ C)$$



Anneal Temp.



More Specificity

Less Specificity

Calculating Annealing Temperature

3`CTTCAGGAACGTAGCAC5`→ *Reverse primer*

Tm = (5+3)X2 + (4+5)X4 = 16 + 36 = 52°C

Ta = 52 – 4 = 48°C

GC content = 9 / 17 = 52.9%

5`CTGATTCCTGTCAACGT3`→ *Forward primer*

Tm = (3+6)X2 + (3+5)X4 = 18 + 32 = 50°C

Ta = 50 – 4 = 46°C

GC content = 8 / 17 = 47.06%

Assure that primers in a set have annealing temperature within 2 ºC – 4 ºC of each other

Online T_m Calculator available in : http://www.appliedbiosystems.com/support/techtools/calc

Tm Calculator

Directions:

To calculate the optimal melting temperatures of your primers, enter each primer's concentration, its 5'-3' sequence, and the salt concentration (K+ and Na+) of your PCR or Real-Time PCR reaction.
Note: If entering info only for Primer #1, ignore result values given for Primer #2.

Primer #1

Salt Concentration (mM) 50 Primer Concentration (µM) .2

5' 3'

Primer #2

Salt Concentration (mM) 50 Primer Concentration (µM) .2

5' 3'

Submit Clear

Tm Calculator Results

Results for these primers are as follows:

Primer	Tm (DegC)	Salt (mM)	Primer (µM)	%GC	Length
Primer #1: CTTCAGGAACGTAGCAC	47.64	50	.2	53	17
Primer #2: CTGATTCCTGTCAACGT	47.45	50	.2	47	17
Tm Delta = 0.20					

ReSubmit

designing primers by prog.

- Available by The National Center for Biotechnology Information
- http://blast.ncbi.nlm.nih.gov/Blast.cgi
- http://www.ncbi.nlm.nih.gov/tools/primer-blast/

```
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      541 tacacccagt acggcaacaa gaactacgat ctggccggta cgaatgtccg caacaagctg
      601 gacacgcagg atatccgcgt cggcatcggc tacaagttct aa
//
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Copy and paste the target seqoence

copy

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ORIGIN
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      541 tacacccagt acggcaacaa gaactacgat ctggccggta cgaatgtccg caacaagctg
      601 gacacgcagg atatccgcgt cggcatcggc tacaagttct aa
//
```

paste

Sequences of primer sets available to the community

DNA Source [info]

Locus: Enter a standard gene name or systematic ORF name (i.e. ACT1, YKR054C)

OR

Enter the DNA Sequence (numbers are OK, but comments should be removed)

```
       481 gctggtgccg gtctcgaagc caagctgacg gacaacatcc tcggccgcgt
tgagtaccgt
       541 tacacccagt acggcaacaa gaactacgat ctggccggta cgaatgtccg
caacaagctg
       601 gacacgcagg atatccgcgt cggcatcggc tacaagttct aa
```

Purpose: PCR or Sequencing [info]

- (•) **PCR [info] or**
- ( ) **SEQUENCING [info]**

Submit Reset

Select the primer options

Location [info]

Select YES to Amplify a region with **EXACT** endpoints of the DNA sequence entered ◉ NO ○ YES
Length of DNA in which to search for valid primers: 35

Melting Temperature [info]

Optimum Tm: 56 Minimum Tm: 52 Maximum Tm: 60
Optimum primer length: 20 Minimum length: 18 Maximum length: 21

Primer Composition [info]

Optimum percent GC content: 45 Minimum GC: 30 Maximum GC: 60

Primer Annealing [info]

Self Anneal: 24 Self End Anneal: 12 Pair Anneal: 24 Pair End Anneal: 12

Submit Reset

Primers for PCR

There were 66 forward primers in the valid range for GC content.
There were 23 reverse primers in the valid range for GC content.
There were 43 forward primers in the valid range for melting temperature.
There were 22 reverse primers in the valid range for melting temperature.
There were 43 forward primers with valid self anneal values.
There were 22 reverse primers with valid self anneal values.
There were 33 forward primers with valid self end anneal values.
There were 22 reverse primers with valid self end anneal values.
Some primers were not evaluated

There were 250 pairs of valid primers.

This is the BEST pair of primers

List of all valid pairs of primers

The best choice and primer data

Primer Pair Data

Primers for 1 (Entered)

These primers are for PCR

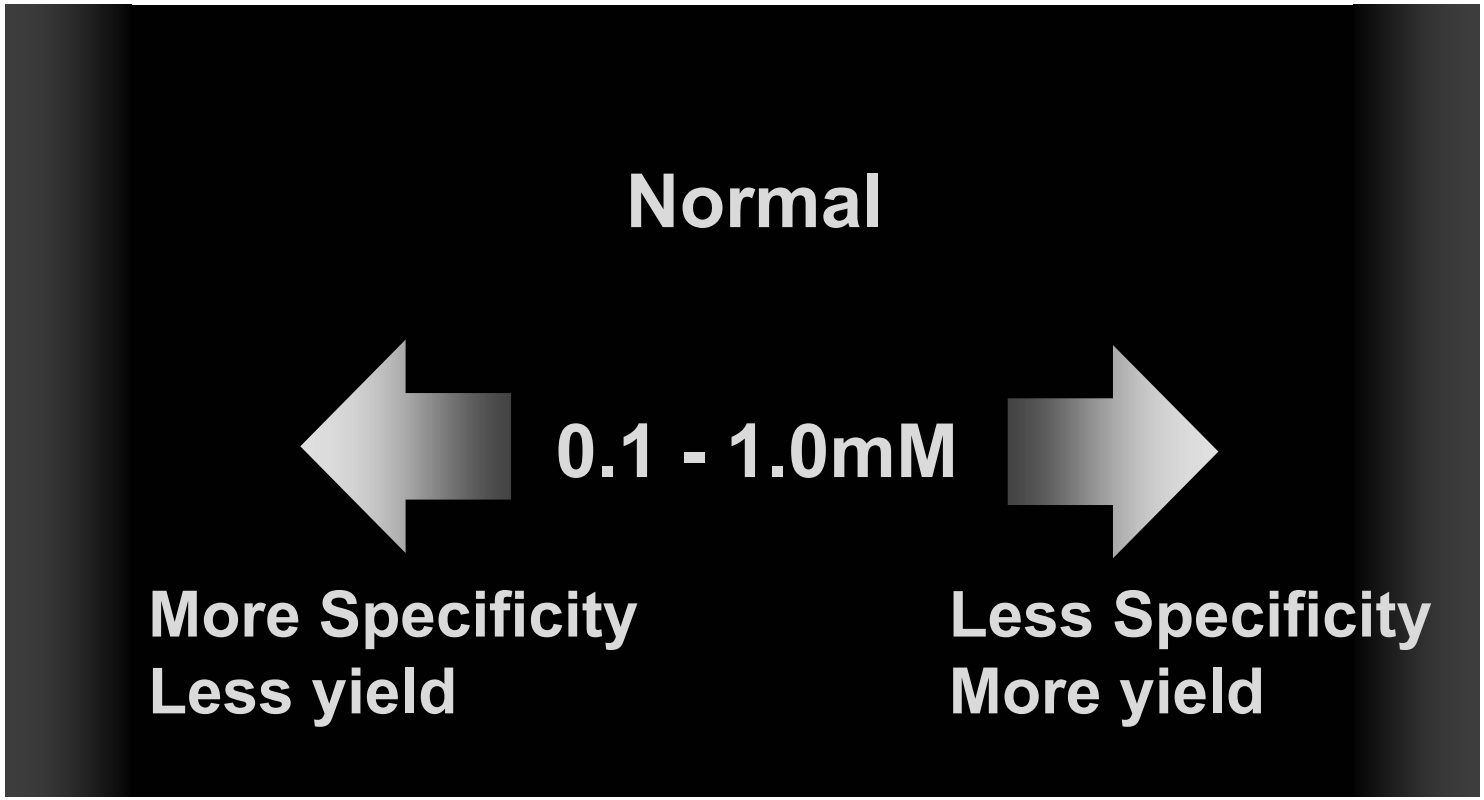
Forward-Primer	ATGCGCACTCTTAAGTCTCT	**Reverse-Primer**	TTAGAACTTGTAGCCGATGCC
Length:	20	Length:	21
Percent GC:	45	Percent GC:	47
Tm:	48	Tm:	53
Self-Anneal:	20	Self-Anneal:	10
End-Anneal:	6	End-Anneal:	4
Offset:	1	Offset:	642

Pair-Anneal: 16
Pair-End-Anneal: 8
Total valid primer pairs: 250

These primers will amplify a fragment of 642 basepairs
The sequence of DNA that will be amplified by these primers is

```
1   ATGCGCACTC TTAAGTCTCT CGTAATCGTC TCGGCTGCGC TGCTGCCGTT
51  CTCTGCGACC GCTTTTGCTG CCGACGCCAT CCAGGAACAG CCTCCGGTTC
```

PCR Primer Concentrations



RAPD-PCR

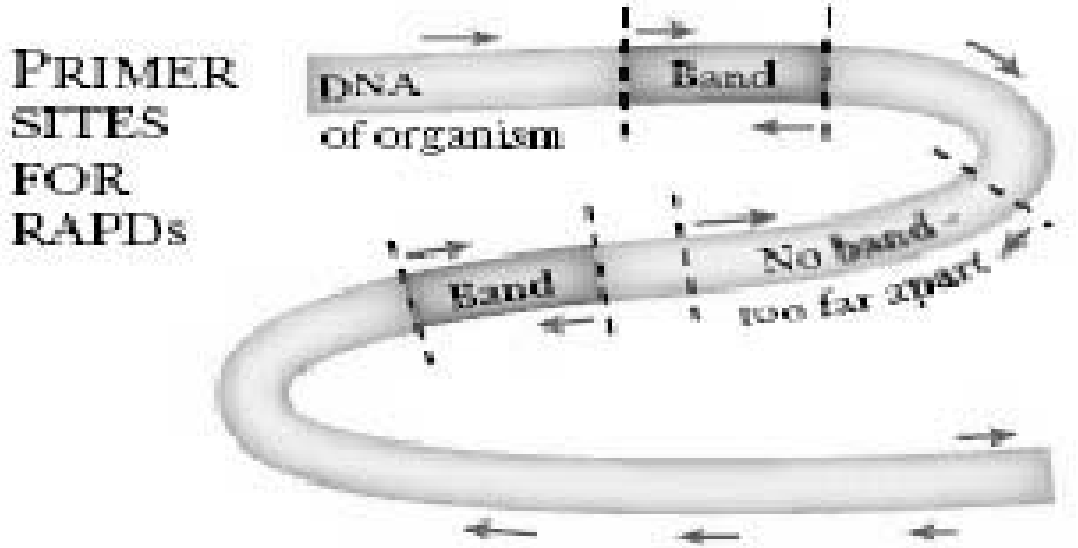
RAPD = Random Amplified Polymorphic DNA

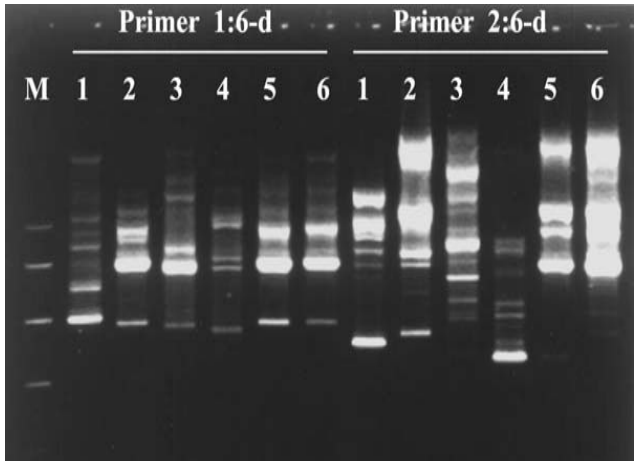
- **Amplify unknown DNA sequences using single, short (10-12 bases), and random primers.**

Example:

OPO-13	**5`-GTC AGA GTC C-3`**
OPO-14	**5`-AGC ATG GCT C-3`**
OPO-16	**5`-TCG GCG GTT C-3`**
OPO-18	**5`-CTC GCT ATC C-3`**

RAPD-PCR





Primer 5:6-d