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How to design primers for your PCR?

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Gene??

- A gene is a section of DNA that codes for a protein.
- Each unique gene has a unique sequence of bases.
- This unique sequence of bases express for a unique protein.

Toxoplasma gondii **strain TgCgCa1 micronemal protein (*mic*2) gene (371 bp)**

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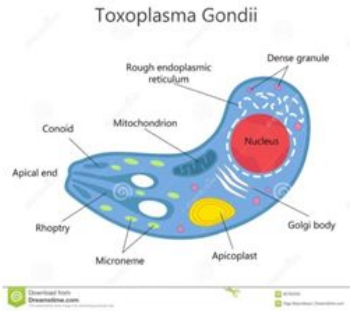
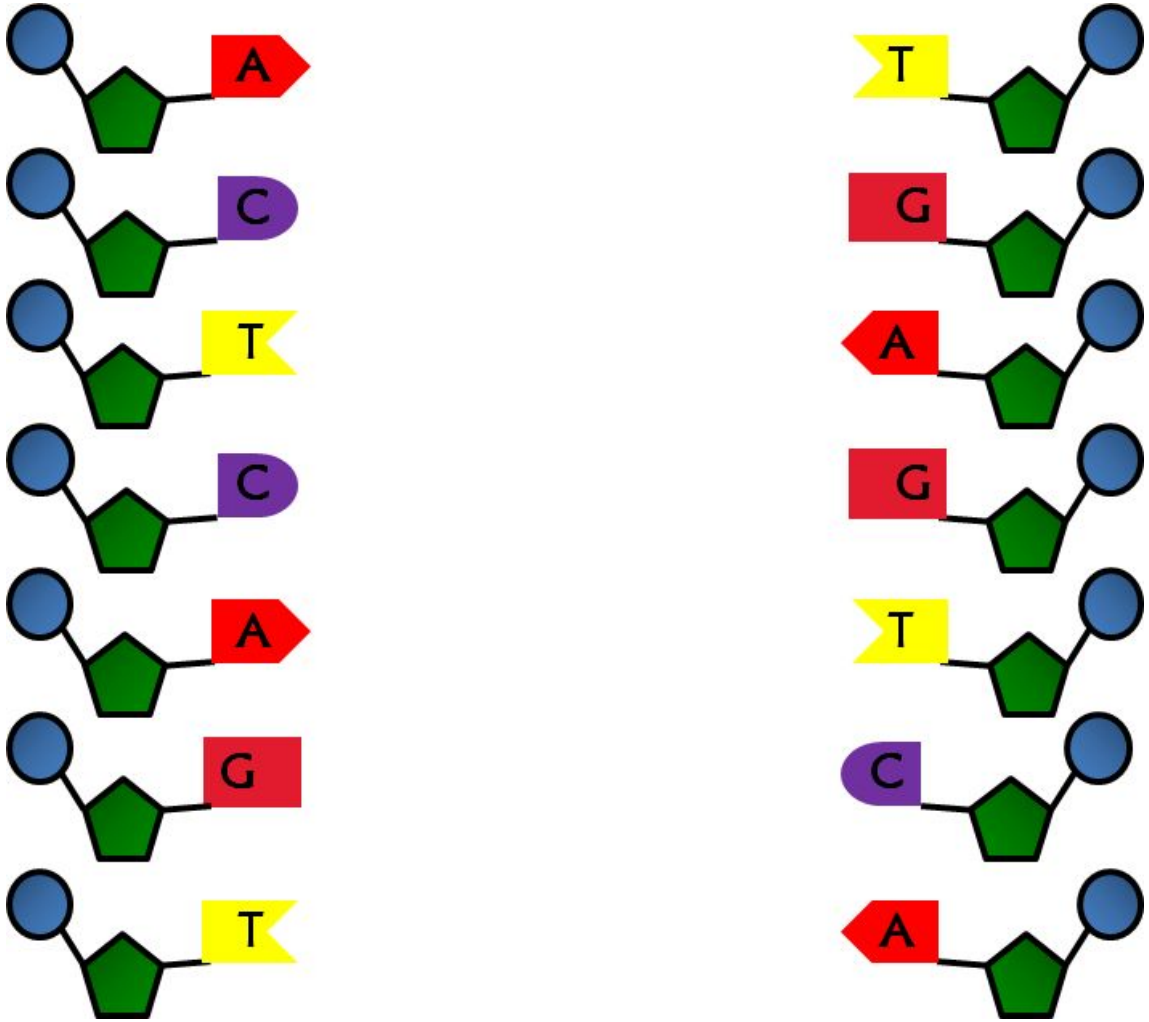
1 aaggttatca gaaaagcagt tgtgtcgcct tgtacgaatg aggcggtatc agaagtcgcg
61 tctgactgta gtgacggtcg aattgtgtag ggatggggag acacagcaag cccagaagca
121 tgaagttgtt ccacgctacc ttgtctgcgc gatcgatttt ctcaaagatc attaggtgca
181 acgcgatatg gcagtcgttg cgggccaaga aaacgaattc tacaaacagg gtgtacaggg
241 gaaactgctc gcacgcgata atttttgaaa tggaagttac ccttggggca gtgctgtttc
301 atcttgcgca ggcataggga tgtttagtat ccgacacgat gagcctccgg gtgtctgttt
361 atctgctctt c
```




A
C
T
C
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A

PCR?

A technique that takes a small amount of DNA with specific sequence and amplifies it to be detected or used for further testing.

PCR

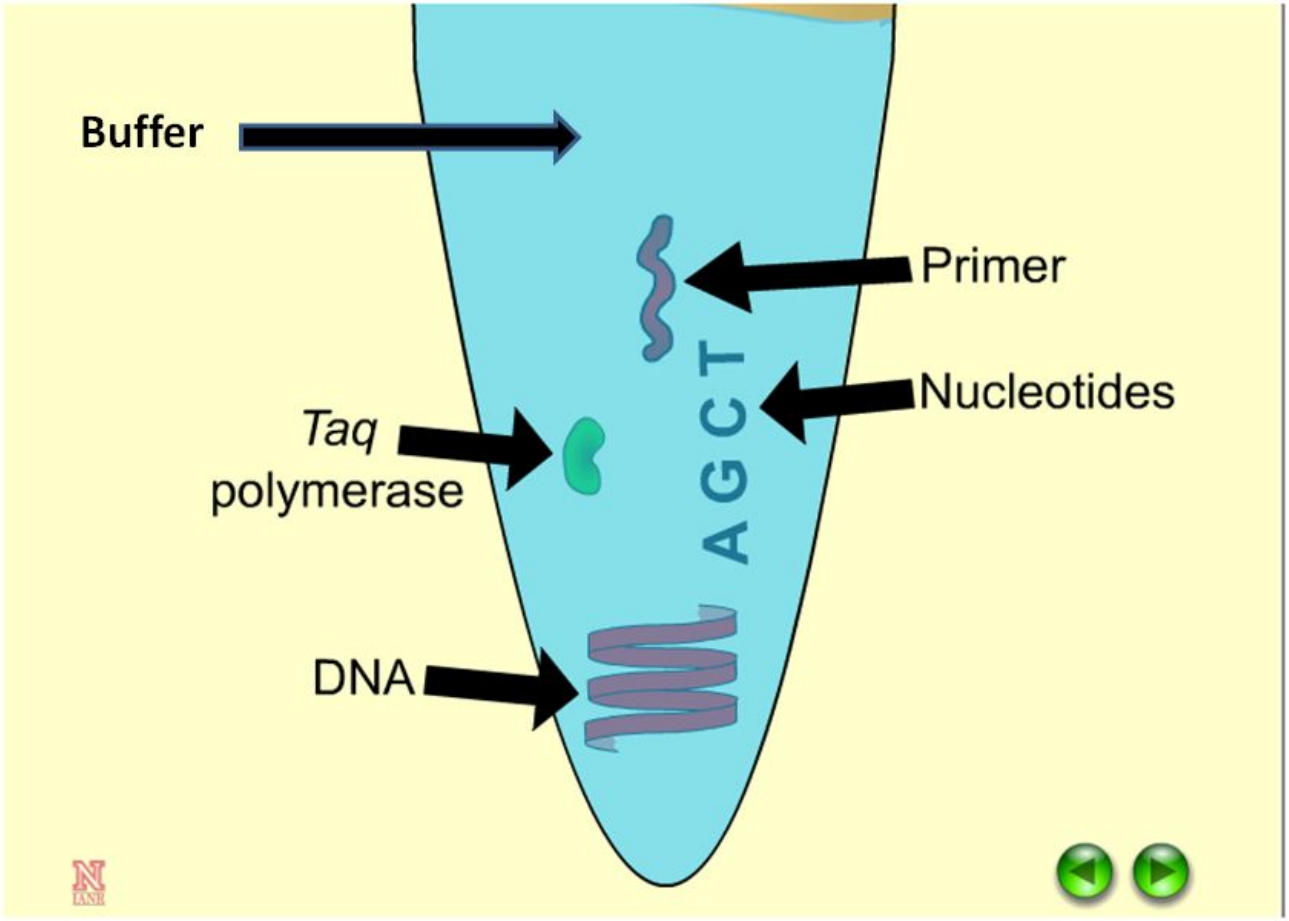
PCR is a laboratory version of DNA Replication in cells.

Invented by an American biochemist Kary Banks Mullis (born1944) in1983 and won Nobel prize in 1993.

Steps of PCR

- Denaturation (DNA denaturation step)
  94°- 95°C.
- Annealing (primer annealing step)
  50°- 65°C

- Extension (polymerase extension step)
  72° C

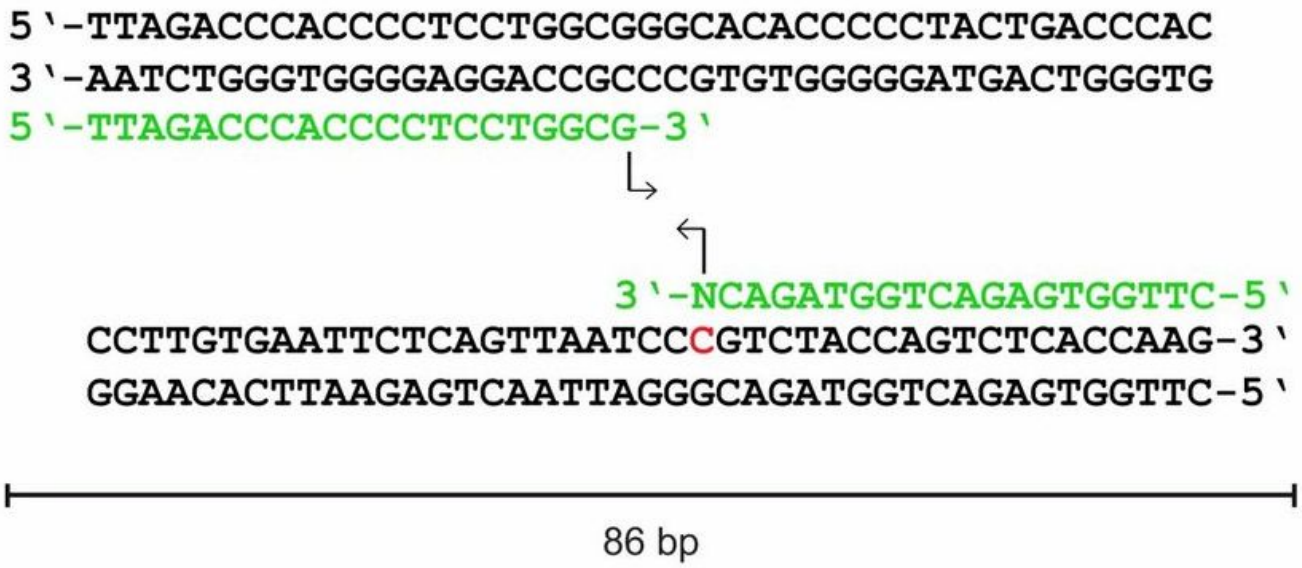


Master mix

Primers

- Single-stranded oligonucleotides, range from 15 to 30 nucleotides.
- Complementary building blocks of the target sequence (the region to be amplified).
- Optimal concentration between 0.1 and 0.6 μM.

How to design a primer?



- Higher primer concentrations may lead to accumulation of non specific product.
- Lower primer concentrations may be exhausted before the reaction is completed, resulting in lower yield of desired product.

- Usually about 18-22 nucleotides in length
- Melting point determined by G-C and A-T content

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

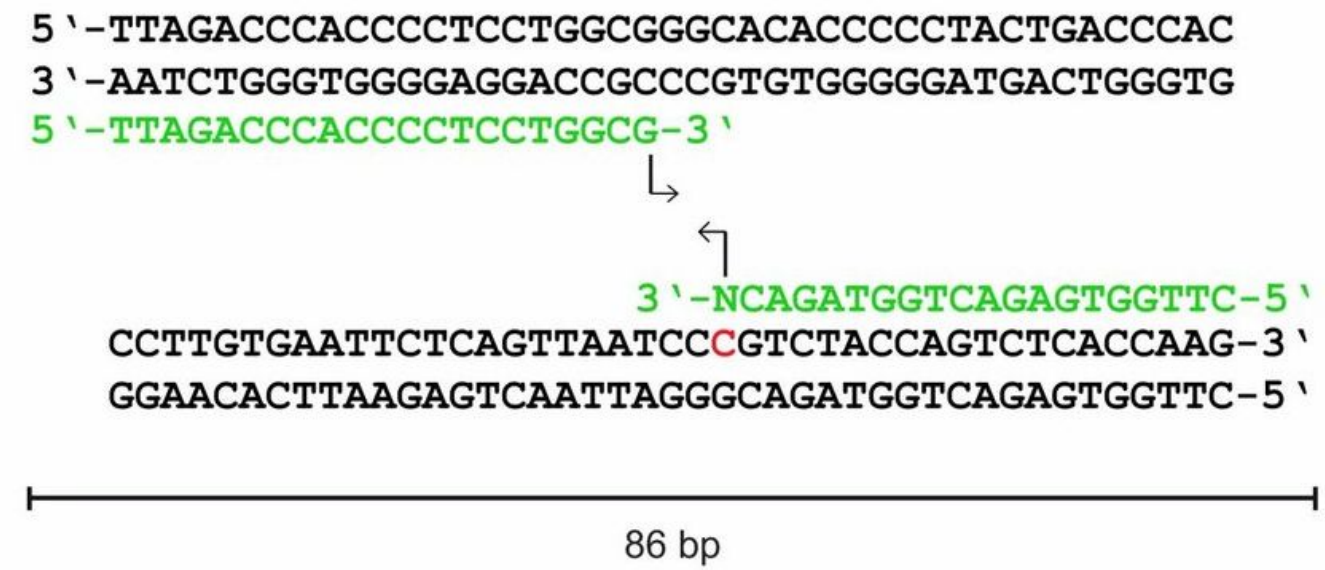
CAAGCTTATGGCGCTCACCT

G+C = 11

A+T = 9

Tm= 62 ^oC

How to design a primer?



NCBI

(National Center for Biotechnology Information)

https://www.google.com/search?client=opera&q=NCBI&sourceid=opera&ie=UTF-8&oe=UTF-8

NCBI

https://en.wikipedia.org/wiki/National_Center_for_Biotechnology_Information

- The **National Center for Biotechnology Information** (**NCBI**) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health (NIH). The NCBI is located in Bethesda, Maryland and was founded in 1988 through legislation sponsored by Senator Claude Pepper.
- The NCBI houses a series of databases relevant to biotechnology and biomedicine and is an important resource for bioinformatics tools and services. Major databases include GenBank for DNA sequences and PubMed, a bibliographic database for the biomedical literature. Other databases include the NCBI Epigenomics database. All these databases are available online through the Entrez search engine.

- Basic Local Alignment Search Tool

References

- https://en.wikipedia.org/wiki/National_Center_for_Biotechnology_Information
- https://www.ncbi.nlm.nih.gov
- http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html


Thank You!