PCR
The polymerase chain reaction

Crick and Watson – structure of DNA

A double helix with two antiparallel strands
“It has not escaped our notice that the specific pairing we have postulated immediately suggests a copying mechanism for the genetic material.”
My gene:
5' TACGACGTTGTAAGAACGTCTATACCGAGCTCCTCTCTCTCTCATAGCTGTTTCCTGTGTGAA 3'

I design custom oligonucleotides to the "sense" strand at the beginning and the "antisense" at the end.

Sense 5' TACGACGTTGTAAGAACGTCTATACCGAGCTCCTCTCTCTCTCATAGCTGTTTCCTGTGTGAA 3'
Antisense 5' GATACTGTGCAGATATGGCTCGAGGAGAGAGAATACGTAAAGGACACACTT 3'

"Forward primer"
5' TACGACGTTGTAAGAACGTCTATACCGAGCTCCTCTCTCTCTCATAGCTGTTTCCTGTGTGAA 3'
"Reverse primer"
3' GATACTGTGCAGATATGGCTCGAGGAGAGAGAATACGTAAAGGACACACTT 5'
PCR: Polymerase Chain Reaction

30-40 cycles of 3 steps:

1. Denaturation
   1 minute 94°C

2. Annealing
   45 seconds 54°C
   forward and reverse primers

3. Extension
   2 minutes 72°C
   add dNTPs

Denaturation: 94°C
DNA hybridization rate is altered by changing:

- Temperature: template dependent
- Salt Concentration: can be standardized
- DNA concentration: can be standardized
- Time: can be standardized

Thermal cycler

- Heated lid (prevents evaporation problems)
- Peltier effect heating/cooling block
- 96-well plate format
- Microprocessor controlled thermal program
Real-time PCR
Quantitative
Fast
High sample processing speed
Very sensitive

Parasitic Worm Inoculation Levels

Violet = 200 worms per root
Red = 50 worms per root
Blue = 20 worms per root