DNA SEQUENCE DATA

-From template DNA to Sequence Alignment...

Case Study:

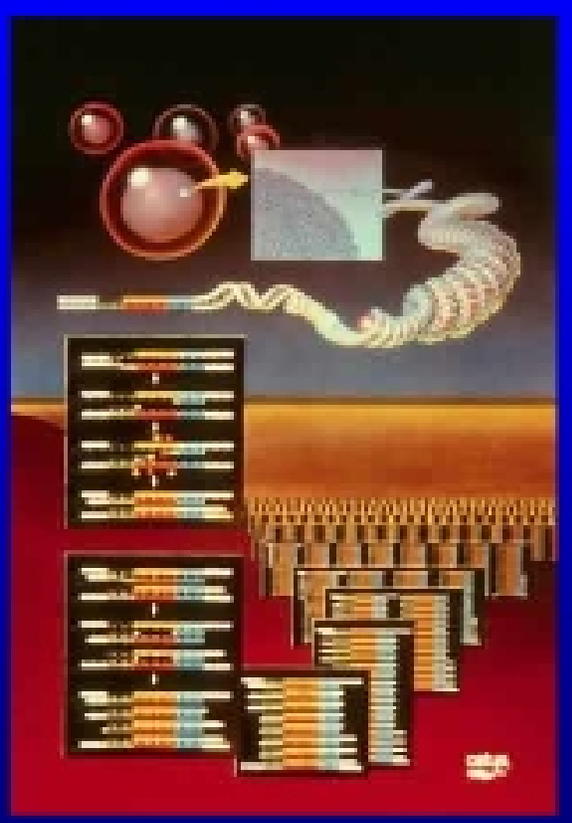
Western Diamondback Rattlesnake (*Crotalus atrox*)

Protocol

1. Collect tissue samples from *C. atrox* individuals and extract tDNA
2. Amplify specific gene using PCR (Polymerase Chain Reaction)
3. Sequence PCR products
4. Align our sequence with published sequences
5. Analyze with phylogenetic software

PCR – Purpose

- Need multiple copies of the gene in order to sequence it
- Primer extension reaction for amplification of specific nucleic acids in vitro

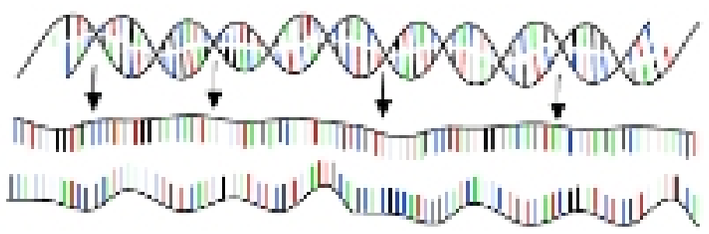


PCR – Reaction Composition

- tDNA
- Sequence specific primers
- dNTP's
- Taq polymerase
- Buffer
- Thermocycler

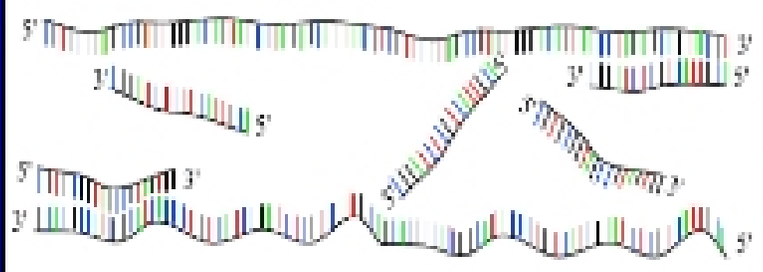
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

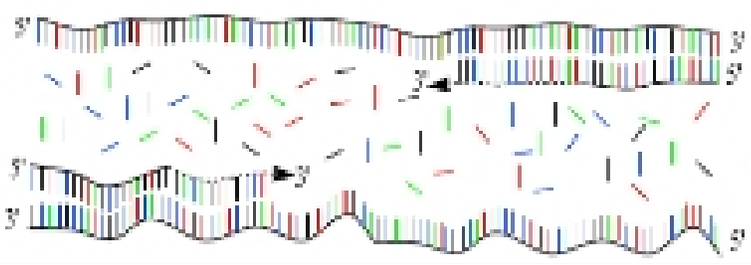
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C

only dNTP's