RAPD

Randomly Amplified Polymorphic DNA

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RAPD

- a method based on PCR developed in 1990.

- RAPD is different from conventional PCR as it needs one primer for amplification. The size of primer is normally short (10 nucleotides), and therefore, less specific.

- the primers can be designed without the experimenter having any genetic information for the organism being tested.

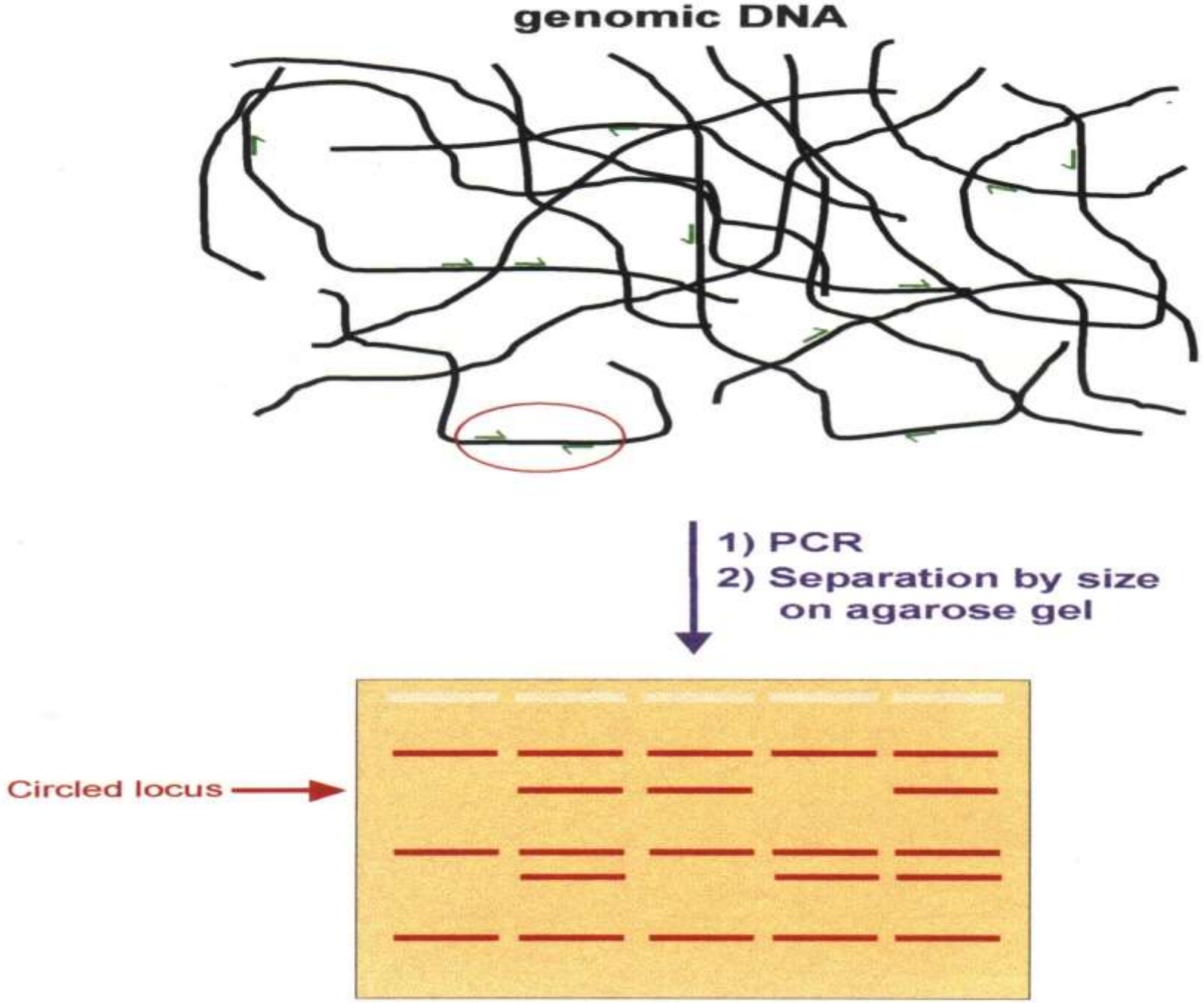
- more than 2000 different RAPD primers can be available commercially.

RAPD

- Genomic DNA normally has complimentary sequences to RAPD primers at many locations.

-If two of these locations are close to each other (<3000bp), and the sequences are in opposite orientation, the amplification will be established. This amplified region is said as a RAPD locus.

-Normally, a few (3-20) loci can be amplified by one single RAPD primer.


genomic DNA
1) PCR
2) Separation by size
on agarose gel
Circled locus

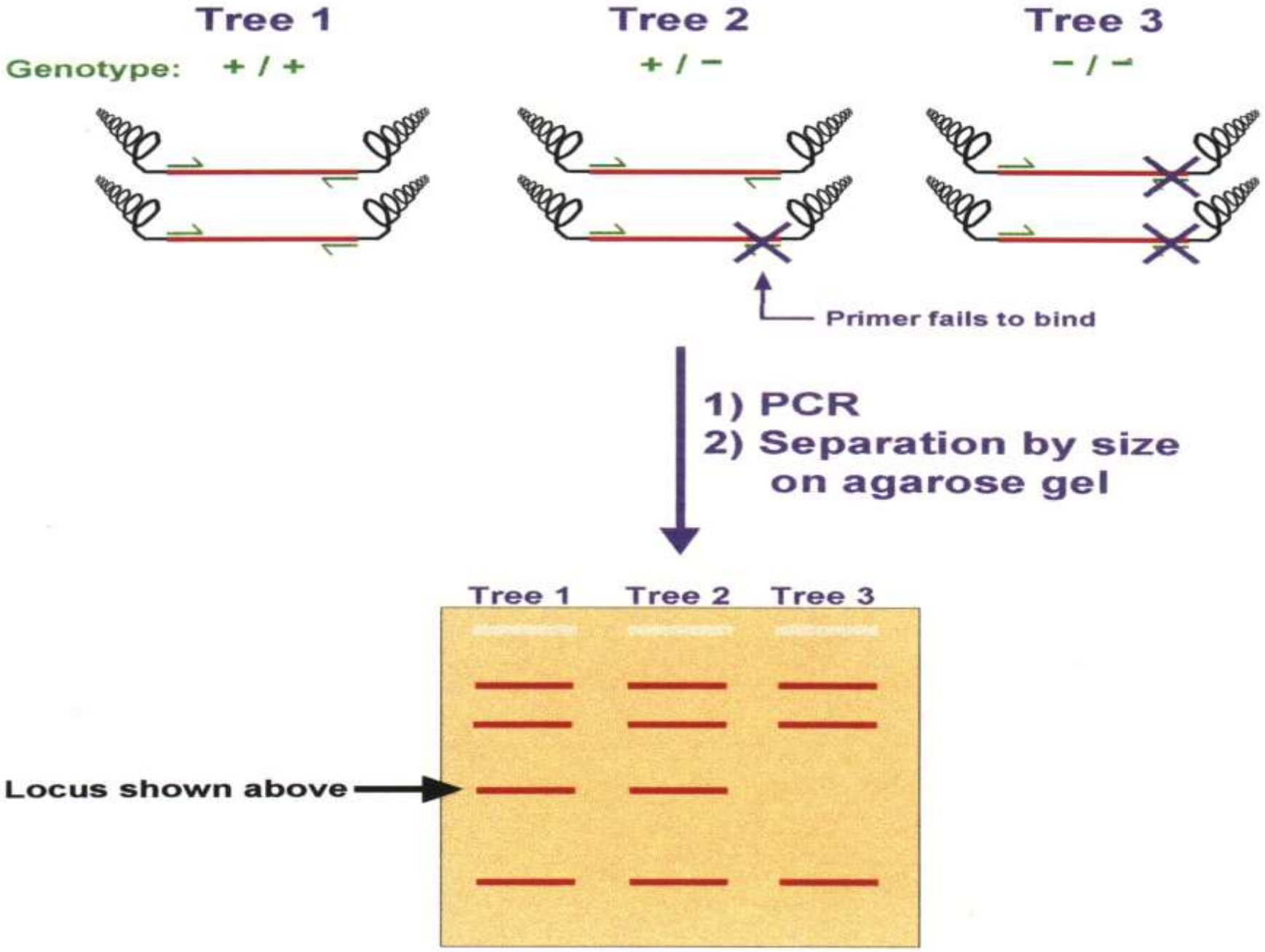
RAPD

Variation DNA detected by RAPD is due to the loss of RAPD loci. The loss of RAPD loci is caused by:

a) change of sequence at primer annealing site in the genomic DNA

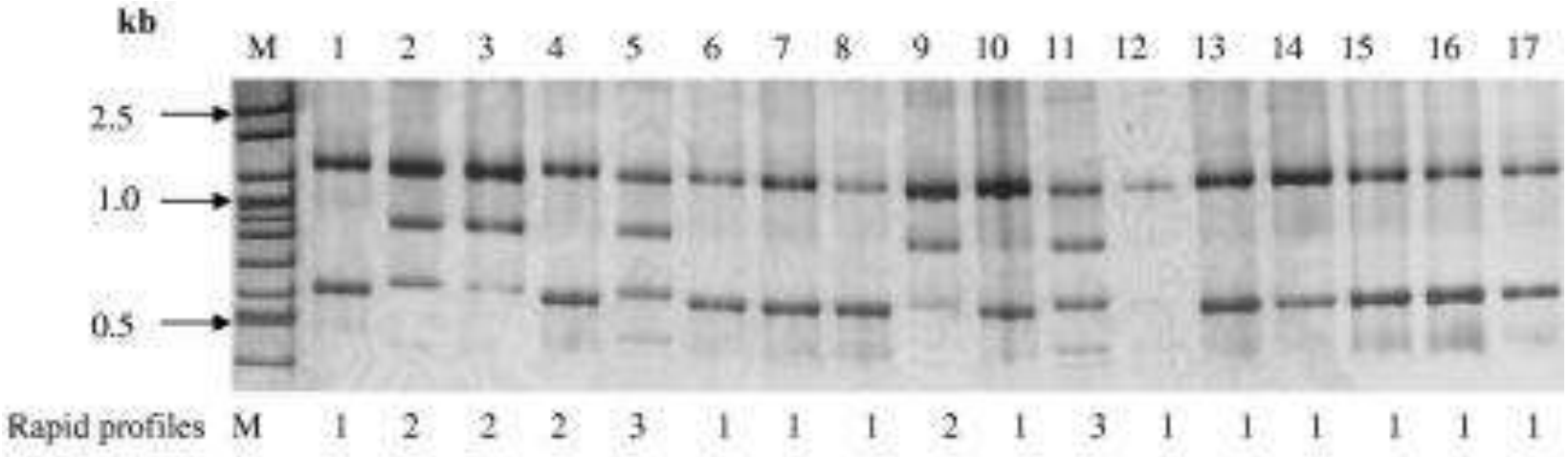
b) deletion of primer annealing site in the genomic DNA

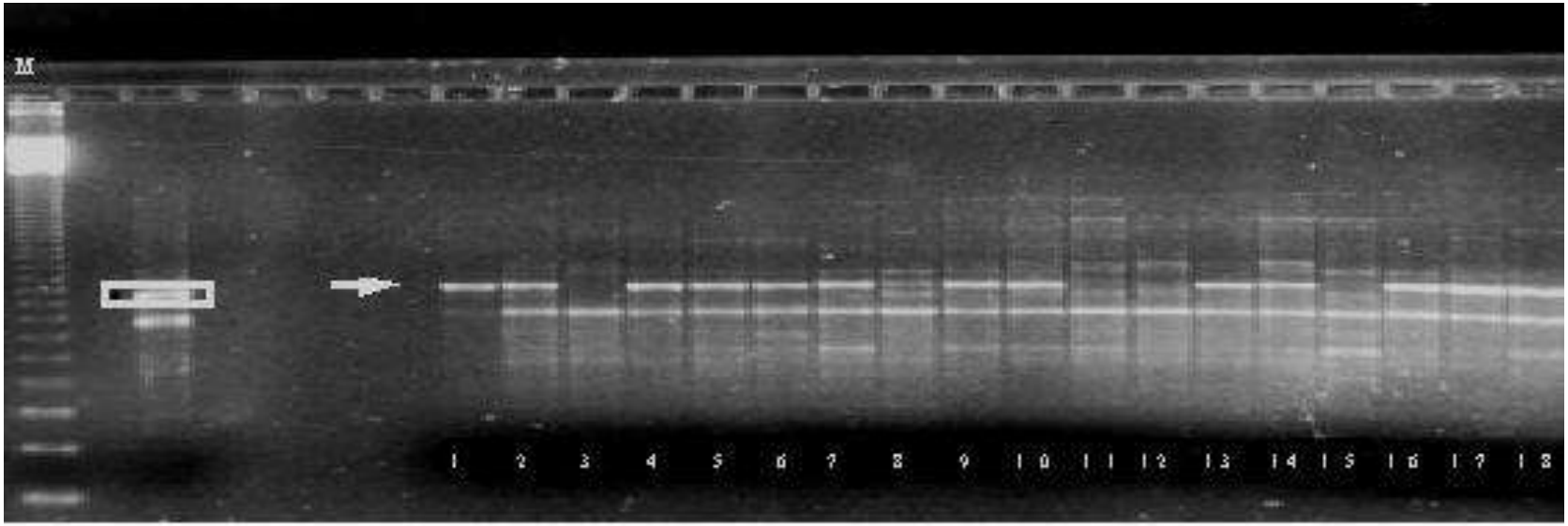
c) large insertion in between two primer annealing sites


Tree 1
Genotype: + / +
Tree 2
+ / −
Tree 3
− / −
Primer fails to bind
1) PCR
2) Separation by size on agarose gel
Tree 1
Tree 2
Tree 3
Locus shown above

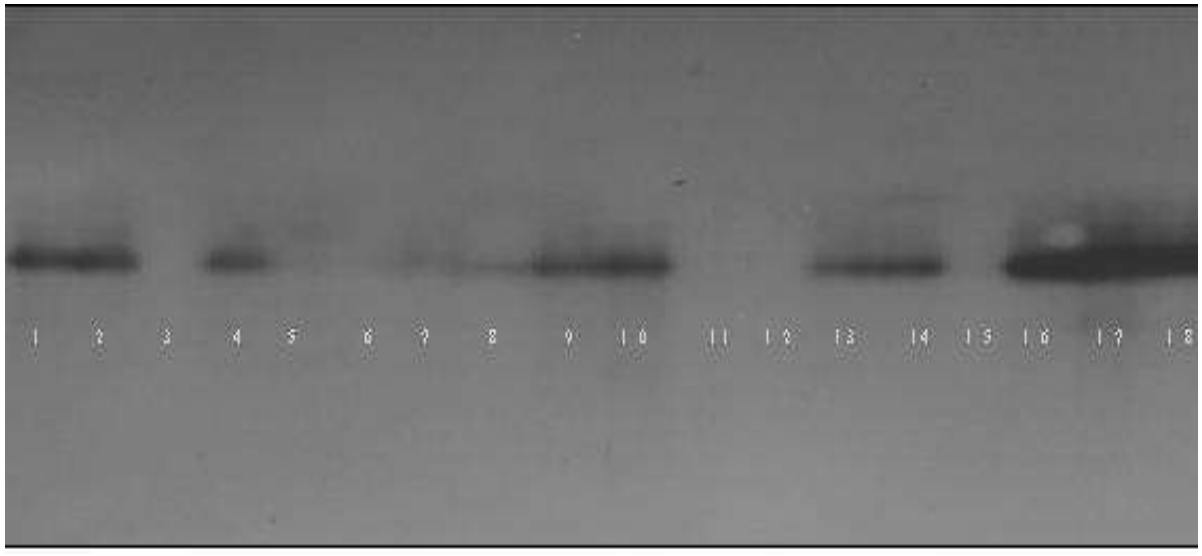
RAPD

Silver-stained polyacrylamide gel showing three distinct RAPD profiles generated by primer OPE15 for *Haemophilus ducreyi* isolates from Tanzania, Senegal, Thailand, Europe, and North America





HOMOLOGY TEST FOR FRAGMENTS OF SIMILAR MOBILITY IN RAPD PROFILES



RAPD

- RAPD marker is a dominant marker.

- Presence of a DNA band is dominant; absence of a DNA band is recessive.

- DNA bands of different sizes are assumed to be amplified products from different RAPD loci.

Modifications of RAPD

Techniques similar to RAPD:

- AP-PCR
- DAMD
- ISSR

AP-PCR

- AP-PCR (Arbitrary Primed PCR).

- similar to RAPD.

- involves two cycles of low-stringency amplification, followed by cycles conducted at higher stringency, using primer of arbitrary sequence.

AP-PCR

- the length of primers is 20-34 nucleotides long.

- the primers used include the Universal M13 sequencing primer, the M13 reverse sequencing primer and the T3 sequencing primer.

DAMD

- DAMD (Directed Amplification of Minisatellite Region DNA)

- technique for detecting polymorphisms using VNTR core sequences as primers for PCR

ISSR

- ISSR (Inter-Simple Sequence Repeat).

- A PCR-based molecular marker assay of genomic sequence lying between adjacent microsatellites (SSRs). Primers carrying, at their 3'-end, sequence complementary to the repeat unit of the microsatellite will amplify this genomic DNA.

Criticism in RAPD

- lack of reproducibility.

- RAPD banding patterns prone to:

i) DNA template concentration and quality

ii) Different Taq DNA polymerases

iii) Different PCR machines or related equipment used in conducting PCR.

Genetic diversity parameters

- Percentage of polymorphic loci
- Shannon diversity index, *H*
  - $H = \Sigma^{n}_{i=1} -\rho_i \ln \rho_i$
- Genetic similarity, F
  - $F = 2m_{xy} / (m_x + m_y)$
- Genetic distance, 1-F