oligonucleotide "10- mer" primer to amplify or not the DNA template. In this type of PCR; usually used more than one primer. The result depends on numbers of present or absent bands on agarose gel after PCR, which represent the polymorphism of the DNA as a result of mutation or any changing in sequences of the DNA. Theses polymorphisms can be represented by dendrogram or phylogeny tree of the organism DNA under different condition " similarity and distance", (8), fig (5).

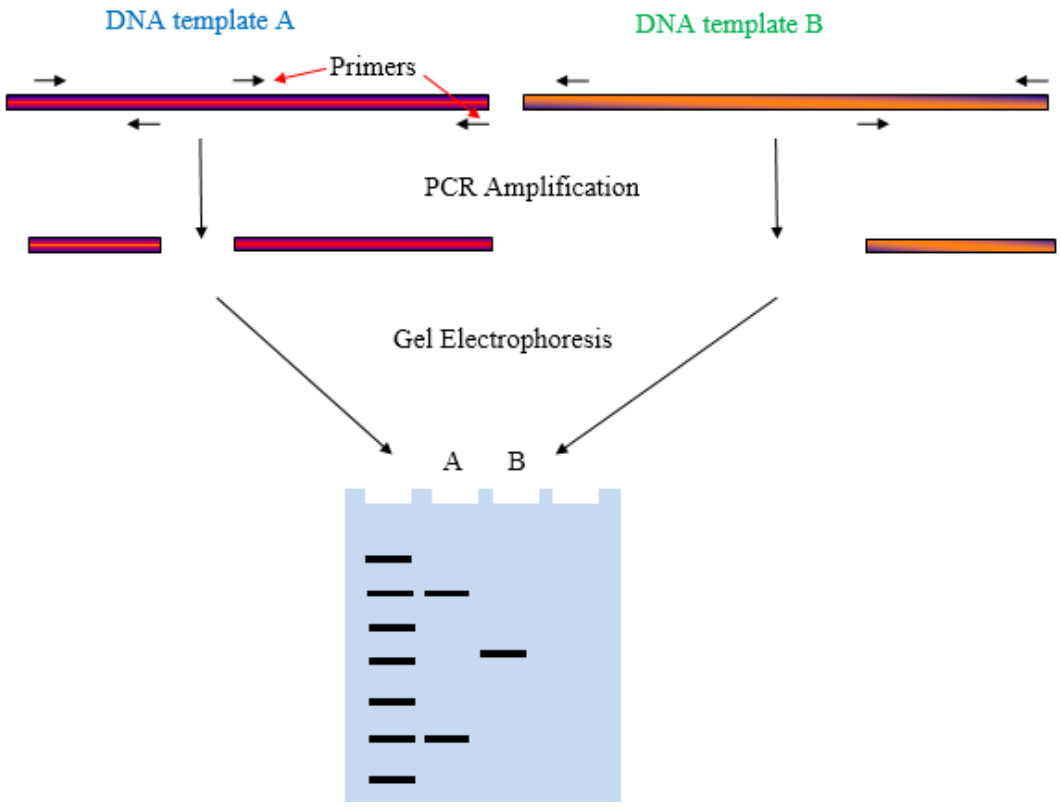

Figure (5): RAPD PCR PCR

SSR-PCR:

It’s an abbreviation of " Simple Sequence repeat anchored polymerase chain reaction"; it’s a technique depend on sequences of primer on the 5` or 3` end of microsatellite " a short repeated sequences of DNA at specific one locus of a chromosome used as fingerprinting in genetics" the nucleotide typically repeated 5-50 times. The primer used a highly annealing temperature. The microsatellite is a highly polymorphic mutation and distribution in eukaryotic genomic DNA, it's not exceed 200 bp which targeting amplifying more one locus in microsatellite DNA, (9), fig (6).

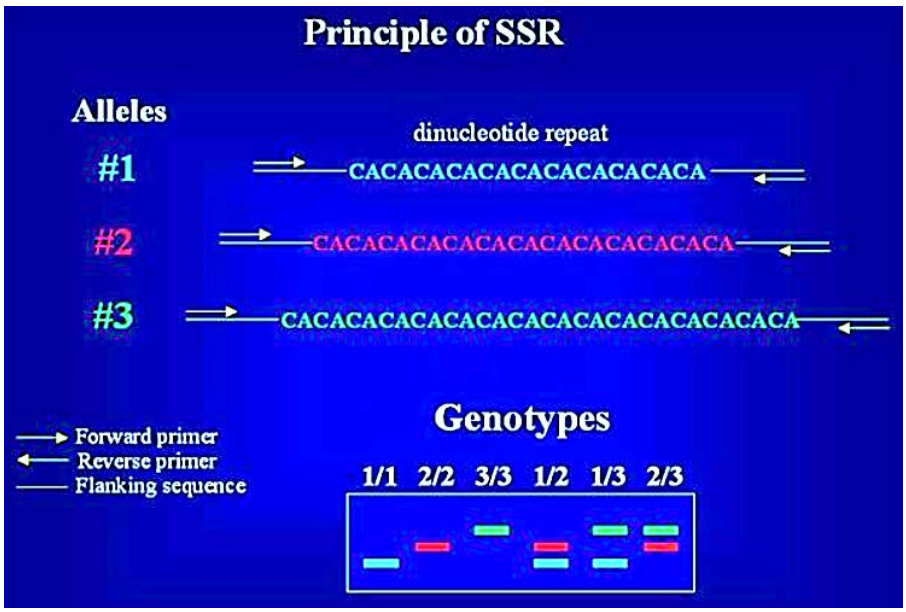

Figure (6): SSR PCR

AFLP and RFLP PCR

AFLP PCR " Amplified Fragment Length Polymorphism " it's used a restriction enzyme to digest the DNA then amplified the digested segment by PCR, this reaction is used to detect the polymorphisms of fingerprinting with related population. While RFLP-PCR " Restriction fragment length Polymorphisms" is used the restriction enzymes to digest the genomic DNA then running the gel electrophoresis and making the blotting by using radioactive labeled probe, (10), fig (7).

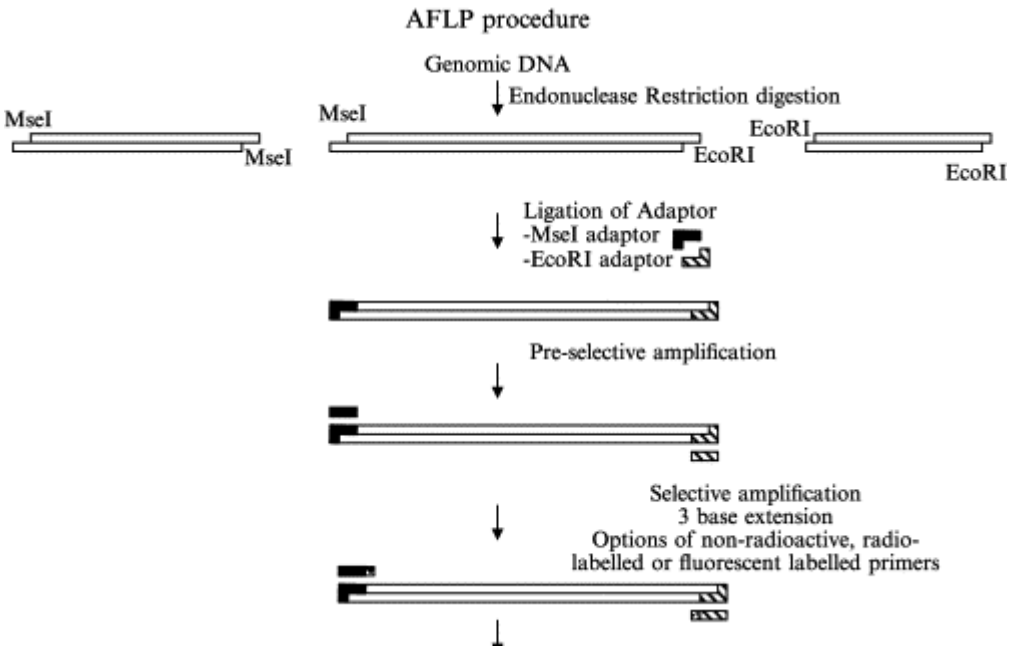

Figure (7): AFLP PCR

ARMS PCR

It's an abbreviation of " Amplification of Refractory Mutation System" which is a technique used to detect a single nucleotide change in the sequence, it's also called " PCR Amplification of specific alleles" or " single nucleotide Polymerase", its used to detect known mutation but not new mutation, its depends on designing a specific primer for two alleles; which is one normal and the other is polymorphisms in single nucleotide.

The two primers are, fig (8), one matches the natural allele and the other match the abnormal allele (mutant) and that is depends on one base in 3` end. In primer design; a modification of primer must be considering to achieve successfully results. At the near 3` end (2 base before the end) of primer a strong mismatch base is added, (C: T, G: A, T: T), this mismatch will alter the annealing temperature, if the template has a complementary base (normal allele) the amplification will have done with presence the strong mismatch, while if there is a non-complementary base in the 3` end of primer (mutant allele) the amplification will terminate,(11), fig (9).

Figure (8): Designing ARMA PCR

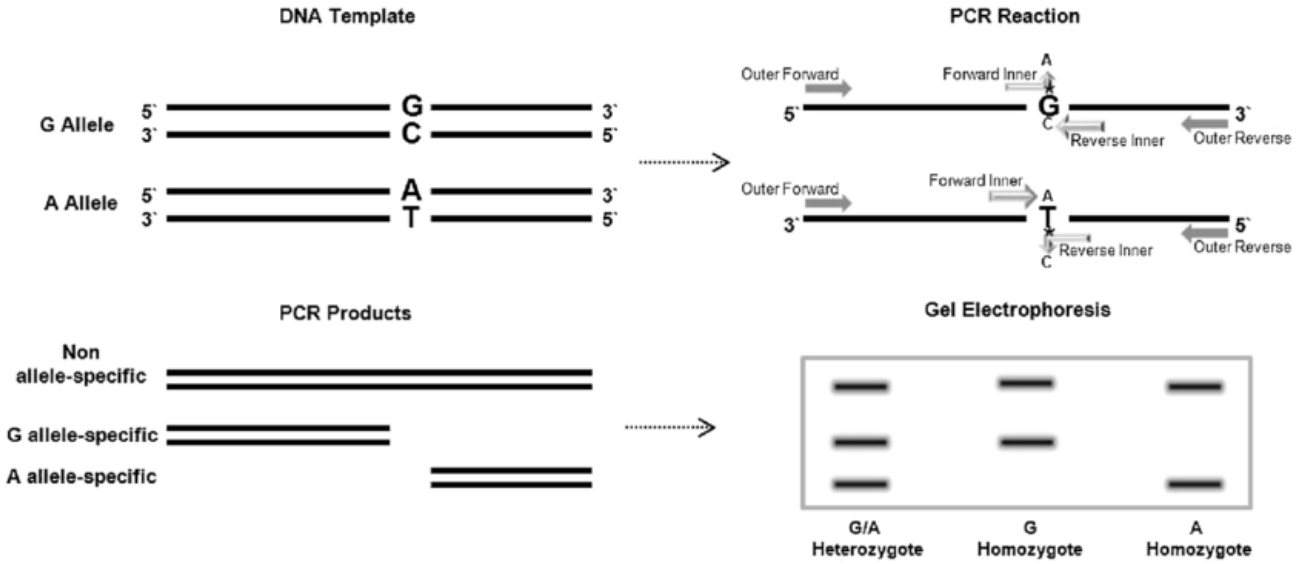

Figure (9): ARMA PCR

References

(1). Laura Doyle, Sherilynn Vogel, Gary W Procop, *Pneumocystis* PCR: It Is Time to Make PCR the Test of Choice, *Open Forum Infectious Diseases*, Volume 4, Issue 4, Fall 2017, ofx193,

(2). Khalil, Mohammad I. , Jaffar, S. and Yousif, B. (2018). Molecular Detecting of fungi and Bacteria in the Blood of Patients With Genital System Inflammatory Infection, Journal of Babylon University/Pure and Applied Sciences/ No.(1), Vol.(26).

(3). Khalil, M. I.(2020).CRISPR/Cas9 design to knockout and knockin the breast cancer gene- BRCA1 in Arabidopsis thaliana, International Conference on Pure Science (ISCPS-2020), Journal of Physics: Conference Series.

(4). Khalil, M. I. (2020).in silico detection of dna methylation in fungi neurospora crassa genes, rid-1 and dim-2, conference: 2nd international conference on materials engineering & science, aip conference proceedings 2213(1):020072

(5). Khalil, M. I. (2020).identification of cladosporium sp. fungi by in-silico rflp-pcr, baghdad science journal 17(1(suppl.)):0220

(6). Khalil, M. I. (2020).effect of smoking on dna methylation by using neurospora crassa fungi as a model, biochemical and cellular archives 20 (2):5575-5580.

(7). Karim Kadri (2019). Polymerase Chain Reaction (PCR): Principle and Applications, Synthetic Biology - New Interdisciplinary Science.

(8). Maria D, Angela P, Chialexei L (2008) Characteristics of RAPD markers inbreeding of Cucumis sativus L. Roum Biotech Lett 13:3843–3850

(9). Livak KJ and Schmittgen TD (2001). Analysis of relative geneexpression data using real-time quantitative PCR and the 2-Ct method. Methods 25: 402-408

(10). Hayden, M.J., T.M., Nguyen, A., Waterman andChalmers, K.J. 2008. Multiplex-ready PCR: Anew method for multiplexed SSR and SNPgenotyping. BMC Genomics. 9:80.

(11). Ye S, Dhillon S, Ke X,et al.(2001) An efficient procedure forgenotyping single nucleotide polymorphisms. Nucleic AcidsRes 29:e88.