severity with which they conform to the repeat pattern. STRs can be further divided into several categories and these categories can be based on the repeat pattern. Not all of these alleles of an STR locus contain complete repeat units. Even simple repeats can also contain non consensus alleles that are in between alleles with full repeat units. Goldstein in 1995 anticipated that STR loci would at last permit a high-resolution portrayal of the human transformative history.

Microsatellite have non-coding or coding region that are present in prokaryotes as well as euchromatin of the eukaryotes. SSR divided into two categories in cereal the first one is repetitive sequence or the second one is unique sequences. In the oats the repetitive sequence is less polymorphic. SSR basically smaller than 12bp nucleotide which have mutation potential SSR is microsatellite. When the mutation rate increase in SSR it led to change in gene expression [15]. The types of microsatellites are Di, tri and tetra nucleotides, Compound. Perfect. Imperfect.

Methodology:

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Our total amount of samples that were four blood samples were collected from university of central Punjab Lahore. We obtained 4ml blood sample in EDTA in sterilized collection vials. The specifics of individuals were noted in a prescribed consent from signed by the volunteer who were participating. One aliquot (300mL) of whole blood sample in micro centrifuge tube was preserved at -70°C. For each sample as backup source and remaining blood was preserved at -20°C. Sterile tubes and micro centrifuge tubes were used for preservation. The extraction of DNA from blood sample is done to extract genomic DNA.

Procedure:

Addition 200µl EDTA blood samples were thawed at 37ºC for 10 minutes and vortex it. Wash with washing buffer (20Mm TRIS HCL with 7.5) for all the removal of material of blood except white blood cells. This removes all red blood cells and serum protein following 20 minutes mix by centrifuge at 3,000 rpm for 15 minutes. Then discard the supernatant and

save the pallet. Repeat the process for 2 to 3 times until the pinkish color of blood is removed. Add 100µl lysis buffer, 15µl proteinase K and 10% SDS. Incubate at 37°C overnight in shaking incubator. Equal volume of PCI is added in Eppendorf tubes followed by centrifugation at 3000 rpm for 15 minutes and save the pallet. Equal volume of Isopropanol and centrifuge for 15 min at 3000rpm.Discard supernatant add 70% chilled ethanol and centrifuge at 5000rpm for 5 minutes. Dry the pellet and add 5micro liter RNASE incubate it and water bath at 60°C for 1:30 hours. If the pallet is visible than add 50µl TE pH 8. For complete suspension place on water bath at 70°C for 10-15 minutes.

- **Electrophoresis by agarose Gel:**

For electrophoresis the gel is made as 0.8% agarose solution. It was prepared in order to confirm the extracted DNA. Mix the extracted DNA with a dye i.e., loading dye and loaded them into the wells of the agarose gel. Now view it under the documentation system to visualize the gel.

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- **PCR Master Mix Composition:**

Master Mix consists of Nuclease Free Water and PCR Master Mix two2X. PCR Master Mix *ie* a combination of two solution containing Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers at most effective concentrations for environment friendly amplification of DNA templates through PCR.

- **Buffers and Solutions Composition:**

1. PCR Master Mix amount 50ml
2. Taq DNA polymerase of pH 8.5 400uM
3. dATP, dGTP. dCTP, dTTP 3mM

- **Polymerase Chain Reaction:**

PCR amplifies the DNA at an exponential rate. It consists of two profiles reaction profile and temperature profile. For the amplification of the CSF1PO was done by using the primers. Amplification was carried out using the master mix, primers and DNA template PCR is performed using a thermo cycler. The DNA concentration in a working solution of about 10 ng/ μL in ddH_20 was confirmed by spectrophotometric evaluation at 260 nm. For optimization, the concentration of the genomic DNA,1X buffer plus MgCl2, STR primers and Taq DNA polymerase were optimized for the STR. The primers were synthesized from genetic research. Taq polymerase, collectively, with 5X PCR buffer, MgCl2 and dNTP were synthesized locally. The amplification was performed once in the thermal cycler PTC-100 for 30 cycles.

Polymerase Chain Reaction Components	
Step 1	PCR tubes were label with permanent marker.
Step 2	PCR tube added PCR water (double distilled water) 3.5 ul
Step 3	Added chilled 12.5ul master mix consist of dnTPs, Taq polymerase, buffer
Step 4	Added 2ul forward primer and 2ul reverse primer
Step 5	We added 5ul of extracted DNA sample
Step 6	Placed PCR tubes into the PCR machine and adjusted the temperature

Table 1: PCR reaction component

Condition of PCR for amplification:

Amplification of the genomic DNA were later confirmed in agarose gel 2.5% stained with ethidium bromide. Due to the small size of the base pair, the alleles did no resolve in the gel agarose. Therefore, charge concentrations for polyacrylamide gel electrophoresis (PAGE) were performed as a function of the brightness of the band. For accurate allele determination, 5-8% PAGE was used. The gel was used using a 0.5X TBE working buffer in an apparatus followed by the silver staining method.

Constituents	Amount	Temperature	Time
Primer forward	3µl	95 °C	3 minutes
Primer backward	3µl	93 °C	45 seconds
Double distilled water	2.5µl	58 °C	45 seconds
Master mix	12.5µl	72 °C	45 seconds
DNA sample	2µl	72 °C	10 minutes
$MgCl_2$	2µl	4 °C	10 minutes
Total	25µl		

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Table 2: Composition profile by changing its annealing temperature

Result:

Sample was collected from a student from university. This study was performed with three other research students' samples. In order to avoid any confusion our sample was named Z1. DNA extraction was carried out of the sample. Extraction of DNA was confirmed using gel electrophoresis method. This was performed to get the conformation. After the extraction of DNA, the samples were amplified in thermo cycler PCR machine by using PCR machine by using STR locus; CSF1PO as shown below.

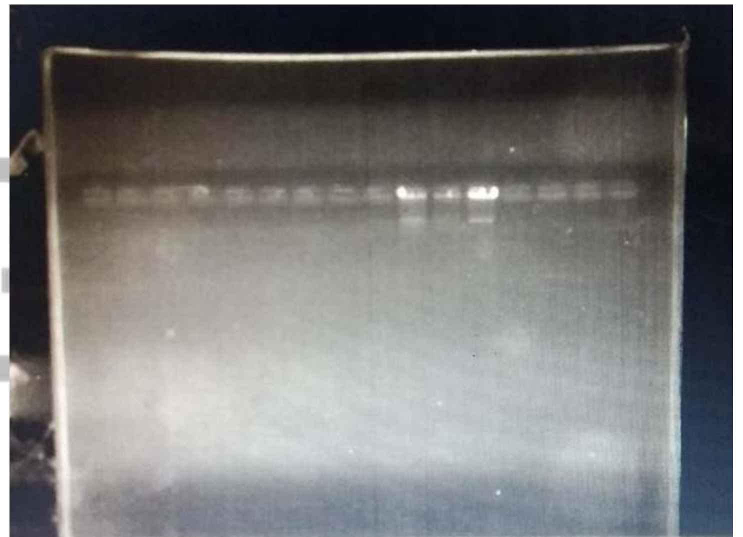

Figure 3: Collection of blood sample **Figure 4: Result of extracted DNA sample**

DNA markers and STR analysis:

For genotyping approaches markers are used. To increase their genetic essential quality in the field of botany, microsatellite is used. Microsatellite have non-coding or coding region, which are present in prokaryotes as well as euchromatin of the eukaryotes. SSR divided into two categories in cereal the first one is repetitive sequence or the second one is unique sequences. In the oats the repetitive sequence is less polymorphic. SSR basically smaller than 12bp nucleotide

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which have mutation potential SSR is microsatellite. When the mutation rate increase in SSR it led to change in gene expression. It has been just about a long time since the 13 hereditary markers that shaped the center of the FBI Research center's Consolidated Ordering Framework (CODIS) were chosen in November 1997. Utilizing DNA to recognize two people is a precarious issue, since near 99.9 percent of our DNA is equivalent to every other person's DNA. DNA that codes for proteins can't change much without rendering the proteins ineffectual. The four nucleotide bases that make up the foundation of DNA give directions to collecting the amino acids in proteins by being in an exact arrangement, with every three-base gathering coding for a particular amino corrosive. In the event that that DNA base succession is modified, the grouping of amino acids in the subsequent protein can likewise be adjusted. Thus, since protein work gets from a particular amino corrosive grouping, the protein may not work. Among the 3 million or so DNA bases that don't code for proteins are areas with numerous duplicates of short rehashing groupings of these bases, which make up the DNA spine (for instance, TATT). These groupings rehash a variable number of times in various people. Such loci are classified "variable number of short tandem repeats," and they are the premise of STR analysis. An assortment of these can give almost certain proof measurably of an individual's character in light of the fact that the probability of two unrelated individuals having a similar number of rehashed arrangements in these districts turns out to be progressively little as more areas are examined. CSF1PO is a STR repeat is tetra nucleotide repeat c-fms proto-oncogene for the CSF-1 receptor on the long arm of chromosome number 5. Usually found alleles contain an AGAT repeat of 5 to 17 repeats [16]. CSF1PO is one of the thirteen core loci used in the CODIS database, and alleles found for this short tandem repeat locus contain 6 to 15 repeats of the tetra nucleotide AGAT.

CSFIPO on a locus	
Location on chromosome	5q33.3-34

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Locus according to GenBank	HUMCSF1PO is Human c-fms proto-oncogene for CSF-1 receptor gene is the locus
Repeat sequence 5'-3'	AGAT (*) is the repeat unit

Table 3: Information about position of CSFIPO on a locus

Conclusion:

At any locus of STR, it is expected to find the presence of two alleles, one inherited maternally and other paternally. These alleles are seen as a two-banded pattern, as seen in a heterozygote, or a single banded pattern as seen in a homozygote. Rarely, a three-banded pattern can be seen at a single locus in a multiplex STR profile and that is not a result of a mixture. This can occur by an additional chromosomal occurrence or a duplication of the locus. Three-banded patterns are already reported at the TPOX, CSF1PO, FGA, D5S818, D21S11, and D18S51 loci. The presence tri-allelic patterns are mostly because of genetic replication in tandem of a short part of chromosomal DNA; or incorrect separation during meiosis or mitosis because of a chromosomal nondisjunction which leads to a trisomy. Autosomal short tandem multiplexed polymerase chain re action (PCR) system for 17 autosomal loci D1S1656, D3S3045, D3S477, D8S1435, D10S1418, D11S2368, D11S2368, D11S2368, D11S2368, D11S2368, D11S268, D11S2368, D11S2368, D19S253 and D22-GATA198B05

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