Detection of the Discriminative Power of Variable Number Tandem Repeats Polymorphism to Be Used After Bone Marrow Transplantation

Doaa M. Abdelaziz[1], Taysir R. Hafiz[2], Khaled O. Abdallah1, Salwa I. Bakr[1], Shahira F. Elfedawy[1], Hala A. Talkhan[1] and Omima M. Hassanin[3]

[1]Department of Clinical and Chemical, Faculty of Medicine, Ain-Shams University Hospitals, Cairo, Egypt
[2]Department of Microbiology and Immunology, Faculty of Medicine Al Azhar University
[3]Molecular Biology Department, Medical Research Center, Faculty of Medicine, Ain-Shams University Hospitals, Cairo, Egypt
dr.taysiralassuty@gmail.com

Abstract: Background & Objective: During the last 3 decades, bone marrow transplantation (BMT) and hematopoietic stem cell transplantation (HSCT) have become the treatment of choice for patients suffering from certain malignant and non malignant hematological disorders. In human allogenic HSCT, complete donor-derived hematopoiesis has been considered essential for sustained engraftment and for the prevention of relapse. The major causes of these treatments failure are disease relapse, graft rejection, and graft-versus-host disease (GVHD).The success of HSCT treatment modality is mainly affected by the recurrence of the underlying disease, here is the advantage of these methods which predict disease relapse after chemotherapy or HSCT and allow early intervention and may result in "salvage" of many patients and improvement in the probability of long-term disease free survivalInce. Variable numbers of tandem repeats (VNTR) are highly polymorphic markers constitute unique sets of genetic markers for individual identification. The purpose of our study to detect polymorphism, discriminative power and establish a logarithm of VNTR to follow up chimerism in patients undergoing BMT. **Material & Methods**: A total of 40 subjects were included in the study [18 plus 11 pairs (donors and their recipients)], (age range: 20 – 34 years, mean 26.15 ± 4.34). The six loc (Apo-B, YNZ-22, D1S80, 33.1, 33.6 and HRAS) were detected in every subject. Apo-B, YNZ-22 and D1S80 were examined in 40 cases (18 + 11 pairs), while 33.1, 33.6 and HRAS were examined in 30 cases (18 + 6 pairs). Blood samples were withdrawn from each subject. After DNA extraction amplification of VNTR loci were performed with PCR. Amplified product of DNA samples were run on 2% agarose, stained with ethidium bromide together with DNA ladder to identify the site of the band. **Results:** Apo-B was the most polymorphic VNTR locus with 5 detected alleles. Followed by YNZ-22, D1S80and 33.6 with 4 detected alleles. Heterozygosity index of Apo-B locus among included cases was 70%; of YNZ-22 locus was 55%; of DIS80 locus was 71.5%; of 33.1 locus was 66.7%; of 33.6 locus was 60%, and of H-Ras locus was 36.7%. Concerning discriminative power of Apo-B, YNZ-22, DIS80, 33.1,33.6 and HRAS VNTR loci among the included paired cases.YNZ-22 had the highest discriminative power (72.7%), followed by 33.1 (66.7%, Apo-B (54.5%) and DIS80 (45.5%), while 33.6 and H-Ras had the lowest discriminative power (33.3%). Nevertheless, Apo-B and DIS80 had a higher non-identical discriminative power, when compared to YNZ-22, 33.1, 33.6 and H-Ras (36.4% vs. 36.4% vs. 18.2% vs. 16.7% vs. 16.7% vs. 0%, respectively). Moreover, combination of more than one loci increase the discrimination power more than use of one separate locus. **Conclusion:** Our results indicate that ApoB, YNZ-22 and D1S80 are very polymorphic in Egyptian. The uses of more than one loci is essential to increase the discrimination power of VNTR loci. Furthermore, the number of segregating alleles and higher heterozygosity in Egyptians further support the usefulness of VNTR loci for population genetic studies, chimerismfollow up linkage analysis and forensic purposes. **The aim of this study** is to establish a logarithm of VNTR to follow up chimerism in patients undergoing BMT**.**

[Doaa M. Abdelaziz, Taysir R. Hafiz, Khaled O. Abdallah, Salwa I. Bakr, Shahira F. Elfedawy, Hala A. Talkhan and Omima M. Hassanin. **Detection of the Discriminative Power of Variable Number Tandem Repeats Polymorphism to Be Used After Bone Marrow Transplantation.** *J Am Sci* 2015;11(8):112-117]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 17

Keywords: VNTR, PCR, HSCT, chimerism.

1. Introduction:

In medicine the phenomenon of co- existence of cells from two different organisms (evolved from two different zygotes) in one body is called chimerism(1). The stage when the patient shows no evidence of recipient cells at any time after transplantation is considered to be complete chimerism (CC*)* ordonor chimerism (DC) as all cells are of donor origin. Mixed

chimerism (MC), where the patient shows both recipient as well as donor cells in the peripheral blood. Mixed chimerism may be increasing or decreasing, also may be transient, stable or progressive [2]. In patients with malignancies, MC is most often transient and conversion to CC after rapid tapering of immuno-suppression[3]. With the emergence of more advanced HSCT procedures include nonmyeloablative stem cell transplantation and depletion of the donor's T-cells from bone marrow grafts and donor lymphocyte infusion (DLI) it becomes most important to detect the exact status of chimerism in patients after HSCT.

Tandem DNA in the human genome shows a wide range of repeat sizes and organization, ranging from microsatellites or short tandem repeat (STR) which are repeats of sequence less than 5 base pairs in length and minisatellites or variable number tandem repeats (VNTRs) or simple sequence repeat which contain longer blocks (10 to 80 bp) to megasatellites which contain up to several kb [4].

VNTR are highly polymorphic markers. Although each VNTR locus is usually associated with a large number of different alleles in any population, each individual carries two alleles only-one on each of the two homologous chromosomes. Due to their high polymorphic content, VNTR constitute useful tools in evaluating hematopoietic chimerism and in determining the origin of leukemic cells in patients with recurrent disease after BMT[5].

Selecting an informative locus is a key step in chimerism analysis, and VNTR/STR markers offer the highest probability of finding an informative marker [3]. As to identify an informative locus, pretransplant samples are screened for informative VNTR and STR loci. An informative locus is one for which at least one recipient allele has a different number of repeats than the donor allele(s). However, the discriminative power of these loci depends on the number of alleles detected and their distribution in the population [6]. Our aim was to study allelic polymorphism of six VNTR loci among Egyptian population to detect the most polymorphic locus to be used as fingerprint for genetic differentiation between Egyptian populations.

2. Subjects and Methods:

1- Subjects:

The current study was conducted at clinical pathology department of Ain Shams University Hospital during the period between June 2009 and January 2011. A total of 40 subjects [18 plus 11 pairs (donors and their recipients)] were included in the study. The mean age of included subjects was 26.15 ± 4.34 years (range: 20 – 34 years). 19 (47.5%) were males, while 21 (52.5%) were females. In order to study the relative distribution of their various VNTR alleles in Egyptian population, in order to be used in chimerism follow up. Apo-B, YNZ-22 and D1S80 were examined in 40 cases (18 + 11 pairs), while 33.1, 33.6 and HRAS were examined in 30 cases (18 + 6 pairs).Six VNTR loci were detected in every subject.

2-Methods

A) Sampling:

Two ml of venous blood were withdrawn aseptically into sterile EDTA vacutainer tubefor VNTR polymorphism testing by PCR.

B) Analytical methods:

DNA extraction was performed using Wizard whole blood genomic DNA extraction kit Supplied by Promega (Promega corporation. 2800 woods Hollow Road. Madison, WI 537 11-5399 USA, www.promega.com). PCR amplification of six different VNTRs loci (Apo-B, YNZ-22, 33.6, 33.1, D1S80 and H-Ras) were performed, all oligonucleotide primers were synthesized commercially (Promega), primer sequences and amplification cycles **(Tables 1, 2)** were obtained from previously published data[7,8,9] with some modification. All reactions were performed in a volume of 50ul containing 25 ul master mix ready to use (Promega), 24 pmol each primer (Promega), 250ng template DNA, 15 ul deionized water and and 2.5 units Taq polymerase (Promega). Products were separated on 2% agarose gel containing ethidiumbromide for 1 hour at 100 volts, visualized using ultra violet transilluminator and photographed. Alleles were characterized by their molecular weight determined relative to a 100bp DNA ladder (Promega) run as a marker.

The sizes of alleles ranged from 435 bp to 1006 bp for Apo-B, from 666 bp to 2375 bp for 33.1, from 364 bp to 993 bp for 33.6, from750 bp to 1500 bp for 33.4, from 238bp to 938bp for YNZ-22, from 488 bp to 768 bp for D1S80 and from 1000 bp to 2500 bp for H-ras (**Figs. 1).**

Statistical analysis:

All qualitative variables were described in the form of number and percentage.Chi –square test was used to compare qualitative variables. $P \geq 0.05$ (NS) Non significant. $P < 0.05$(S) Significant.

Table (1): VNTR PCR primers

Gene	Sequence	Reference
APO-B	5' CCTTCTCACTTGGCAAATAC 3' 5' ATGGAAACGGAGAAATTATG 3'	(Ref.8)
33.1	5' CGTGTCACCCAC_AAGCTTCT 3' 5' TGCTTTCTCCACGGATGGGA 3'	(Ref.10)
YNZ-22	5' GGTCGAAGAGTGAAGTGCACAG 3' 5' GCCCCATGTATCTTGTGCAGTG 3'	(Ref.10)
33.6	5' TGTGAGTAGAGGAGACCTCAC 3' 5' AAAGACCACAGAGTGAGGAGC 3'	(Ref.10)
D1S80	5'GAAACTGGCCTCCAAACACTCCCCGCCG 3' GTCTTGTTGGAGATGCACGTGCCCCTTGC 3'	(Ref.7)
HRAS	5' TTGGGGGAGAGCTAGCAGGG 3' 5' CCTCCTGCACAGGGTCACCT 3'	(Ref.10)

Table (2): PCR conditions.

VNTR Locus	Denaturation	Annealing	Extension	Cycles
Apo-B	94ºC, 1min	58ºC, 6min		26
33.1, 33.6 and H-ras	95ºC, 30 seconds	65ºC, 1min	72ºC, 30 seconds	25
YNZ-22	95ºC, 1min	59C, 1min	72ºC,2 min.	30
D1S80	94ºC, 1min	66ºC, 1min	70ºC, 5min.	28

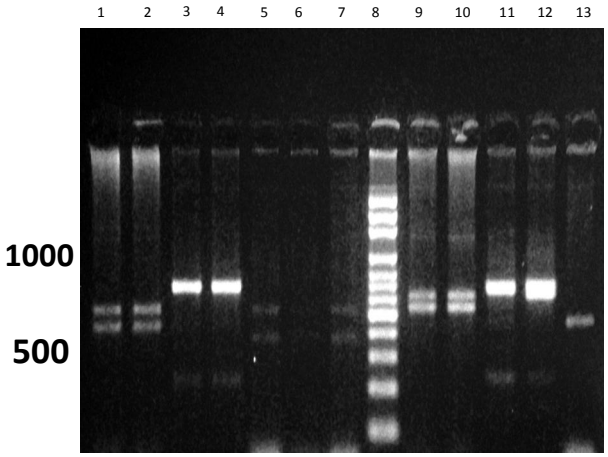

Figure (1) photograph of 2% agarose showing 5 pairs after transplantation showing full chimerism
Lane (8): DNA molecular weight 100bp ladder (rang from 100bp-3000bp).
Lane 1,2 (1 st pair) testing for Apo-B(435bp-1006bp).
Lane 3,4 (2nd pair) testing for D1S80(488bp-768bp).
Lane 5,7 (3rd pair) testing for D1S80(488bp-768bp).
Lane 9,10 (4th pair) testing for 33.6 (238bp-938).
Lane 11,12 (5th) testing for 33.1 (666bp-2375).

3. Results:

1. A total of 40 subjects from outpatient clinic [18 plus 11 pairs (donors and their recipients)]. The six VNTR loci were detected in every subject.
2. Apo-B, YNZ-22 and D1S80 were examined in 40 cases (18 + 11 pairs); the number of alleles detected were 5,4,2 respectively. While 33.1, 33.6 and HRAS were examined in 30 cases (18 + 6 pairs); the number of alleles detected were 3,4,4 respectively (**Table 3).**
3. Heterozygosity index of Apo-B locus among included cases was 70%; of YNZ-22 locus was 55%; of DIS80 locus was 71.5%; of 33.1 locus was 66.7%; of 33.6 locus was 60%, and of H-Ras locus was 36.7%. There was no significant difference between detected and previously reported heterozygosity index of different VNTR loci **(Table 4).**
4. Concerning discriminative power YNZ-22 had the highest discriminative power (72.7%), followed by 33.1 (66.7%, Apo-B (54.5%) and DIS80 (45.5%), while 33.6 and H-Ras had the lowest discriminative power (33.3%). Nevertheless, Apo-B and DIS80 had a higher non-identical discriminative power, when compared to YNZ-22, 33.1, 33.6 and H-Ras (36.4% vs. 36.4% vs. 18.2% vs. 16.7% vs. 16.7% vs. 0%, respectively) (**Tables 5, 6).**

Table-3. Numbers of VNTR Alleles Detected in Egyptians

VNTR Locus	Cases Examined	No. of Alleles Detected	No. of Alleles Reported
Apo-B	40 (18 + 11 pairs)	5	14**(Ref.2)**
YNZ-22	40 (18 + 11 pairs)	4	14**(Ref.11)**
DIS80	40 (18 + 11 pairs)	4	16**(Ref.2)**
33.1	30 (18 + 6 pairs)	3	10**(Ref.11)**
33.6	30 (18 + 6 pairs)	4	13**(Ref.11)**
H-Ras	30 (18 + 6 pairs)	2	11**(Ref.11)**

VNTR variable number of tandem repeats

Table-4. Heterozygosity Index of Measured VNTR Loci

VNTR Locus	Detected HeterozygosityIndex	Reported Heterogeneity Index	*P**	*S*
Apo-B	28/40 (70%)	79%	0.257	(NS)
YNZ-22	22/40 (55%)	54%	0.915	(NS)
DIS80	29/40 (72.5%)	81%	0.268	(NS)
33.1	20/30 (66.7%)	66%	0.946	(NS)
33.6	18/30 (60%)	67%	0.479	(NS)
H-Ras	11/30 (36.7%)	-		

VNTR variable number of tandem repeats * Analysis using corrected Chi-squared test
S significant, NS non-significant

Table-5. Discriminative Power of Measured VNTR Loci

VNTR Locus	No. of Pairs Tested	Non-Discriminative cases No (%)	Discriminative cases		
			Total No(%)	Haplo-Identifcal No(%)	Non-Identical No(%)
Apo-B	11	5 (45.5%)	6 (54.5%)	2 (18.1%)	4 (36.4%)
YNZ-22	11	3 (27.3%)	8 (72.7%)	6 (54.5%)	2 (18.2%)
DIS80	11	6 (54.5%)	5 (45.5%)	1 (9.1%)	4 (36.4%)
33.1	6	2 (33.3%)	4 (66.7%)	3 (50%)	1 (16.7%)
33.6	6	4 (66.7%)	2 (33.3%)	1 (16.7%)	1 (16.7%)
H-Ras	6	4 (66.7%)	2 (33.3%)	2 (33.3%)	0 (0%)
		X^2=4.29*, p=0.508 (NS)		X^2=7.22, p=0.205 (NS)	

VNTR variable number of tandem repeats Haploidentical: Sharing one allele.
Non-identical: No shared alleles. * Analysis using Chi-squared Test
NS non-significant

Table-6. Combined Discriminative Power of Measured VNTR Loci

	Discriminative Power
One VNTR Locus	33.3% –72.7%
Combined 2 VNTR Loci	50% – 83.3%
Combined 3 VNTR Loci	50% – 100%
Combined 4 VNTR Loci	50% – 100%
Combined 5 VNTR Loci	66.7% - 100%
Combined 6 VNTR Loci	100%

4. Discussion:

Allogenic HCST has become the main treatment in many malignant and nonmalignant hematological diseases in adults and children[(11)].

Chimerism detection is the key stone in following up patients after HSCT[(5)]. Chimerism monitoring can identify and predict HCST outcome.

Various techniques as cytogenetic analysis, fluorescent in situ-hybridization, restricted fragment length polymorphism, STR/VNTR analysis andreal-time quantitative PCR are used for chimerism analysis post-transplantation[(2)]. However, previous techniques had many limitation which open the gate to find the most suitable method which can be applied to all cases Nowadays, more sensitive methods for determining chimerism based on real-time PCR. However, STR/VNTR still keeping its importance and its privacy as dependable method in comparison to real-time PCR. ***Kletzel et al.,*** compare between the two methodsand found complete correlation between them [(12)].

STR and VNTRs are highly polymorphic with high heterozygozity rate in populations, not surprisingly, longer VNTR tend to show greater polymorphism and greater utility for genotyping[(1)].The human genome contains a large amount of highly repetitive DNA sequences including many variable number of tandem repeats (VNTRs)[(5)].

In the present study six VNTR loci (ApoB, 33.1, 33.6, YNZ-22, HRAS and D1S80) were studied in Egyptians, aiming to use several informative loci due

to differences in the efficiency of amplification of the alleles at each locus. The use of more than one marker is also needed for consecutive donor chimerism evaluation, since loss of specific chromosomal regions during clonogenic evolution has occurred in several haematological malignancies[13].

In the present study number of alleles in seven loci were 5 alleles in Apo-B compared to 12 alleles were detected by ***Kamel et al.***[2] in Egyptians in a study done at National Cancer Institute, Cairo University. Of overall 14 alleles were detected by ***Helio***[13] in their studied group. 4 alleles were found in YNZ-22 compared to 9 alleles were detected by ***Kamel et al.,*** [2] in Egyptians of overall 14 alleles were detected by ***Ugozzoli et al.***[10] in their studied group**.** 4 alleles were found in 33.1 compared to 10 alleles were detected by ***Kamel et al.,***[2] in Egyptians of overall13 alleles were detected by ***Ugozzoli et al.,***[10] in their studied group. 4 alleles were found in D1S80 compared to 9 alleles were detected by ***Kamel et al.,***[2] in Egyptians of overall 16 alleles were detected by ***Budowle et al.,***[7] in their studied group, 24 different alleles were identified in the Iranian populations.

Regarding HRAS polymorphism, only 2 allele were detected in our population, one of them is present in 85% of individuals, compared to 7 alleles were studied by ***Kamel et al.,***[2] of overall 11 alleles were detected ***by Ugozzoli et al.,*** [10]. So it's the least one to be performed in the population studies and chimerism detection as it depends on alleles polymorphism and their distribution among populations. Similarly, ***Kamel et al.,*** [2] found that one allele was present in 60% of case so they also exclude it from chimerism detection thereafter.

The lower number of alleles detected in our series compared to those reported in the literature [10] may be attributed to missing larger alleles due to preferential amplification of small ones as in YNZ-22**,** interalleles as in DIS80, indirect result of the different PCR conditions or the subsequent detection steps utilized, due to small sample size or due to ethnic variations. Another explanation related to selection of population as in population study the sample must be unrelated, but we choose the subjects as relatives (brothers) because the main aim of this study is to establish a logarithm of VNTR to follow up chimerism in patients undergoing BMT.

In the present study, the heterozygosity index of different VNTR loci among Egyptians were investigated. The study showed that 28 subjects were heterozygous for Apo-B of overall 40 subjects, that means that the heterozygosity index for APO-B is 70%. These is in accordance with ***Ugozzoli et al.,*** [10] who found it 79%, and with ***Kamel et al.,***[2] who found it 84%.While in D1S80 loci, 29 subjects were heterozygous of overall 40 subjects, and heterozygosity index is 72.5 %, in accordance with these result ***Budowle et al.***[7] and ***Kamel et al.***[2] found that heterozygosity index was 81% and 79 % respectively.

Regarding 33.1, 20 subjects were heterozygous of overall 30 subjects, and heterozygosity index is 66.7 %.

Similarly ***Jeffreys et al.,*** [11] and ***Roux et al.,*** [9] found the same percentage 66%. While ***Kamel et al.,***[2] found it 73 %.

Concerning 33.6, 18 subjects were heterozygous of overall 30 subjects, and heterozygosity index is 60. %, which is in accordance with ***Jeffreys et al.,*** [11]***and Kamel et al.,***[2] who found that heterozygosity index was 66% and 68 % respectively.

Concerning YNZ-22, 22 subjects were heterozygous of overall 40 subjects, and heterozygosity index is 55 %, accordance with ***Deka et al.,*** [17] who found it 54%. In contrast to ***Kamel et al.,*** [2] found it 28 %, which was attributed to the misclassification of a heterozygous individual as homozygous as a cause of high incidence of homozygosity observed with YNZ-22, as larger alleles fail to amplify efficiently under standered conditions when present in heterozygosity with another much smaller alleles. ***Deka et al.,***[17] have designed several modified PCR protocolsto improve amplification of relatively large alleles, these modifications based onsmaller amount of genomic DNA template, lower temperature and longer time of the extension step.

Concerning HRAS, only 11 subjects individuals were heterozygous of overall 30 subjects, and heterozygosity index is 36.7 %, which is in contrast to ***Ugozzoli et al.,*** [10] who report it as 70%.

There are many factors that suggest high heterozygosity of VNTRs loci in Egyptians, include Egypt place in African continent, a history of longtime wars with foreign nations, and marked immigration from neighboring countries. On the other hand, relative ethic purity has been maintained by the natural geographic borders within Egypt and by an ancient culture that encourages familiar marriages that suggest low heterozygosity.

Concerning population studies every allele is important to be detected so the undetected alleles will be of value, unlike in the study of chimerism the aim is to find a discriminative locus whether or not we are missing an allele so the undetected alleles may not be of value [11]**.** Concerning the discriminative power of Apo-B, YNZ-22, DIS80,33.1,33.6 and HRAS VNTR loci among the included paired cases.YNZ-22 had the highest discriminative power (72.7%), followed by 33.1 (66.7%), Apo-B (54.5%) and DIS80 (45.5%), while 33.6 and H-Ras had the lowest discriminative power (33.3%). The combining of 6 loci increase the discriminative power to 100%; combination of 5, 4, 3 VNTR loci had discriminative powers ranging between 66.7 – 100%, 50 – 100%, 50 – 100% respectively.

While combination of 2 VNTR loci had a discriminative powers ranging between 50 – 83.3%. These confirm that uses of more than one loci is essential to increase the discriminative power of VNTR loci.

As we mentioned that the main aim of our study is to establish a logarithm of VNTR to follow up chimerism in patients undergoing BMT. So from our results we can conclude that the best loci can be used in chimerism follow up after HSCT are Apo-B followed by YNZ-22, then D1S80. The uses of more than one loci is essential to increase the discriminative power of VNTR loci which is essential in detection chimerism status.

Acknowledgment
Many thanks to grant office Ain Shams University.

References

1. Jeffery V (2006): Methods of Genotyping. In: Genetic Analysis of Complex Diseases. Jonathan H L and Vance M P (eds.), 2nd edn. New York; 167-92.
2. Kamel A, Mossallam G, Mahmoud H, Hamdy N (2002): Variable number tandem repeat polymorphism as a tool of chimerism detection in allogeneic stem cell transplantation.Journal of the Egyptian Nat. Cancer Inst., Vol. 14, No. 4, December: 275-287.
3. Khan F, Agrawal S (2004): Genetic variation of APOB 3 hyper variable region polymorphism among Brahmins of North India. Curr Sci 2004; 86:697-701.
4. Ames D, Murphy N, Helentjaris T, Sun N and Chandler V (2008): Comparative analyses of human single- and multilocus tandem repeats. Genetics; 179:1693-704.
5. Babushkina N and Kucher A (2011): Functional Role of VNTR Polymorphism of Human Genes, Russian Journal of Genetics, Vol. 47, No. 6, pp. 637-645.
6. Muniz E, Plassa F, Amselem S, Goossems M and Vernant J (1994): Molecular analysis of polymorphic loci to study chimerism after allogeneic bone marrow transplantation. Transplantation, 57: 451-456.
7. Budowle B, Chakraborty R, Giusti A, Eisenberg A and Allen R (1991): Analysis of the VNTR locus D1S80 by PCR followed by high resolution PAGE.American Journal of Human Genetics, 48: 137-144.
8. Boerwinkle E, Xiong W, Fourest E and Chan L (1989): Rapid typing of tandemly repeated hypervariableloci by PCR: application to apolipoprotein B 3' hypervariable region. Proceedings of the National Academy of Science of USA, 86: 212-216.
9. Roux E, Chapius B, Jeannet M and Roosnek E (1992): Evolution of mixed chimerism after allogeneic bone marow transplantation as determined by granulocytes and mononuclear cells by polymerase chain reaction. Blood, 79: 2775-2783.
10. Ugozzoli L, Yam P, Petz L, Ferrara G, ChamplinR, Forman S, Koyal D and Wallace R (1991): Amplification by the polymerase chain reaction of the human genome for evaluation of chimerism after bone marrow transplantation. Blood, 77: 1607-1615.
11. Jeffreys A, Wilson V, Thein S (1985). Hypervariableminisatellite regions in human DNA. Nature; 314:67.
12. Kletzel M, Huang W, Olszewski M, and Khan S (2013):Validation of chimerism in pediatric recipients of allogeneic hematopoietic stem cell transplantation (HSCT) a comparison between two methods.Real-time PCR (qPCR) vs. variable number tandem repeats PCR (VNTR PCR). Chimerism. 2013 Jan 1; 4(1): 1–8.
13. Mossallam G, Smith A, Mcfarland C (2005):Comparison of variable number tandem repeat and short tandem repeat genetic markers for qualitative and quantitative chimerism analysis post allogeneic stem cell transplantation. Journal of the Egyptian Nat. Cancer Inst., Vol. 17, No. 2, June: 103-113.
14. Helio T (1991): Concept of VNTR alleles: Comparison of apolipoprotein B 3' hypervariable region genotyping results obtained by three methods. Biochem. Biophy. Res. Comm., 181: 846-851.
15. Mahdieh N, Tafsiri E, Karimipour M, Akbari M T(2005): Heterozygosity and allele frequencies of the two VNTRs (ApoB and D1S80) in Iranian population Indian Journal of Human Genetics, Vol. 11 Issue 1.
16. Peter Warburton E, Dan Hasson, Flavia Guillem, Chloe Lescale, Xiaoping Jin and Gyorgy Abrusan (2008): Analysis of the largest tandemly repeated DNA families in the human genome. BioMed Central Ltd; 509-33.
17. Deka R, De Croo S, Yu L and Ferrell R (1992): Variable number of tandem repeat (VNTR) polymorphism at locus D17S5 (YNZ22) in four ethnically defined human populations. Human Genetic, 90: 86-90, 1992.

10/2/2015