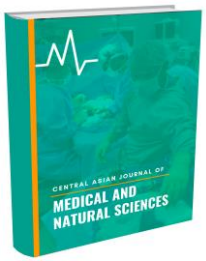

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Article

The Usefulness and Discrimination Power of DYS391 Locus Using Power Plex ® Fusion and Power Plex®23Y Systems in Paternity Testing

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Abstract: Thirty random cases of Iraqi families contain data of father/son DNA haplotypes have been analyzed between 2017-2022 to find out the usefulness of DYS 391 Locus in paternity testing using power-plex ®fusion and power-plex ®Y23systems. Scientifically, all cases were confirmed and officially reported as an absolute cases of exclusion paternity. A combination of the two systems should permit effective analysis with sufficient diversity and discrimination power of the locus. The results show significant match (i.e., inclusion) at the DYS391 locus between alleged fathers and corresponding sons for both kits in all the cases meanwhile there were no match (i.e., exclusion) in many autosomal DNA loci and the rest Y-haplotypes other than DYS391. Furthermore, the results revealed only three DYS391 allele polymorphisms; Allele number 10,11and 9 in a percentage of 69.23%, 27.692% and 3.076% contained in all 30 cases respectively. Allele frequencies, homozygosity, haplotype diversity (HD), match probability and the power of discrimination of DYS391 locus (PD) were estimated. The observed allele frequencies were (0.692, 0.276 and 0.030) for allele numbers 10, 11 and 9 respectively. Homozygosity was found to be (0.478, 0.0761 and 0.0009) respectively too. Measurement of the upper bound confidence interval (CI) for each corresponding allele was (2.642, 2.234 and 1.989). Haplotype diversity was (0.451) and combined power of discrimination for DYS391 Locus was (0.4442). As a conclusion, there is a limitation in certifying DYS391 locus as an exclusive locus useful Y-STR in testing paternity and male lineage in autosomal STR kits because of little allele variants, poor power of discrimination and gene diversity.

Keywords: Paternity testing, male lineage, DYS391 locus, Power plex ®fusion system, Power plex ®Y23syste
Power of Discrimination

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1. Introduction

Paternity and male lineage are the most important task considerations in forensic issues. This is especially in communities that look at the consequences of judicial ruling issued against them such as honor crimes, incest, rape, adoption and inherited property cases. Iraq as an eastern society is not far from this concept and such communities.

Kinship Laboratory of MLD/Baghdad has the responsibility to generate DNA profiles successfully analyzed in a rate high 93%. More than 60% of work is related to establishing parents' names for the purpose of issuing identity cards while other casework involves questioned paternity such as losing or kidnaping children, newborn exchange in hospitals and or husband's doubts about the child's lineage [1], [2].

Off course as it is a daily routine work, the purpose and circumstances for refereeing families are usually documented besides forensic report based on DNA Profiles among the trio (Father, Mother and son/daughter). Obviously, when there is an absolute denial of paternity in initial examination, more than once test should be repeated, preferably with different initial kit for deciding the paternal genetic tree. Therefore, in such case (power-plex ®Y23) system is used with Power-Plex® Fusion System (Promega) for further confirmation.

Power Plex® Fusion System consists of 24 autosomal locus multiplexes including amelogenin plus male-specific additional marker which is DYS391 locus [3]. As it is known, the Y chromosome has a useful STR markers used in forensic purposes so as autosomal one but yet is being limited since it descended from father to son without recombination. However, the properties of male haplotypes specific DNA Kit like in power-plex ®Y23system make it becoming a key part of studies of human identity testing, forensics genetic, male lineage and paternity testing [4], [5].

In the two a above mentioned kits, DYS391 Locus is being contained in both systems. DYS391 Locus is considered as a single copy Y STR known as a "minimal haplotype " which has been also included with the "extended haplotype " used in Europe. [6-7].

This locus is a tetrameric repeat region found in a Y-chromosome location Yq11.21 i.e. at the long arm far from the amelogenin, so far its pattern in families reflects a (Y-specific linked locus) besides a (polymorphic X locus) with an X-linked pattern of inheritance. The repeat category or motif is TCTA. In addition, it's allele range is between (5-17) therefore, the allele range and gene diversity were described as relatively narrow and low for males. However, DYS391 locus is not affected by degradation of DNA so it's known to be stable [8], [9], [10].

Also, to improve specificity and applicability of DYS391 locus to be of high Y- specificity, the primer had been designed and developed for amplification using a novel sequence (GATA) as a repeat tetrameric sequence for the characterization and identification of STRs in DYS391 locus as well as the whole Y systems later. [9], [11], [12].

However, based on what have been mentioned, a common sense question arises by participant in training or new examiners about the feasibility of existence and the uniqueness of DYS391 locus in an autosomal STR system 24-Fusion kits in testing paternity? Now, whatever the causes of case reference, number and types of kits nor primers or repeat sequence of DYS391genetic locus, data has been accumulated annually for each locus and allele in the Kinship laboratory of MLD. The review of such data helps study of variable forensic parameters each locus, one of them is estimation of power of discrimination and gene diversity besides other forensic parameters to assess the usefulness of DYS391 STR in paternity male lineage testing.

The aim of the present study is to track the usefulness of DSY391 Locus as a single Y Chromosome haplotype included in autosomal 24-Fusion power plex kit in testing paternity for Iraqi Arab Population. Now to attain such goal, some important forensic parameters and indices must be estimated which should permit effective confidential analysis for the locus.

2. Materials and Method

A retrospective study of thirty (30) work cases of randomly chosen families have been selected between (2017-2022) in the laboratory Kinship of MLD/Baghdad. All work cases had been processed, confirmed and reported as mis-matched DNA Profiles between alleged fathers and their sons under suspicion.

All the families had identified themselves as (Arabs) originated from different Iraqi provinces where the majority lived in the middle and south of Iraq. All fathers and mothers were consanguineously unrelated.

2.1. Sampling

Blood samples were collected with informed consent from 30 families (27 work cases were composed of a trio of father, mother and son) and (3 cases were composed of Father, Mother and 2 sons). Therefore, the total number of samples was for 65 fathers and sons. All the blood samples were collected from their finger 's tip by lancet, then stored and labeled on FTA™ Classic Cards, Whatman™ (GE Healthcare, UK) until analysis.

2.2. Method

Direct amplification (PCR) was performed on the punched dried blood on FTA Cards for two systems: power plex ®fusion and power-plex ®Y23 (Promega) according to the manufacture protocol for each kit aiming comparison and confirmation. A Gene Amp® PCR System 9700 (Applied Biosystems with serial No.805S1241621) thermal cycler was used.

2.3. Genetic Electrophoresis and Analysis

The PCR products as mentioned in the technical manual were run on 3130 xl Genetic Analyzer® (Applied Biosystems, USA. with serial No.18222-027). The data were analyzed with Gene Mapper ID Analysis Software (Applied Biosystems, USA.v3.2). Power plex ®Fusion and Power-plex ®Y23systems were used for investigation, therefore the data were analyzed for establishing the DNA and Y Profiles for each case.

2.4. Statistical analysis

Counting method and some mathematical equations have been used to extract the forensic parameters such as allele frequencies, homozygosity, random match probability, haplotype diversity, upper bound confidential interval, power of discrimination and match probability of the combined DYS391 loci.

3. Results

The outcome results of Autosomal STR DNA Profiles of Father/Sons for all 30 studied families including DYS391locus are shown below in table-1.

All designated haplotypes of Y Chromosomes STR including DYS391 locus for all studied Father/Sons are shown below in table-2.

Being concentrating on DYS391 locus, there are three allele polymorphisms have been documented in this study; Allele number 10 (69.23%), allele number 11 (27.692%) and to the lesser extent allele number 9 which has been identified in only (3.076%) of all 30 work cases as it's shown in table-3.

Few useful forensic parameters of DYS391 Locus (number of allele occurrence, observed allele frequencies, homozygosity, random match probability, upper bound confidential interval, haplotype diversity, match probability and combined power of discrimination) have been calculated and listed with their formulae in table-3 below.

Table 1. The Autosomal loci of 30 families amplified by power plex® Fusion kit are shown in bold lines revealing the mismatch between the alleged fathers and corresponding sons. DYS391 locus haplotypes have been shaded in pink color.

Fam.1	strangers																								
father		15,16	16,17	13,14	15,17	10,12	5,12	9,10	12,15	17,17	12,12	11,14	2,5	15,16	30,32	10,13	10,11	8,8	11	15,16	17,21	13,14	19,23	14,16	11,12
son		15,16	16,17	11,13	13,15	11,12	10,12	12,13	13,15	22,23	11,12	8,11	4,4	14,16	29,30	8,12	10,13	7,8	11	15,17	19,21	14,16	20,21	15,17	13,17
Fam.2	strangers																								
father		14,17	17,20	11,14	15,16	11,13	10,12	11,13	13,16	22,24	12,13	9,11	4,8	16,16	29,30	11,11	11,12	8,8	11	15,16	20,23	13,16	22,24	16,16	
son		13,15	17,17	11,14	13,17	9,12	9,12	11,13	13,15	19,22	11,12	9,11	3,3	14,16	29,30	10,11	11,15	8,8	11	15,16	20,22	11,16	23,26	13,17	
son2		13,13	16,17	11,11	13,15	11,11	12,12	13,13	14,15	17,18	11,11	11,11	3,6	17,17	30,30.2	8,10	10,11	8,8	11	15,16	20,22	11,15	23,23	13,17	
Fam.3	strangers																								
father		15,17	16,16	13,14	15,16	11,12	10,12	11,13	12,15	18,22	12,13	10,11	3,9	14,16	30,30.2	10,13	10,11	8,11	11	15,16	19,21	13,14	23,25	15,16	
son1		14,17	16,17	11,13.2	15,16	11,13	11,12	11,13	14,15	19,22	11,12	9,11	3,4	14,16	29,30	10,13	11,11	8,11	11	15,16	19,20	13,16	20,23	15,16	
son2		14,17	16,17	14,14	15,16	11,17	10,11	11,11	14,15	17,21	12,13	10,11	3,6	14,16	29,30	10,13	10,12	8,8	11	15,16	19,21	13,16	20,24	15,16	
Fam.4	strangers																								
father		15,15	15.3,16.3	11,14	13,16	10,11	10,10	12,12	14,15	18,20	12,12	10,11	3,3	15,17	30,30.2	13,13	12,12	8,8	11	15,16	21,23	13,15.3	23,25	15,16	
son		14,17	17,18	11,12	13,15	12,13	12,14	13,15	15,18	19,20	12,13	10,11	3,3	15,16	29,30	13,13	12,13	11,12	11	15,16	19,21	13,16	23,24	15,16	
Fam.5	strangers																								
father		14,17	15,16	11,14	13,14	9,10	7,11	9,13	15,17	17,20	12,15	10,13	2,3,5,6	14,15	30,30	9,13	10,11	8,8	10	9,13	19,21	14,14	23,25	15,16	
Fam.6	strangers																								
father		15,17	16,17.2	11,17	15,15	11,13	7,11	10,12	15,15	18,20	11,12	10,14	2,6	15,17	29,32.2	13,13	12,13	8,10	11	15,16	22,22	15,16	23,26	15,16	
son		15,16	16,17.2	11,14	13,17	9,10	10,12	11,11	13,16	17,20	11,12	10,13	2,3	14,15	29,30	8,11	11,13	8,11	11	15,17	19,22	13,16	20,24	15,17	
Fam.7	strangers																								
father		14,17	15,17	11,14	13,14	9,10	7,12	10,13	13,15	17,18	12,13	10,13	2,9	15,17	30,30	12,12	10,13	7,9	11	15,16	19,21	13,16	23,25	15,16	
son		15,16	15,16	11,14	13,14	9,10	7,11	11,11	13,14	18,20	12,13	9,14	2,7	15,17	30,30.2	10,13	9,12	8,10	11	15,16	19,22	13,14	22,24	15,16	
Fam.8	strangers																								
father		14,17	15,17	11,13.1	14,14	11,13	10,12	11,13	13,15	17,20	12,13	10,12	3,4	15,16	30,30.2	9,11	9,10	8,11	11	15,16	19,20	14,16.3	23,24	15,16	
son		15,16	15,16	11,14	14,14	11,12	12,13	12,13	13,15	19,19	11,12	9,11	3,4	15,17	29,30.2	9,11	10,11	8,8	11	15,16	19,20	14,16	23,24	15,16	
Fam.9	strangers																								
father		15,15	15,17	12,13	13,14	9,12	6,10	12,13	15,18	18,20	12,13	9,10	2,3,5	15,16	29,30	8,9	10,11	8,8	10	15,17	19,24	13,14	23,24	15,16	
son		15,17	15,17	12,13	13,14	9,12	6,10	11,12	14,14	18,20	12,13	9,11	2,3,5	15,16	29,30.2	8,10	10,12	8,8	11	15,17	19,22	13,14	21,23	15,16	
son2		14,17	15,17	12,13	13,14	9,12	6,10	11,12	14,14	18,20	12,13	9,11	2,3,5	15,16	29,30.2	8,10	10,12	8,8	11	15,17	19,22	13,14	22,24	15,16	
Fam.10	strangers																								
father		15,16	17,18	12,13	13,13	9,11	9,10	9,13	13,15	20,22	12,13	9,10	2,3,5	14,16	30,31.2	9,11	9,11	8,8	11	15,16	19,21	13,16.3	23,24	15,16	
son		15,16	15,17	12,13.1	13,14	11,12	9,11	11,14	13,13	18,20	11,12	9,11	3,4	15,17	29,30.2	11,13	12,13	8,11	11	15,17	19,21	13,14	23,24	15,16	
Fam.11	cousins																								
father		15,15	15,17.2	11,11	14,14	12,12	12,13	13,13	13,15	19,20	13,13	10,14	3,5	17,17	28,28	9,11	9,13	8,8	11	15,16	18,19.3	14,15.1	21,22	15,18	
son		15,16	15,16	12,13	13,14	11,13	12,13	13,13	13,15	22,24	12,13	9,10	2,3	16,16	29,30	11,12	12,13	8,8	11	15,15	21,23	13,15	23,26	15,16	
Fam.12	strangers																								
father		14,16	17,17	11,14	13,14	10,10	8,11	9,13	14,15	18,20	11,13	9,11	3,4,5	15,16	30,30.2	9,11	11,13	8,11	10	15,16	19,21	13,14	22,23	15,16	
son		14,16	17,18.2	14,16	13,15	11,13	10,11	11,13	14,15	18,20	11,13	9,11	3,4	17,17	30,31.2	11,12	11,13	8,8	10	15,16	19,21	13,14	23,24	15,16	
Fam.13	strangers																								
father		15,17	15,16	14,15	13,15	9,11	10,11	11,13	15,17	17,17	12,13	9,10	2,3,5	15,16	30,30.2	8,9	9,11	8,12	10	15,16	19,22.2	13,14.2	23,24	15,16	
son1		15,15	16,17.2	11,13.2	13,15	10,13	9,11	9,13	12,14	17,20	12,13	9,11	2,7	15,16	29,30	8,10	10,12	8,8	10	15,16	19,21	14,16	23,24	15,16	
son2		15,17	16,17.2	11,14	13,15	10,12	10,11	9,12	14,15	18,18	11,13	11,12	3,4	14,16	29,30.2	11,11	10,12	8,8	10	15,16	21,23	13,16	24,25	15,15	
Fam.14	distant relatives																								
father		14,17	17,20.2	13,16	13,15	11,13	10,12	9,11	14,15	17,20	11,13	9,11	2,4	14,16	30,31.2	8,8	11,12	8,8	10	15,16	21,24	14,16	23,24	15,16	
son		14,16	17,20.2	14,14	13,15	11,12	12,13	12,13	14,20	18,20	11,13	10,11	4,4	15,16	30,31	8,11	11,13	8,8	10	15,16	19,22	14,16	21,23	15,16	
Fam.15	strangers																								
father		15,15	16,17.2	11,11	13,15	11,13	10,12	11,13	13,15	18,20	12,13	9,11	2,3	15,16	29,30.2	13,13	11,12	8,8	11	15,16	19,21	13,14	23,24	15,16	
son		15,16	15,17	11,13	14,15	12,13	12,13	11,11	13,15	18,20	12,13	9,10	2,3	15,16	29,30.2	9,11	11,13	8,8	11	15,16	19,22	13,16	22,24	15,17	
Fam.16	strangers																								
father		15,16	17,18	12,13	13,15	9,11	10,12	12,13	13,15	18,20	12,13	9,11	2,3,5	15,16	30,30.2	9,12	9,11	8,11	11	15,16	19,21	13,14	23,24	15,16	
son1		15,17	16,17	11,13.2	13,15	9,11	9,12	11,13	13,15	17,20	12,13	9,11	2,3,5	15,16	30,31	8,10	11,13	8,8	11	15,16	19,21	13,14	23,24	15,16	
son2		14,17	15,16	11,11	14,14	11,12	10,12	11,13	14,15	17,20	12,13	9,11	2,3,5	14,16	29,30.2	8,11	10,12	8,11	11	15,16	19,21	14,16	23,24	15,16	
Fam.17	distant relatives																								
father		17,17	14,15	10,11	14,16	11,12	5,15	9,13	15,21	18,18	12,12	2.2,9	6,7	14,17	30,32.2	10,11	10,11	8,8	11	15,15	17,19	13,14	19,23	14,13	
son		15,17	11,16	11,16	13,13	8,9	12,14	12,13	13,14	19,20	10,12	9,11	6,7	17,20	29,29	8,10	9,12	8,8	11	14,14	18,20	14,16	19,23	15,11	
Fam.18	strangers																								
father		16,18	12,18.3	11,14	15,17	11,11	10,14	10,11	14,15	24,24	12,12	8,9	6,6	18,18	30,31.2	10,10	11,13	6,10	10	14,14	21,22	14,14.2	22,23	11,11	
son		16,17	14,16	11,12	14,15	11,12	12,12	8,12	10,15	17,24	12,12	9,13	6,6	16,16	29,32.2	12,12	12,12	8,11	10	11,12	20,23	12,16	20,26	16,17	
Fam.19	strangers																								
father		14,15	14,15	11,11	14,14	12,12	11,15	11,11	16,16	17,25	12,12	13,13	7,9	18,18	27,31	9,10	12,12	8,11	10	12,15	17,22	15,15.2	23,23	15,13	
son		17,17	13,16	10,11	14,15	12,12	12,16	11,11	15,18	19,20	10,12	13,13	8,6	14,17	29,32.2	10,11	10,11	8,11	10	16,16	19,22	12,13	22,23	15,13	
Fam.20	cousins																								
father		16,17	15,16	11,12	14,16	12,14	7,12	9,12	12,17	24,25	11,12	9,10	6,8	16,18	28,31.2	8,11	11,13	9,11	11	12,14	21,21	13,16	22.2,23	15,15	
son		16,17	16,16	11,12	14,15	8,11	13,18	9,12	16,19	19,23	12,12	11,14	6,9	17,18	30,30	11,12	9,11	8,8	11	14,15	18,18	11,12	19,20	15,16	
Fam.21	strangers																								
father		15,15	15,16.3	13,14	13,14	11,11	10,17	12,13	12,18	17,19	11,12	9,10	6,9.3	17,17	28,29.2	8,8	11,12	8,8	10	13,14	21,24	14,14	23,24	15,12	
son		15,17	15,15	11,11	15,16	9,10	13,17	9,10	15,17	20,20	12,12	13,15	9,9.3	17,19	30,31.2	8,11	11,13	8,9	10	10,11	18,22	13,16	21,21	15,15	
Fam.22	strangers																								
father		15,16	16,17.3	10,11	14,15	11,12	11,13	11,13	14,19	20,22	11,12	9,13	7,9	18,18	30,31.2	11,11	11,13	8,11	10	11,16	18,21	14.2,16	20,22	15,15	
son		16,18	12,15	10,11.3	13,13	13,14	10,10	8,12	14,15	17,23	12,12	13,15	6,8	18,19	28,28	11,11	12,13	8,8	10	13,14	18,20	9,15	22,22	15,15	
Fam.23	strangers																								
father		16,16	15,16	11,11	15,15	11,12	10,15	11,12	15,16	17,17	11,12	13,13	6,9	17,17	30.2,33.2	13,13	12,12	10,11	11	11,12	22,22	11,16	23,24	14,19	
son		15,19	15,16.3	14,14	15,15	11,12	7,11	10,13	11,15	22,25	11,12	9,13	7,9.3	15,16	30,31	8,10	11,13	8,9	11	15,15	18,18	14,14	21,23	16,17	
Fam.24	strangers																								
father		15,16	16,17.3	10,11	14,15	11,12	11,13	11,13	14,19	20,22	11,12	9,13	7,9.3	18,18	30,31.2	11,11	11,13	8,11	10	11,16	18,21	14.2,16	20,22	15,15	
son		16,18	12,15	11,11.3	13,13	13,14	10,10	8,12	14,15	17,23	12,12	13,15	6,8	18,19	28,28	11,11	12,13	8,8	10	13,14	18,20	13,15	22,22	15,15	
Fam.25	strangers																								
father		16,18	12,16	11.3,14	14,16	8,11	11,12	12,12	17,17	21,24	10,10	9,13	6,6	18,18	28,29	8,11	12,13	8,8	11	13,14	18,18	15,16	20,22	11,13	
son		16,17	14,16	14,15	13,13	11,12	7,9	11,13	12,15	20,25	10,10	8,10	6,9.3	16,18	29,30	8,10	11,13	8,11	11	13,15	15,19	11,14	24,24	16,15	
Fam.26	strangers																								
father		16,17	15,16	11,12	14,16	12,14	7,12	9,12	12,17	24,25	11,12	9,13	6,8	16,18	28,31.2	8,11	11,13	9,11	11	12,14	21,21	13,16	22.2,23	15,15	
son		16,17	16,16	11,12	14,15	8,11	13,18	9,12	16,19	19,23	12,12	11,14	6,9	17,18	30,30	11,12	9,11	8,8	11	14,15	18,18	11,12	19,20	15,16	
Fam.27	strangers																								
father		15,16	11,13	11,11.3	13,14	8,11	10,13	11,11	14,15	19,24	11,12	9,9	6,9.3	16,17	28,29	10,11	8,9	8,10	11	13,13	17,21	13,16.2	22,22	15,16	
son		15,15	12,13	11,12	14,15	11,12	7,12	10,13	14,20	17,24	10,12	10,15	9,10	16,17	29,30	10,12	11,12	8,8	11	8,10	18,21	13,14	23,24	15,15	
Fam.28	strangers																								
father		15,17	14,17	11,14	14,15	11,12	11,12	11,13	14,15	17,18	11,12	13,13	7,9	16,17	28,29	10,10	10,10	8,11	10	12,13	19,20	12,14.2	21,23	11,11	
son		15,16	16,16.2	11,3,11	14,14	11,12	12,12	12,13	12,12	17,16	11,12	9,13	6,7	14,16	28,29	10,12	10,12	8,8	10	15,13	17,24	13,14.2	21,21	14,16	
Fam.29	strangers																								
father		16,16	17.3,19.3	11,12	15,15	8,10	12,16	9,14	13,17	19,20	10,12	9,10	6,7	14,16	29,31.2	12,12	11,13	8,11	11	11,14	17,18.3	13,15.2	21,22	16,15	
son		16,18	15,15.3	11,15	15,17	11,13	12,13	9,11	13,13	16,19	11,15	9,9	7,8	17,19	27,28	8,11	10,12	8,11	11	10,13	18,21	13,14	20,21	15,17	
Fam.30	strangers																								
father		17,17	14,16	14,14	15,15	12,12	5,7	9,11	14,16	22,25	10,12	9,11	9,9	14,17	30.2,31.2	9,10	9,9	8,10	10	12,13	18,23	14,14	20,21	11,15	
son		16,17	13,16	11,11	14,15	10,13	11,13	10,10	12,17	20,23	10,12	9,11	6,7	14,15	32.2,33.2	8,11	12,13	8,10	10	14,15	21,22	14,14.2	24,25	12,15	

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Table 2. The Y Chromosome loci of 30 families amplified by power-plex® 23 Y kit are shown in bold lines revealing the mismatch between the alleged fathers and corresponding sons. DYS391 locus haplotypes have been shaded in pink color.

Famliy No.	DYS576	DYS389 I	DYS448	DYS389 II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437	DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385	DYS456	YGAT	AH4
Fam.1																							
father	20	13	20	30	16	10	25	13	11	10	14	17	21	23	11	11	9	13	18.2	13,18	15		11
son	17	12	19	28	14	10	23	13	11	10	15	15	23	22	11	14	10	11	15	12,17	15		11
Fam.2																							
father	10	13	18	29	15	10	21	13	10	9	16	18	21	24	12	11	9	12	17	13,17	13		10
son1	18	13	22	32	15	10	21	13	10	10	15	19	21	21	11	11	12	14	18	13,14	16		12
son2	18	13	20	30	14	10	26	13	10	10	14	18	21	23	11	11	9	12	18.2	13,19	14		11
Fam.3																							
father	17	14	20	31	14	10	22	13	12	10	14	17	21	24	12	11	11	13	18	19,20	15		13
son1	18	12	21	29	16	10	22	12	11	10	16	17	21	23	13	11	12	13	16	15,17	15		12
son2	16	13	18	29	15	10	21	13	10	9	15	18	21	23	12	11	9	12	17	13,17	13		10
Fam.4																							
father	16	13	20	29	14	10	25	12	11	10	14	17	21	22	11	11	8	12	18.2	12,19	13		11
son	17	13	19	30	15	10	24	13	11	10	14	16	18	22	10	11	13	15	18	13,21	15		10
Fam.5																							
father	18	13	20	29	14	11	23	12	11	10	14	17	21	23	12	11	9	12	18.2	13,20	14		11
son	17	13	20	31	16	11	30	11	12	10	16	17	23	24	12	11	10	13	17	14,16	15		11
Fam.6																							
father	18	13	20	29	14	10	24	12	11	10	14	18	21	23	11	11	9	12	18.2	13,17	14		11
son	18	13	20	30	15	10	23	12	12	11	14	18	23	25	10	11	10	13	16	11,14	15		13
Fam.7																							
father	18	13	20	29	14	11	23	12	11	10	14	18	21	23	11	11	9	12	18.2	13,19	14		11
son	17	13	20	29	14	11	24	12	12	12	15	18	23	23	12	13	10	12	17	12,15	17		13
Fam.8																							
father	18	13	19	29	13	10	26	11	11	11	14	16	22	22	12	15	12	13	18	13,16	14		10
son	16	14	19	31	14	10	22	15	12	9	14	18	21	23	11	13	10	12	19	14,16	16		11
Fam.9																							
father	18	12	19	29	15	10	24	13	12	9	14	18	21	25	11	11	9	12	18	13,15	15		11
son1	17	13	20	31	15	10	23	12	12	11	14	18	23	25	10	11	10	13	16	11,14	15		13
son2	17	13	20	31	15	10	23	12	12	11	14	18	23	25	10	11	10	13	16	11,14	15		13
Fam.10																							
father	18	13	19	30	15	10	23	12	12	11	15	17	23	23	11	13	10	13	18	12	15		12
son	19	13	21	30	14	10	22	13	11	10	14	20	20	24	11	11	12	13	18	18	15		13
Fam.11																							
father	16	14	20	31	14	10	22	13	11	10	14	19	20	24	12	11	13	13	16	18,19	15		12
son	17	14	19	31	13	10	25	12	11	11	14	16	22	22	12	15	12	13	17	14,17	16		10
Fam.12																							
father	16	13	18	29	15	10	21	14	10	9	15	18	21	24	12	11	9	12	17	13,17	13		10
son	16	13	18	29	15	10	21	13	10	9	15	18	20	24	12	11	9	12	17	13,17	13		10
Fam.13																							
father	19	13	19	31	14	10	23	12	12	9	15	17	21	24	12	11	9	12	18	14,16	17		11
son1	17	14	21	30	15	10	23	12	11	9	15	15	23	23	11	11	11	13	16	15,16	15		11
son2	18	14	20	30	14	10	23	12	13	9	15	17	24	23	11	11	10	13	17	13	15		11
Fam.14																							
father	18	13	20	30	13	9	26	13	12	10	14	16	21	23	12	11	12	14	14	16,18	15		12
son	17	14	20	31	14	9	25	14	11	10	14	18	22	24	12	11	13	13	15	16,17	15		12
Fam.15																							
father																							
son																							
Fam.16																							
father																							
son1																							
son2																							
Fam.17																							
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Fam.29																							
father																							
son																							
Fam.30																							
father																							
son																							

Table 3. The presentation of the important DYS391 forensic parameters values plus their representation and formulae. [6], [13], [14]

N=65 No. of samples(profiles)for all families ***(X)=****No. of Occurrence allele*	Observed allele frequency of y-locus ***p=X/N***	Hardy-Weinberg Law (**HWE**) ***P²=****Homozygote*	Random match probability (RMP) = ***1/profile frequency***	Upper Bound Confidence Interval (CI) $\boldsymbol{P + 1.96\sqrt{(P)(1}}$	Haplotype Diversity HD= $n(1-\sum p^2)/(n-1)$	Match probability $PM= \sum_{K=1}^{M} P^2\ k$	Power of Discrimination of DYS391 ***PD=1-PM***
Allele (9) X=2	P1=0.030	0.0009	Profile frequency =0.032793x10¬³	1.9899	HD=0.4511	*p²1+p²2+p²3=0.5558*	*PD=0.4442*
Allele (10) X=45	P2=0.692	0.4788	RMP=30.494331	2.6421		*PM=0.5558*	
Allele (11) X=18	P3=0.276	0.0761		2.2344			

Three families out of the total 30 work cases were chosen from table -1 and -2 to demonstrate DYS391 alleles variants in figures. Those families were designated as Family Number; (No.)1, 14 and 29. As it is shown from Figures - A1, A2 and A3 below, alleles No. 10 ,11 and 9 have been illustrated by a red circle each, whereas some autosomal alleles were being pointed by blue arrows in DNA Profiles of Father (a) and Son (b) which prove the biological exclusion of Father's son relationships in Families No. 1, 29 and 14 respectively.

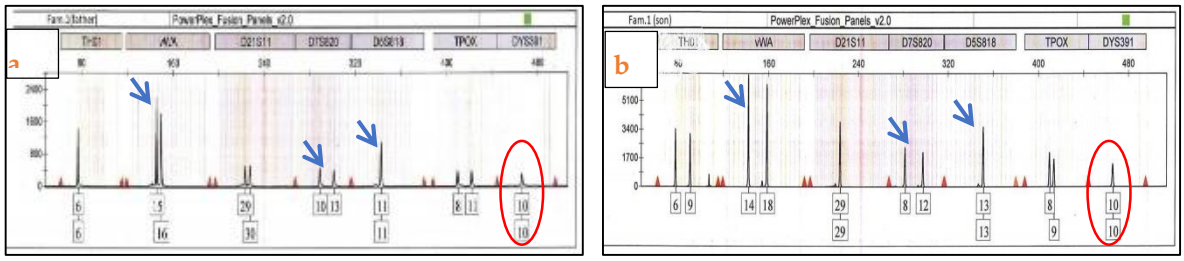

Figure A1 [a &b]: Comparison of some autosomal loci (blue arrow - mismatch) plus DYS391 allele 10 in (red cycle- match) in DNA Profiles of Father / Son Family No. 1 of power-plex ®fusion system.

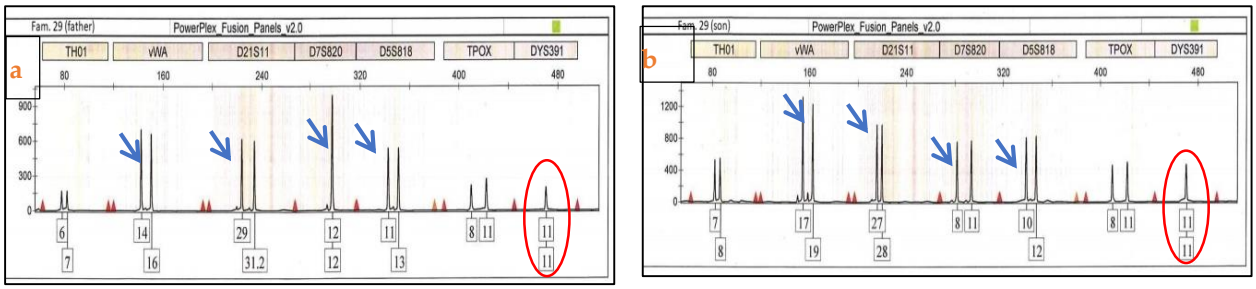

Figure -A2 [a &b]: Comparison of some autosomal loci (blue arrow - mismatch) plus DYS391 allele 11 (red cycle- match) in DNA Profiles of Father / Son Family No. 29 of power-plex ®fusion system.

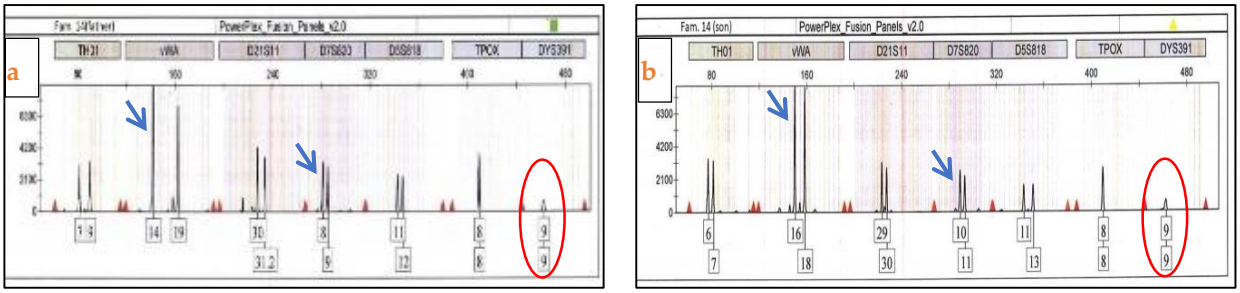

FigurA3 [a &b]: Comparison of some autosomal loci (blue arrow- mismatch) plus DYS391 allele 9 (red cycle- match) in DNA Profiles of Father / Son Family No. 14 of power-plex ®fusion system.

For further confirmation as it is shown in Figures -B1, B2 and B3- below, alleles number 10 ,11 and 9 have been illustrated by a red circle each, whereas some Y Haplotypes were being pointed by blue arrows in both Y Profiles of Father (c) and Son (d) which prove the male lineage exclusion of Father's child relationships in Families No. 1, 29 and 14 respectively too.

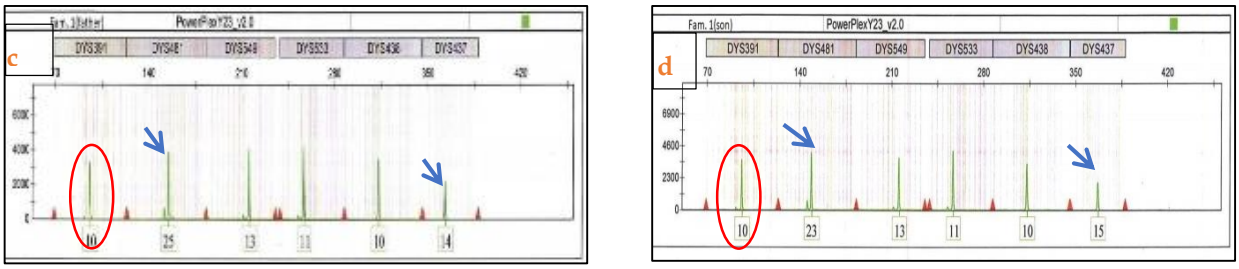

Figure-B1 [c & d]: Comparison of some Y haplotype loci (blue arrow- mismatch) plus DYS391 allele 10 (red cycle -match) in Y -Profiles of Father / Son Family No. 1 of power-plex ®23 Y.

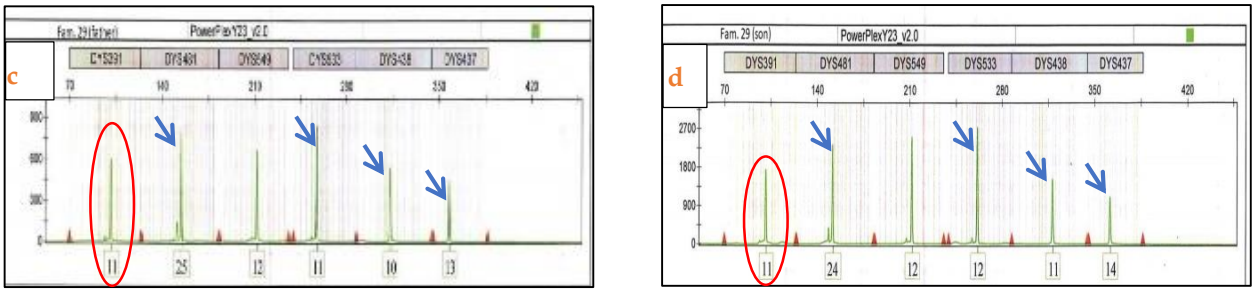

Figure-B2 [c & d]: Comparison of some Y haplotype loci (blue arrow- mismatch) plus DYS391 allele 11 (red cycle -match) in Y -Profiles of Father / Son Family No.29 of power-plex ®23 Y.

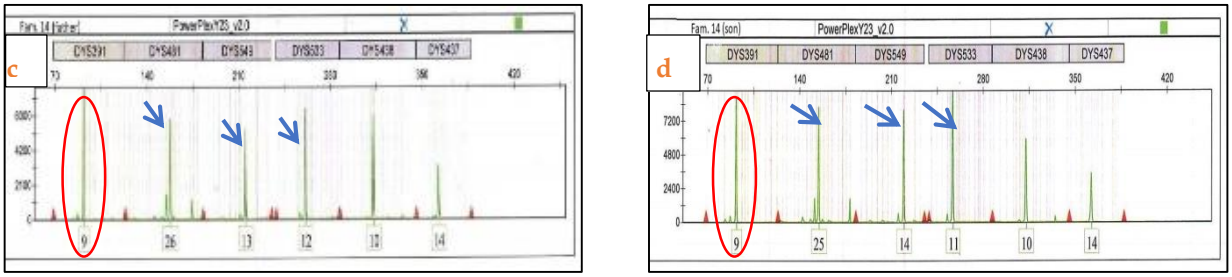

Figure -B3 [c & d]: Comparison of some Y haplotype loci (blue arrow- mismatch) plus DYS391 allele 9 (red cycle- match) in Y -Profiles of Father / Son Family No. 14 of power-plex ®23 Y.

The outcome results confirm the complete match in DYS391 locus between all alleged father/son pairs in all family's despite of mismatch father/ son pairs using two different kits.

4. Discussion

The DNA paternity test provides a typology and a facilitative mechanism for men about their paternity or non-paternity [15].

Obviously, the greater the variety and independent assortment of alleles in DNA, the greater the ability to distinguish father /child primary relationship. Hence, allele ranges, locus characteristics and probability of identity (PI) of different STR loci had been studied thoroughly by forensic scientists' community to help assess the benefits of adding loci to the common 13 CODIS core loci. Moreover, empirical tests of association independence of Y and autosomal markers and a theoretical framework for determining a joint match probability was recommended in many previous studies [9,16,17].

Therefore, a part from what have mentioned above, here we will discuss the usefulness of using the single copy locus DYS391 in testing father/son relationship obtained from Arab Iraqi families work cases.

Unexpectedly, the show results in table (1) and table (2) revealed perfect match in DYS391 between alleged fathers and the corresponding sons in all cases despite of absolute denials of paternity, such congruence calls into question about the strength and effectiveness of such locus in paternity!

The results also revealed only three allele polymorphisms for DYS391 in 65 tested samples i.e. little allele variants; the commonest allele was allele No. 10 in a percentage of 69.23%, then allele No.11 (27.692%) and to lesser extent allele No. 9 in only (3.076%). In

addition, the observed allele frequencies as its shown in table (3) were (0.692, 0.276 and 0.030) for allele numbers 10, 11 and 9 with 95% upper bound confidence interval (CI) of (2.64, 2.23 and 1.98) respectively. Surprisingly, our result is compatible with many other population genetics whose allele No.10 was the commonest allele whether in Iraq or many countries near or far from it. [9,18,19,20,21]. Nevertheless, in another study which was being performed for Iraqi population in 2020, allele No.10 was found to be in second sequence after allele No.9 in a data of 1032 total samples [22], perhaps this is due to the fact that the current study relies on small number of families rather than large scale numbers of sample or data.

On the other hand, the characteristic of different kits like power plex ®fusion and power plex Y systems is expected to be similar for DYS391 at allele numbers correlate and the number of repeats. [7,23]. Therefore, the forensic indices of DYS391 locus have been estimated for the combined three alleles. In table (3), hemizygosities were 0.4788, 0.0761 and 0.0009 for allele No. 10, 11 and 9 respectively. Random match probability (RMP); was found to be of value 30 which considered high among the selected families of 65 samples.

Furthermore, the match probability of combined alleles in DYS391 locus (PM) was 0.5558 i.e., the probability to find different genotypes is low. Meanwhile the gene diversity was 0.451 and the power of discrimination was low to 0.4442. These values reflect the poor haplotype diversity and discrimination ability of the DYS391 locus in this study. Although, in Paternity and male lineage test it's important to remove any suspicion about identity of child's father but in the present study, 30 families examined for DYS391locus, not much different in genotypes neither much specific information has been found. This result is due to the low polymorphisms, narrow allele range features besides the lack of heterozygosity and the probability for the locus of being exclusively not highly discriminated nor much useful for paternity purpose for Iraqi cases under investigation.

Fortunately, the abundance of genetic markers in 23 Y power-plex system perhaps has boosted the strength of identification of non-paternity work cases to the strength of autosomal STR one. But we must say, because of the prevalence of consanguineous and overlap marriages between tribes and clans that characterizing Iraqi society, autosomal STR examination is mandatory to establish paternity here. Our study on families also confirms the suggestion of using well organized two-stage approach in human identification to save cost and is useful in mother side deficiency cases. [7,9,10,24].

5. Conclusion

There is a limitation in certifying DYS391 locus as a useful Y-STR in paternity and male lineage in Arab Iraqi population because of low allele variants. practically, substitution of a highly polymorphic Y STR locus instead in association with autosomal markers is recommended for paternity. Finally, Further studies for evaluation of the usefulness of DYS391 STR in forensic purposes other than paternity are recommended here such as in mixed samples of rape and degraded DNA in Mass Grave cases.

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Ethical Consideration:
Research protocol has been performed according to the ethical guide for implementing health research in Iraqi Ministry of Health (Iraqi National Code of Ethics 2018) with protected data approved by the Scientific and Ethical research committees in MLD/Baghdad in 25/9/2022.

Source of funding: Governmental/personal

Conflict of Interest: None

References:

1) Ammirah J.Omer,et al. "Analytical Study of Paternity Test Cases in Iraq during the period 2002 -2007" .Iraqi Postgraduate Med.J.,2010,Vol.9 No.4, pp:449-456
2) Ammirah J.Omer,et al. "Evaluation of DNA Facilities at Medical Legal Institute: by Sir William Goodwin ". Letter to Editor, Iraqi Med.J ,2014; Vol.60No.2, pp:190-192
3) Kathryn Oostdik et al. "Developmental validation of the PowerPlex1 Fusion System for analysis of casework and reference samples: A 24-locus multiplex for new database standards". F.Sci.I.:Genetics ,12(2014),pp;69-76
4) Jobling, Mark A., and Chris Tyler-Smith. "Human Y-chromosome variation in the genome-sequencing era." Nature Reviews Genetics 18.8 (2017), pp: 485-497.
5) Josephine Purps a, , Sabine Siegert b, Sascha Willuweit et al "A global analysis of Y-chromosomal haplotype diversity for 23 STR loci" F.Sci.I.,12(2014),pp:12-23
6) John M.Butler, Richard S.Petter Vallone, et al "Y Chromosomal DNA Testing" In:Advanced Topics in Forensic DNA Typing : Methodology. Chapte13, AP Academic Press.2011, pp:378
7) John M., Butler et al "A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers",FSI 129 (2002),pp:10-24
8) Wiegand, P., and M. Kleiber. "Less is more–length reduction of STR amplicons using redesigned primers." International journal of legal medicine. 114.4 (2001),pp: 285-287.
9) John M., Butler, Carolyn R.(Becky)Hill, David L. Duewer et al. "Characteristics of 24 Commonly Used Autosomal STR Loci."National Institute of Standard and Technology , Poster#38 at 22nd International Symposium of Human Identification. National Harbor, MD,October4-5,2011. (NIST).STRBase:http://www.cstl.nist.gov/biotech/starbase/pub-pres/ButlerISHI2011poster
10) - Faruk A., Fatih A., Ümit Ç. et al. "Allele and haplotype frequencies of Y-short tandem repeat loci in Turkey." Croat. med J. 44 (2003), pp: 310-314.
11) D Carvalho-Silva and S.D Pena "Molecular characterization and population study of an X chromosome homolog of the Y-linked microsatellite DYS391". Gene.2000 Apr 18;247(1-2), pp:233-40. doi: 10.1016/s0378-1119(00)00085-8.
12) L Gusmão , A González-Neira, P Sánchez-Diz, et al "Alternative primers for DYS391 typing: advantages of their application to forensic genetics" Forensic Sci Int.2000. Jul 24;112(1), pp:49-57. doi: 10.1016/s0379-0738(00)00171-7

13) William Goodwin, Adrian Linacre, and Sibte Hadi. " An introduction to forensic genetics". Second Edition, Wiley-Black Well, Appendix A, 2011: pp181-182
14) Coble, Michael D., Carolyn R. Hill, and John M. Butler. "Haplotype data for 23 Y-chromosome markers in four US population groups." Forensic Science International: Genetics 7.3 (2013),pp: e66-e68
15) Turney, Lyn. "Contested paternity: Why, and to whom, genetic paternity testing matters." Proceedings of the Annual Conference of the Australian Sociological Association. 2004.
16) Imen A., Nadia M.L., Hafedh M. et al. " Combining autosomal and Y-chromosomal short tandem repeat data in paternity testing with male child: methods and application". J Forensic Sci. 2007 , pp:52(5):1068-72
17) Bruce W. Alan J. R. and Michael F. Hanner. " Joint Match Probabilities for Y Chromosomal and Autosomal Markers". F.Sci.I. Volume:174 Issue: 203, 2008 pp: 234-238
18) Imad H., Mohammad J and Aamera O. "Genetic variation of 17 Y-chromosomal short tandem repeats (STRs) loci from unrelated individuals in Iraq". International Journal for Biotechnology and Molecular Biology Research.2013, Vol. 4(8), pp: 119-129
19) Tie, Jian, et al. "Polymorphisms of six Y-chromosome STRs in a Chinese population." Legal Medicine 6.1 (2004), pp: 41-46.
20) Peričić M., Barac-Laucet L., Martinović I.et al "Y chromosome haplotypes in Albanian population from Kosovo. " Forensic Science International. 2004, 146(1), pp:61-4
21) Jeffrey T. Lell, Rem I. Sukernik, Yelena B.et al "The Dual Origin and Siberian Affinities of Native American e Y Chromosomes" Am. J. Hum. Genet. 2002, 70,pp:192–206
22) Hanan K. Mahmood, Nadia F. Salman, Khalifa M. Salih et al "Frequency of Y-chromosome STRs using PowerPlex® Y23 System in Iraqi population". Egyptian Journal of Forensic Sciences 2020 ,10(12),pp:2-8
23) Kayser, Manfred, et al. "Evaluation of Y-chromosomal STRs: a multicenter study." International journal of legal medicine 110.3 (1997),pp: 125-133.
24) R N Oliveira 1, F D Nunes, E K Anzai, E. et al. "Population studies of the Y-chromosome of loci DYS390, DYS391 and DYS393 in Brazilian subjects and its use in human identification" J Forensic Odontostomatol. 2002 Jun;20(1),pp:6-9