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Looking for a reliable criteria for the establishment of solid STR profiles using ancient critical samples from 3000 to 5000 years ago

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ABSTRACT

Through this research we look for the best criterion to establish STR (Short Tandem Repeats) profiles from LTD (Low Template DNA) samples. Five different criteria have been proposed and evaluated for the interpretation of partial STR profiles from LTD samples, thus trying to establish the most reliable one. Specifically, 82Chalcolithic and Bronze Age samples have been employed. The 5 different criteria have been established, taking into account information such as the height of the peaks and the reproducibility of the results.

Secondly, the kinship analysis among individuals buried together has been carried out. In this second part, it has also been taken into account the mitochondrial DNA (mtDNA) analysis, to consolidate the STR results.

1. Introduction

When we perform STR analysis in critical samples, usually we obtain partial profiles that usually lead to LTD problems.

There are many recommendations for the analysis of critical DNA sequences, but there is no any guideline for the LTD partial STR profiles interpretation [2]. Therefore, through this work we look for the formulation of a solid criterion to establish consensus STR profile drawn out of partial profiles.

2. Materials and methods

We analysed 82 samples of 39 individuals from 6 archaeological sites located on the Iberian North Sub-plateau, between 3000 and 5000 BP.

DNA extraction was performed following a silica based protocol [1]. To amplify autosomal STRs we used three different kits: AmpFISTR® MiniFiler, AmpFISTR® NGM™ and PowerPlex® ESX. STR analysis was performed in an ABI PRISM® 3730 Genetic Analyzer, with GeneMapper® 4.0 software. In order to complement STR results we amplified HVI and HVII regions of the mtDNA.

The whole process was performed following the authenticity ancient DNA criteria [2].

In order to assign peaks to a profile, stutters, peaks out of the ladder, allelic disequilibrium or allelic dropout were considered. When a marker showed disbalanced peaks, we only considered as valid the highest peak unless the lowest one reached at least the 50% of the height of the highest one. Also we have taken into account the probability of allelic [3].

Regarding the presence of peaks off the ladder, given that analyzed samples were ancient, there could be “extinct” alleles that are not present in nowadays population. We did not consider these peaks, unless the same result was obtained through different amplifications.

The 5 criteria proposed to the establishment of a consensus profile considered:

1. Peaks above 100 rfus.
2. Peaks above 75 rfus.
3. Peaks above 50 rfus.
4. Every peak above 150 rfus irrespective of the obtaining of replicated results or not. Also consider peaks above 75 rfus.
5. Every peak above 150 rfus irrespective of the obtaining of replicated results or not. Also consider peaks above 50 rfus.

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Table 1
Relation of the number of markers taken into account to establish the consensus profile by the different criteria. In the last column on the right it is possible to see the average number of markers considered in the consensus profile following each criterion, and also the probability of finding of allelic drop-out and drop-in phenomena.

	Archaeological sites																																							
		A				B								C						D				E					F											
	Values\individual	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	Average/ probability
Criterion 1	Number of markers	14	6	4	15	9	2	3	14	1	0	0	1	2	5	0	2	0	2	2	17	0	5	4	18	1	4	1	1	3	2	0	0	1	4	0	0	0	0	0	3,405
	Possible allelic drop-out	3	0	0	2	6	0	1	3	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0,59(59%)
	Possible allelic drop-in	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,051(0,51%)
Criterion 2	Number of markers	16	10	8	15	9	2	5	15	3	2	0	1	2	5	0	2	0	2	3	18	1	7	4	18	1	6	2	3	3	2	0	1	3	4	0	0	0	0	3	4,190
	Possible allelic drop-out	3	1	0	1	4	0	1	3	1	0	0	0	0	0	0	0	0	0	1	2	0	0	1	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0,54 (54%)
	Possible allelic drop-in	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,051 (0,51%)
Criterion 3	Number of markers	18	11	11	16	12	3	6	15	5	2	0	4	4	8	0	2	0	3	3	18	1	9	10	19	1	8	3	6	3	3	0	2	3	6	1	1	2	0	4	5,310
	Possible allelic drop-out	1	0	1	0	2	0	0	2	0	0	0	0	0	1	0	0	0	0	1	0	0	0	5	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0,44(44%)
	Possible allelic drop-in	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0,13 (13%)
Criterion 4	Number of markers	19	13	10	17	12	5	10	17	6	4	1	3	4	12	1	3	1	9	5	19	3	11	10	19	2	9	5	5	4	2	4	3	3	4	1	0	0	3	3	6,238
	Possible allelic drop-out	1	2	1	1	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	2	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0,36 (36%)
	Possible allelic drop-in	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0,21(21%)
Criterion 5	Number of markers	19	13	12	17	14	6	10	17	7	4	1	6	6	13	1	3	1	9	6	19	3	12	11	19	2	11	6	7	4	3	4	3	3	6	2	1	2	3	4	6,905
	Possible allelic drop-out	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,026 (0,26%)
	Possible allelic drop-in	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0,28(28%)

Table 2
Relation of kinships analyzed among the different individuals from each burial. In the first column it is possible to see the kind of relationship and the individuals involved. Second, third, fourth, fifth and sixth columns show the different LRs obtained for each kinship depending on the criterion selected. Also, at the three last columns it is possible to see the number of common alleles considered in each kinship analysis and the compatibility of the relationship with the mtDNA haplotype of the individuals.

Kind of kinship (between individuals)	LR obtained by criterion 1	LR obtained by criterion 2	LR obtained by criterion 3	LR obtained by criterion 4	LR obtained by criterion 5	Common alleles	Same mtDNA	mtDNA incompatibility
Brotherhood (1–3)	6,06725	543,692	26472,7	326,443	688,911	10	Yes	No
Maternity (1–3)	3,91	47,54	0	1427,47	14284,71	10	Yes	No
Same individual (5–10)	0	107682	3931,61	26,052	26,052	2	No	Yes
Brotherhood (5–10)	0	20547,2	61,6714	51,5868	51,5868	2	No	Yes
Brotherhood (10–12)	0	0	0	25830,9	25830,9	2	No	Yes
Same individual (5–6)	0	0	0	6307,81	13459,062	3	No	Yes
Same individual (20–22)	68,97	82575,5	24361,8	$5{,}9106e^{+010}$	$1{,}74379e^{+010}$	8	Yes	No
Brotherhood (20–22)	299,805	11146,5	53560,5	$2{,}72003e^{+006}$	$2{,}26805e^{+007}$	10	Yes	No
Same individual (31–36)	0	0	0	0	107682	1	*	*
Brotherhood (31–36)	0	0	0	0	20547,2	1	*	*
Same individual	0	0	107682	0	4748,9	1	*	*
Brotherhood	0	0	20547,2	0	71,9214	1	*	*
LR average	31,563	11158,5	19714,123	$4{,}92573e^{+09}$	$1{,}45506e^{+09}$			
False kinship	0 cases	2 cases	0 cases	1 cases	2 cases			

In all criteria, peaks (100, 75 or 50 rfus) had to show the same result at least in two amplifications. The lowest height taken into account was 50 rfus because this is the lowest value considered by the great majority of laboratories [4]. The highest height contemplated was 150 rfu, because many laboratories consider that any height above 150rfu is free of LTD phenomena [4].

To evaluate the effect of each criterion on kinship analysis, we have established familiar relationships using *Familias* 3.0 software.

3. Results

The global results of using each criteria are resumed in Table 1. In Table 2 it is possible to visualize different kinship analysis results obtained using each criteria. Also in this table it has been included the compatibility of the kinship with mtDNA results.

4. Discussion

We tried to establish which of the five purposed criteria was the best, considering that it should provide more quantity of information, without generating errors.

We can appreciate that the number of markers considered for the consensus profile increases as the criterion is less restrictive (being the criterion 1 the most restrictive and the criterion 5 the least) (Table 1). Furthermore, this criterion displays also the lower probability of including allelic drop-out phenomena. Therefore, it is possible to consider that criterion 5 is a good option, because provides more complete profiles without providing allelic drop-out. This criterion has, however, the highest level of allelic drop-in probability.

If we analyse the direct consequences of choosing each one of the criteria for establishing familiar relationships, it is possible to appreciate that the average LR increases as the criterion is less restrictive (lower LR values in criterion 1 and higher values in criterion 5) (Table 2). Nevertheless there are some exceptions, maybe because of the absence of peaks above 150 rfu (Table 2).

On one hand, the first criterion is too restrictive, and in many cases it hides kinships which appear when using another criterion more permissive. Something similar occurs in the case of criterion 2. On the other hand, the third criterion allows the appearing of relationships that were masked when we used first and second one, without generating false positives. The LR average increases when applying the fourth and fifth criteria, being relevant for example in the case of the maternity between individuals 1 and 3. Nevertheless, criteria 4 and 5 also increase the false positive risk, so that the exclusive use of criteria 4 and 5 without taking into account other factors, like the number of alleles, or mtDNA, could provide wrong results. In fact, we observe the cases highlighted on Table 2, where there are many false kinships.

Finally it is possible to conclude that criterion 3 is the best selection, because it does not hide familiar relationships like criteria 1 and 2, and do not generates false positives as far as we have observed. On the other hand, it is possible to use criteria 4 and 5 but maximizing precautions, comparing with criterion 3 and taking into account (1) the number of markers considered and (2) the possibility of analysing other type of genetic markers.

Conflict of interest

None.

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