tion reaction combined with further improvements in labeling and flow cytometric analysis may allow the rapid detection and quantification in interphase nuclei of individual chromosomes with chromosome-specific DNA sequence probes (*25–27*), amplified genes, or viral sequences.

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20. *Nuclei isolation and dimethylsuberimidate (DMS) treatment*: Nuclei were isolated from the thymocytes of 6- to 8-week-old BC3 female mice as described (*15–17*). For DMS treatment, K_2CO_3 and DMS were added (from a 5× concentrated stock solution, mixed immediately before use) to a suspension containing 5×10^6 nuclei/ml. Final K_2CO_3 and DMS concentrations were 20 m*M* and 3 m*M*, respectively. After 15 minutes at 25°C, the *p*H was adjusted from 10 to 8.0 by the addition of 50 μl of 100 m*M* citric acid per milliliter of suspension. Nuclei were washed once by centrifugation (300*g*, 10 minutes, 4°C) in IB (50 m*M* KCl and 5 m*M* Hepes buffer, at *p*H 8.0) containing 10 m*M* $MgSO_4$ (IB + M). *In situ hybridization in suspension*: To 2×10^4 DMS-treated nuclei [130 ng total DNA (*28*)] suspended in 20 μl IB in 1.5 ml Eppendorf tubes, the following solutions were added: 28 μl 100 percent formamide, 5.6 μl 20× SSC, 2 μl of a solution of salmon sperm DNA (10 mg/ml), and 1 μl AAF-modified DNA probe in 10 m*M* tris-HCl, 1 m*M* EDTA, at *p*H 7.6. The stock solutions of AAF-modified mouse satellite DNA isolated from liver nuclei (*9*) and of AAF-modified human DNA isolated from placenta contained 265 ng and 250 ng DNA/μl, respectively, resulting in a probe to complementary sequence ratio of 20:1. The mixtures of nuclei and probe were denatured by incubation at 70°C for 10 minutes. Hybridization was carried out at 37°C for 16 hours. Nuclei were washed by the addition of 1.0 ml hybridization buffer (50 percent formamide, 2× SSC, and 20 m*M* KCl, in 2 m*M* Hepes buffer) at 25°C and 100 μl DMS-treated mouse erythrocytes (RBC, see below). After 15 minutes agitation at 25°C, the nuclei were centrifuged for 2 minutes (8000*g*). The pellet was resuspended in 1.5 ml hybridization buffer by repeated syringing through a 23-gauge needle on a 1-ml syringe, centrifuged, and washed in 1.5 ml of IB + M. *Immunofluorescent labeling*: After hybridization, the nuclei were incubated for 60 minutes at 25°C in 200 μl of IB + M containing 2 percent normal goat serum (NGS; Nordic, Tilburg, The Netherlands) and a 1:500 dilution of a rabbit anti-AAF (*21*). The nuclei were washed once in IB + M and then incubated 60 minutes at 25°C in 200 μl of IB + M containing 2 percent NGS and a 1:50 dilution of a TRITC-conjugated goat anti-rabbit IgG (Nordic). The nuclei were washed once and then resuspended in 1.0 ml of IB + M by syringing five times. They were then filtered through 50-μm nylon mesh and stained with Hoechst 33258 (2 μg/ml). *DMS-treated erythrocytes*: Blood was collected into heparin by cardiac puncture from BC3 female mice. Washed erythrocytes (RBC), from which serum and white blood cells were removed by centrifugation, were suspended in physiological salt at a concentration of 10^8 per milliliter and treated three times with DMS. Final DMS concentrations during each treatment were 3 m*M*, 10 m*M*, and 10 m*M*. Additional adjustment of the *p*H to 9 to 10 with 100 m*M* K_2CO_3 was required during the last two treatments. The RBC were washed once in IB and resuspended in IB at a concentration of 10^8/ml. *Dual beam flow cytometry*: A FACS II flow cytometer modified for dual beam excitation, in which the fluorescent signals from the two beam spots are spatially and chromatically separated, was used. The nuclei and RBC were first illuminated by 300-mW UV light (multiline 351–364 from Spectra Physics laser model 171, Mountain View, Calif.) for Hoechst 33258 excitation. Hoechst fluorescence was measured through a KV418 (Schott) filter. Electronic gates were set to select single nuclei and exclude RBC, clumps, and debris from further analysis. The second laser (Coherent model CR 6) produced 100-mW light with a wavelength of 515 nm for TRITC excitation. The TRITC fluorescence of each particle identified as a nucleus by its Hoechst fluorescence was collected through a KV 580 (Schott) filter.
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A Human Y-Linked DNA Polymorphism and its Potential for Estimating Genetic and Evolutionary Distance

Abstract. *A human DNA sequence ($p12f_2$), derived from a partial Y-chromosome genomic library and showing homology with the X and Y chromosomes and with an undetermined number of autosomes, detected two Y-specific restriction fragment length variants on male DNA that had been digested with Taq I and Eco RI. These variants may have been generated through a deletion-insertion mechanism and their pattern of holoandric transmission indicates that they represent a two-allele Y-linked polymorphism (RFLP). By means of DNA from patients with inborn deletions in chromosome Y, this polymorphic DNA site was mapped to the interval Yq11.1–Yq11.22. The frequency of the rarest allele was about 35 percent in Algerian and Sardinian human males, whereas it was only 4 percent among Northern Europeans. The $p12f_2$ probe also detected Y-specific DNA fragments in the gorilla and chimpanzee. In view of the monosomy of the Y chromosome in mammalian species, Y-linked RFLP's may prove to be more useful than autosomal or X-linked markers in estimating genetic distances within and between species.*

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The application of restriction enzyme analysis and DNA recombinant technology to the study of human variation has uncovered a new class of genetic markers that are distributed among families and populations according to the rules of Mendelian inheritance [restriction fragment length polymorphisms (RFLP's)]. A total of 152 human RFLP's have been reported, 42 of which were detected with cloned genes and 110 with random nonrepeated genomic DNA sequences (*1*). This type of genetic variation has been found throughout the human genome with the exception of the Y chromosome (*2*). The recent construction of DNA libraries from the Y chromosome in mouse (*3*) and human (*4*) systems has facilitated the search for Y-linked RFLP's in these species. The detection of strain-specific restriction fragment variants by means of a Y-derived probe has been reported in the mouse (*5*). Our report describes two human Y-linked RFLP's and stresses their potential in studies of human population genetics and mammalian evolution.

The genomic human Y-DNA probe $p12f_2$ is a derivative of a p12f genomic probe that was previously isolated from a partial human Y-DNA library (*4*). Digestion of the p12f clone with Bgl II gave rise to a 2.3-kilobase (kb) fragment (12f2), which was isolated from 1 per-

cent agarose gels and subcloned into a Bam HI site of pBR322. To search for Y-linked RFLP's, the probe ($p12f_2$) and the isolated fragment (12f2) were labeled with ^{32}P to a specific activity greater than 10^8 count/min per microgram of DNA by nick translation (*6*). In order to confirm that the probe was derived from the Y chromosome and to determine the subregional location of the corresponding genomic site, we hybridized the probe to Eco RI–digested DNA from normal males or females as well as from individuals (or cell lines) with sex-chromosome abnormalities. These included fibroblastic or lymphoid cells derived from patients with numerical aberrations of the sex chromosomes (*7*) or exhibiting a deletion along the short or long arm of the Y chromosome (*8*).

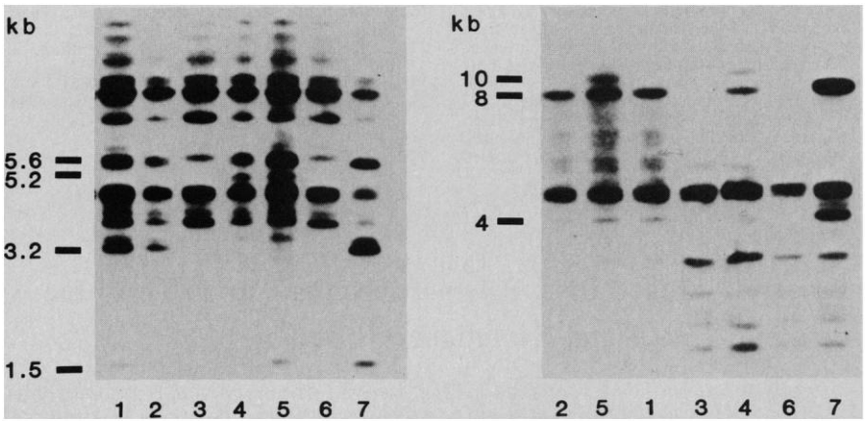
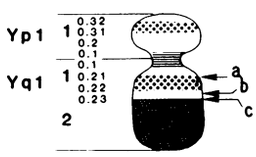


Fig. 1. Eco RI (left) and Taq I (right) patterns detected by the probe $p12f_2$ in DNA from normal human males and females and from individuals with numerical aberrations of the sex chromosomes (49XYYYY). The extraction of DNA from peripheral blood cells and from freshly harvested cultured cells was as previously described (*21*). DNA fragments after restriction endonuclease digestion was separated by electrophoresis on 0.8 percent agarose gels in a buffer containing 40 m*M* tris-acetate and 1 m*M* EDTA. The DNA was then depurinated in 0.3*M* HCl for 7 minutes at 25°C, denatured twice in 0.5*M* NaOH plus 0.5*M* NaCl for 15 minutes, and neutralized twice with 1*M* tris-HCl, *p*H 7.4, plus 3*M* NaCl for 15 minutes each time. The DNA was transferred to a Zetapor membrane by passive diffusion (*22*) in 20× standard saline citrate (SSC) and, prior to the hybridization step, the filters were incubated for at least 4 hours at 42°C in a solution of 50 percent formamide, 5× SSC, 2× Denhardt's solution, 50 m*M* phosphate buffer (*p*H 7.0), 0.5 percent sodium dodecyl sulfate (SDS), and denatured salmon sperm DNA (100 ug/ml). The washings were performed at stringent conditions twice for 30 minutes at 65°C in 2× SSC plus 0.1 percent SDS and once for 30 minutes at 65°C in 0.1× SSC plus 0.1 percent SDS. The filters, still wet, were exposed to autoradiographic films at −80°C in the presence of an intensifying screen. (Lanes 1, 2, 4, and 5) Normal male, 46 XY; (lanes 3 and 6) normal female, 46XX; and (lanes 7) male with four Y chromosomes, 49XYYYY.



Sex complement and phenotype of cell donors	Eco RI fragments retained (kb)		
	5.6	5.2	1.5
46XY M	+	+	+
46XX F	−	−	−
46XYp− F	+	+	+
46XYq− M q11-21 breakpoint (a)	−	−	−
46XYq− M breakpoint(b) up to q11-23	+	+	+
46XYq− M q11-23 breakpoint(c)	+	+	+

Fig. 2. Description of the Y-deleted human cell lines used for the subregional localization of the Y-specific DNA sequences homologous to probe $p12f_2$ and assignment of the DNA sequences detected by the probe to the interval Yq11.1–Yq11.22. The sketch of the Y chromosome indicates the relative position of the two faint bands (coarse stippling) and of the strong fluorescent band (intense stippling) seen after quinacrine staining of prometaphase chromosome preparations [Magenis *et al.* (*8*)].

Under conditions of high-stringency washing, $p12f_2$ detected several Eco RI and Taq I fragments on DNA digests prepared from normal unrelated individuals of either sex (Fig 1). Some of these fragments were Y-specific as indicated by their complete or partial absence in female DNA and the positive correlation between fragment intensity and the number of Y chromosomes present in the male DNA preparations. This dosage effect was seen for the 5.6- and 1.5-kb Eco RI fragments, which were present in every male DNA tested; it also occurred with the 5.2- and 3.2-kb fragments, whose presence was mutually exclusive (Fig. 1, left). Thus, the most common Eco RI patterns (or haplotypes) detected by probe $p12f_2$ were 5.6/5.2/1.5 kb and 5.6/3.2/1.5 kb. For the conditions of stringency used in these experiments (Fig. 1), the 3.2 and 1.5-kb Eco RI fragments were absent in females. The 5.6-kb fragment was fainter in the females and disappeared almost completely when the washing was done at a slightly higher stringency.

On Taq I–digested DNA, the Y chromosome–dependent dosage effect was seen for the fragments of 10, 8, and 4 kb; however, the 10-kb fragment was absent in a variable proportion of males and its absence was associated with a twofold increase in the intensity of the 8-kb fragment (Fig. 1, right). This additional type of dosage effect—confirmed by densitometric studies—indicates that the most common patterns identified by probe $p12f_2$ after Taq I digestion were 10/8/4 kb and 8/8/4 kb. The Eco RI pattern 5.6/5.2/1.5 kb corresponded in 21 out of 21 cases to the Taq I pattern 10/8/4 kb; the Eco RI pattern 5.6/3.2/1.5 kb corresponded to the Taq I pattern 8/8/4 kb in eight out of eight cases. The χ^2 value for deviation from independence was 40.1 with 1 df ($P < 0.001$). This suggests that the size variations of the Y-specific Eco RI and Taq I DNA fragments detected by the probe $p12f_2$ were generated by the same event, probably through an insertion-deletion mechanism involving a 2-kb DNA sequence.

The hybridization of the $p12f_2$ probe to Eco RI–digested DNA derived from subjects bearing different types of chromosome Y deletions indicates that all tested Y-specific DNA fragments map to the region included between Yq11.1 and Yq11.22 (Fig. 2). Other fragments detected by the $p12f_2$ probe were probably autosomal as suggested by their presence in both sexes and the lack of a dosage effect in DNA preparations with different numbers of X or Y chromosomes.

An independent confirmation of the Y-linked inheritance of the length variants has been sought through segregation studies carried out in five pedigrees where the holoandric transmission from father to all sons occurred in 40 informative meioses. Initial population studies indicate that the distribution of this Y-linked RFLP may vary among different ethnic groups. In particular , the Eco RI haplotype 5.6/3.2/1.5 kb occurred in only 4 percent of males from metropolitan France, but in 34 to 38 percent of the males from southern Sardinia and northern Algeria (Table 1); it was not found among 15 black males of African descent.

The two invariant Eco RI fragments of 5.6 and 1.5 kb and most of the autosomal fragments detected by probe $p12f_2$ were present (Fig. 3) in Eco RI–digested DNA from four male chimpanzees and one male gorilla, thus suggesting the conservation of these sequences among primates.

The existence of an extensive DNA sequence homology among Y, X, and other human chromosomes is well established (*9*). The dispersion of homologous DNA sequences throughout the human genome might be the result of small translocation events after nonhomologous chromosomal interchanges, possibly mediated by transposable elements or retroviruses (*9*). In particular, there seem to be two kinds of DNA homology between the Y and X mammalian chromosomes. One is exemplified by the existence of homologous genes in the pairing chromosomal regions at the tip of the short arms of the human X and Y chromosomes (*10*) and at the distal termini of the murine X and Y chromosomes (*11*). The other kind of homology is probably generated by rare and erratic events involving nonpairing chromosomal regions that lead, in the absence of lethality, to the one-way transfer of DNA sequences between the sex chromosomes or between these and the autosomes. The Y/X/autosomal homology detected by our probe $p12f_2$ is an example of the latter category; the Y-RFLP found among human males is probably due to the occurrence of a relatively recent (in evolutionary terms) insertion of a 2-kb DNA fragment into a Y-specific DNA site.

With the exception of the loci controlling the expression of two cell surface antigens (*10, 12*), the human Y chromosome is apparently devoid of regularly expressed genes (*13*) and, above all, of polymorphic genetic markers (*2*). Thus, in spite of its homology with autosomal DNA sequences, the Y-chromosome–derived probe $p12f_2$ should be useful for population and evolution studies and also for detecting interstitial deletions in the proximal portion of the human Y chromosome long arm.

Table 1. Distribution of the Eco RI and Taq I Y-specific RFLP's in random samples of human male DNA representative of the populations of metropolitan France (F), northern Algeria (A), and southern Sardinia (S).

Population	Distribution* of RFLP's	
	5.6/5.2/1.5	5.6/3.2/1.5
F	25(18.9)	1(7.1)
A	21(24.8)	13(9.2)
S	21(23.3)	11(8.7)

*The distribution is shown as the number observed (number expected in a random distribution). The χ^2 values (1 df) for F versus A, F versus S, and A versus S were 9.86 ($P < 0.01$), 8.22 ($P < 0.01$), and 1.00 ($P > 0.3$), respectively. For the overall distribution, χ^2 (2 df) was 9.20 ($P \simeq 0.01$).

The distribution of the Y-linked RFLP's suggests a close resemblance between Sardinians and Algerians. This finding is at variance with previous reports that showed no resemblance on the basis of the distribution of classical examples of genetic polymorphisms (*14*). The similarity between males of southern Sardinia and northern Algeria agrees with historic and archeological data that document the presence over long periods of time of Phoenicians and Carthaginians along the coastal settlements of southern Sardinia prior to the Roman colonization. The extension of these studies to the rest of the island population is expected to contribute information on the relative role played by migration and selection on the genetic structure of the now existing Sardinian isolates (*15*).

The Y chromosome exists in a state of perpetual monosomy and appears to be normally excluded from regular recombination [with the possible exception of its short-arm telomeric region (*10, 16*)]. Thus, the genetic distances between human isolates or populations may be more accurately measurable in terms of their specific Y haplotype distributions as soon as new examples of Y-linked RFLP's are discovered in the same and other subregions of the Y chromosome (*17*). Studies on the Y-RFLP distribution within and between species should complement the estimation of genetic distances in terms of mitochondrial DNA polymorphisms (*18*). Extensive analysis supports the contention that all human ethnic groups were derived from the same ancestor population, which contained three fundamental mitochondrial DNA types (*19*). Moreover, a new evolutionary tree, based on mitochondrial DNA genetics, suggests that *Homo sapiens* appeared before the split of chimpanzee from gorilla (*20*). Comparisons among measurements of intraspecies and interspecies distances based on Y-DNA genetics, on mitochondrial data, on data from traditional genetics, and on DNA or protein sequencing will provide an opportunity for assessing the relative value of these various types of molecular data for studies of evolution.

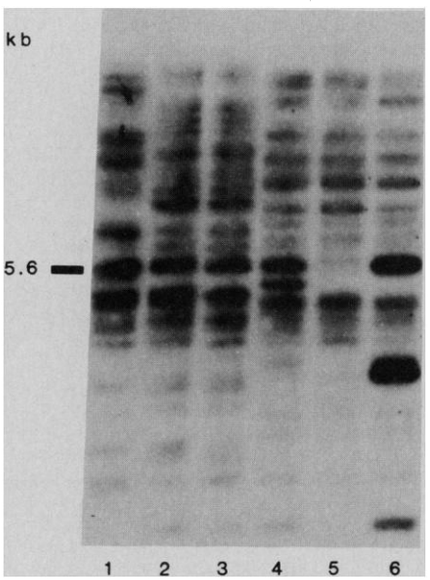


Fig. 3. Comparison of the Eco RI pattern detected by probe $p12f_2$ in human, gorilla, and chimpanzee DNA. (Lane 1) Male gorilla; (lanes 2 and 3) male chimpanzee; (lane 4) normal male human; (lane 5) female human; and (lane 6) male human, 49XYYYY.

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Rapid Mutations in Mice?

Fitch and Atchley (*1*) analyzed genetic variation among inbred mouse strains and concluded that an extraordinarily increased mutation rate occurred. They hypothesized that early in the derivation of the lines there was selection for heterozygosity or increased mutation and concluded that classical population genetic theory cannot explain their data. It may be more simply and realistically concluded that inbred mice do not have extraordinarily high mutation rates for the reasons (i) that a biased sample of loci was used to establish the model for high rates, (ii) that an unbalanced comparison of inbred strains and natural populations was employed, and (iii) that appropriate consideration was not given to mutation rates determined by direct observation.

Fitch and Atchley's model (*1*) is based on the assumption that the loci they compared among inbreds represent a random sample of the mouse genome. Thus, the loci that they did not consider are presumed to vary in frequency and pattern in accordance with loci for which they present data. By their own calculations 300 or more invariant loci among the strains examined would invalidate their model. Several direct analyses of mouse strains by two-dimensional (2-D) electrophoresis indicate that the number of nonvariable proteins among inbreds may easily exceed 300 (*2*). The loci encoding these proteins were not included in the analysis (*1*). Furthermore, the data used by Fitch and Atchley are from a list of loci polymorphic among inbred strains (*3*); it does not contain monomorphic loci. Use of this list (*3*) to compare variation among strains will therefore show a high proportion of loci contributing to interstrain differences, but the proportion will be uninformative in terms of the mouse genome.

The most recent list of mouse loci (*4*), contains 47 reserved gene symbols indicating genetic variation known in man or other organisms but not yet documented in inbred mouse strains. Because there is generally great interest in mouse models it is unlikely that mouse strains have not been examined for a great many more specific loci than those reported to vary among the strains. Understandably, there has simply not been much incentive to maintain a formal documented list of monomorphic loci among inbred mice.

Another basis for the model of Fitch and Atchley is the statement that inbred strains are more variable than wild populations; however, they do not restrict their analysis of variation among inbreds to the same loci that have been studied in wild mice. The bias introduced into the analysis by comparing variabilities between different sets of loci is exemplified by the *H-2* complex, which was included in Fitch and Atchley's data for inbred strain but was not included in the studies cited by Fitch and Atchley for wild mice. The ten strains analyzed by Fitch and Atchley display five different *H-2* haplotypes, and divergence appears among the related C57BL/6, C57BR, and C58 strains as well as between the closely related DBA/1 and DBA/2 strains. This is a greater amount of variability than shown by typical allozymic loci. However, the same *H-2* loci have recently been typed in wild mice (*5*), and these mice also show an extremely high level of polymorphism. The average frequency of a given haplotype within a population was only 0.025 and more than 90 percent of the animals tested were heterozygous at *H-2*.

Similarly, restricting the comparison to loci other than *H-2*, and for which there are corresponding data from wild or ancestral populations and inbreds (Table 1), shows no evidence for an increase in the number of alleles, heterozygosity, or percent polymorphism in the inbreds (*3*, *6*). Thus, the inbreds contain homozygous subsets of the same alleles found in nature, just as expected from ordinary sampling and inbreeding. Small samples from nature with subsequent inbreeding sufficiently explain the high level of apparent parallel (or back) substitution observed by Fitch and Atchley in their phylogenetic analyses of the data (51.7 percent or 71 out of 145 substitutions).

Electrophoretic techniques by which the biochemical variants of inbred mouse strains are recognized have been used for the direct measurement of mutation rates in strains C57BL/6J and DBA/2J

Table 1. Allelic variants at polymorphic loci in both inbred strains and wild populations of *Mus musculus*. Inbred strain data are from Staats (*3*). Data for wild populations are given in (*6*). Total number of alleles in inbred strains, 33; total number of alleles in wild populations, 52; ND, no data available.

Protein locus	Inbred strains analyzed by Fitch and Atchley										Wild mice
	A/HE	AKR	BALB/c	CBA	C3H	C57BL	C57BR	C58	DBA/1	DBA/2	
Akp-1	b	b	b	b	b	a	a	a	a	a	a, b
Es-1	b	b	b	b	b	a	a	b	b	b	a, b, c
Es-2	b	b	b	b	b	b	b	b	b	b	a, b, c, d
Es-3	c	c	a	c	c	a	a	a	c	c	a, b, c, d
Es-5	b	b	b	b	b	b	b	b	b	b	a, b
Es-6	a	a	a	a	a	a	a	a	ND	a	a, b
Gpi-1	a	a	a	ND	b	b	ND	a	ND	a	a, b
Got-1	a	a	a	a	a	a	a	a	ND	a	a, b
Got-2	a	a	a	a	a	a	a	a	ND	a	a, b
Gpt-1	a	a	a	a	a	a	a	a	a	a	a, b, c
Gr-1	a	a	a	a	a	a	a	ND	ND	a	a, b
Hbb	d	d	d	d	d	s	s	s	d	d	s, d, p
Idh-1	a	b	a	b	a	a	b	a	b	b	a, b, c
Ldr-1	a	a	a	a	a	a	a	a	a	a	a, b
Mod-1	a	b	a	b	a	b	b	b	a	a	a, b
Mpi-1	b	b	b	b	ND	a	b	ND	ND	b	a, b
Np-1	a	a	a	a	a	a	ND	ND	a	a	a, b
Pgd-1	a	a	a	a	a	a	a	a	a	a	a, b
Pgm-1	a	a	a	a	b	a	a	a	b	b	a, b
Pgm-2	a	a	a	a	a	a	a	a	ND	a	a, b
Pre-2	b	a	a	a	b	b	a	ND	ND	a	a, b
Tsf	b	b	b	a	b	b	b	b	b	b	a, b