Volume 276, number 1,2, 138–142 FEBS 09203 December 1990

Human tRNA^{Glu} genes: their copy number and organisation

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Received 28 September 1990

The tRNA^{Glu} gene copy number, determined by genomic blot analysis of human placental DNA, is approximately thirteen. These studies, using several probes and DNA digested with several restriction enzymes singly or in combination, show that most of these tRNA^{Glu} genes are flanked by DNA of very similar sequence for at least 5 kb. This conclusion is supported by the close similarity of the restriction maps of two λ Charon-4A recombinants of human genomic DNA containing two different tRNA^{Glu} genes.

Recombinant DNA; tRNA^{Glu} gene; λ Charon4A; Copy number; Genomic blot; Multigene family

1. INTRODUCTION

There are approximately 1200 tRNA genes and pseudogenes per human haploid complement [1], so that the 60 different tRNAs are each represented by 10 to 20 genes. The organisation of these genes in this superfamily varies at 3 levels. At the chromosome level, genes for the same tRNA often appear to be on the same chromosome [2–4] but some are scattered [4]; with respect to their proximity to other tRNA genes, some occur as 'solitary' genes while others are clustered with genes for other tRNAs; finally, the immediate flanking sequences of different genes for the same tRNA are sometimes highly homologous but sometimes unrelated [6,7]. Although about 30 tRNA genes have been characterized so far [5–9], relatively little is known about the organisation of copies of genes for the same acceptor human tRNA family – the 12 tRNA$_i^{Met}$ genes are widely scattered in the genome [6] and tRNA^{Val} genes, which show no homology in their flanking sequences [7] and occur as dispersed (non-tandem) repeats.

Two recombinants, selected from a human foetal liver DNA library by screening with a mixed human placental [^{32}P]tRNA probe [8], were each found to contain a single tRNA^{Glu} gene and also showed a high sequence homology for at least 1 kb of flanking sequence (Gonos and Goddard, submitted). We have therefore mapped the two recombinants and used a tRNA^{Glu} gene specific probe in genomic blot analysis to investigate whether this similarity of sequence environment for two tRNA^{Glu} genes was a common feature for all the tRNA^{Glu} genes and whether it extended far beyond the sequenced regions.

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Abbreviations: bp, base pair(s); kb, kilobases or 1000 bp; tRNA, transfer RNA; nt(s), nucleotides; p, plasmid; SDS, sodium dodecyl sulphate

2. MATERIALS AND METHODS

λht137 and λht190, each containing a single copy of a tRNA^{Glu} gene within a 2.4 kb *Hin*dIII fragment, were isolated as previously described [8] from a bank of fragments of human foetal liver DNA (12–20 kb) cloned in λ Charon 4A[10], the gift of Dr T. Maniatis. pLB4 is a pAT153 recombinant of the above 2.4 kb *Hin*dIII fragment from λht137. The *Hae*III fragment containing 74 bp of the 5′ flank and 63 bp of the tRNA^{Glu} gene (residue 282–419 of ref. [8]) was subcloned in both orientations into M13mp18 to yield MtGlu(282/419) and MtGlu(419/282). MtGlu(301/1) contains the 301 bp *Sma*I–*Sst*I fragment from the 5′ flank (−54 to −355) of the gene of pLB4 cloned in M13mp19.

The following single stranded DNA probes were prepared by primer extension [11]: (a) probe 1, containing 63 of the 71 nts of a tRNA^{Glu} gene and 74 nts of 5′-prime flanking sequence, was made by extension of M13 universal primer annealed to MtGlu(282/419) and cutting with *Eco*RI; (b) probe 2 was prepared from the same recombinant using as primer CCTGTGTGTCCCTGGTG, corresponding to residues −8 to +9 of the tRNA^{Glu} gene and cutting with *Ava*II. This contained only 63 of the 71 nts of the tRNA^{Glu} gene and some M13 vector sequence; (c) probe 3, containing only 301 nts (−54 to −355) of 5′-flanking sequence, was prepared using universal primer with MtGlu(301/1) and cutting with *Eco*RI. The λ recombinants were digested with one or two of the restriction enzymes indicated in Fig. 1 and fragment sizes determined. Alignment of fragments was based largely upon Southern [14] blot hybridisation with specific restriction fragment probes, labelled with ^{32}P [15]. All hybridisations were performed as described in Fig. 2, but omitting the last high stringency wash. The location of tRNA^{Glu} genes, using a 'periodate-treated' tRNA[^{32}P]pCp probe[8], also served to confirm the derived restriction maps.

Human genomic DNA, prepared from fresh placentae by the method of Bowtell [14], was digested with *Hin*dIII by incubation with 5 units *Hin*dIII per μg genomic DNA for 2.5 h. Under these conditions 0.1 vol. of the reaction mixture completely digested 0.5 μg λ

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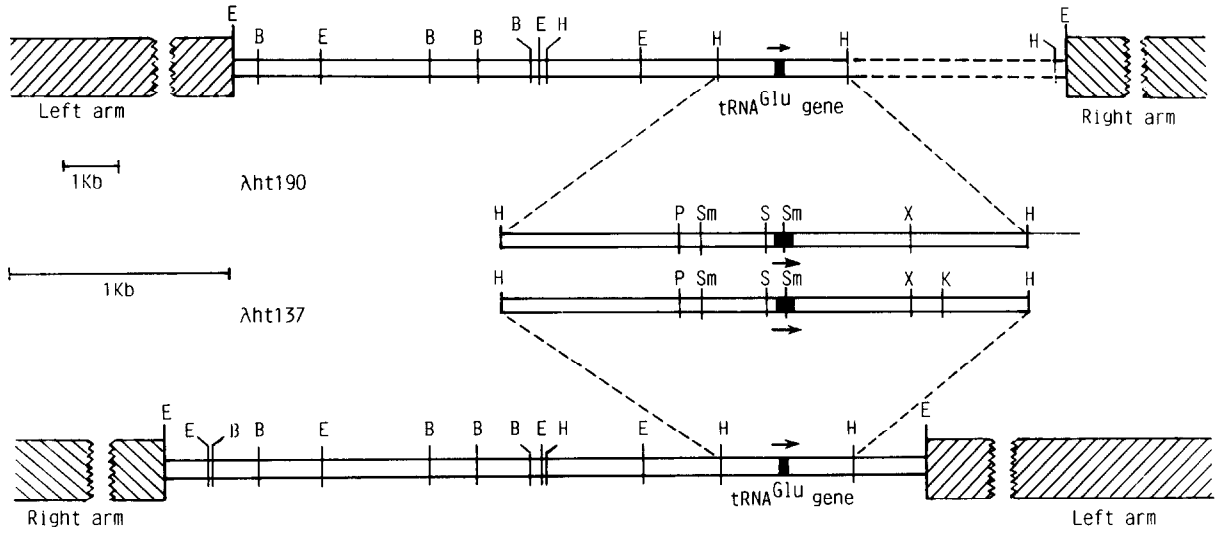

Fig. 1. Restriction maps of λht137 and λht190. The extent of homology in λht137 and λht190 and the positions of the tRNA^{Glu} gene copies are shown. The broken region of λht190 has not been mapped.

DNA. To ensure complete digestion of the genomic DNA, the incubation was repeated after addition of a further 5 units of *Hin*dIII per μg DNA. The resulting fragments were electrophoresed in parallel with known amounts of a single copy tRNA^{Glu} gene, obtained as a 2.4 kb *Hin*dIII fragment from recombinant λht 190, blotted and hybridised versus probe 1. The membrane was stripped, the experiment was repeated with probe 2 and the resulting autoradiographs were scanned.

3. RESULTS

The similarity of the restriction fragment profiles of λht137 and λht190 helped in the construction of their restriction maps, shown in Fig. 1. The two recombinants share identical restriction sites for *Eco*RI, *Bam*HI and *Hin*dIII for a region of at least 10.7 kb. To determine whether this similarity between the two fragments was typical of the tRNA^{Glu} gene family Southern blots of genomic DNA were performed. The results shown in Fig. 2a are unusual for tRNA genes in that hybridisation is confined to relatively few bands. For example the majority of the hybridisation to *Hin*dIII-digested genomic DNA is to one band, indicating that most of the copies are located on *Hin*dIII fragments of 2.4 kb and implying similarity of environment for the different gene copies. Gene copy number was therefore first estimated by measuring the extent of hybridisation of probe 1 (containing gene and immediate 5′ flanking sequence) to the genomic DNA *Hin*dIII digests relative to that of known amounts of single copy tRNA^{Glu} gene (Fig. 2b). Nine copies of the gene for tRNA^{Glu} are in 2.4 kb *Hin*dIII fragments (Fig. 2b), while one copy is in 5.5 kb *Hin*dIII fragment, two copies in 8.2 kb *Hin*dIII fragments and one is in a very large (approx. 30 kb) *Hin*dIII fragment (Fig. 2a), thus indicating that the copy number for the human tRNA^{Glu} gene family is thirteen per haploid genome complement. The same results were obtained using probe 2, which contained only 63 nucleotides of the tRNA^{Glu} gene (data not shown).

The restriction maps of two gene copies, in λht137 and λht190, resembled each other not only in the relative positions of *Hin*dIII sites but also in having common sites for several other restriction enzymes (Fig. 1), suggesting that many of the other tRNA^{Glu} gene copies might also be in very similar sequence environments. We therefore extended the studies to genomic DNA cut with one or two of seven different restriction enzymes, including *Kpn*I, *Pvu*II, or *Sst*I which are found within the 2.4 kb *Hin*dIII fragment from recombinants λht137 and λht190. The results of some of these experiments are shown in Fig. 2a,c,d. The copy number represented by each band was calculated by reference to known amounts of single-copy and tabulated (data not shown but available on request). In total 33 such estimates were made after hybridisation of probe 1, 2 or 3 to blots representing fourteen different single or double digests of genomic DNA. The estimated copy number was in the range 11–15, with a mean of 12.85 ± 1.06. One notable feature was that in every case except for *Hin*dIII the extent of hybridisation in at least two bands represented a gene copy number of 3 or more. These multiple (≥ 3) copy bands represented 7.42 ± 2.55 gene copies.

The similarity of the restriction maps of the two cloned gene copies (Fig. 1), the results from the *Hin*dIII-cut genomic DNA (Fig. 2b) and the occurrence of multiple bands with other digests (Fig. 2a,c,d) encouraged us to attempt to construct the likely disposition of restriction sites occurring within the remaining unmapped tRNA^{Glu} gene copies, shown in Fig. 3. Starting with the λht137 and λht190 maps it was clear, for example, that seven other copies share the *Hin*dIII sites (a) and (b)

and that the single copy 5.5 kb *Hin*dIII fragment would arise by loss of *Hin*dIII site (b) (copy 3, Fig. 3). Such a positively identified 'lost' site is represented by X. In several instances some uncertainties remain, particularly for regions distant from the gene and the probe sequences used. Only those sites which may be positively inferred from the data are shown so that the omission of a site from any of the copy maps (e.g. *Sst*I site (d) in copies 2, 4, 6–9) does not imply its absence unless it is denoted by X.

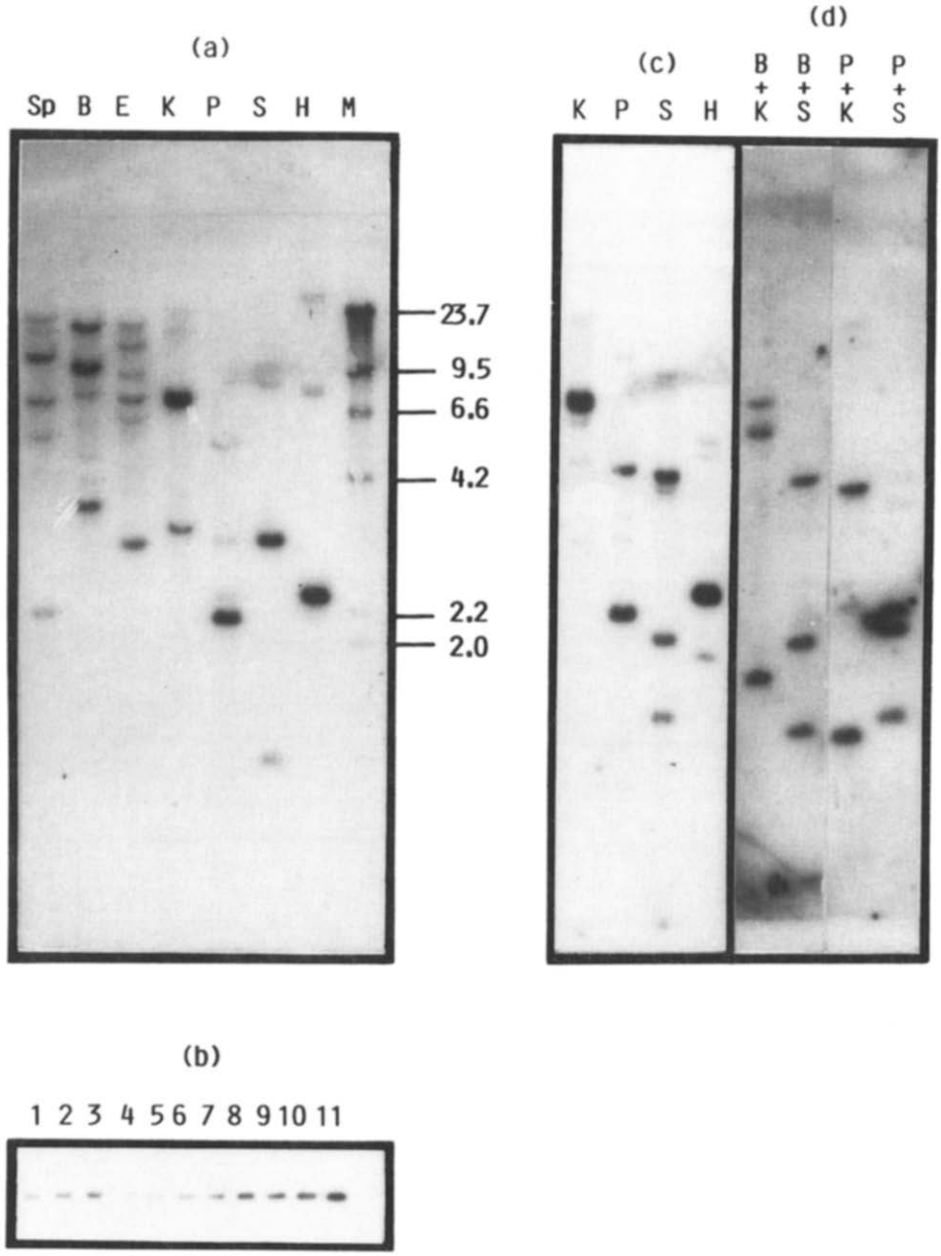

Fig. 2. (a) Hybridisation of probe 1 to *Sph*I (Sp), *Bam*HI (B), *Eco*RI (E), *Kpn*I (K), *Pvu*II (P), *Sst*I (S) and *Hin*dIII (H) fragments of human genomic DNA (2 μg). After pre-hybridization (6 h; 42°C) in 0.2% polyvinyl-pyrrolidone; 0.2% ficoll; 0.2% bovine serum albumin; 50 mM Tris-HCl, pH 7.5; 1 M NaCl; 0.1% sodium pyrophosphate; 1% SDS; 10% dextran sulphate; 50% deionised formamide; 1 mg denatured salmon sperm DNA and hybridisation in pre-hybridisation buffer containing 20 ng probe 1 (see section 2) at 42°C for 16 h, the membrane was washed (2 × 100 ml of 2 × SSC for 5 min room temperature; 2 × 200 ml of 2 × SSC and 1% SDS for 30 min at 65°C; 2 × 200 ml of 0.1 × SSC and 1% SDS for 30 min at 68°C) and autoradiographed on preflashed Fuji RX film for 7 days at −70°C in the presence of two intensifying screens. Densitometric analysis of autoradiographs was performed using a Laser densitometer (220$\frac{2}{0}$ Ultrascan, LKB). (b) The autoradiograph of the 2.4 kb band following hybridisation of probe 1 (see section 2) to three different amounts of human genomic DNA digested with *Hin*dIII (1 = 1 μg, 2 = 1.3 μg, 3 = 2 μg) electrophoresed in parallel with 8 different amounts of pTC51 (4 = 10 pg, 4 = 15 pg, 6 = 20 pg, 7 = 25 pg, 8 = 30 pg, 9 = 35 pg, 10 = 40 pg and 11 = 50 pg) digested with *Hin*dIII in the presence of 0.1–0.5 μg wild-type λ DNA 'carrier'. (c) Hybridisation of probe 3 to *Kpn*I, *Pvu*II, *Sst*I and *Hin*dIII, *Bam*HI + *Kpn*I (B + K), *Bam*HI + *Sst*I (B + S), *Pvu*II + *Kpn*I (P + K) and *Sst*I + *Kpn*I (S + K) fragments of human genomic DNA. The radiolabelled marker (M = λ DNA/*Hin*dIII) fragment sizes are indicated.

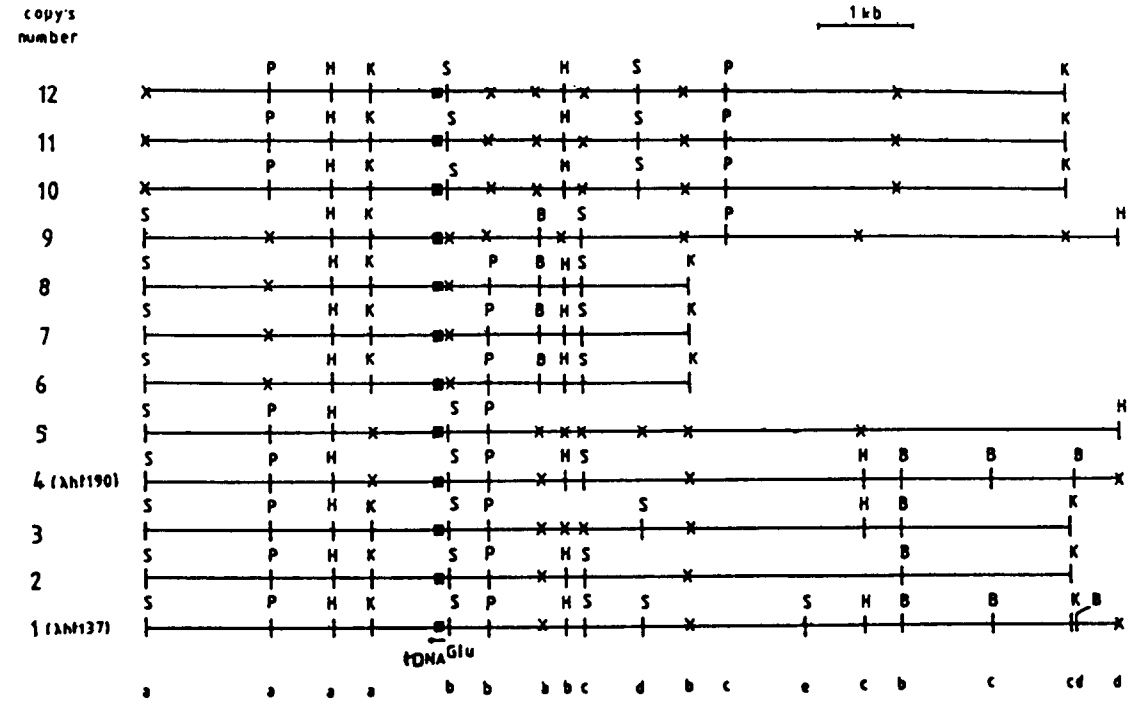

Fig. 3. Potential restriction maps of 12 human genomic DNA fragments (for sites H = *Hin*dIII, S = *Sst*I, K = *Kpn*I, P = *Pvu*II, B = *Bam*HI) containing $tRNA^{Glu}$ genes are shown. Positively identified 'lost' sites are represented by X. The postition and orientation of the $tRNA^{Glu}$ genes 1 to 12 are indicated. Multiple sites identified for the same restriction enzyme are identified (a), (b), (c) etc.

4. DISCUSSION

The calculated copy number of thirteen $tRNA^{Glu}$ genes per human haploid complement is remarkable only for its close similarity to those determined for other human tRNA genes - approximately 12 for $tRNA_i^{Met}$ [6], at least 13 for $tRNA^{Val}$ [7], at least 12 for $tRNA^{Tyr}$ [16] and approximately 14 for $tRNA_3^{Lys}$ [17]. Thus with the exception of the single copy gene and pseudogene for the suppressor phosphoserine tRNA [9,18] and a recent estimate of approximately 60 genes for $tRNA^{Asn}$ [3], estimates of the gene copy number of specific tRNAs are remarkably similar and suggestive of a general constancy of gene copy number for different tRNAs.

Fig. 3 shows the most likely disposition of restriction sites occurring within the remaining unmapped $tRNA^{Glu}$ gene copies. While not complete in every detail, they permit some general conclusions which are unlikely to be changed in subsequent refinement. The 5′ flanking sequence probe 3 gave very similar hybridisation results to probe 2, containing only the gene. Common hybridising bands represented 10.10 ± 1.60 of the 13 gene copies, suggesting that most of the copies have a similar flanking sequence for at least 350 bp upstream of the gene. Inspection of Fig. 3 suggests that copies share common restriction sites with each other over a much larger region, which is consistent with high sequence homology between copies in that region. If each copy shares 97% sequence homology with any other copy, as found for 1.1 kb in λht137 and λht190 (Gonos and Goddard, submitted), then one would expect each copy to share on average 4 of 5 common 6 bp recognition sites or 80% of the copies to share a common restriction site. A situation approximating to this is found for copies 1–12 in Fig. 3. The 5.5 kb region in the right half of the maps is less complete but again shows many conserved sites between copies.

This present study is, to our knowledge, the first evidence for a family of human tRNA genes where the majority of members share homologous flanking sequences. tRNA gene copies which share homologous flanking sequences probably have evolved by a series of duplications of a large (>10kb) ancestral sequence, followed by base insertions, deletions or substitutions rather than by RNA-mediated transposition [4]. Different flanking sequences for eukaryotic tRNA gene families confer tissue specific expression [19,20] presumably by providing sites for binding of tissue-specific factors. In tRNA gene families having extensive flanking sequence homology, expression may be regulated by the abundance of TFIIIB [21].

Acknowledgements: The work was supported by a grant from the Science and Engineering Research Council to J.P.G. We thank Mr Tom Carr for technical assistance and Dr R.L.P. Adams for discussions. E.S.G. gratefully acknowledges the award of a Glasgow University Scholarship during part of this work.

Table I

	*Kpn*I			*Pvu*II			*Sst*I			*Hin*dIII		
	Size (Kb)	P1 or P2 Rel. no.	P3 Rel. no.	Size (Kb)	P1 or P2 Rel. no.	P3 Rel. no.	Size (Kb)	P1 or P2 Rel. no.	P3 Rel. no.	Size (Kb)	P1 or P2 Rel. no.	P3 Rel. no.
	22.5	1*	1	15.2		1	10		1	>25	1	
	14.8	1*	1	11.5		1	8.5	2		12		1
	7.3	6*	6	10.3	1		7.4		1	8.2	2	
	6.2		1	5.2	1		4.55	(1) 4*	4	5.5	1*	1
	4.85		1	4.75	3*	3	4.10		1	4.9		1
	3.55		1	3.45	1*	1	3.45	1		2.4	9*	9
	3.3	3*	1	3.15	1*	1	3.15	5		1.8		2
	3.1		1	2.35	1		2.00		4			
				2.15	5*	5	1.4		3			
							1.2	2				
Total copies detected by probes 1 & 2		**11**			**13**			**14**			**13**	
No. fragments (*) common to all probes		**9**			**10**			**4**			**10**	
No. fragments only with probe 3		**4**			**2**			**10**			**4**	

	*Bam*HI + *Kpn*I			*Pvu*II	+ *Kpn*I		*Bam*HI + *Sst*I		*Sst*I + *Kpn*I	
	Size (Kb)	P1 Rel. no.	P3 Rel. no.	Size (Kb)	P1 Rel. no.	P3 Rel. no.	Size (Kb)	P3 Rel. no.	Size (Kb)	P3 Rel. no.
	8.8	1*	1	20	1*	1	4.10	4	4.80	1
	7.3	3*	3	14.5		1	2.60	1	4.30	1
	5.5	3*	3	3.7	4*	4	2.20	1	3.70	1
	1.95	1*	1	3.4	1		2.00	4	2.10	4
	1.75	4*	4	2.15	2*	2	1.40	3	2.00	4
				1.8		1			1.40	3
				1.2	6*	6				
Total copies detected		**12**			**14**			**13**		**12**
No. fragments (*) common to all probes		**12**			**13**					
No. fragments only with probe 3		**0**			**2**					

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