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Technical Progress Report
DOE Grant # DE-FG02-91ER61136

An Improved Method for Producing Radiation Hybrids Applied to Human Chromosome 19

DOE/ER/61136--2

DE93 003156

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STUDIES CONDUCTED DURING THE CURRENT BUDGET PERIOD 3/1/92 - 2/28/93

Since our technical progress report for the second year of funding was submitted February 1992 this represents the progress we have made over the last seven months.

MOLECULAR AND CYTOGENETIC CHARACTERIZATION OF THE RADIATION HYBRIDS

At the initiation of the grant we had just produced radiation hybrids from a monochromosomal microcell hybrid containing human chromosome 19 as its only human component (PK87-19). During the first year of funding we initiated analysis of a panel of hybrids for markers in known locations on human chromosome 19. We also initiated fluorescent in situ hybridization analysis of the hybrid cell lines using biotinylated total human DNA as a hybridization probe to metaphase chromosomes prepared from the hybrids cell lines.

Marker Analysis

We have continued to analyze our panel of 94 hybrids for additional markers obtained from the literature, or the genome data base as well as to complete the analysis of any hybrids not yet scored for the markers in the table. In addition, we have started to analyze the hybrids for the Index markers for human chromosome 19 compiled by Dr. James Weber and published by the genome center in March, 1992. The hybrid panel had already been tested for apolipoprotein C_2. We have tested the radiation hybrids for D19S177 (mfd 120), D19S178 (mfd 139) and HRC (histidine rich calcium binding protein). In addition we have also analyzed for the presence of slow troponin1 (TNNT1) and GPI (glucose phosphate isomerase). These markers were assayed using the PCR

method described in our continuation submitted last year. Many of the new markers currently being isolated are dinucleotide repeat markers which are highly polymorphic and therefore extremely useful for genetic linkage. These markers consist of primer sequences which amplify a fragment using PCR typically around 100bp long. This type of marker has not been trivial to analyze in the radiation hybrids using direct detection of the PCR product by agarose gel electrophoresis. Several of them preferentially amplify hamster DNA under the recommended PCR conditions and the human band is irreproducibly faint. All of the index markers with the exception of 2 markers are dinucleotide repeat sequences. Those markers for which changing the PCR conditions does not improve the signal upon amplification will be assayed using the incorporation of α^{32}P dCTP and run on sequencing gels. Figure 1 shows the markers tested and their approximate location on the chromosome. The current status of our characterization is shown in Table 1. As we test more markers it is becoming evident which of the hybrids have more than 1 fragment of chromosome 19 and which are likely to contain a single fragment.

In Situ Hybridization Studies

In situ hybridization is certainly one of the most direct methods of gene mapping in general and the most direct method to visualize the number of chromosome fragments in a hybrid.

We have utilized fluorescent in situ hybridization (FISH) to determine the number of human fragments contained in each radiation hybrid cell line. This methodology is definitely an improvement over the nonfluorescent in situ protocols that we employed in the past, prior to the acquisition of a fluorescent microscope for the Cytogenetics Laboratory.

Until several months ago, all FISH experiments were performed using standard procedures and total human genomic DNA labeled with biotin as described in last year's continuation. Table 2 lists the hybrids for which we have prepared metaphase

chromosome spreads via conventional cytogenetics harvesting techniques and those hybrids which have been tested by FISH.

Of the 24 hybrid cell lines analyzed to date by FISH, most have only a single chromosome fragment. Some of the cell lines have 2 fragments such as hybrid 8A-303 and hybrid 4A-102. Hybrid 4A-102 shows no marker discontinuity but shows 2 pieces by FISH. Hybrid 8A-303 is positive for a single long arm marker S9 and probably contains a small fragment from the long arm in addition to the fragment containing the selectable marker. This is a higher fraction of hybrids containing single fragments than we expected. Additional fragments can be randomly carried along and we expected to produce cell lines with multiple fragments as well as single fragments. This has been our experience with radiation hybrids for chromosome 9 and in the literature as well. Chromosome 19 is a much smaller chromosome and therefore the total number of breaks on the chromosome would be less. Also we have only analyzed 24 hybrids. Over the next year we hope to finish the analysis on the rest of the hybrids which will provide a better estimate of the fraction of hybrids that contain 1 fragment vs. multiple fragments.

The details of cell culture, harvest and slide processing have been reported elsewhere (Mark et al., 1992, manuscript accepted by Applied Cytogenetics; also see Jackson et al., 1992).

During the past several months, we have also been testing the reverse protocol for in situ hybridization, utilizing both biotinylated Alu-PCR products as well as biotinylated total hybrid DNA as probes for hybridization on normal human peripheral blood slides.

Dr. Mark's laboratory has a constant supply of human blood slides and this can potentially result in a savings in time and effort since slides do not have to be prepared from each hybrid. It also has other advantages as discussed elsewhere (Mark et al., 1992).

Although this newly tested protocol clearly needs additional refinement, our preliminary results are encouraging. Figure 2 is an example of FISH where there was

insufficient suppression using labeled Alu-PCR products. Banding patterns similar to R-bands can be seen. In Figure 3 Alu-PCR products from hybrid #13 (2-108) were hybridized to human metaphase chromosomes and regions of chromosome 19 can be seen showing the positive signal following this alternative protocol.

Over the next year we will be testing different primers described for Alu PCR to determine the optimum combination. Our current studies were done with primer Alu 559 and primer A1S.

In addition to the FISH protocols described above, we have also tested various other in situ techniques. The Cytogenetics Laboratory routinely uses FISH as an adjunct to conventional cytogenetics and is interested in testing various probes for potential use. Figure 4 (a, b & c) is a representative metaphase after "chromosome painting" using a chromosome 9 probe, acridine orange stain and DAPI counterstain. Photomicrographs of additional hybrids that we have recently analyzed are also enclosed (Figure 5).

PLANS FOR THE UPCOMING FISCAL YEAR

Our plans for the upcoming fiscal year do not deviate significantly from the specific aims in the original proposal or the original time table proposed.

Characterization of the Radiation Hybrids

We plan to analyze the hybrid panel for the rest of the markers on the genome index marker map as well as additional markers that become available.

In addition to testing the hybrids for chromosome 19 markers, we plan to continue to characterize each hybrid cell line by fluorescent in situ hybridization (Figure 5a, b &c). We have demonstrated that total human DNA can be used successfully as a probe for hybridization to metaphase chromosome spreads of the hybrids. We now will continue to test the technique of chromosome painting using labelled hybrid DNA as probes. We will have to determine whether biotinylated Alu PCR products or biotinylated total hybrid DNA make a better probe. Our preliminary experiments indicate that Alu PCR products

are better but we need to do further studies. We plan to test other additional Alu PCR primers to determine the best combination for FISH.

We plan to continue to keep up with the constantly evolving fluorescent in situ hybridization technology so that newer FISH protocols can be applied to improve our research.

FISH using the rescued phage clone as a probe to specifically localize the retroviral insertion site has not yet been performed. The preliminary localization was p13.2-q12 using a somatic cell hybrid panel. We plan to perform the necessary FISH experiments for final localization.

Lastly, we plan to publish our results upon completion of the above analyses and make the hybrids available to the scientific community.

Radiation Hybrids with Additional Markers

We are proceeding with the production of a series of hybrids with a G418 resistance marker done in collaboration with Dr. Jude Samulski as described in last year's continuation. Currently we are analyzing the $G418^r$ clones obtained from the infection of PK87-19 with an AAV-2 viral vector containing the neomycin resistance gene. We are now using a PCR assay to verify that the vector has inserted into the long arm of chromosome 19. The assay utilizes one primer from AAV and a second primer from chromosome 19. Once we have verified that the AAV has integrated into chromosome 19 we will make radiation hybrids Instead of using a range of doses as I described previously we plan to make hybrids at 8000 rads. By isolating a large number of hybrids (100-200) from a single dose, we will be able to utilize some of the computer programs designed for analysis of radiation hybrids using the mathematical relationship between dose of radiation and order of markers.

Lastly, Dr. Hugh Keeping here at Rhode Island Hospital is developing a hygromycin containing retroviral vector which will be available for our use to infect PK87-19.

Tables

Legends for Figures

Figure 1 Chromosome 19 and markers

Figure 2 Fluorescent in situ hybridization using biotinylated probe with inadequate suppression.

Figure 3a Fluorescent in situ hybridization with suppression, Alu-PCR protocol.

Figure 3b Another metaphase spread, fluorescent in situ hybridization with suppression, Alu-PCR protocol.

Figure 4a A metaphase spread showing whole chromosome painting using a commercial chromosome 9 probe and "Spectrum Orange".

Figure 4b Same metaphase using DAPI counterstain.

Figure 4c Same metaphase above, doubly exposed.

Figure 5a Fluorescent in situ hybridization of radiation hybrid 19-8A-302 containing a single chromosome 19 fragment.

Figure 5b Fluorescent in situ hybridization of radiation hybrid 19-4A-104 #26 containing a single chromosome 19 fragment.

Figure 5c Fluorescent in situ hybridization of radiation hybrid 19-4B-202 #14 containing a single chromosome 19 fragment.

Hybrid	D19 S177	INSR	F10190	D19 S11	LDLR	HIS	52	F2085	EF2	D19 S7	D1379	D1430	MSUD	RYR	APO C2	D19 S9	CKMM	XRCC	FTL	CGB	SNRP	GPI	HRC	TNNT	D19 S178
PK87-19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+
1A-102	+	+		+	+	+			+				+	+	+		+	+	+	+	+	(+)	+	+	+
1B-204	+	-		+	+	+	+		+				+	+	+		+	+	?/+	+	+	+	+	+	+
2-201	+	-	+	+	+	+	+	+	+		+	+	-	-	-		-	-	+	-	-	-		+	-
2-204	+	+	+	+	+	+	+	+	+		+	+	-	-	-		+	-	+	-	-	-		-	-
4B-101	(-)	-	+	+	+	+	+	+	-	+	+	+	-	-	-		+	-	-	-	-	-		-	-
4B-103	(-)	-	-	-	+	+	+	-	-		-	-	+	-	+		+	-	-	-	-	-		-	+
6-204	(-)	+	+	+	+	+	+	+	-		+	+	-	-	-		-	-	-	-	-	-		-	-
6-304		-			-				+				-	-	-			-	?/-	-		(-)			
8B-201	(-)	+	+	+	+	+	+	+	-		+	+	-	-	-		+	-	-		-	-			-
8B-204	(-)	-	-	-	-	+		-	-	+	-	-	-	-	-		+	-	-	-	-	-		-	-
1A-104	+	+		+	+	+	+		+				+	+	+		+	+	+	+	+	+	+	+	+
1B-203	+	+		+	+	+	+		+				+	+	+		+	+	+	+	+	+		+	+
2-108	+	+	+	+	+	+	+	+	+		+	+	-	-	-		-	-	+	-	-	(-)			-
4B-202	+	+	+	+	+	+	+	+	+		+	+	+	+	-		+	-	?/+	-	-	+			
4A-301	+	+	+		+	+	+	+	+		+	+	-	-	-			-	?/+	-	-	(-)	+		
6-202	+	+			+				+				-	-	-			-	?/+	-	-			-	-
6-203		+	+		+	+	+	-	-		+	+	-	-	-		+	-	-		-	-			-
8B-304	+	+	+		+	+	+	+	-		+	+	-	-	-		+	-	-	-	-	-			-
8B-303	+	+	-			+	+	-	+		+	+	-	-	-			-	+	-	-	-			-
1A-202	+	+			+	+	+		+	-			-	-	+		+	+	+	+		-		+	-
1B-103	+	+		+	+	+	+		+	-			+	+	+		+	+	+	+		+	+	+	+
2-108	-	-	+		+	+	+	+	-	+	+	+	-	-	-			-	-	-	-			-	-
2-301	+	+	+		+	+	+	+	+	-	-	-	+	+	+		+	+	+	+		+	+	+	+
6-201	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-		-	-	+	-	-	-			-
6-303	(+)	+	+		+	+	+	+	+	+	+	+	-	-	-			-	+	-	-	-			
4A-104	-	-	-		-	+	+	+	-	+	-	-	-	-	-		-	-	?/-	-	-	-			-
4B-104	(+)	+	+		+	+	+	+	-	-	-	-	+	+	+		-	+	-	-	-	+			+
8A-102	+	+	+		+	+	+	+	-	-	+	+	-	-	-		+	-	-	-	-	-			
8B-203	+	+	+		+	+	+	+	-	+	+	+	-	-	-		-	-	-	-	-				
1A-305	+	+			+				+				+	+	+		+	+	+	+	+	+		+	-
2-303	+	+			+				+				+	+	+		+	+	+	+	+	+	+	+	
2-101	+	+	+		+	+	+	+	+	+	+	+	-	-	-		-	-	-	-	-				
2-303	+	+	+		+			+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	(-)
4A-103		+	+		+	+	+	+	+	+	+	+	-	-	-			-	+	-	-				(-)
4B-302		-	-		-	+	+	+	-	-	-	-	-	-	-			-	-	-	-				(+)
6-103		+	+		+	+	+	+	+	-	+	+	-	-	-			-	-	-	-				
6-302		-	-	+	+	+	+	+	-		-	-	+	+	+		+	+	-	+	+	+	+	+	+
8A-302		+	+		+	+	+	+	+	-	+	+	-	-	-			-	+	-	-				
8B-101		+			+				+				+	+	+		+	+	+	-	-				
1A-203		+			+	+				+			-	-	-		-	-	+	-		+			+
1B-303		-			+	+				+			-	-	-	-	-	+		+	-				
2-107		+	+		+			-	+		+	+	+	+	+		+	+		+					
4A-304		-	-		+	+		+	-	+	-	-	-	-	-	+	-	-	-	+					
4B-204		+	+		+	+		-	+	-	+	+	-	-	-	-	-	-	+	-					
6-101		+	+		+	+		+	+	+	+	+	-	+	-	-		-	+	-					
6-104	-	-	-		-	+		+	-	+	-	-	-	-	-	-		-	-	-		+			
8A-103	(+)	-	-			+		-	-	-	-	-	-	-	-	-		-	-	-					
1A-201	(-)	+			+	+			+	-			-	-	-	-		-	(-/+)			(-)		+	

1B-302	+	+			+	+			+	-			+	-	-	+	-	(-/+	+	[illegible]		[illegible]	
4A-105	+	+	+		+	+		+	+	+	+	+	-	-	+	-	-	+	-	(-)			
1B-202	+	+	+		+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
1B-202	+	+			+	+			+	+			+	+	+	+	+	+	+	+		+	
2-106		+	+		+	+		+	+	+	+	+	-	-	-		-	+	-			+	
1B-2G2		+			+	+			+	+			+	-	+	-	-	-	-	-	+	+	+
1A-303		+			+	+			+	+			+	+	+	+	+	+	+	+		+	+
8A-303		-	+		+	+		+	-	-	-	-	-	-	-	+	-	-	-	-		+	
9B-202		+	+		+	+		+	-	+	-		-	-	-	-	-	(-/+	-	-			
8B-302		+	+	-	+		+	-	+	+	+	+	-	-	-		-	(-/+	-				
6-103		+	+	+	+	+	+	+	+	-	+	+	-	-	-		-	+	-	-			
4B-203		+	+	+	+	+	+	+	+	+	+	+	-	-	-		-	(-/+	-				
6-301		-	+	+	+	+	+	+	-	+	-	-	-	-	-		-	-	-				(-)
8B-301	-	-	-	+	-	+	+	-	-	-	-	-	+	+	+		+	-	-			+	+
8A-304	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-		-	-	-				
1A-302	+	+		+	+	+	+		+	+			-	-	-		-	+	-				(+)
4A-102	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-		-	-	-				(-)
1A-103	+	+		+	+	+	+		+				+	+	+		+	+	+		+	+	+
8A-302	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+		+	+	+		+	+	+
8B-102	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-		+	-	-				+
1A-304	+	+		+	+	+	+		+	+			+	+	+		+	+	+		+	+	+
4B-102	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-		-	+	-			+	(-)
1B-104		+			+	+	+		+	+			+	+	-	+	+	+	-				
8A-205		-	-		-	+	+	+	+	-	-	-	-	-	-	+	-	+	-				(-)
4A-302	+	+	+		+	+	+	+	+	+	+	+	-	-	-	+	-	+	-				(-)
8B-103	+	+	+		+	+	+	-	+	-	-		-	-	-	-	-	+	-				(-)
8A-301		-	-		+	+	+	-		+	-	-	+	-		-	-	-	-				+
8B-104	+	+	+		+	+	+	+	+	+	+	+	-	-	-	-	-	+	-				(-)
1B-201	+	+			+	+	+		+	-			+	-	-	+	-	+	-			+	+
4B-201	+	+	+	-	+	+	+	-	+	-	+	+	-	-	-	-	-	+	+			+	(-)
1A-105	+	+			+	+	+		+	+			-	-	-	-	-	+	+			+	+
4B-303	+	+	+		+	+	+	+	+	+	+	+	+	+	-	-	-	+	-				(-)
8A-206	+	-	+		+	+	+	+	+	+	-	-	-	-	-	+	-	+	-				(-)
4A-202	-	-	-		-	+	+	+	-	+	-	-	-	-	-	+	-	-	-				(-)
8A-101	-	+	+		+	+	+	+	+	-	-	-	-	-	-	-	-	-	-				(-)
1B-101	+	+		+	+	+			+				-	-	-	-	-	+	-			+	(+)
4A-303	-	-		+	+				-				+	+	+		+	-	+			+	+
4A-203	+	+		-	+				+				-	-	-		-	+	-			+	(-)
8A-101	-	-		+	-				-				-	-	-		-	-	-				(-)
4B-304	-	-		+	+				-				-	+	-		-	-	+			+	(-)
4A-205	+	+		+	+								-	-	-		-	+	-				(-)
8A-104	-	+		+	+								-	-	-		-	-	-				(-)

Table 1

Table 2

NO.	HYBRID	METAPHASE SLIDES PREPARED	RESULTS
	PK87-19		
13	2-108	yes	okay, 1 positive signal in cells
3	2-201	yes	good, 1 signal translocated onto hamster, or 2 signals, 1 on hamster plus 1 sm piece
4	2-204	yes	ok, 1 sm signal
66	4A-102	yes	good, low % pos signals, 2 small frag in pos cells (3 lg cells w/2 pos signals)
26	4A-104	yes	good, 1 sm signal on sm hamster chromosome in most cells, a few cells with 2 signals
15	4A-301	yes	poor prep, mostly one F size signal stands alone - need better pictures
27	4B-104	yes	good, only cells with one small signal
14	4B-202	yes	ok, mostly 1 sm signal on sm hamster chromosome, some 2 and 3 signals also
16	6-202	yes	
7	6-204	yes	good, most cells one signal, some cells two signals
62	6-301	yes	
25	6-303	yes	good, sm signal alone? one signal in most, a few cells 2 signals
28	8A-102	yes	some bkg, most metaphase 1 signal (1 cell w/3 signals)
48	8A-103	yes	repeat, poor (PI) uptake
82	8A-206	yes	good, small fragment onto medium hamster
84	8A-208	yes	good, most metaphase 1 signal (2 cells w/4 signals)
38	8A-302	yes	poor prep. 1 sm signal on hamster chromosome, might need better pictures
57	8A-303	yes	good, 2 sm signals on one large hamster chromosome
64	8A-304	yes	ok, only cells with single human piece
69	8B-102	yes	
9	8B-201	yes	poor prep, very sparse, only saw one sm signal on lg hamster chromosome
29	8B-203	yes	poor, 1, 2 or 3 signals
10	8B-204	yes	good (PI) uptake, no human signal, repeat
63	8B-301	yes	slides dark, sparse, 1 very small pos signal
59	8B-302	yes	good, looks like 3 sm signals on 1 chromosome, mostly 3 and 4 counts for signal
19	8B-303	yes	sm fragment on hamster, 1, 2, 3 and 4 signals
18	8B-304	yes	good, only one positive signal

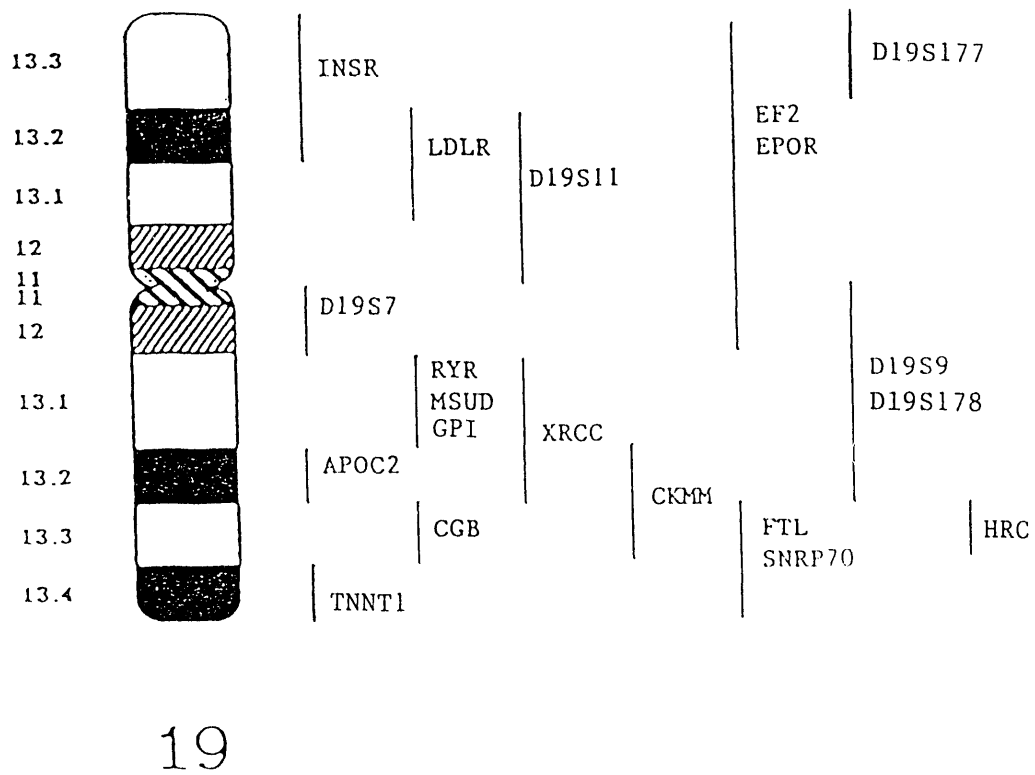

Figure 1 - Chromosome 19 and markers

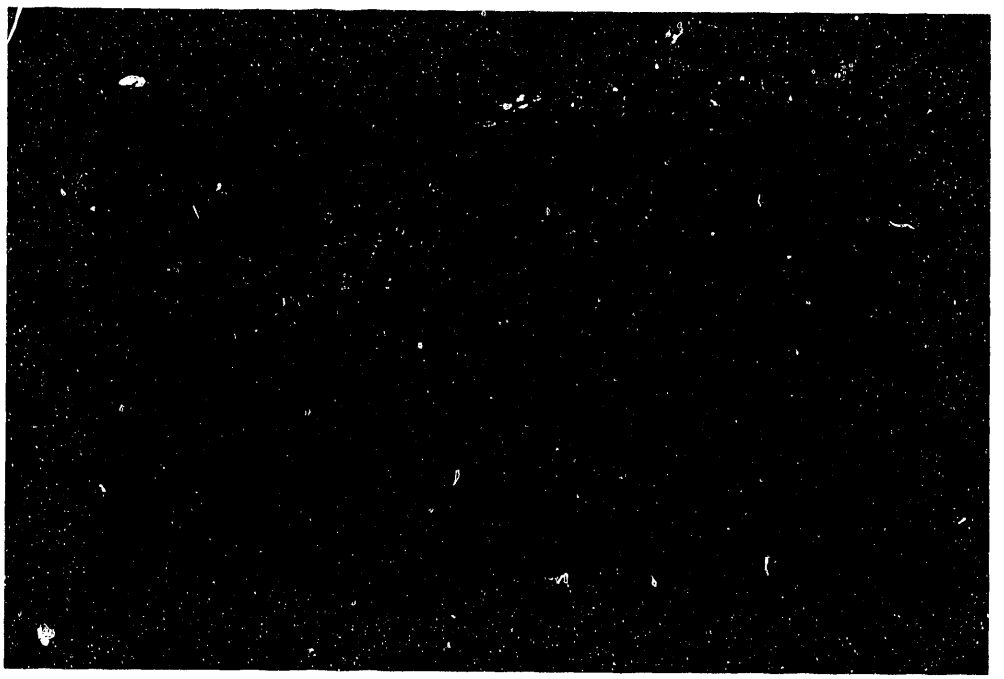

Figure 2. Fluorescent in situ hybridization with inadequate suppression.

Figure 4a. Fluorescent in situ hybridization "Whole Chromosome Painting" using chromosome 9 probe and "Spectrum Orange".

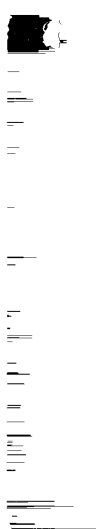

Figure 4b. Same metaphase using DAPI counterstain.

Figure 4c. Same metaphases, doubly exposed.

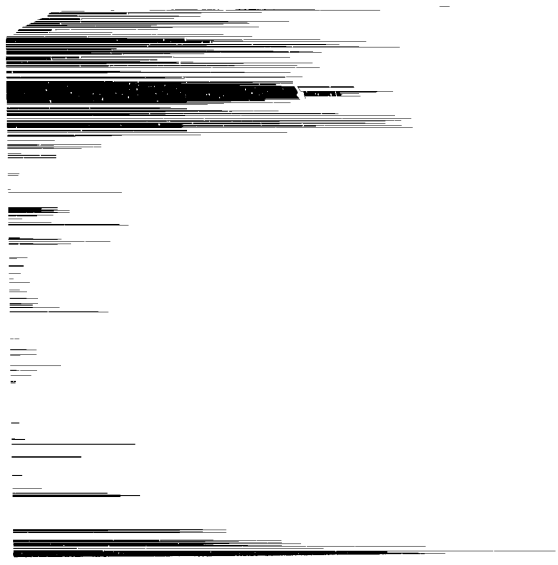

Figure 5a. Fluorescent in situ hybridization of radiation hybrid 19-8A-302 containing a single chromosome 19 fragment.

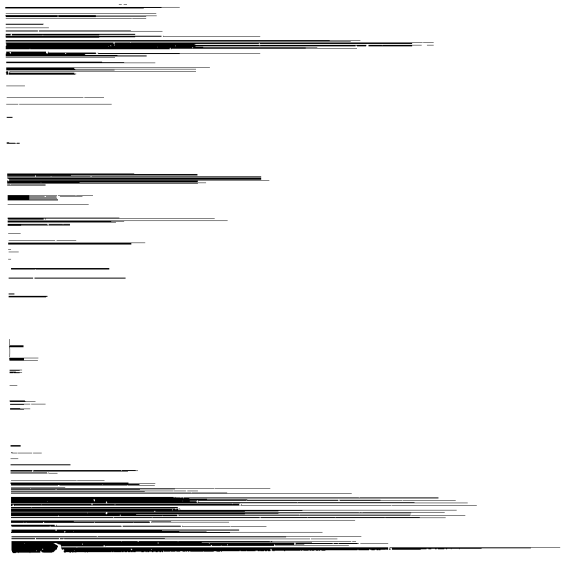

Figure 5b. Fluorescent in situ hybridization of radiation hybrid 19-4A-104 #26 containing a single chromosome 19 fragment.

Figure 5c. Fluorescent in situ hybridization of radiation hybrid 19-4B-202 #14 containing a single chromosome 19 fragment.

DATE
FILMED
12/10/92