Chromosome Research **8**: 319–334, 2000.

Human monochromosome hybrid cell panel characterized by FISH in the JCRB/HSRRB

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Received 3 January 2000; received in revised form and accepted for publication by M. Schmid 28 February 2000

Key words: FISH, human monochromosome hybrid cell panel, JCRB (Japanese Collection of Research Bioresources), mouse A9 cells, reverse FISH

Abstract

The human monochromosome hybrid cell panel in the Japanese Collection of Research Bioresources (JCRB) consists of 23 mouse cell clones, each containing a different human chromosome (the Y chromosome is not yet included). The panel is currently distributed by the Human Science Research Resources Bank (HSRRB) in Osaka. In order to determine the state of the human chromosomes and to supply the information to investigators, we characterized the cells by fluorescence *in-situ* hybridization (FISH) with corresponding human chromosome-specific painting probes, and, in part, by reverse FISH with the hybrid total DNA hybridized onto human metaphase spreads. Here, we report the frequency of intact human chromosomes maintained in each hybrid and the retained subregions of corresponding human chromosomes with relative frequencies estimated by fluorescent intensity. We used specific painted patterns to classify each hybrid into tentative types with their frequencies showing the nature of each hybrid and the state of rearrangements. This characterization will provide valuable information to investigators using the panel.

Introduction

The human monochromosome hybrid cell panel was established by Oshimura and his colleagues and deposited in the Cell Bank of the JCRB (Japanese Collection of Research Bioresources)/HSRRB (Human Science Research Resources Bank). By means of microcell-mediated chromosome transfer, single chromosomes derived from normal human cells were introduced into mouse A9 cells, except for chromosome 9, which was introduced into CHO cells. The hybrids containing transferred single human chromosomes, which were tagged with a dominant marker gene for resistance to neomycin,

blasticidin, hygromycin, or histidinol, were selected and cloned (Koi *et al.* 1989a, 1989b). The collection of cell lines is registered as JCRB2201–2223 and each human chromosome except Y is represented in one of the lines (Table 1). Total genomic DNAs extracted from those cells, which were frozen in ampules and listed on the board of the JCRB Cell Bank, are available through JCRB/HSRRB Gene Bank ⟨http://www.nih.go.jp/yoken/genebank/⟩. Human–rodent somatic cell hybrids have been generally used for construction of genomic libraries, assignment of genes, or isolation of DNA markers. Most of them, however, consist of several hybrids containing partial human chromosomes or more complicated composites, except for NIGMS mapping panel 2 (Ledbetter *et al.* 1990, Warburton *et al.* 1990, Ning *et al.* 1992, 1993, Drwinga *et al.* 1993, Dubois & Naylor 1993, Leonard *et al.* 1997, 1998, 1999). In contrast, the panel presented here consists of a set of hybrid cells with each single human chromosome; only the Y chromosome is not yet included. This will provide a valuable resource for functional analyses of human chromosomes with regard to specific chromosomal or subchromosomal regions. Indeed, utilization of the panel has already led to information on many aspects of gene function, including DNA repair, ageing, and tumor suppression (Ejima *et al.* 1990, Kugoh *et al.* 1990, Yamada *et al.* 1990, Tanaka *et al.* 1991, Kurimasa *et al.* 1994, Katoh *et al.* 1995, Jongmans *et al.* 1996, Matsuura *et al.* 1997, Kodama *et al.* 1998, Uzawa *et al.* 1998, Cui *et al.* 1999). A remarkable advance is the 'trans-chromosomic mouse' project in which Tomizuka and his colleagues have utilized A9 hybrids containing a human chromosome 2 (IgL kappa), 14 (IgH), or 22 (IgL lambda) and have successfully introduced these human chromosomes into mouse embryonic stem cells via microcell-mediated chromosome transfer (Tomizuka *et al.* 1997, 2000). These mice stably express human-type immuno-

Table 1. Human monochromosome hybrid cell panel.

Cell no.	Line	Human chromosome	Selection	Origin[a]	Intact chromosomes (%)[b]	Signals (%)[c]	Rearranged (%)[d]
JCRB2201	A9(Neo1)	1	G418	An	45.4	95.4	50.0
JCRB2202	A9(Neo2)	2	G418	An	90.8	93.9	3.1
JCRB2203	A9(Neo3)	3	G418	NTI-4	84.9	84.9	0
JCRB2204	A9(Neo4)	4	G418	MRC-5	100	100	0
JCRB2205	A9(Neo5)	5	G418	Mo	94.1	94.1	0
JCRB2206	A9(Neo6)	6	G418	An	98.1	98.1	0
JCRB2207	A9(Neo7)	7	G418	NTI-4	100	100	0
JCRB2208	A9(Neo8)	8	G418	Mo	100	100	0
JCRB2209	CHO(His9)	9	Histidinol	Primary	0	100	100
JCRB2210	A9(Bsr10)	10	Blasticidin	Primary[e]	32.4	97.1	64.7
JCRB2211	A9(Neo11)	11	G418	MRC-5	100	100	0
JCRB2212	A9(Neo12)	12	G418	NTI-4	0	100	100
JCRB2213	A9(Hygro13)	13	Hygromycin	Primary	80.9	80.9	0
JCRB2214	A9(Hygro14)	14	Hygromycin	Primary	96.1	96.1	0
JCRB2215	A9(Neo15)	15	G418	An	100	100	0
JCRB2216	A9(Neo16)	16	G418	Primary	100	100	0
JCRB2217	A9(Neo17)	17	G418	Primary	77.8	87.0	9.2
JCRB2218	A9(Neo18)	18	G418	Mo	100	100	0
JCRB2219	A9(Neo19)	19	G418	MRC-5	100	100	0
JCRB2220	A9(Neo20)	20	G418	Mo	68.5	93.2	24.7
JCRB2221	A9(Hygro21)	21	Hygromycin	Primary	96.0	100	4.0
JCRB2222	A9(Hygro22)	22	Hygromycin	Primary	0	100	100
JCRB2223	A9(BsrX)	X	Blasticidin	Primary[e]	16.7	100	83.3

[a] Cells from which human chromosome was derived.
[b] Frequency of intact chromosomes detected by whole chromosome painting (WCP) probes.
[c] Frequency of any signals detected by respective WCP probes.
[d] Frequency of chromosome rearrangement (c minus b).
[e] These two hybrids contain a chromosome derived from fibroblasts of the same Japanese male.

globulins in a proper tissue-specific manner. This novel procedure is expected to have therapeutic applications.

In the present study, we report the results of a detailed analysis of the JCRB panel aimed at an extensive characterization as a process of quality control identifying the human chromosomal regions by fluorescence *in situ* hybridization (FISH) with corresponding human chromosome-specific painting probes, which are called whole chromosome painting (WCP) probes, and in part combined by reverse FISH (revish) with total DNA of the hybrid onto normal human metaphase spreads. We believe these results provide useful information for investigators to study further functional genomics.

Materials and methods

Cells and chromosome preparation

Cells registered as JCRB2201–2223 in the JCRB Cell Bank were used (detailed information of each cell line is referred to in the web site ⟨http://cellbank.nihs.go.jp/⟩). Metaphase spreads of each cell line were obtained by the standard procedure. Briefly, cells were cultured in medium containing 0.02–0.04 μg/ml colcemide (Gibco BRL) for 2–4 h, exposed to hypotonic solution (0.2% trisodium citrate + 0.06 mol/L potassium chloride) for 20 min, and fixed with Carnoy's fixative. Cell suspension was dropped onto glass slides, air-dried, and stored in a freezer until use.

Fluorescence in situ hybridization (FISH) and reverse FISH (revish)

SpectrumGreen-labeled WCP probes specific for human chromosomes 1, 5, 9 and X were obtained from Vysis Inc., and Cy3-labeled WCP probes specific for all other human chromosomes were obtained from Amersham, Co. Ltd. All probes were used according to the manufacturers' instructions. Approximately 50 metaphases per probe were analyzed. Revish procedure was basically the same as that previously described (Tanabe *et al.* 1995). In brief, total genomic DNAs of cell lines were obtained by the GenTle solution kit (Takara), labeled with biotin-16-dUTP (Boerhinger) by nick translation, and hybridized onto the normal human metaphase spreads with a 20-fold excess of human CotI DNA (Gibco BRL). After hybridization, the biotinylated DNA probe was visualized by FITC-conjugated avidin and biotinylated anti-avidin antibody combination system (Vector). The slides were counterstained with DAPI (0.05 μg/ml) in antifade medium.

Fluorescence microscopy and imaging

Fluorescent images were observed by a Zeiss Axiophot2 epifluorescence microscope equipped with an Apochromato ×63 objective lens and the filter set for DAPI, FITC, and TRITC, captured by a Photometrics PXL cooled CCD camera, and colorized by the MultiFluor Electronic Photography system (Biological Detection, Inc.). In revish, fluorescent intensities along with the painted chromosomes were analyzed by Optimas 5.1 and graphically presented. The chromosomal deleted regions were estimated by the data of relative fluorescent intensities from approximately 10 metaphases per probe.

Results

First, metaphases were examined by means of QFH-banding analysis. In some hybrids the human chromosome was cytogenetically normal, but in others there were remarkable rearrangements, including interstitial deletions and/or translocations between human and host chromosomes, although the rearrangement frequencies were relatively low (Nakagawa *et al.* 1996). To characterize them more precisely, we used WCP probes to determine the frequency of cells that retained the human chromosome. As shown in Table 1, a relatively high percentage of rearrangements was found in the hybrids containing human chromosomes 1, 9, 10, 12, 20, 22, or X. Even though, in highly rearranged chromosomes, it is difficult to determine which human chromosomal regions are retained by WCP analysis, nevertheless, the revish technique enables such determination with precision, and their relative frequency as well. We have assigned tentative types corresponding to different patterns of WCP signals in each hybrid and our efforts to incorporate information from detailed characterization are described in Table 2, and photomicrographs are shown in Figures 1 and 2.

Table 2. Summary of WCP analysis and assignment of a tentative type of human chromosome in each hybrid.

JCRB2201	A	1 region (intact #1)	28	43.8	-	M	
	B	2 regions (intact #1 x 2)	1	1.6	-	M	
	C	1 region (partial deletion of 1q)	23	35.9	+	SM	del(1)(q?41→q44)
A9(Neo1)	D	2 regions (partial deletion of 1q x 2)	1	1.6	+	SM	del(1)(q?41→q44) x 2
	E	1 region (t and del; only 1p?34→1p10 remained on p-arm)	8	12.5	+	SM	der(host)(?::1p?34→1p10::?)
	F	No signals	3	4.6	-		
		Total	64	100			
		Frequency of metaphases with intact #1 (A+B)	29	**45.4**			Lot: 960418
		Frequency of metaphases with any signals (excluding F)	61	**95.4**			
JCRB2202	A	1 region (intact #2)	53	81.6	-	SM	
	B	2 regions (intact #2 x 2)	6	9.2	-	SM	
	C	1 region (partial deletion of 2p)	1	1.5	-	SM	del(2)(p25→p?14)
A9(Neo2)	D	2 regions (fission)	1	1.5	-	T	fis(2)(p10)
	E	No signals	4	6.2	-		
		Total	65	100			
		Frequency of metaphases with intact #2 (A+B)	59	**90.8**			Lot: 960615
		Frequency of metaphases with any signals (excluding E)	61	**93.8**			
JCRB2203	A	1 region (intact #3)	39	73.6	-	M	
	B	2 regions (intact #3 x 2)	4	7.5	-	M	
	C	1 region (host chr. was added to 3q)	2	3.8	+	SM	der(3)add(3)(q29)
A9(Neo3)	D	No signals	8	15.1	-		
		Total	53	100			
		Frequency of metaphases with intact #3 (A+B+C)	45	**84.9**			Lot: 960619
		Frequency of metaphases with any signals (excluding D)	45	**84.9**			
JCRB2204	A	1 region (intact #4)	47	92.2	-	SM	
	B	2 regions (intact #4 x 2)	4	7.8	-	SM	
	C	No signals	0	0	-		
A9(Neo4)		Total	51	100			
		Frequency of metaphases with intact #4 (A+B)	51	**100**			Lot: 960516
		Frequency of metaphases with any signals (excluding C)	51	**100**			
JCRB2205	A	1 region (intact #5)	16	31.4	-	SM	
	B	2 regions (intact #5 + rearranged #5)	30	58.8	-	SM	del(5)(q?34→q35)
	C	3 regions (intact #5 + rearranged #5 x 2)	2	3.9	-	SM	del(5)(q?34→q35) x 2
A9(Neo5)	D	No signals	3	5.9	-		
		Total	51	100			
		Frequency of metaphases with intact #5 (A+B+C)	48	**94.1**			Lot: 960522
		Frequency of metaphases with any signals (excluding D)	48	**94.1**			

Table 2.—continued

Cell Name	Type	WCP Painted Regions / Cell	No of Cells	%	T[a]	M[b]	Notes
JCRB2206	A	1 region (intact #6)	34	64.1	-	SM	
	B	2 regions (intact #6 x 2)	13	24.5	-	SM	
A9(Neo6)	C	4 regions (intact #6 x 4)	2	3.8	-	SM	
	D	2 regions (intact #6 + t and del; whole arm deletion of 6p)	1	1.9	+	SM	der(6)t(6;host)del(6)(p25→p10)
	E	3 regions (intact #6 x 2 + t and del; whole arm deletion of 6p)	2	3.8	+	SM	der(6)t(6;host)del(6)(p25→p10)
	F	No signals	1	1.9	-		
		Total	53	100			
		Frequency of metaphases with intact #6 (A+B+C+D+E)	52	**98.1**			Lot: 960414
		Frequency of metaphases with any signals (excluding F)	52	**98.1**			
JCRB2207	A	1 region (intact #7)	33	62.2	-	SM	
	B	2 regions (intact #7 x 2)	17	32.1	-	SM	
A9(Neo7)	C	3 regions (intact #7 x 3)	1	1.9	-	SM	
	D	4 regions (intact #7 x 4)	1	1.9	-	SM	
	E	2 regions (intact #7 + t and del; small region)	1	1.9	+	SM	der(host)t(7;host)del(7)(p22→q?31)
	F	No signals	0	0	-		
		Total	53	100			
		Frequency of metaphases with intact #7 (A+B+C+D+E)	53	**100**			Lot: 960515
		Frequency of metaphases with any signals (excluding F)	53	**100**			
JCRB2208	A	1 region (intact #8)	8	15.1	-	SM	
	B	2 regions (intact #8 x 2)	45	84.9	-	SM	
A9(Neo8)	C	No signals	0	0	-		
		Total	53	100			
		Frequency of metaphases with intact #8 (A+B)	53	**100**			Lot: 960529
		Frequency of metaphases with any signals (excluding C)	53	**100**			
JCRB2209	A	10 regions (greater part of #9 was shuffled)	1	1.9	+	SM	del(9)(q?33-q34) X 2~4 + der(host)t(9;host)
	B	12 regions (greater part of #9 was shuffled)	4	7.5	+	SM	idem
	C	13 regions (greater part of #9 was shuffled)	2	3.8	+	SM	idem
CHO(His9)	D	14 regions (greater part of #9 was shuffled)	35	66.0	+	SM	idem
	E	15 regions (greater part of #9 was shuffled)	2	3.8	+	SM	idem
	F	16 regions (greater part of #9 was shuffled)	6	11.3	+	SM	idem
	G	17 regions (greater part of #9 was shuffled)	1	1.9	+	SM	idem
	H	18 regions (greater part of #9 was shuffled)	2	3.8	+	SM	idem
	I	No signals	0	0	-		
		Total	53	100			
		Frequency of metaphases with intact #9	0	**0**			Lot: 960902
		Frequency of metaphases with any signals (excluding I)	53	**100**			

Table 2.—continued

Cell Name	Type	WCP Painted Regions / Cell	No of Cells	%	T[a]	M[b]	Notes
JCRB2210	A	1 region (intact #10)	20	29.4	-	SM	
	B	2 regions (intact #10 x 2)	1	1.5	-	SM	
	C	3 regions (intact #10 x 2 + t and del; 10?p11→?q21 remained)	1	1.5	+	SM+A	der(10)t(10;host)(?q21;host)del(10)(p15→?p11)
A9(Bsr10)	D	1 region (partial del of 10q or highly condensed intact #10?)	39	57.3	+	SM	del(10)(q?21→q26)
	E	2 regions (t and del; two small regions remained)	1	1.5	+	SM+A	der(host)t(10;host)(?q24;host)
	F	2 regions (partial del of 10q x 2)	3	4.4	+	A	del(10)(q?21→q26) x 2
	G	2 regions (t and del; iso 10p? + partial del of 10q)	1	1.5	+	M+A	i(10)(?p10) + del(10)(q?21→q26)
	H	No signals	2	2.9	-		
		Total	68	100			
		Frequency of metaphases with intact #10 (A+B+C)	22	**32.4**			Lot: 960805
		Frequency of metaphases with any signals (excluding H)	66	**97.1**			
JCRB2211	A	1 region (intact #11)	50	98.0	-	SM	
	B	2 regions (intact #11 x 2)	1	2.0	-	SM	
	C	No signals	0	0	-		
A9(Neo11)		Total	51	100			
		Frequency of metaphases with intact #11 (A+B)	51	**100**			Lot: 960413
		Frequency of metaphases with any signals (excluding C)	51	**100**			
JCRB2212	A	1 region (isochromosome of 12p)	37	68.5	-	M	i(12)(p10)
	B	2 regions (isochromosome of 12p x 2)	13	24.1	-	M	i(12)(p10) x 2
	C	3 regions (isochromosome of 12p x 3)	4	7.4	-	M	i(12)(p10) x 3
A9(Neo12)	D	No signals	0	0	-		
		Total	54	100			
		Frequency of metaphases with intact #12	0	**0**			Lot: 960604
		Frequency of metaphases with any signals (excluding D)	54	**100**			
JCRB2213	A	1 region (intact #13)	31	66.0	-	A	
	B	2 regions (intact #13 x 2)	5	10.6	-	A	
	C	3 regions (intact #13 x 3)	2	4.3	-	A	
A9(Hygro13)	D	No signals	9	19.1	-		
		Total	47	100			
		Frequency of metaphases with intact #13 (A+B+C)	38	**80.9**			Lot: 960706
		Frequency of metaphases with any signals (excluding D)	38	**80.9**			
JCRB2214	A	1 region (intact #14)	44	86.3	-	A	
	B	2 regions (intact #14 x 2)	3	5.9	-	A	
	C	3 regions (intact #14 x 3)	2	3.9	-	A	
A9(Hygro14)	D	No signals	2	3.9	-		
		Total	51	100			
		Frequency of metaphases with intact #14 (A+B+C)	49	**96.1**			Lot: 960704
		Frequency of metaphases with any signals (excluding D)	49	**96.1**			

Table 2.—continued

Cell Name	Type	WCP Painted Regions / Cell	No of Cells	%	T[a]	M[b]	Notes
JCRB2215	A	1 region (intact #15)	12	24.0	-	A	
	B	2 regions (intact #15 x 2)	38	76.0	-	A	
A9(Neo15)	C	No signals	0	0	-		
		Total	50	100			
		Frequency of metaphases with intact #15 (A+B)	50	**100**			Lot: 960617
		Frequency of metaphases with any signals (excluding C)	50	**100**			
JCRB2216	A	1 region (intact #16)	43	86.0	-	M	
	B	2 regions (intact #16 x 2)	7	14.0	-	M	
A9(Neo16)	C	No signals	0	0	-		
		Total	50	100			
		Frequency of metaphases with intact #16 (A+B)	50	**100**			Lot: 960601
		Frequency of metaphases with any signals (excluding C)	50	**100**			
JCRB2217	A	1 region (intact #17)	37	68.5	-	SM	
	B	2 regions (intact #17 x 2)	5	9.3	-	SM	
	C	1 region (t and del; pter region of submetacentric)	4	7.4	+	SM	der(host)t(17;host)(?q24;host)
A9(Neo17)	D	1 region (t and del; qter region of acrocentric)	1	1.8	+	A	der(host)t(17;host)(?q24;host)
	E	No signals	7	13.0	-		
		Total	54	100			
		Frequency of metaphases with intact #17 (A+B)	42	**77.8**			Lot: 960625
		Frequency of metaphases with any signals (excluding E)	47	**87.0**			
JCRB2218	A	1 region (intact #18)	5	9.4	-	SM	
	B	2 regions (intact #18 x 2)	35	66.0	-	SM	
	C	3 regions (intact #18 x 3)	10	18.9	-	SM	
A9(Neo18)	D	4 regions (intact #18 x 4)	2	3.8	-	SM	
	E	5 regions (intact #18 x 5)	1	1.9	-	SM	
	F	No signals	0	0	-		
		Total	53	100			
		Frequency of metaphases with intact #18 (A+B+C+D+E)	53	**100**			Lot: 960618
		Frequency of metaphases with any signals (excluding F)	53	**100**			
JCRB2219	A	1 region (intact #19)	50	98.0	-	M	
	B	3 regions (intact #19 x 3)	1	2.0	-	M	
	C	No signals	0	0	-		
A9(Neo19)		Total	51	100			
		Frequency of metaphases with intact #19 (A+B)	51	**100**			Lot: 960828
		Frequency of metaphases with any signals (excluding C)	51	**100**			

Table 2.—continued

Cell Name	Type	WCP Painted Regions / Cell	No of Cells	%	T[a]	M[b]	Notes
JCRB2220 A9(Neo20)	A	1 region (intact #20)	13	17.8	-	M	
	B	2 regions (intact #20 x 2)	1	1.4	-	M	
	C	1 region (t and del; whole arm del of 20q)	17	23.3	+	M	der(20)t(20;host)del(20)(q10→q13)
	D	2 regions (t and del; whole arm del of 20q + small region)	1	1.4	+	M	idem + der(host)t(20;host)(?q13;host)
	E	2 regions (intact #20 + t and del; whole arm del of 20q)	32	43.8	+	M	der(20)t(20;host)del(20)(q10→q13)
	F	3 regions (idem with type E + small region)	1	1.4	+	M	idem + der(host)t(20;host)(?q13;host)
	G	3 regions (intact #20 x 2 + t and del; whole arm del of 20q)	3	4.1	+	M	der(20)t(20;host)del(20)(q10→q13)
	H	No signals	5	6.8	-		
		Total	73	100			
		Frequency of metaphases with intact #20 (A+B+E+F+G)	50	**68.4**			Lot: 960707
		Frequency of metaphases with any signals (excluding H)	68	**93.2**			
JCRB2221 A9(Hygro21)	A	1 region (intact #21)	48	96.0	-	A	
	B	1 region (whole del of 21p)	2	4.0	-	T	del(21)(p13→q?11)
	C	No signals	0	0	-		
		Total	50	100			
		Frequency of metaphases with intact #21 (A)	48	**96.0**			Lot: 960813
		Frequency of metaphases with any signals (excluding C)	50	**100**			
JCRB2222 A9(Hygro22)	A	1 region (t and del; partial del of 22pter→q?12)	48	96.0	+	A	der(host)t(22;host)del(22)(p13→q?12)
	B	2 regions (t and del; partial del of 22pter→q?12 + small region)	2	4.0	+	A	idem + der(hdst)t(20;host)(?q13;host)
	C	No signals	0	0	-		
		Total	50	100			
		Frequency of metaphases with intact #22	0	**0**			Lot: 960816
		Frequency of metaphases with any signals (excluding C)	50	**100**			
JCRB2223 A9(BsrX)	A	1 region (intact #X)	7	14.6	-	SM	
	B	1 region (isochromosome of Xp?)	3	6.2	-	M	?i(X)(p10)
	C	1 region (t and del; partial del of Xp?11.2→qter)	35	72.9	+	SM	der(host)t(X;host)del(X)(p?11.2→qter)
	D	1 region (t and del; small region)	1	2.1	+	SM	der(host)t(X;host)del(X)(?p22→?q26)
	E	2 regions (intact #X x 2)	1	2.1	-	SM	
	F	2 regions (deletion; small acrocentric x 2)	1	2.1	-	A	der(X) x 2
	G	No signals	0	0	-		
		Total	48	100			
		Frequency of metaphases with intact #X (A+E)	8	**16.7**			Lot: 960814
		Frequency of metaphases with any signals (excluding G)	48	**100**			

[a] T indicates human/rodent (host) translocated chromosome.

[b] M means morphology of human chromosomes or human/rodent rearranged chromosome. M=metacentric, SM=submetacentric, A=acrocentric, T=telocentric.

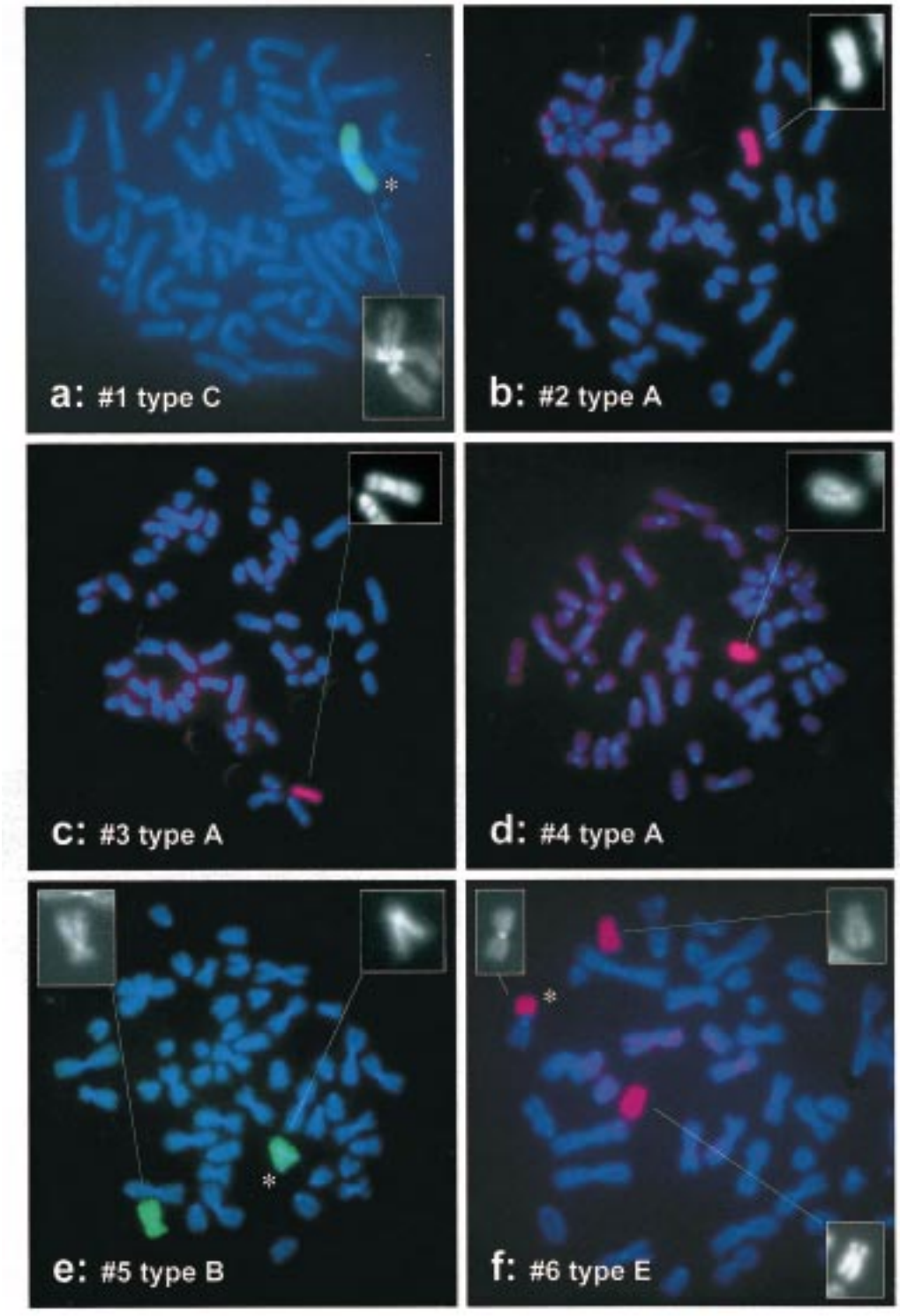
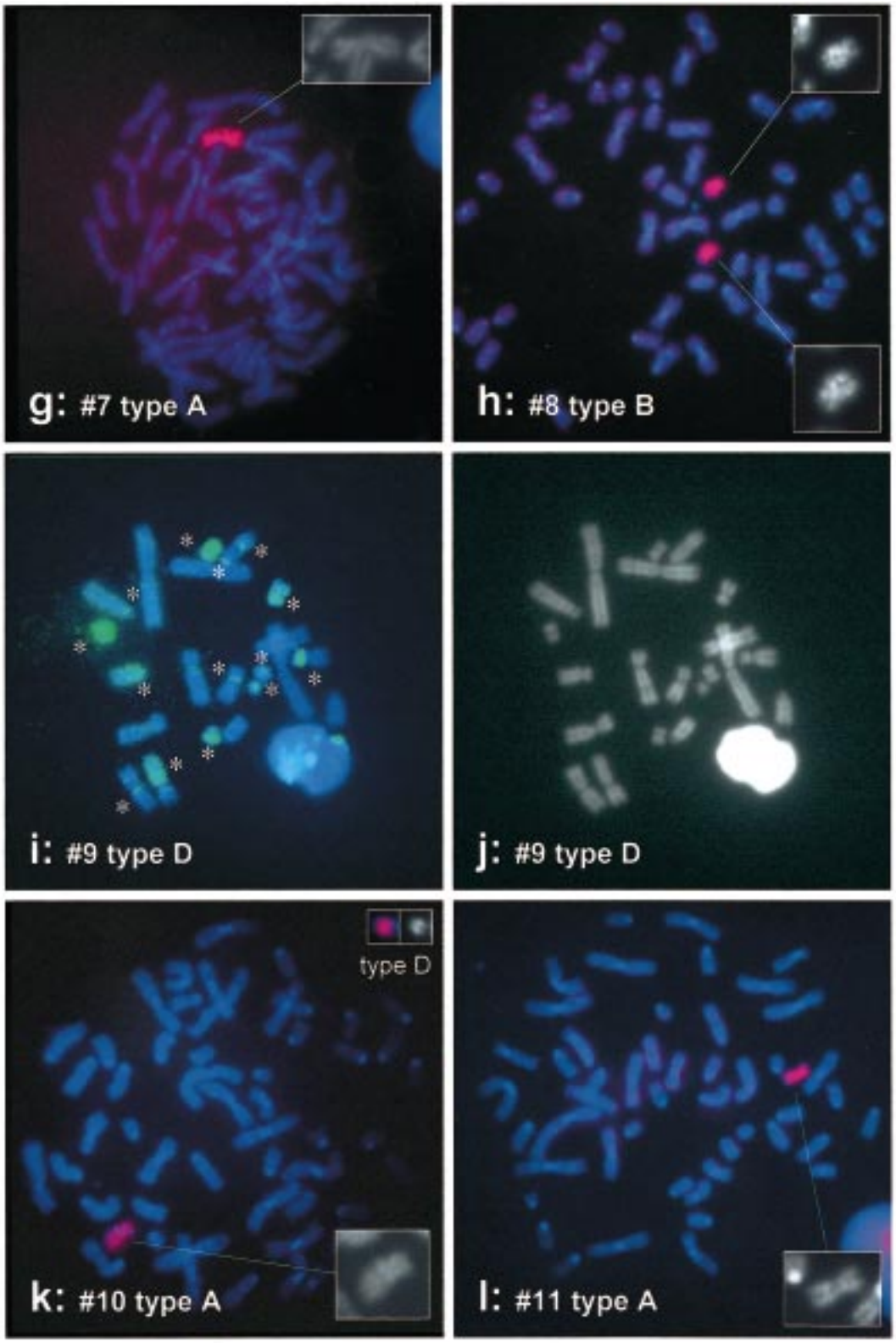
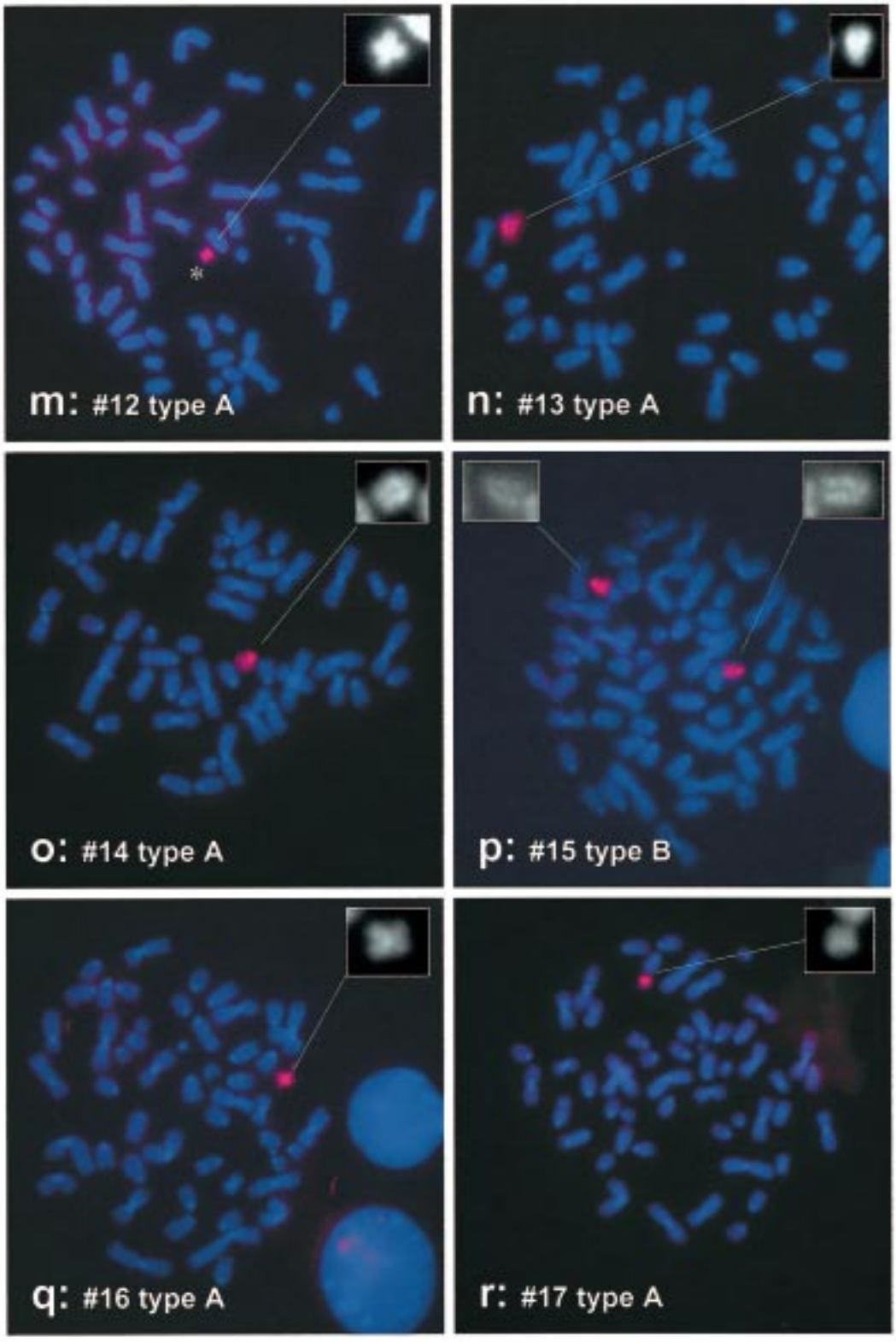
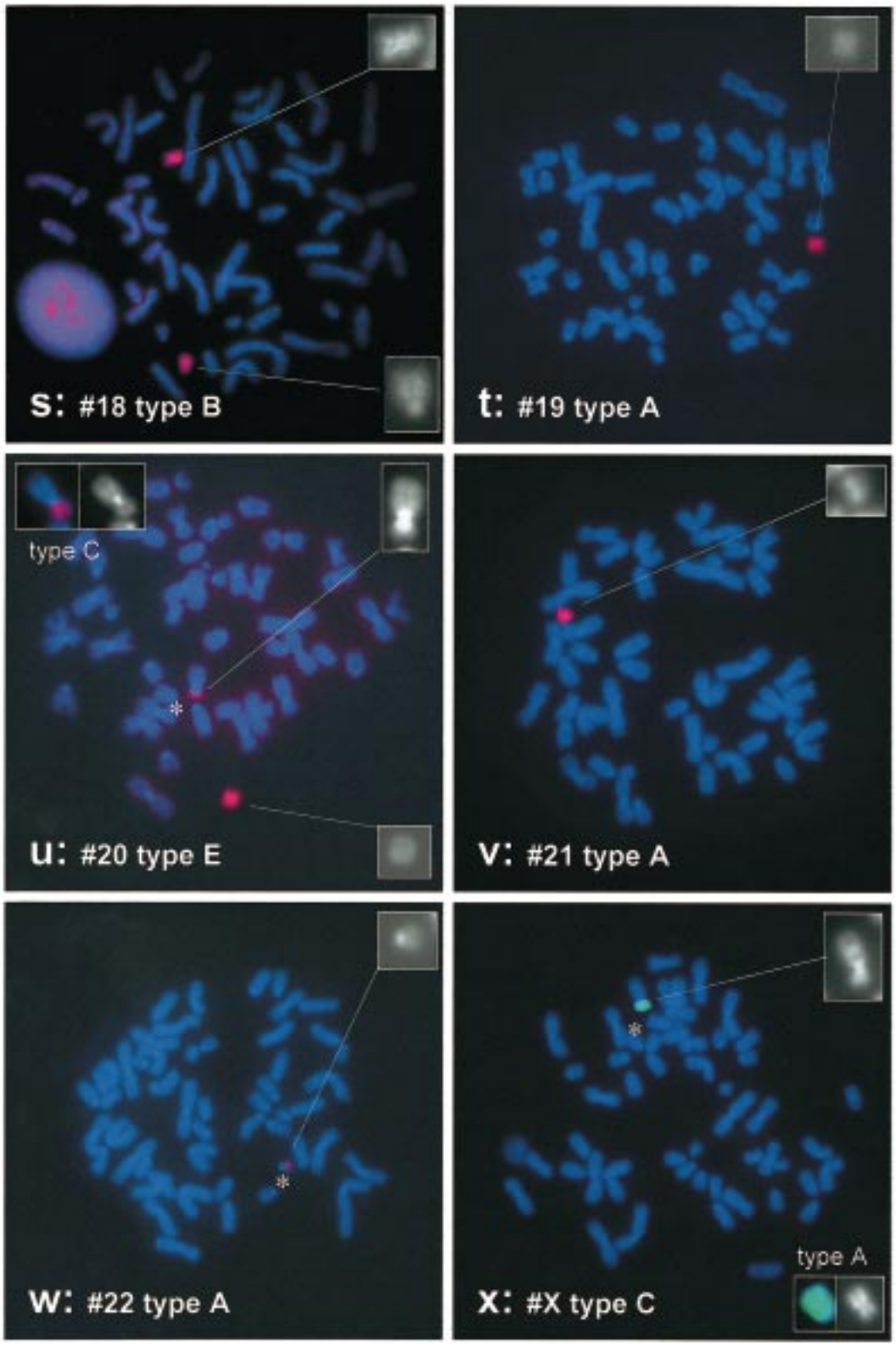


Figure 1. Representative metaphase images in each A9 cell hybrid after FISH with corresponding WCP probes and their tentative types. Insets show the DAPI image of the painted chromosomes. Asterisks show the rearranged chromosome of interest revealed by WCP signals. (**j**) shows a DAPI-stained image of the same metaphase as in (**i**).


g: #7 type A
h: #8 type B
i: #9 type D
j: #9 type D
type D
k: #10 type A
l: #11 type A


m: #12 type A
n: #13 type A
o: #14 type A
p: #15 type B
q: #16 type A
r: #17 type A


s: #18 type B
t: #19 type A
type C
u: #20 type E
v: #21 type A
w: #22 type A
type A
x: #X type C

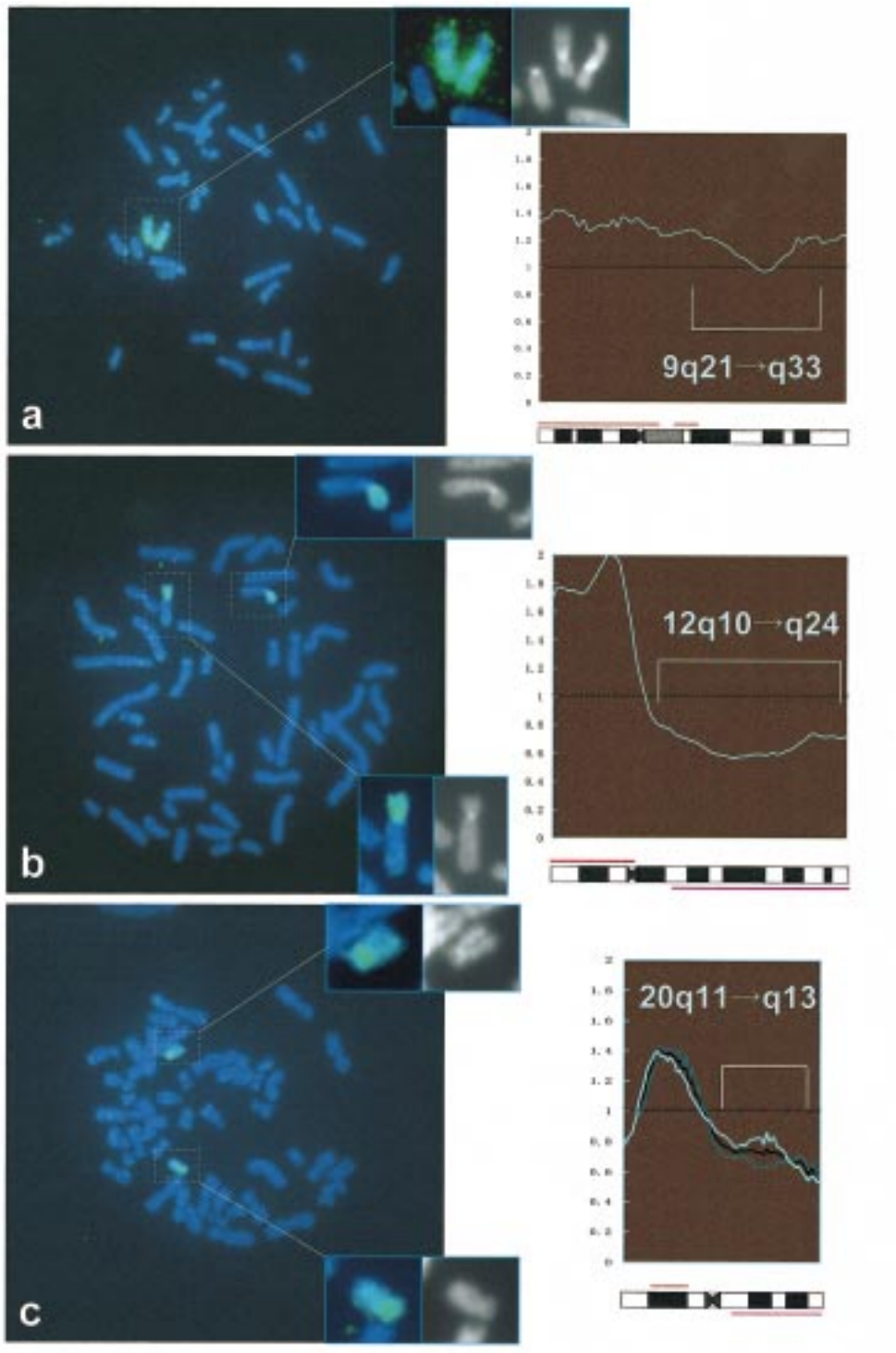


Figure 2. Summary of revish results of (**a**) CHO(His9), (**b**) A9(Neo12), and (**c**) A9(Neo20). Normal human metaphase images are shown after FISH with total genomic DNAs obtained from each hybrid. Insets show the painted chromosomes with their DAPI image. Fluorescent intensities along with the painted chromosomes are graphically presented by Optimas 5.1, and deleted subregions are shown between the bars.

Discussion

The majority of the hybrids contained intact human chromosomes at relatively high frequencies. Those with human chromosomes 1, 9, 10, 12, 20, 22, and X, however, contained a high frequency of rearrangements. Therefore, new clones should be isolated by recloning procedures, or different hybrids should be prepared by microcell-mediated chromosome transfer. The observed instabilities may be due to relationships between the host genome and endogeneous factors on the introduced human chromosomes, but it is also possible that the telomere lengths of the human chromosomes are shorter than those of the mouse chromosomes, resulting in the earlier unstable structures which occurred on the human chromosomes, although those instabilities are constrained under the genetic regulation of telomerase in multiple pathways (Kipling & Cooke 1990, Oshimura *et al.* 1996). Moreover, specific genes on the introduced human chromosomes could have affected the chromosomal stability. For example, CDKN2A (cyclin-dependent kinase inhibitor 2A) at 9p21 mediates the rapid growth-arrest response associated with human chromosome 9 (England *et al.* 1996). When chromosome 9 is present in proliferating clones of A9 hybrids, it either carries microdeletions involving the CDKN2A region or the region is epigenetically silenced (the former is twice as frequent). Thus, A9 hybrids with human chromosome 9 are prone to induced chromosomal deletion involving 9p21 regions leading to high rearrangements, rather than the epigenetic silencing of CDKN2A (England *et al.* 1996). As for chromosome 12, isochromosome 12p is commonly detected in fibroblasts of Pallister–Killian syndrome patients (Raffel *et al.* 1986, Ohashi *et al.* 1993, Dutly *et al.* 1998, Struthers *et al.* 1999) and an A9 hybrid containing i(12p) as a sole human chromosome was constructed a decade ago (Zhang *et al.* 1989). A9(Neo12) of the present panel with its high frequency of i(12p) will provide a suitable model for the study of the way isochromosomes form and operate.

We believe that results characterizing the present panel molecular cytogenetically are prerequisite for any further scientific work, in particular for the purpose of functional genomics or for investigation of fundamental cellular mechanisms, as is coming in the post-human genome era. Actually, several studies have already been reported in telomerase activity and cellular sensescence (Horikawa *et al.* 1998, Tanaka *et al.* 1998, 1999, Uejima *et al.* 1998, Kugoh *et al.* 2000), gene expression (Suzuki *et al.* 1997, Mitsuya *et al.* 1999), and genomic imprinting (Meguro *et al.* 1997, Mitsuya *et al.* 1998, Kugoh *et al.* 1999, Lee *et al.* 1999). In addition, via microcell-mediated chromosome transfer from A9 hybrids to chicken DT40 cells, which have a high homologous recombination proficiency, chromosome modification by telomere-directed truncation of human chromosomes transferred back to A9 hybrids has now become a realistic technique (Koi *et al.* 1997, Kuroiwa *et al.* 1998, Mills *et al.* 1999).

The human chromosomes in all the hybrids discussed here tended to have internal rearrangements or translocations with the host chromosomes. Accordingly, investigators using the hybrids have to pay attention to this property and avoid inappropriate culture conditions. Whether the JCRB hybrids contain the intact human chromosome with high frequency or not, it is important to characterize the resource as extensively as possible and to provide the information to investigators. Thus, we have made our data available at the web site ⟨http://cellbank.nihs.go.jp/⟩ and we hope it will be of value.

Note: Correspondence requesting panel cell lines should be addressed to: HSRRB in Osaka Branch of National Institute of Health Sciences, 1-1-43, Hoen-Zaka, Chuo-ku, Osaka 540-0006, Japan; c/o Dr. Toho Yoshida; Tel: +81-6-945-2869; Fax: +81-6-945-2872; E-mail: hsrrb@nihs.go.jp; URL:⟨http://cellbank.nihs.go.jp/⟩.

Acknowledgements

This study was supported in part by the grant-in-aid for the human genome and gene therapy program from the Ministry of Health and Welfare of Japan and a grant-in-aid from the Japan Health Science Foundation.

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