

図 11. STR マーカーおよび BAC CGH 解析による TK6 細胞変異体の欠失部位

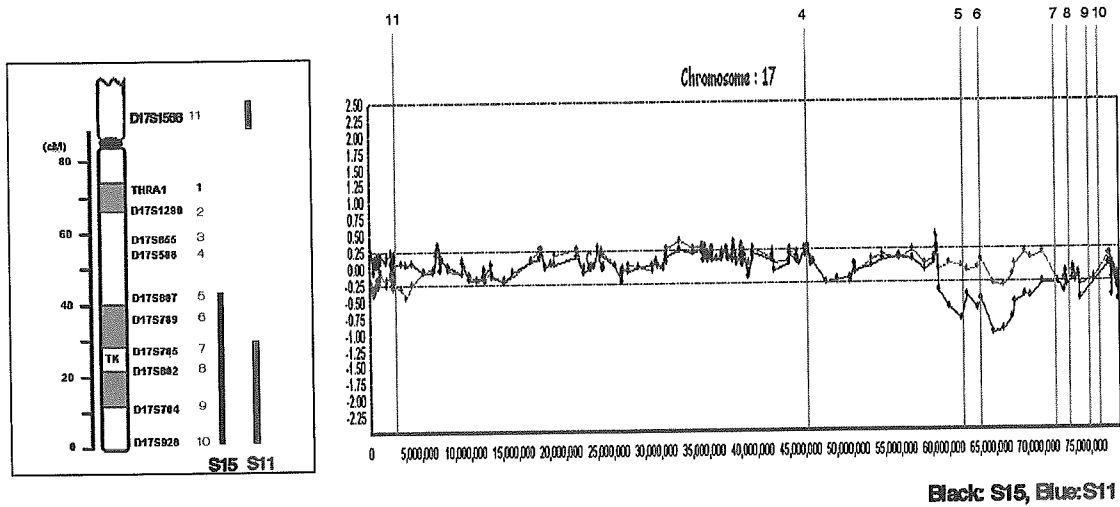


図 12 TK6 細胞株の 17 番染色体の SNP call の比較 (黒枠は LOH の範囲)

Pter	Position TK6 S11 S15				Cent	Position TK6 S11 S15				Qter			
	TK6	S11	S15	TK6		S11	S15	TK6	S11		S15		
	3565047	BB	BB	BB		22802254	AB	AB	AB	49029601	AB	AB	AB
	3565280	AA	AA	AA		22839015	AA	AA	AA	49253291	AB	AB	AB
	3616376	AB	BB	AB		23026451	BB	BB	BB	49427482	AB	AB	AB
	3899851	BB	BB	BB		23092869	AB	AB	AB	49668269	AA	AA	AA
	4358578	BB	BB	BB		23092917	AB	AB	AB	50007384	BB	BB	BB
	5806239	AA	AA	AA		24488142	AA	AA	AA	50052312	BB	BB	BB
	6211340	AA	AA	AA		25122345	AB	AB	AB	50052572	AA	AA	AA
	6227250	AA	AA	AA		25755541	BB	BB	BB	50112164	BB	BB	BB
	6498735	AB	AB	AB		26887999	AB	AB	AB	50185576	AA	AA	AA
	7478517	BB	BB	BB		26955730	AA	AA	AA	50405024	AB	AB	AB
	8524543	AA	AA	AA		27014232	BB	BB	BB	51292515	BB	BB	BB
	8551316	AB	AB	AB		28713731	AA	AA	AA	51454698	NoCal	BB	BB
	8644145	BB	BB	BB		28813156	AA	AA	AA	52197554	AA	AA	AA
	9385044	AA	AA	AA		28813215	BB	BB	BB	52536345	BB	BB	BB
	9705256	AB	AB	AB		28942170	AB	AB	AB	54792947	AA	AA	AA
	9756951	AB	AB	AB		28942222	BB	BB	BB	55320513	AB	AB	AB
	9876974	AB	AB	AB		29183029	AB	AB	AB	57029085	AA	AA	AA
	10484743	AA	AA	AA		29615779	AA	AA	AA	57159092	AA	AA	AA
	10484854	BB	BB	BB		29706619	AB	AB	AB	57230707	BB	BB	BB
	10822776	BB	BB	BB		30539277	AA	AA	AA	57734309	AB	AB	AB
	10884111	BB	BB	BB		30711529	AB	AB	AB	59136652	AA	AA	AA
	10884321	AA	AA	AA		30770914	AB	AB	AB	61578821	AA	AA	AA
	11076318	AB	AB	AB		31041457	BB	BB	BB	61670208	AB	AB	BB
	11076427	AB	AB	AB		32663038	AA	AA	AA	61700898	AB	AB	AA
	11168833	AA	AA	AA		33248878	BB	BB	BB	62034036	AB	AB	NoCa
	11169314	AB	AB	AB		34169959	BB	BB	BB	62512579	BB	BB	BB
	11310562	BB	BB	BB		34693022	BB	BB	BB	63694336	AB	AB	AA
	11660397	AB	AB	AB		36182069	BB	BB	BB	63940203	AA	AA	AA
	12271548	AB	AB	AB		36518826	AA	AA	AA	64213939	BB	BB	BB
	12472410	BB	BB	BB		36815436	AA	AA	AA	64214235	BB	BB	BB
	12472708	AA	AA	AA		36902470	BB	BB	BB	64914787	AA	AA	AA
	12549702	BB	BB	BB		39115918	BB	BB	BB	65148453	AA	AA	AA
	12549963	AA	AA	AA		40352675	BB	BB	BB	65388241	AB	AB	AA
	12558426	BB	BB	BB		40668676	BB	BB	BB	65762031	AB	AB	AA
	12934094	BB	BB	BB		41412603	AB	AB	AB	65762135	AB	AB	BB
	12980517	BB	BB	BB		41412669	AB	AB	AB	65942534	AB	AB	BB
	13485944	BB	BB	BB		42522367	BB	BB	BB	65942588	BB	BB	BB
	13668116	AA	AA	AA		43381030	BB	BB	BB	65942621	AA	AA	NoCa
	14304430	BB	BB	BB		44162133	BB	BB	BB	66103923	AB	AB	NoCa
	14432937	AA	AA	AA		45647724	AB	AB	AB	66313271	AA	AA	AA
	14433113	BB	BB	BB		47396100	AA	AA	AA	66467656	BB	BB	BB
	14515369	AA	AA	AA		47542451	AA	AA	AA	66657008	AB	AB	AA
	14691500	AB	AB	AB		47810325	AB	AB	AB	66848285	BB	BB	BB
	15045841	NoCal	AB	NoCal		47925935	AB	AB	AB	66940099	BB	BB	BB
	15053487	AA	AA	AA		47934288	AA	AA	AA	66952872	AA	AA	AA
	15108700	AB	AB	AB		48020820	BB	BB	BB	67801683	AB	AB	AA
	15186911	AA	AA	AA		48076070	AA	AA	AA	68095922	BB	BB	BB
	15237661	AB	AB	AB		48168557	AB	AB	AB	68880877	AA	AA	AA
	15618609	BB	BB	BB		48416713	AB	AB	AB	69271555	AA	AA	AA
	17350285	BB	BB	BB		48497919	BB	BB	BB	70276810	AB	AB	BB
	20147237	AB	AB	AB		48498517	AA	AA	AA	70734460	AA	AA	AA
	22436842	AA	AA	AA		48561694	BB	BB	BB	71725370	BB	BB	BB
	22760245	AA	AA	AA		48608529	NoCal	BB	BB	72151125	AB	AA	AA
	22760442	BB	BB	BB		48608764	BB	BB	BB	74409070	BB	BB	BB
	22802254	AB	AB	AB		48730410	BB	BB	BB	74635244	BB	BB	BB
	22839015	AA	AA	AA		49029601	AB	AB	AB	74687946	AB	BB	BB
						49253291	AB	AB	AB	76399520	AB	AA	AA

図 13 CGH および SNP チップによる切断点解析

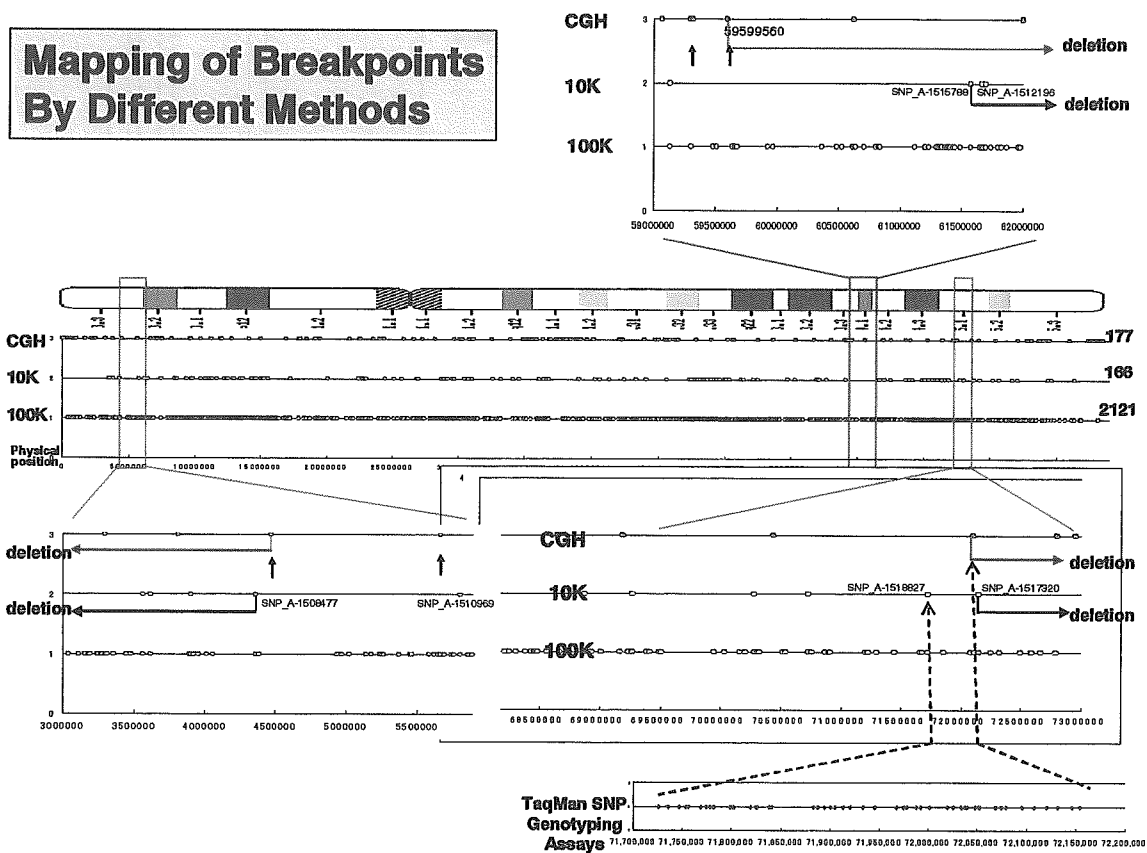


図 14 HL60 および HL60-RG の m-FISH による染色体解析

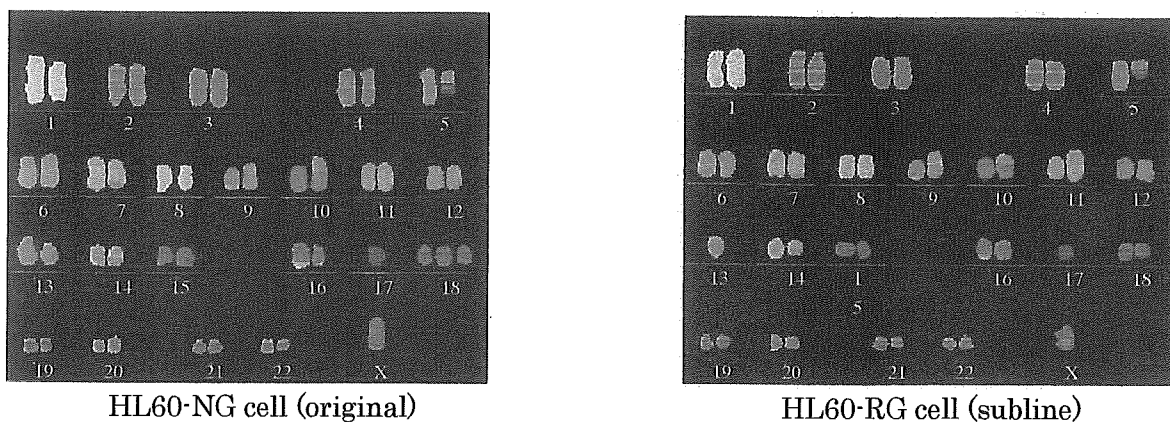


図 15 SNP call による HL-60RG 株での LOH の検出例

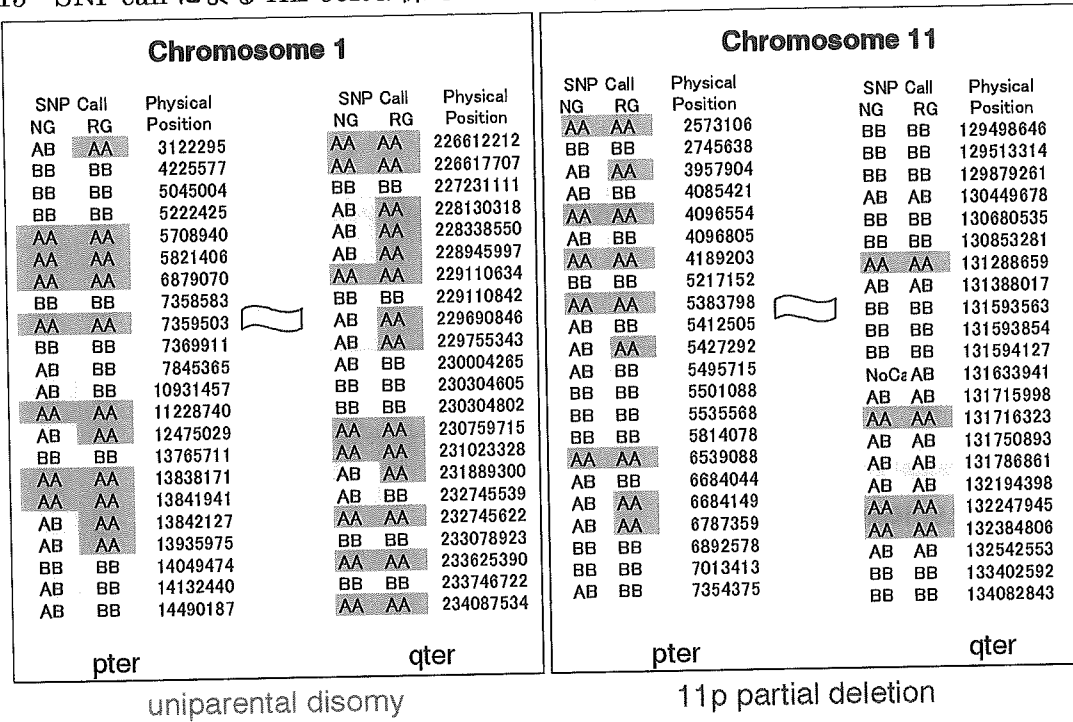


図 16A d-Chip ソフトウェアによるコピー数の解析結果と CGH アレイによる結果の比較

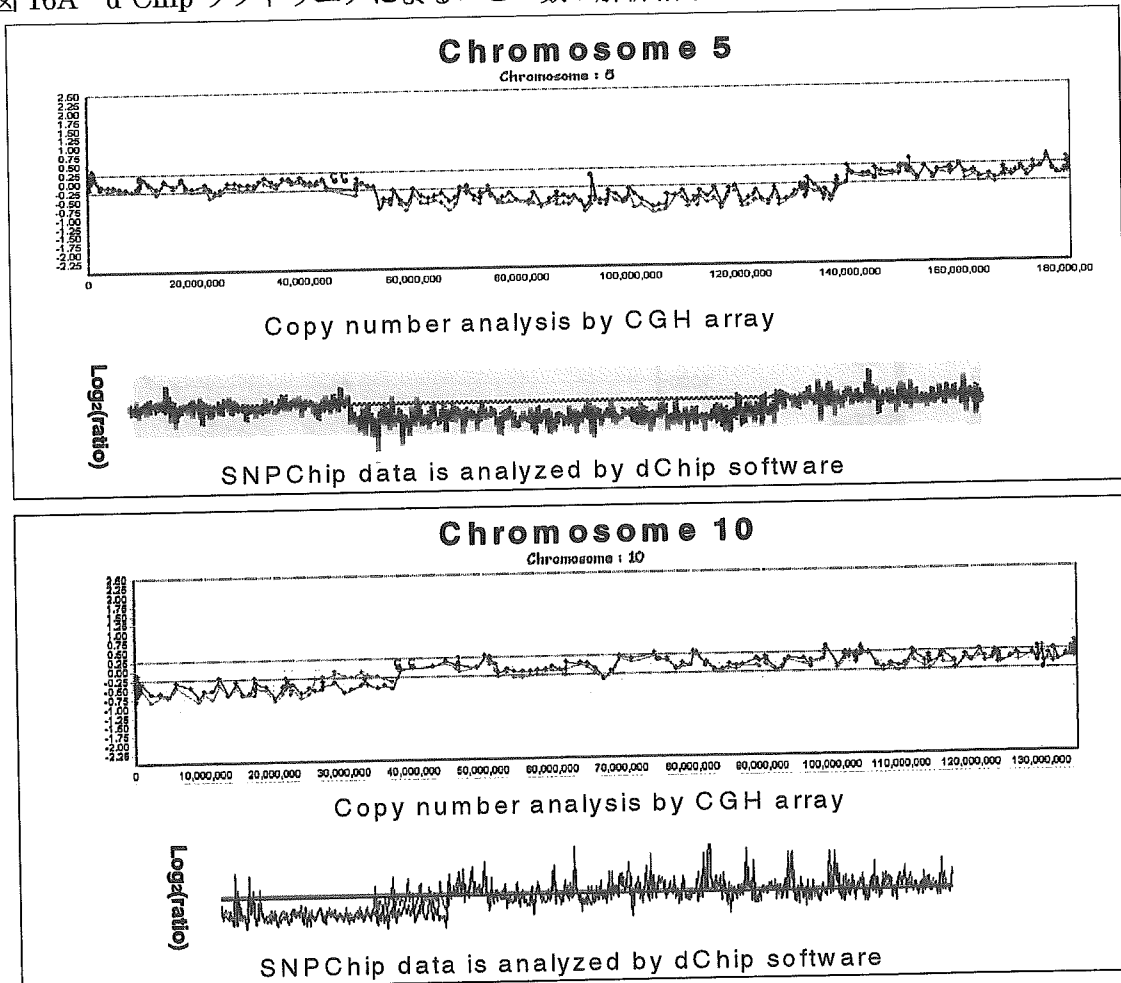


図 16B c-myc 遺伝子近傍の増幅領域

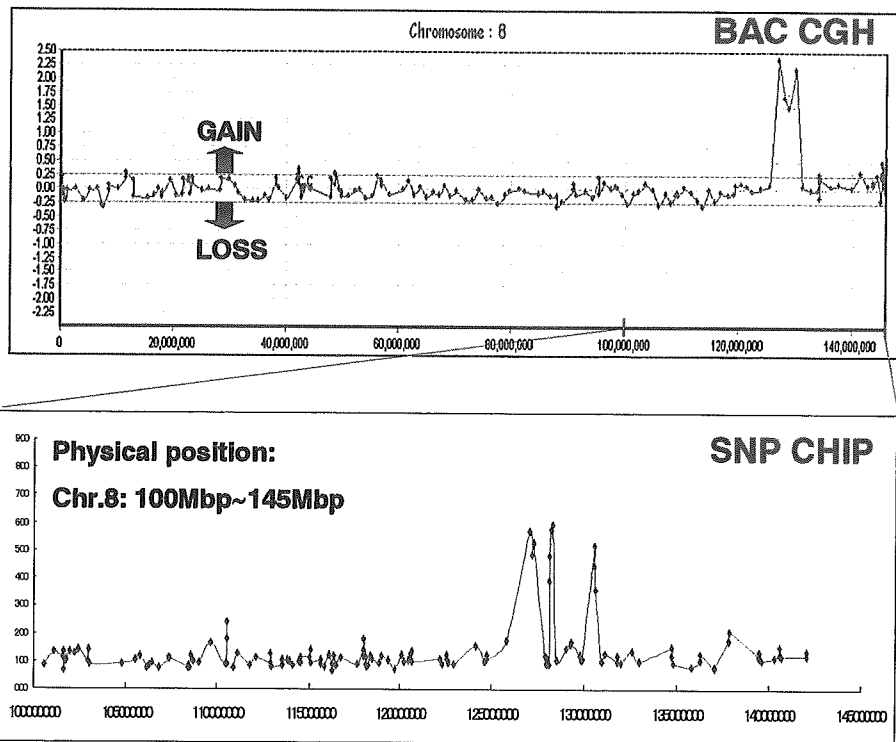


図 17 発現解析データを利用した欠失アレルに関する検討

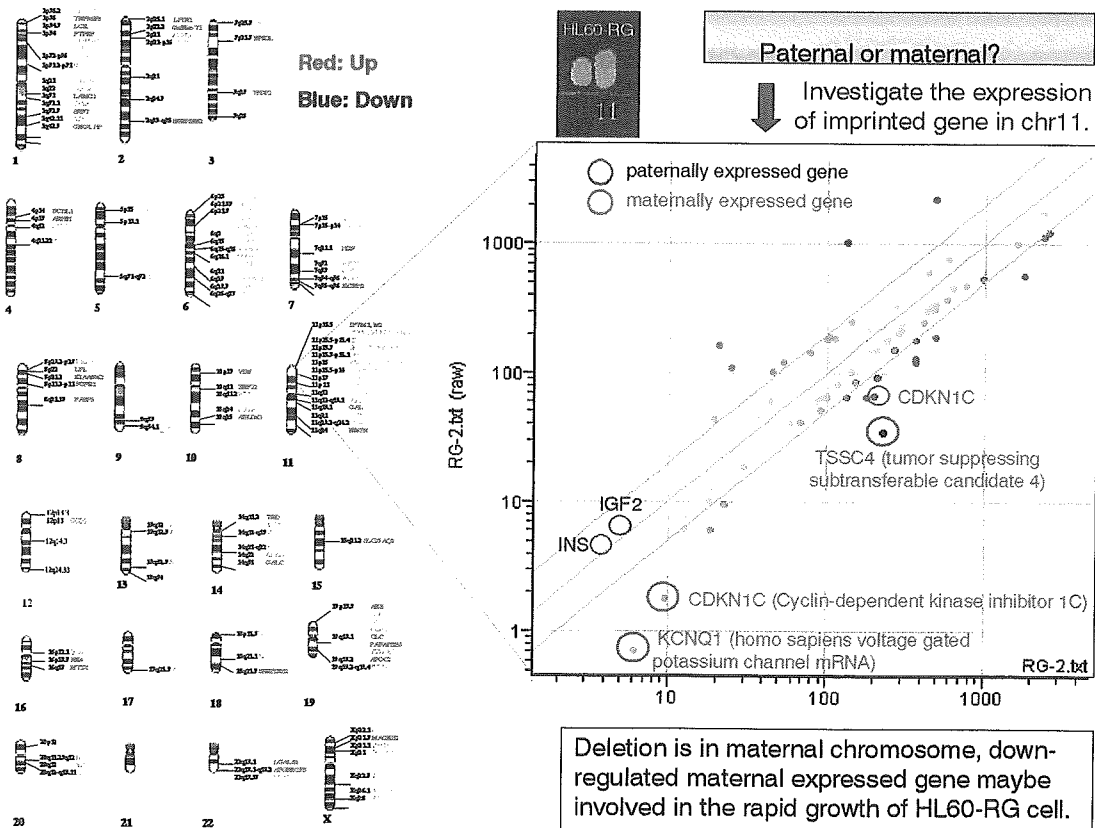


表3 HL60-RG細胞株において1番染色体上で発現変化した遺伝子群

NG-1	NG-2	RG-1	RG-2	Ratio-1	Ratio-2	Gene Name	Map	Description
(1p36)								
881 P	1128 P	25 A	14 A	0.03	0.01	PABPC4	1p32-p36	poly(A)-binding protein, cytoplasmic 4 (inducible form)
114 P	97 P	969 P	1112 P	8.15	8.19	TNFRSF8	1p36	Tumor necrosis factor receptor superfamily, member 8
10 A	33 A	4 A	8 A	0.41	0.18	T1A-2	1p36	lung type-1 cell membrane-associated glycoprotein
75 A	50 A	32 A	29 A	0.46	0.42	DHRS3	1p36.1	short-chain dehydrogenase/reductase 1
117 A	95 A	40 A	22 A	0.36	0.17	RHCE	1p36.11	Rhesus blood group CcEe antigens
27 A	29 A	10 A	13 A	0.41	0.32	RHCE	1p36.11	Rhesus blood group CcEe antigens
33 A	30 A	12 A	23 A	0.56	0.54	RHD	1p36.11	Rhesus polypeptide (RhIII)
129 A	61 A	30 A	23 A	0.25	0.27	DJ462C23.2	1p36.12-p35.1	hypothetical protein DJ462C23.2
90 A	68 A	9 A	12 A	0.10	0.13	PADI4	1p36.13	peptidyl arginine deiminase, type V
94 A	97 A	3 A	4 A	0.04	0.03	PADI4	1p36.13	PADI-H protein
458 P	341 P	123 A	255 A	0.29	0.54	SLC2A5	1p36.2	solute carrier family 2, member 5
117 A	119 A	10 A	13 A	0.09	0.06	SLC2A5	1p36.2	solute carrier family 2, member 5
40 A	42 A	19 A	22 A	0.52	0.37	KIAA1026	1p36.21	KIAA1026 protein
102 A	42 A	11 A	11 A	0.11	0.19	ODA	1p36.2-p35	cytidine deaminase
128 A	135 P	37 A	89 A	0.31	0.47	FCR	1p36.2-p36.1	Cardner-Rasheed feline sarcoma viral oncogene homolog
83 A	65 A	19 A	53 A	0.25	0.56	EGFL3	1p36.3	EGF-like domain, multiple 3
446 P	324 P	870 P	1039 P	2.02	2.30	BACH	1p36.31-p36.11	Brain acyl-CoA hydrolase
124 A	83 A	37 A	31 A	0.32	0.24	C1QA	1p36.3-p34.1	complement component 1, q subcomponent, alpha polypeptide 1
160 P	219 P	7 A	22 A	0.05	0.07	TNFRSF1B	1p36.3-p36.2	tumor necrosis factor receptor superfamily, member 1B
(1q21)								
289 P	187 P	34 A	53 A	0.13	0.23	S100A6	1q21	S100 calcium-binding protein A6 (calycylin) (S100A6)
1594 P	1581 P	92 A	68 A	0.06	0.03	S100A9	1q21	S100 calcium-binding protein A9 (calgranulin B)
5921 P	3289 P	85 A	97 A	0.02	0.02	S100A8	1q21	S100 calcium-binding protein A8 (calgranulin A)
45 A	53 A	1 A	4 A	0.03	0.05	S100A8	1q21	S100 calcium-binding protein A8 (calgranulin A)
262 A	110 A	23 A	22 A	0.09	0.14	S100A10	1q21	S100 calcium-binding protein A10 (annexin II ligand, calpactin 1)
56 A	69 A	20 A	24 A	0.33	0.25	GBA	1q21	glucosylase, beta, acid (includes glucosylceramidase)
217 P	125 P	60 P	87 P	0.29	0.50	S100A12	1q21	S100 calcium-binding protein A12 (calgranulin G)
89 P	68 A	175 P	240 P	2.12	2.54	SH2D2A	1q21	SH2 domain protein 2A
312 P	272 P	156 P	172 P	0.54	0.45	PEA15	1q21.1	phosphoprotein enriched in astrocytes 15
9 A	8 A	3 A	6 A	0.36	0.48	KCNK3	1q21.3	potassium intermediate/small conductance calcium-activated channel
47 A	10 A	15 A	8 A	0.34	0.53	FL-21919	1q21.3	FL5B1.24 PRO2179
37 A	14 A	10 A	10 A	0.30	0.53	APOA2	1q21-q23	apolipoprotein A-II

図18 TNFRSF1B (TNF receptor 2) と TNFRSF8 (CD30) 遺伝子の配置と CpG アイランド

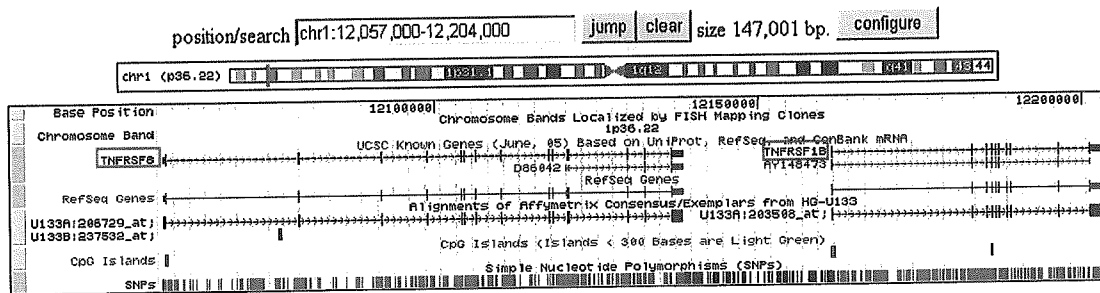


図 19A SV40NLS-EGFP-TRF1 フラグメント融合タンパク質

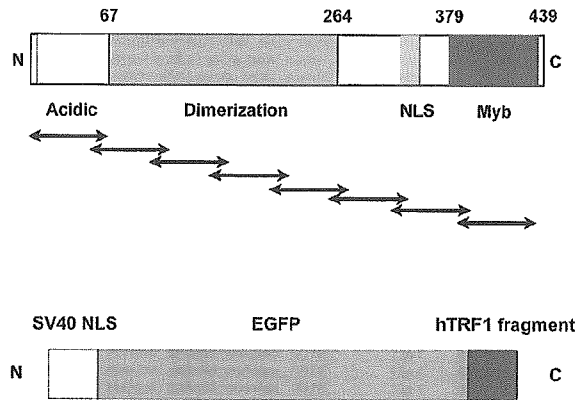
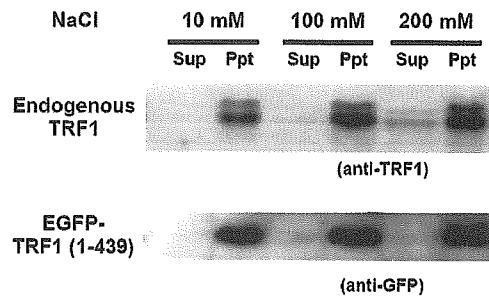


図 19B SV40NLS-EGFP-TRF1 フラグメント融合タンパク質の細胞内局在

EGFP-TRF1 (1-439)発現細胞の解析



EGFP-TRF1 (56-126)発現細胞の解析

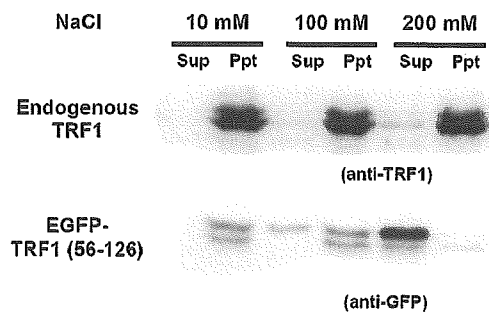


図20

TRF1は細胞の不死化に伴って誘導されるが、
Telomerase活性やテロメア配列の長さ、
不死化の原因とは無関係である

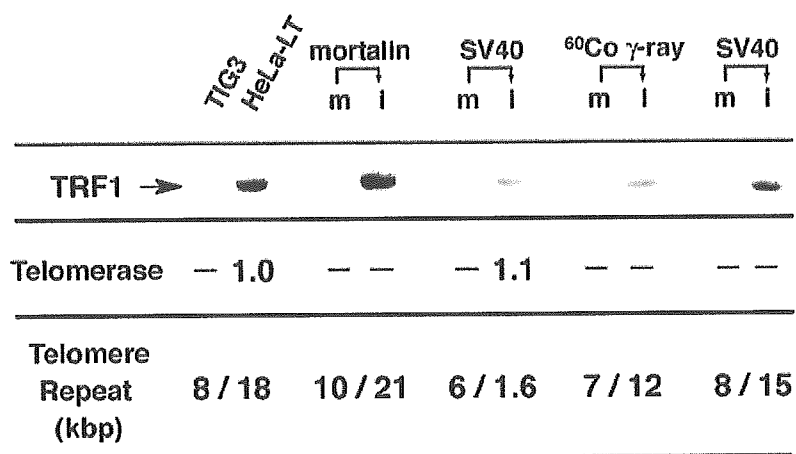


表4 不死化細胞および正常細胞における
TRF1遺伝子とTERT遺伝子のプロモーター活性

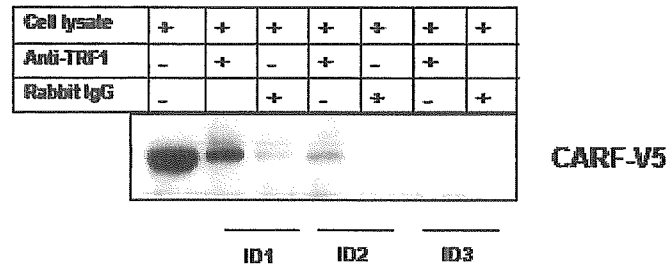
	TRF1	TERT	Promoter-Less	Mock
HeLa	14.067	78.159	1.675	0.003
TIG (primary Fibroblast)	1.367	0.195	0.039	0.003

(x 1000 RLU/μg)

図21 CARFとTRF1との結合および細胞内局在

A

Interaction between CARF-V5 and TRF1



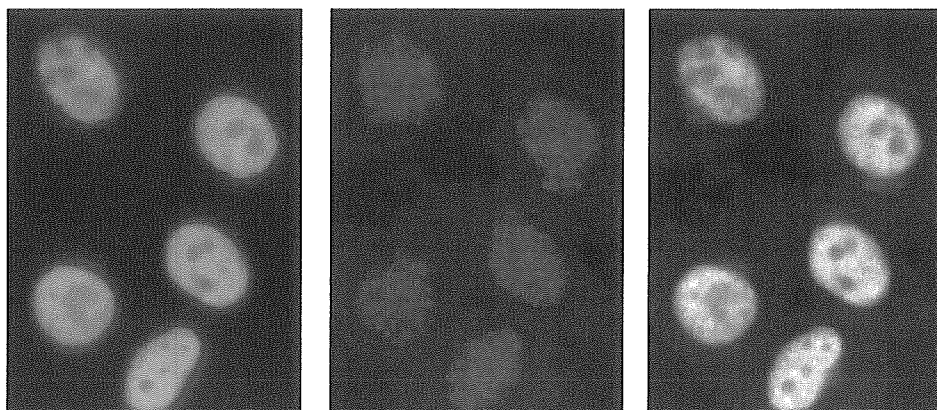
IP: anti-TRF1

IB: anti-V5

Results: TRF can pull down CARF-V5 from cell lysate

B

Double staining for CARF and TRF1



CARF

TRF1

Merge

図22. AC133陽性細胞をSCFやTPO共存下で産生される細胞の増殖
とCD31強陽性細胞の解析

(B) EPCのFACSによる解析

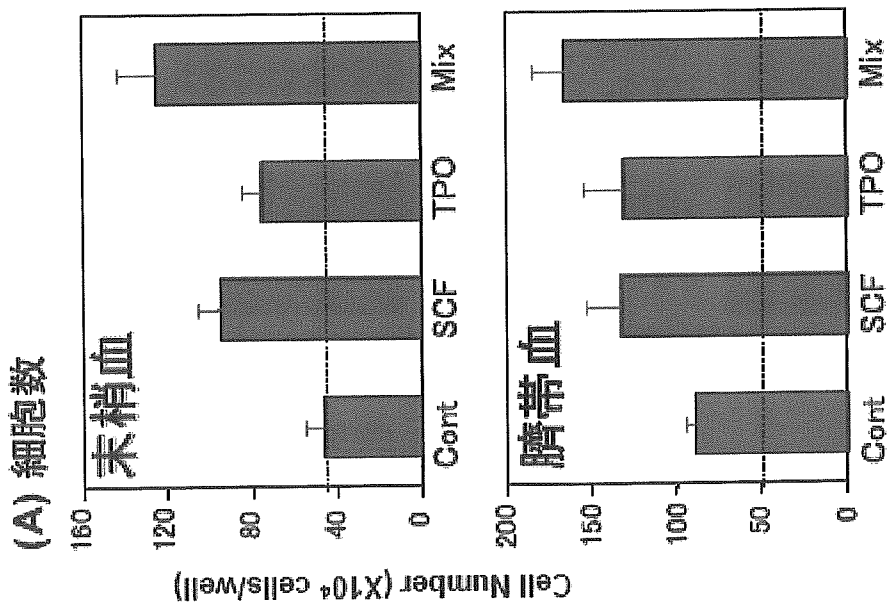
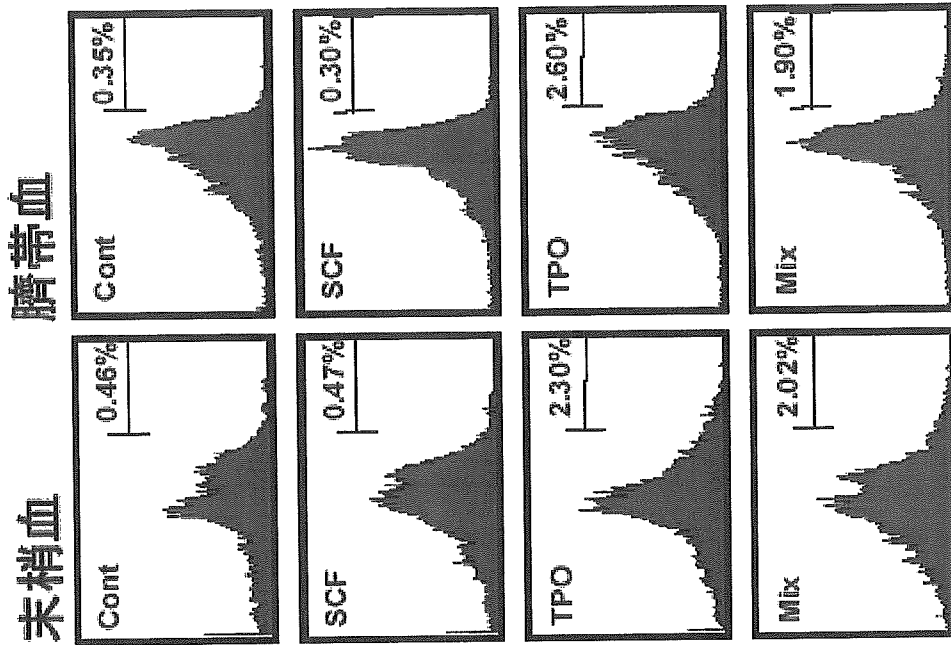


図23. AC133陽性細胞をTPO共存下で産生される
CD31強陽性細胞の絶対数

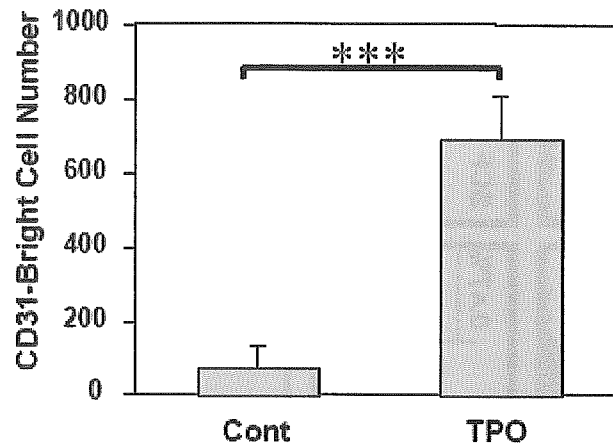
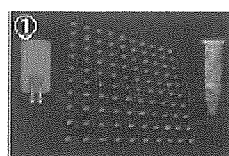
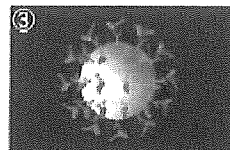


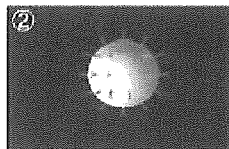
図24. サスペンション・アレイ・テクノロジーの原理



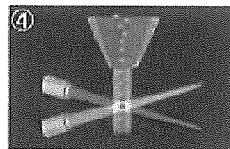
① 2種類の蛍光色素の量比を利用して、ビーズを100種類に色分けする。



② さらにビオチンつき二次抗体、アビジン蛍光物質を結合させる。



③ 1種類のビーズに1種類の抗体を結合させる。



④ フローサイトメリーの技術を利用し、ビーズを一つずつ、検出部に送る。

培養7日目のAC133陽性細胞におけるCD31発現

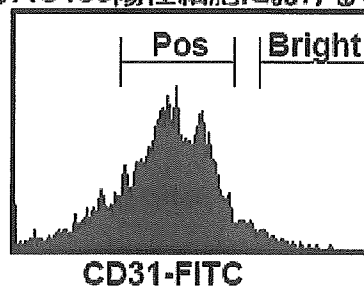
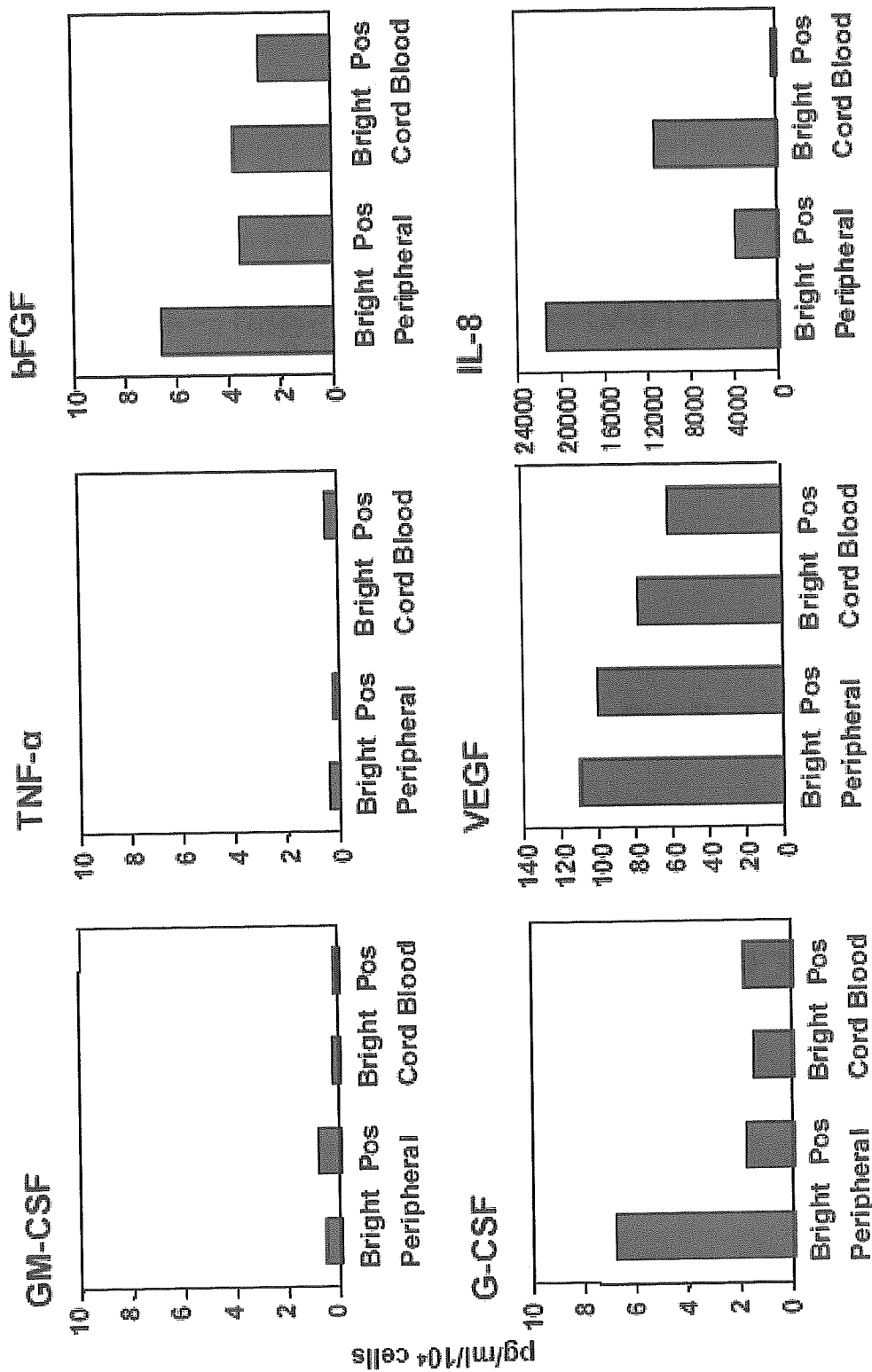
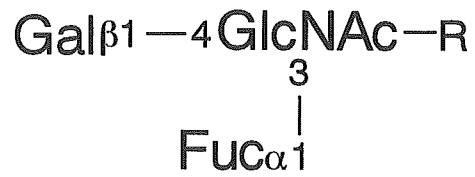


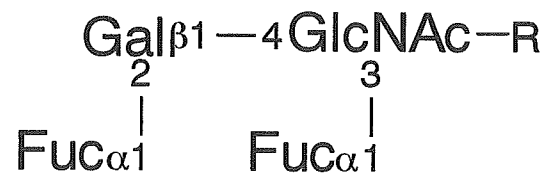
図25. CD31強陽性・陽性細胞におけるサイトカインの放出



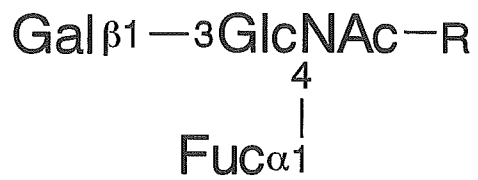
Lewis x (Le^x)



Lewis y (Le^y)



Lewis a (Le^a)



Lewis b (Le^b)

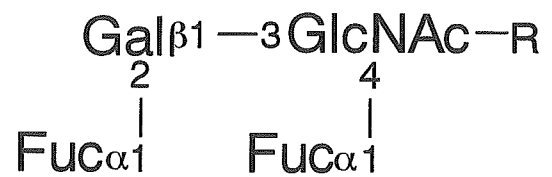


図26 Le^x, Le^y, Le^a 及び Le^b糖鎖の構造

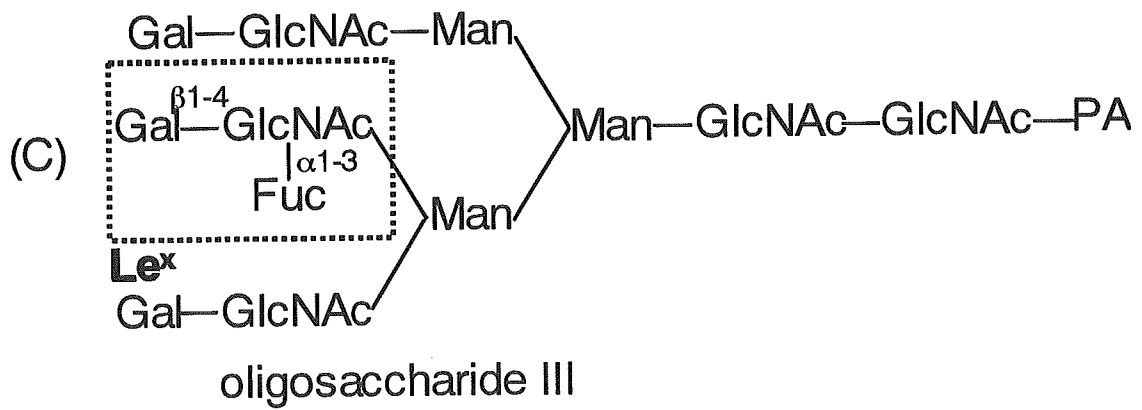
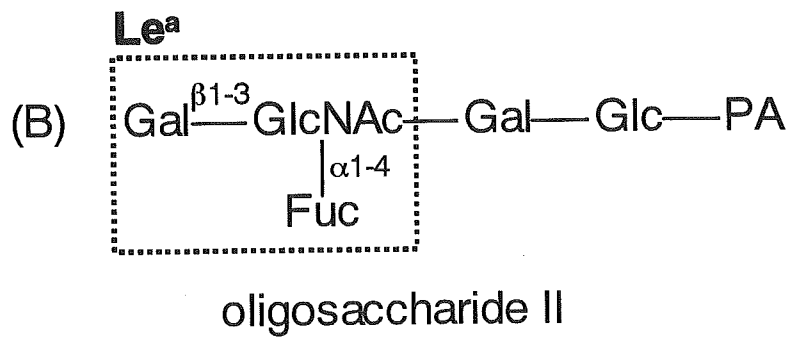
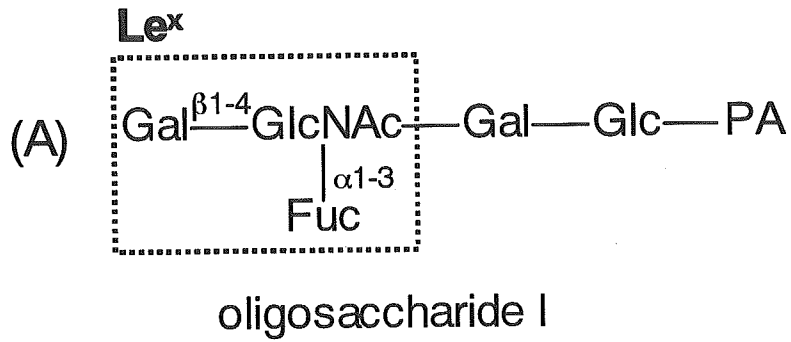


図27 Le^x及びLe^aモデル糖鎖

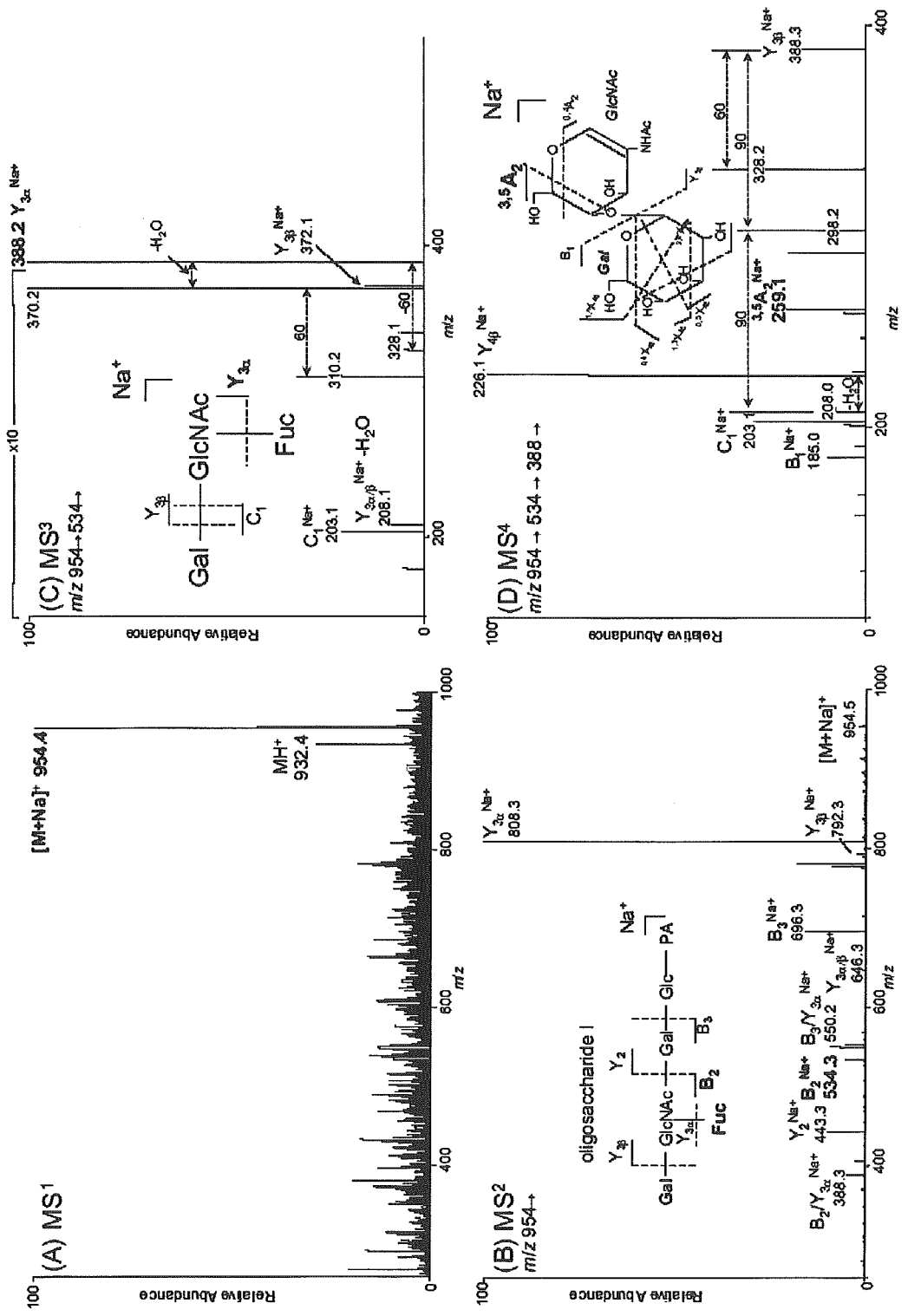


図28 Lexマニル(糖鎖)のMS¹⁻⁴スペクトル

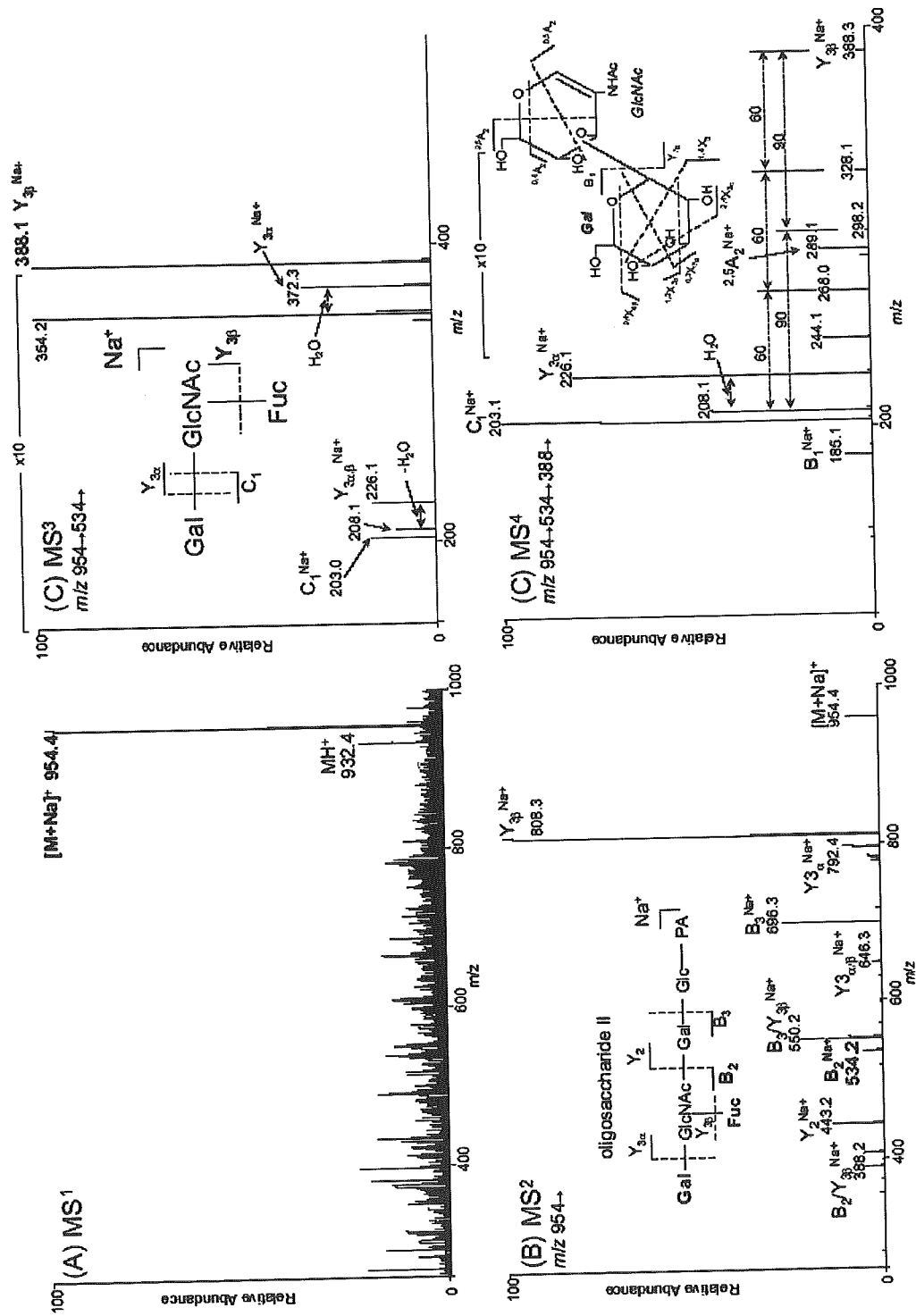


図29 Le^aモデル(糖鎖II)のMS¹⁻⁴スペクトル

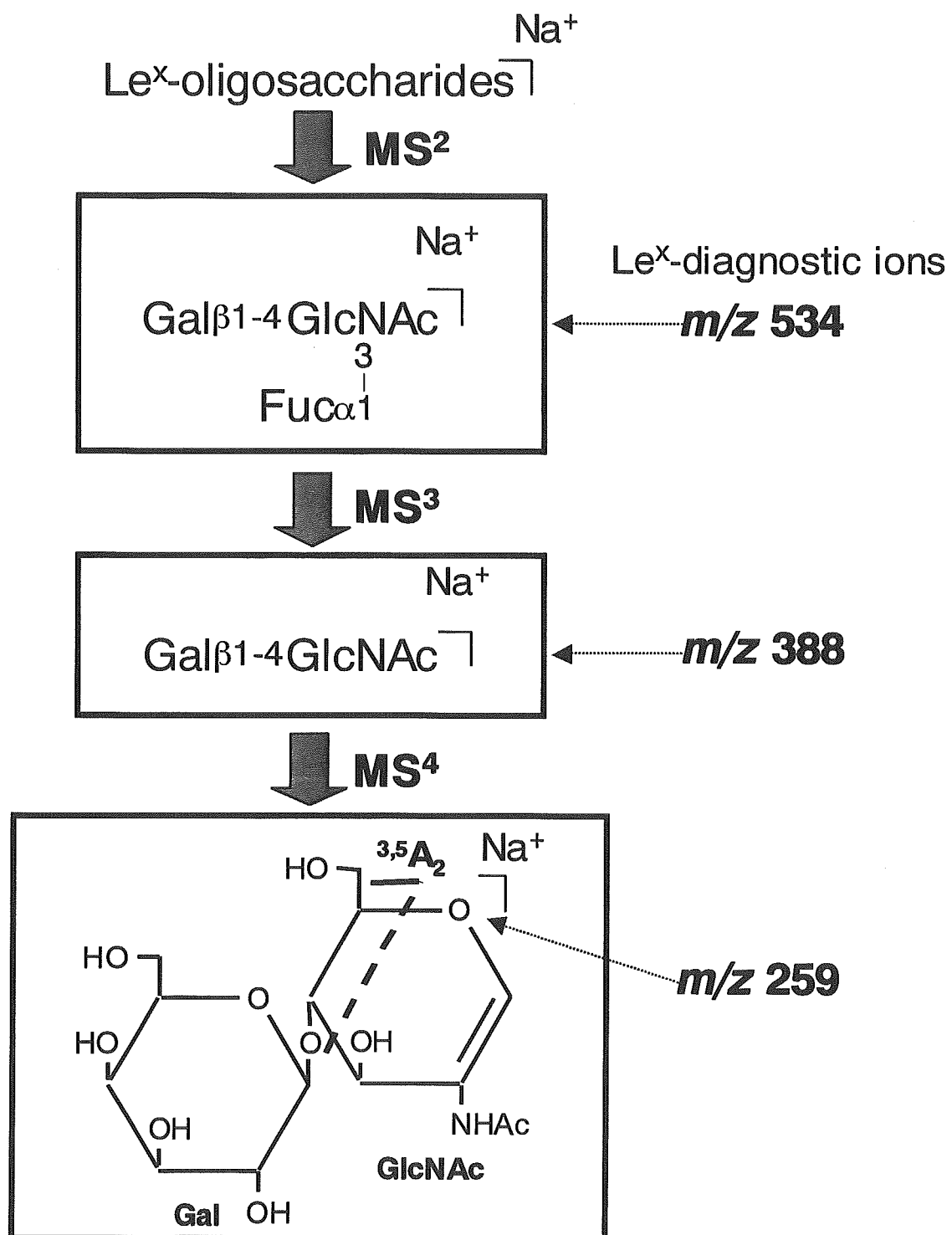


図30 Le^x糖鎖の開裂とLe^x診断イオン

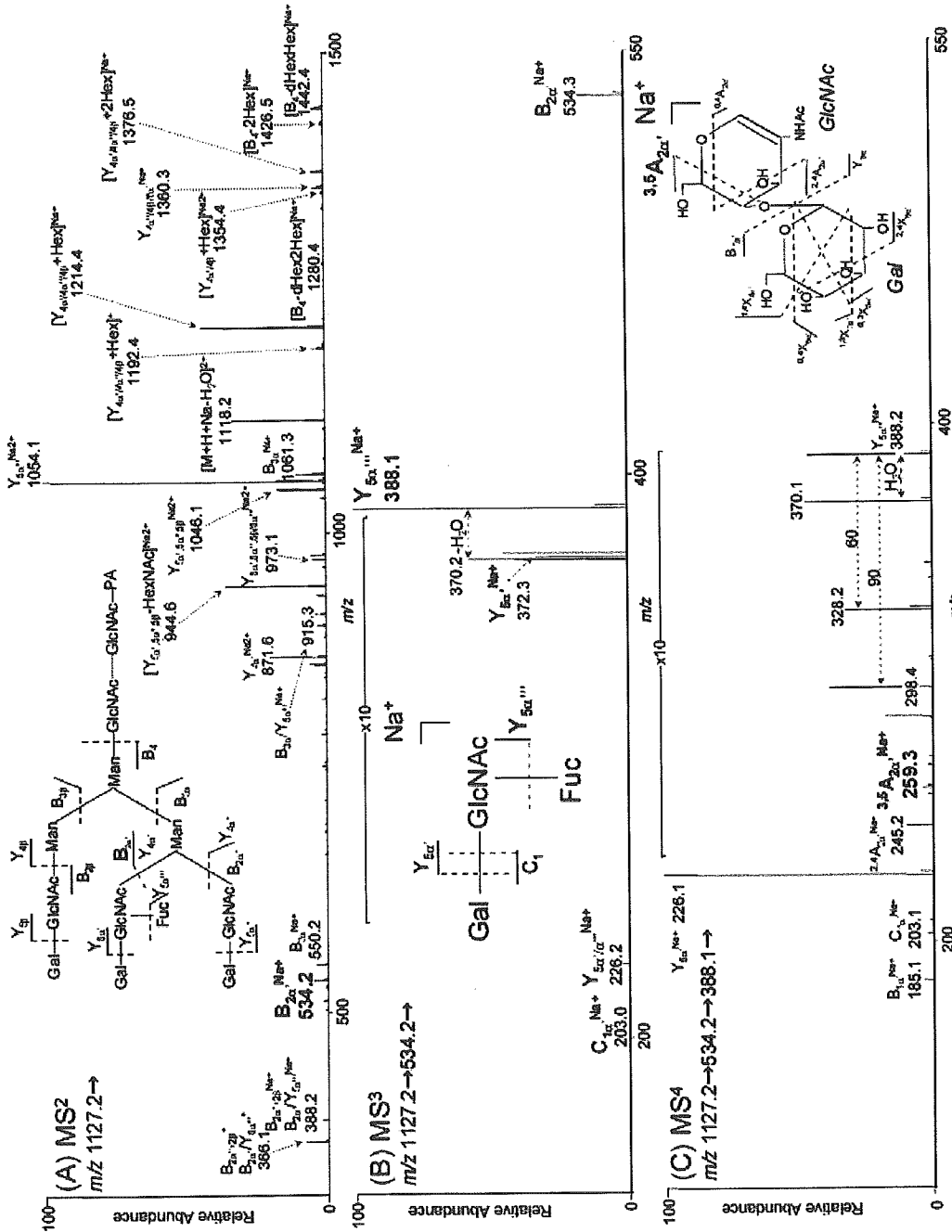


図31 Le^xモデル(糖鎖II)のMS²⁻⁴スペクトル

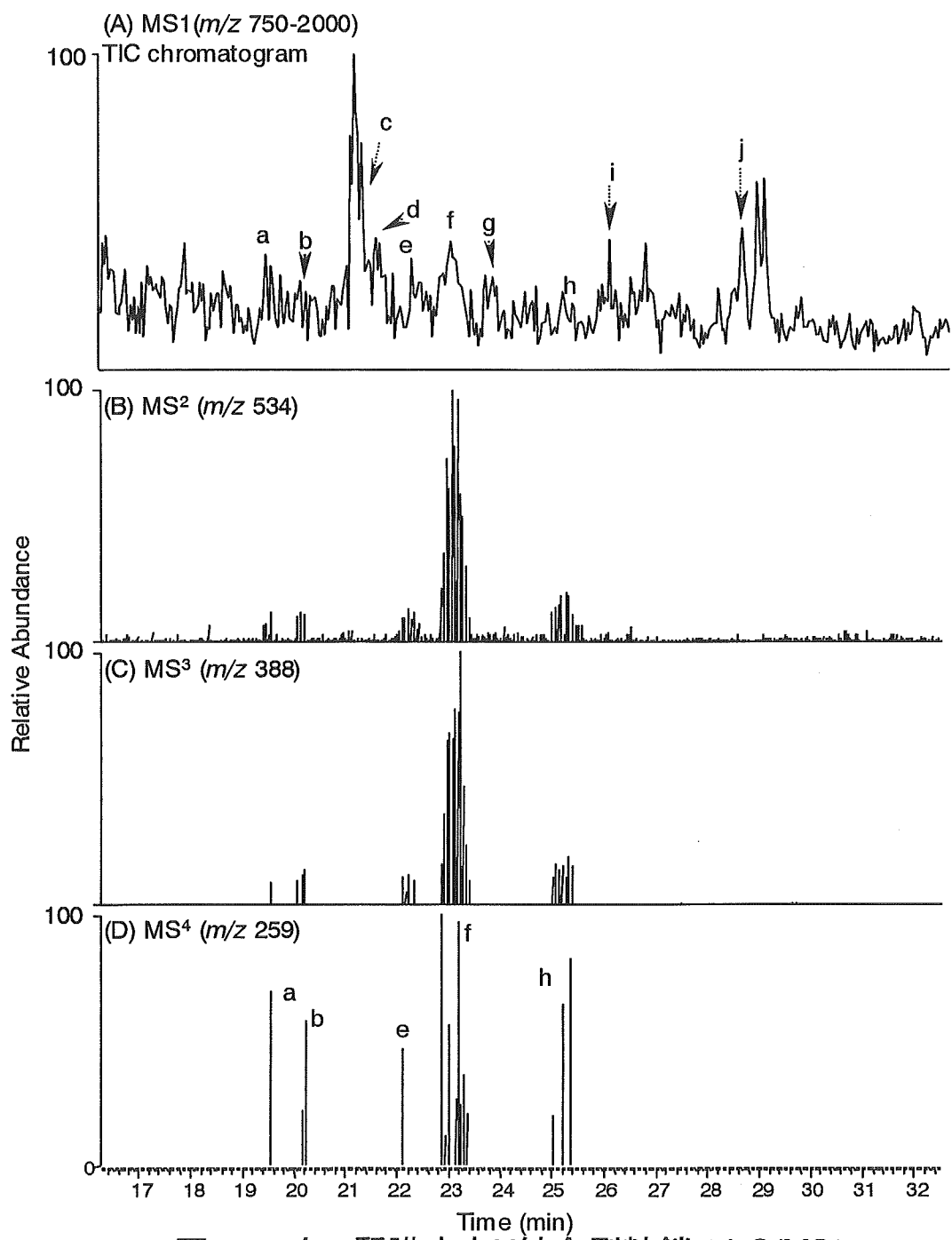


図32 マウス腎臓由来N結合型糖鎖のLC/MS¹⁻⁴

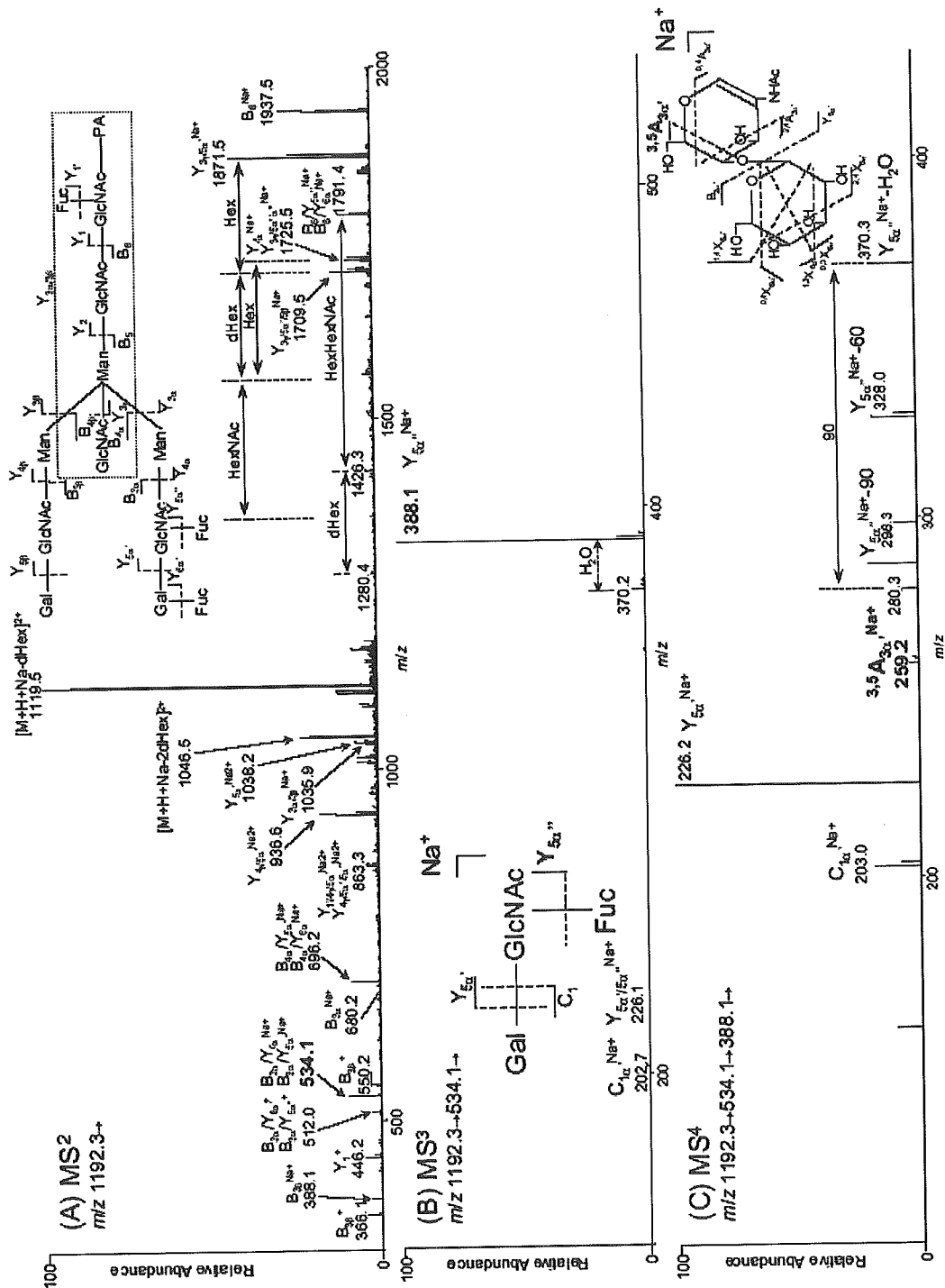


図33 マウス腎臓由来糖鎖 (ピーク①)のMS²⁻⁴スペクトル

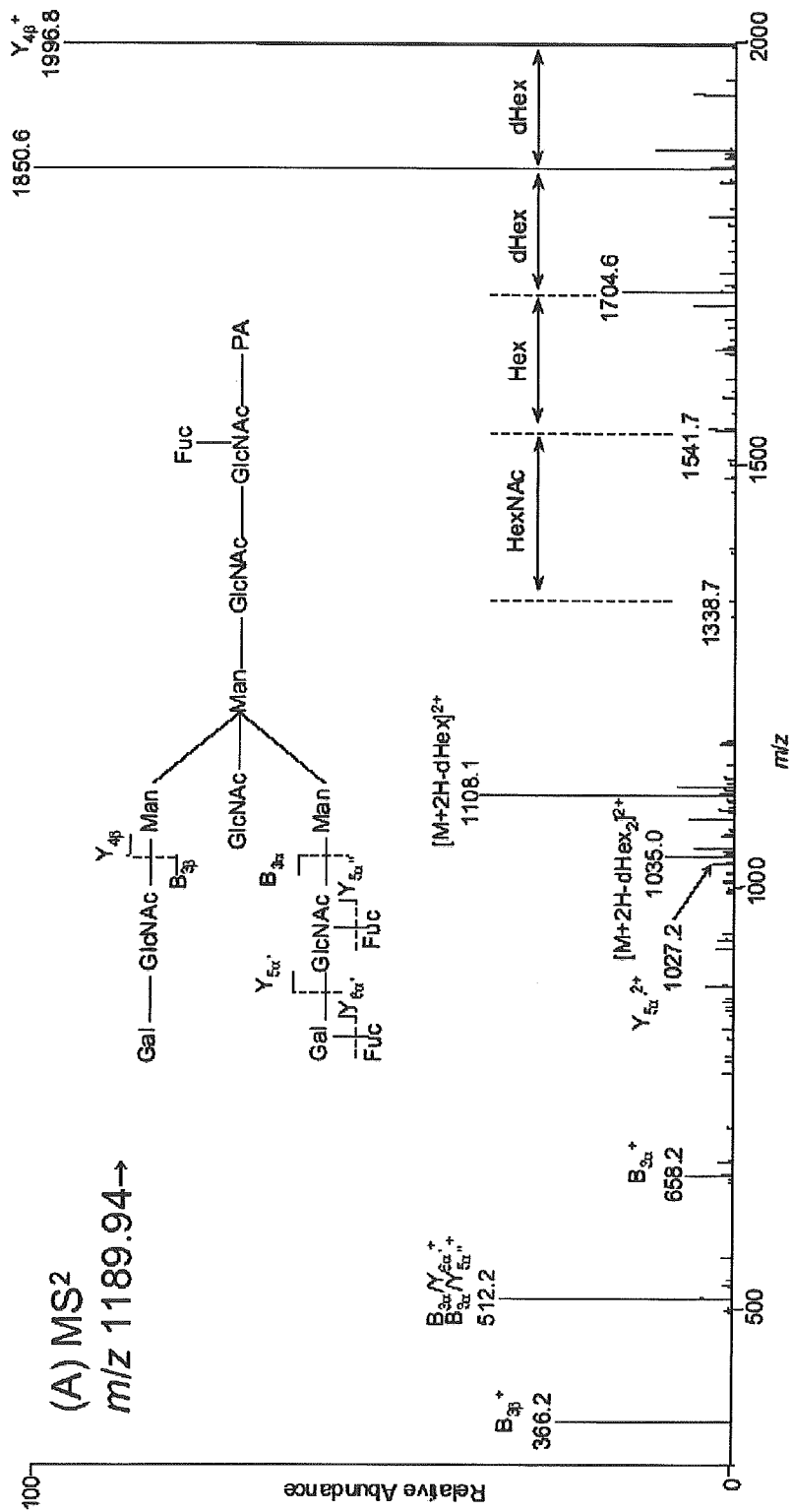


図34 Ley糖鎖のMS²スペクトル