

直貴、藤田卓也、山本昌、早川堯夫、水口裕之；35型アデノウィルスベクターを用いた遺伝子導入におけるβ1インテグリンの関与に関する検討；第21回日本DDS学会（東京）；2006年7月7-8日

61) Fuminori Sakurai, Kenji Kawabata, Takao Hayakawa, Hiroyuki Mizuguchi; Adenovirus serotype 35 vector-mediated transduction in mouse hematopoietic stem cells isolated from human CD46-transgenic mice; 9th Annual Meeting of American Society of Gene Therapy ; 2006年6月 (Baltimore, USA)

H. 知的財産権の出願・登録

H-1 特許取得

特願 2006-109854 「細胞の心筋細胞分化活性検出用マーカー」出願日：平成 18 年 4 月 12 日、発明者：佐藤陽治、長谷川哲也、山口照英、細野哲司、佐藤光利

H-2 実用新案登録 なし

H-3 その他 該当事項なし

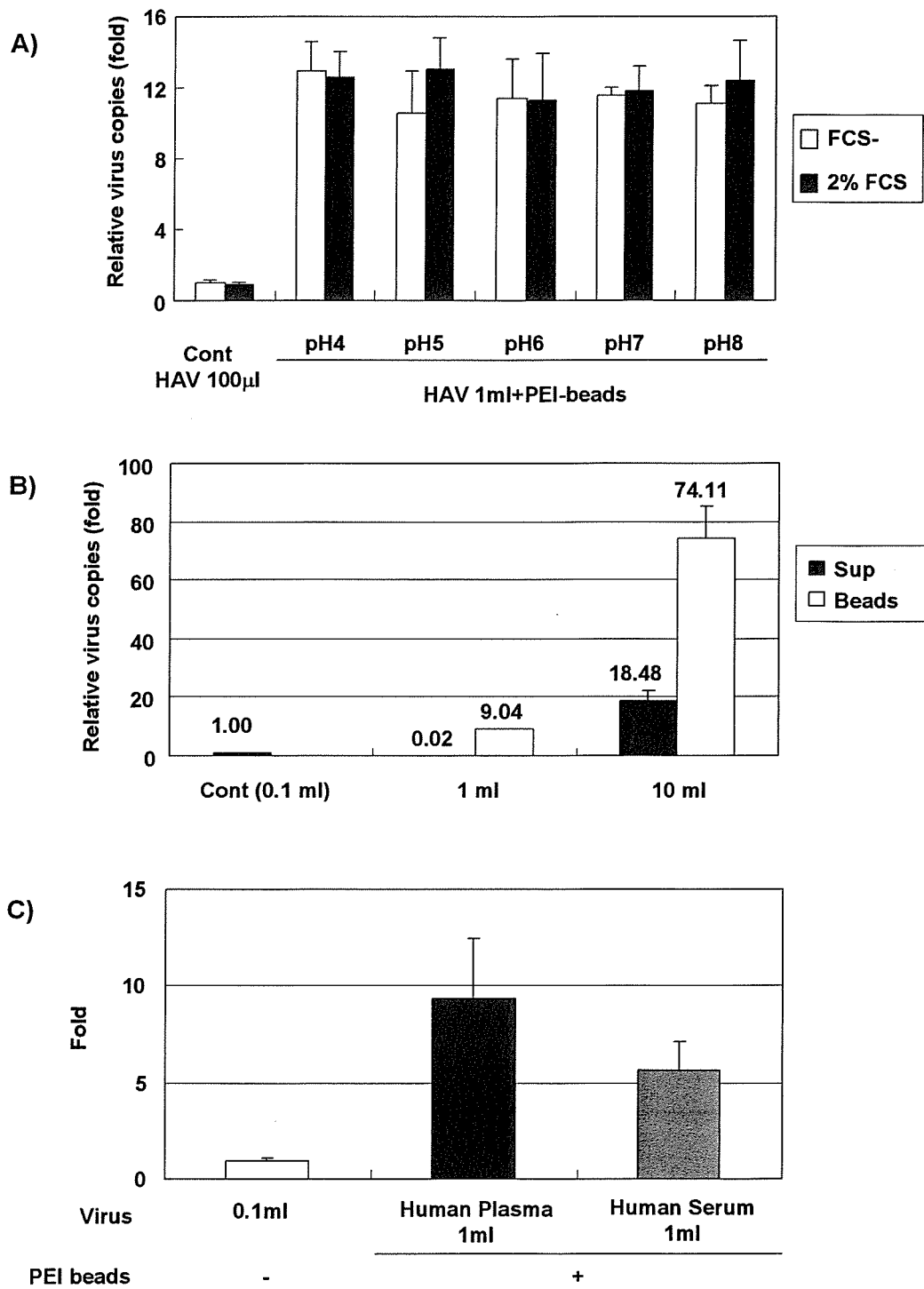


Fig.1 PEIビーズによるHAVの濃縮

A) pH, FCSの影響; B) 大容量からの濃縮; C) ヒト正常血漿、血清の影響

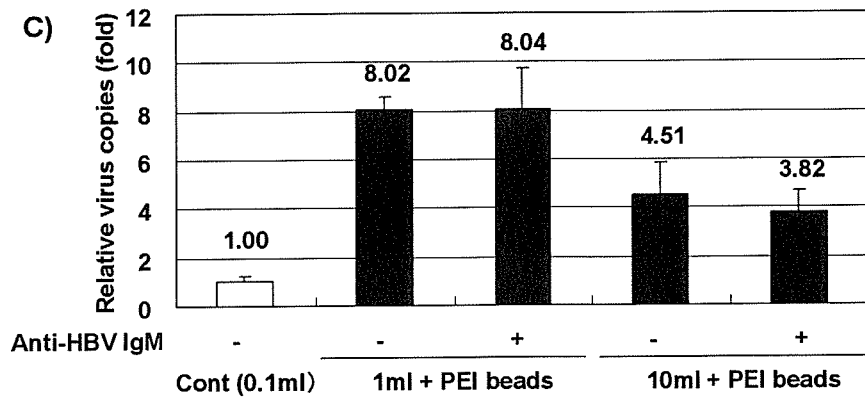
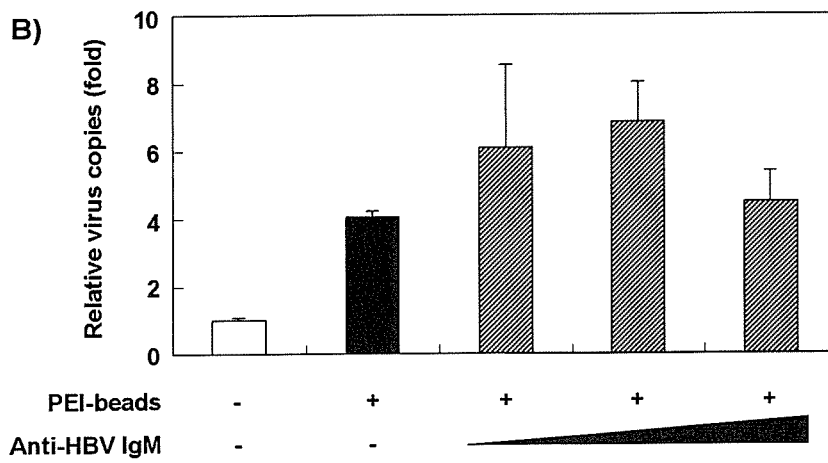
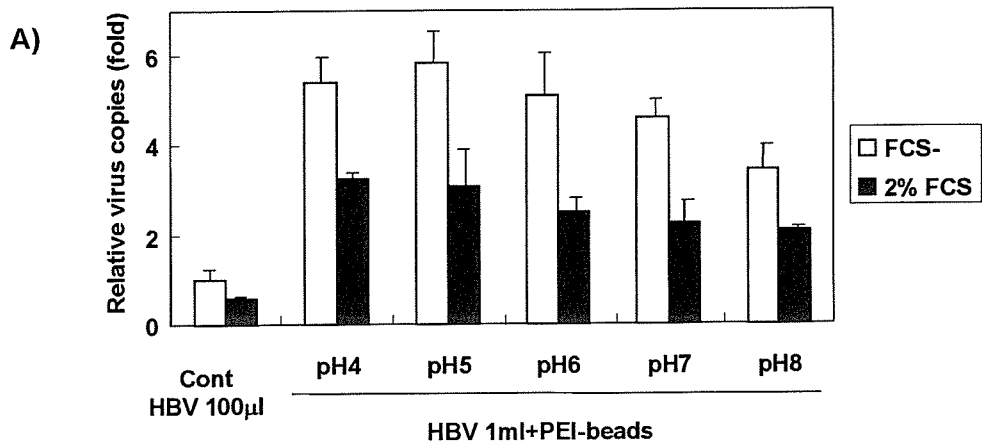


Fig.2 PEIビーズによるHBVの濃縮

A) pH, FCSの影響; B) 抗HBV-IgM抗体の影響; C) 大容量からの濃縮

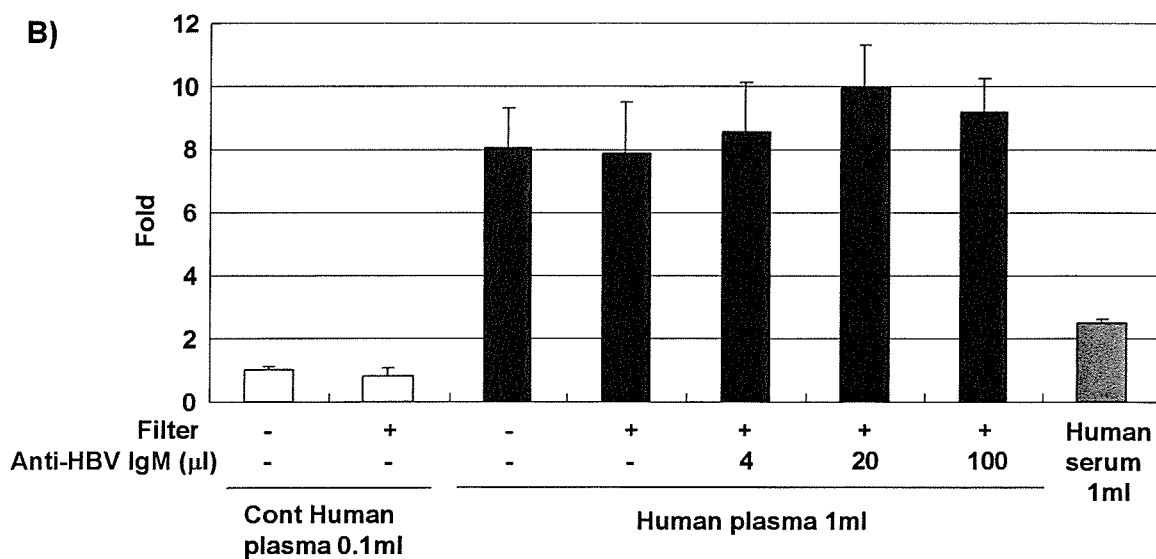
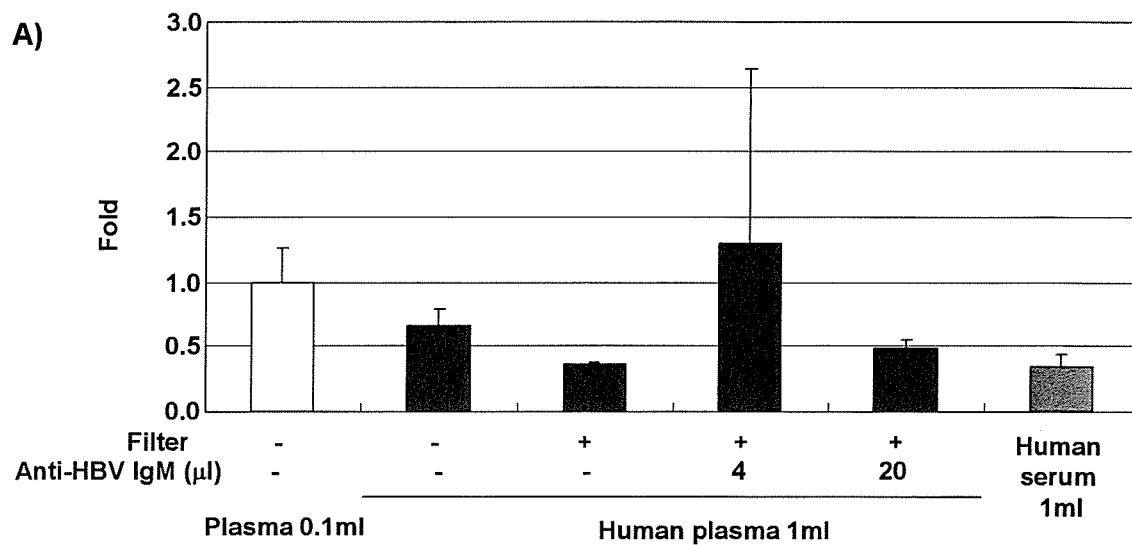


Fig.3 PEIビーズによるHBV濃縮に及ぼすヒト正常血漿、血清の影響

A) HBV標準品; B) HBVジェノタイプパネル

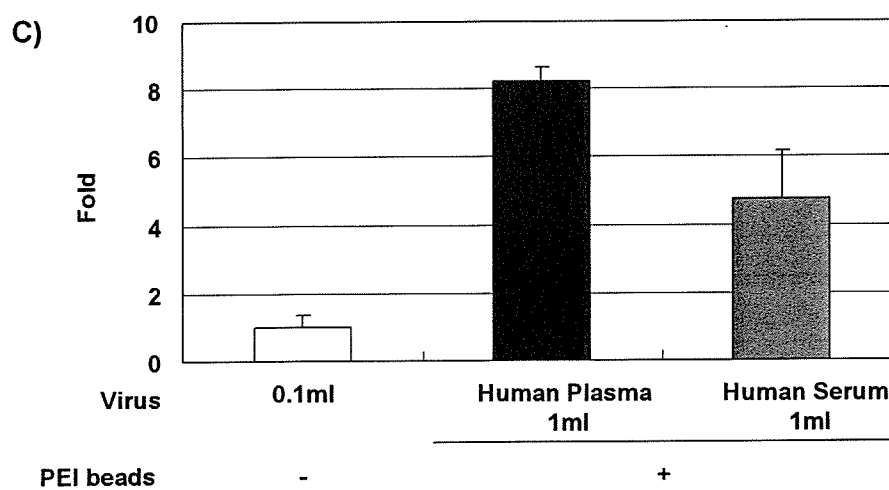
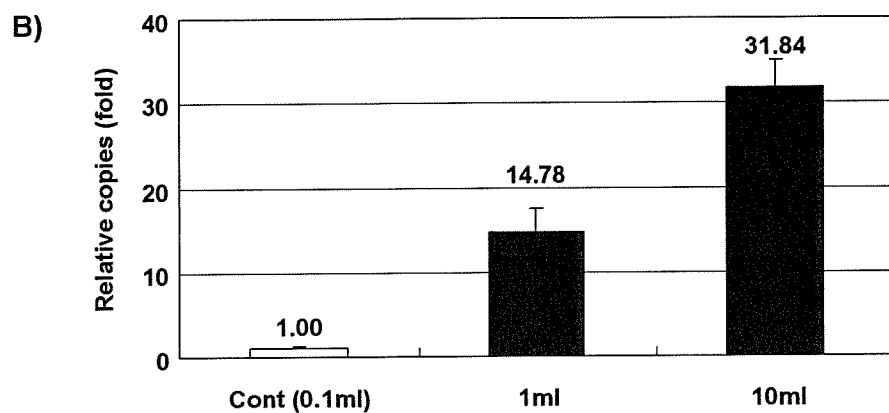
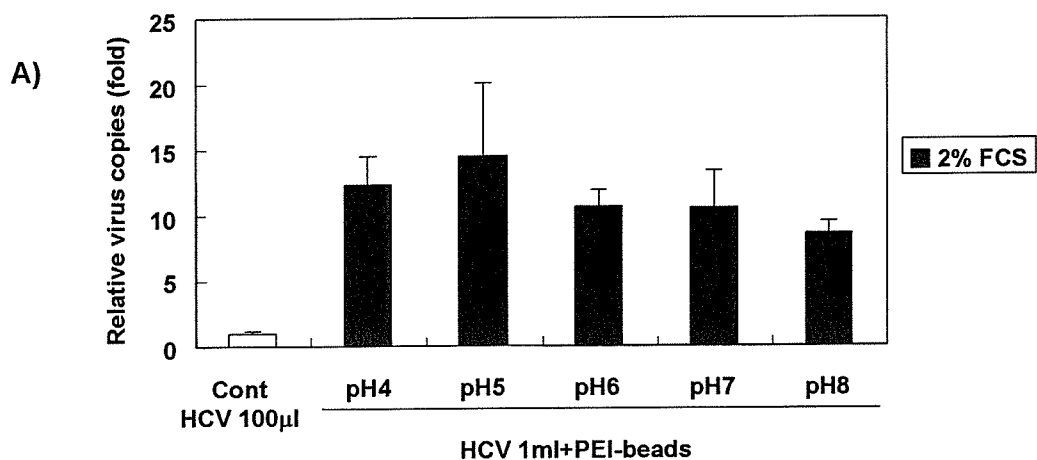
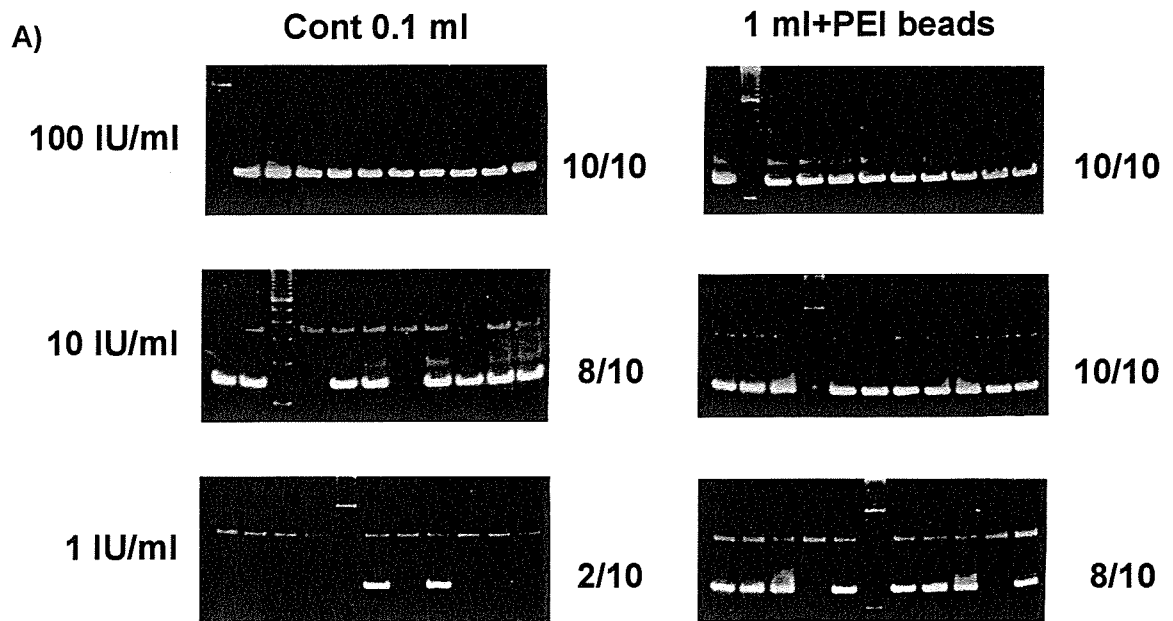


Fig.4 PEIビーズによるHCVの濃縮

A) pH, FCSの影響; B) 大容量からの濃縮; C) ヒト正常血漿、血清の影響

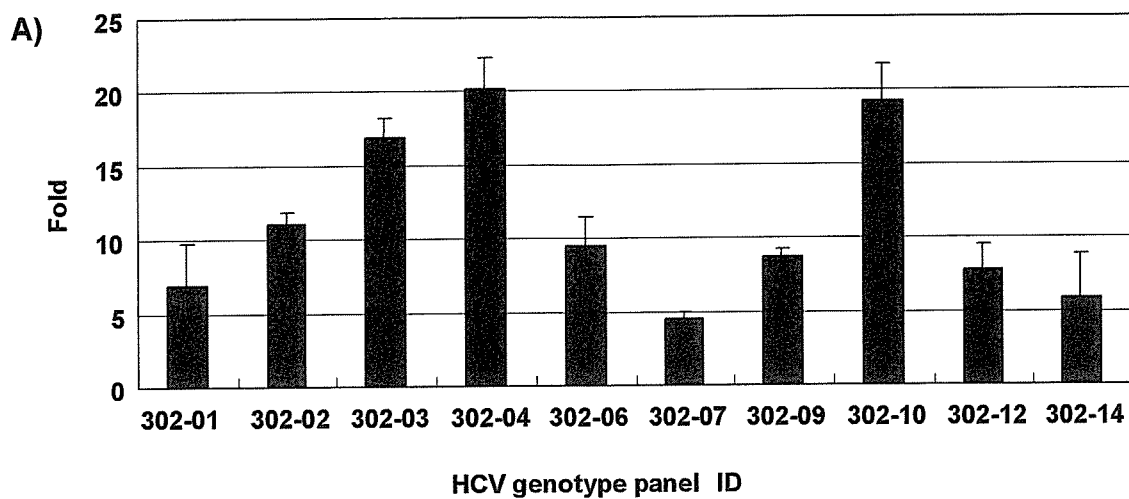


B)

HCV conc. (IU/ml)	Control 0.1ml			1ml + PEI beads		
	#1 (Copies)	#2 (Copies)	Mean (Copies)	#1 (Copies)	#2 (Copies)	Mean (Copies)
100	7.7×10^1	8.6×10^1	8.2×10^1	5.5×10^2	5.9×10^2	5.7×10^2
10	8.3×10^0	2.3×10^1	1.6×10^1	8.5×10^1	7.2×10^1	7.9×10^1
1	-	-	-	-	6.8×10^0	3.4×10^0

Fig.5 PEIビーズによるHCVの濃縮とHCV検出感度

A) Nested PCR; B) 定量的RT-PCR
HCVはヒト正常血漿で希釈して使用。



B)

Genotype ID	Origin	HCV genotyping	RNA copies/ml
302-01	China	1b	3.1×10^6
302-02	Thailand	1	1.1×10^6
302-03	South Africa	1b	4.0×10^5
302-04	China	2a	2.5×10^6
302-06	China	3b	6.6×10^6
302-07	USA	3a	8.2×10^5
302-09	Egypt	4a	1.0×10^6
302-10	Egypt	4	8.8×10^4
302-12	Unknown	5a	6.5×10^5
302-14	Unknown	6a	1.6×10^6

Fig.6 PEIビーズによるHCV ジェノタイプパネルの濃縮

A) ジェノタイプパネル 1mlからのPEI磁気ビーズ濃縮

B) ジェノタイプパネルの詳細

各試料はヒト正常血漿で希釈して使用。

Table 1 カスタム oligo-CGH アレイのプローブデザイン

	Physical position	Size (Mb)	No. of probes	Interval (kb)
MYC amplification area				
1	sense: 125,800,000-130,950,000	5.15	46377	continuous
2	sense: 130,950,000-137,800,000	6.85	5341	1
3	sense: 137,800,000-138,000,000	0.2	1688	continuous
4	sense: 138,000,000-146,214,826	8.27	6326	1
5	sense: 146,214,826-146,274,826(telomere)	0.06	181	continuous
6	antisense: 125,822,452-127,032,094	1.21	11230	continuous
7	antisense: 127,252,004-127,923,887	0.67	5187	continuous
8	antisense: 128,116,770-128,139,631	0.02	239	continuous
9	antisense: 128,306,003-128,519,403	0.21	2031	continuous
10	antisense: 129,909,921-130,528,321	0.62	5955	continuous
11	antisense: 130,591,690-130,945,617	0.35	2653	continuous
total			87408	
Chr.10				
1	25,000,000-26,000,000	1	8995	continuous
2	1-200,000	0.2	1166	continuous
3	200,000-25,000,000; 26,000,000-38,000,000	36.8	28389	1
total			38550	
Chr.5				
1	53,310,000-53,360,000	0.05	573	continuous
2	139,300,000-139,600,000	0.3	3285	continuous
3	53,360,000-139,300,000	85.94	15122	5
total			18980	
Chr.9				
1	11,600,000-12,000,000	0.4	3422	continuous
2	32,500,000-32,900,000	0.4	3515	continuous
3	12,000,000-32,500,000	20.5	3609	5
4	101,000,000-104,500,000 (Novel small deletion)	3.5	32446	continuous
total			42992	
Chr.11				
1	1-200,000	0.2	1292	continuous
2	2,380,000-2,880,000	0.5	5906	continuous
3	44,800,000-45,200,000	0.4	4072	continuous
total			11270	
Chr.16				
1	83,500,000-84,500,000	1	11583	continuous
2	88,767,254-88,827,254	0.06	557	continuous
3	84,500,000-88,767,254	4.27	3575	1
total			15715	
Chr.17				
1	1-60,000	0.06	639	continuous
2	60,000-20,000,000	19.94	3655	5
3	20,000,000-22,500,000	2.5	20759	continuous
total			25053	
Chr.15				
1	28,200,000-29,200,000	1	9861	continuous
total			9861	
Chr.14				
1	62,600,000-64,600,000	2	19386	continuous
2	80,500,000-81,000,000	0.5	4661	continuous
3	64,600,000-80,500,000	15.9	2890	5
total			26937	
Chr.18 (1-76,117,153)				
1	sense	-	13468	5
2	antisense	-	13468	5
total			26936	
Chr.22				
1	sense		6365	5
total			6365	
Chr.X				
1	sense		23884	5
total			23884	
Total:338961				

*Chr10 および Chr11 の末端領域を、1-200,000 に変更して設計しました。
*Chr22 および ChrX を、5kbp のインターバルにて追加設計しました。

Table 2 6 継代および 26 継代目の hMSC 細胞の G-banding による核型解析

Mode	40	41	42	43	44	45	46	47	Total
Cell No. of Passage-6							50		50
Cell No. of Passage-26	1	3	1	2	2	6	14	21	50

		Cell No
Passage-6	46 XX	10
Passage-26	A 47, XX, +7, +der(7) del (7)(p?)del(7)(q?), der(7) (7pter→7p15::7?::cen::17?),	9
	B 46, idem, dic(21,22)(p13;p11)	1

Fig.7 HL60 細胞 8 番染色体 8q24 c-myc 近傍領域の CGH 解析の結果

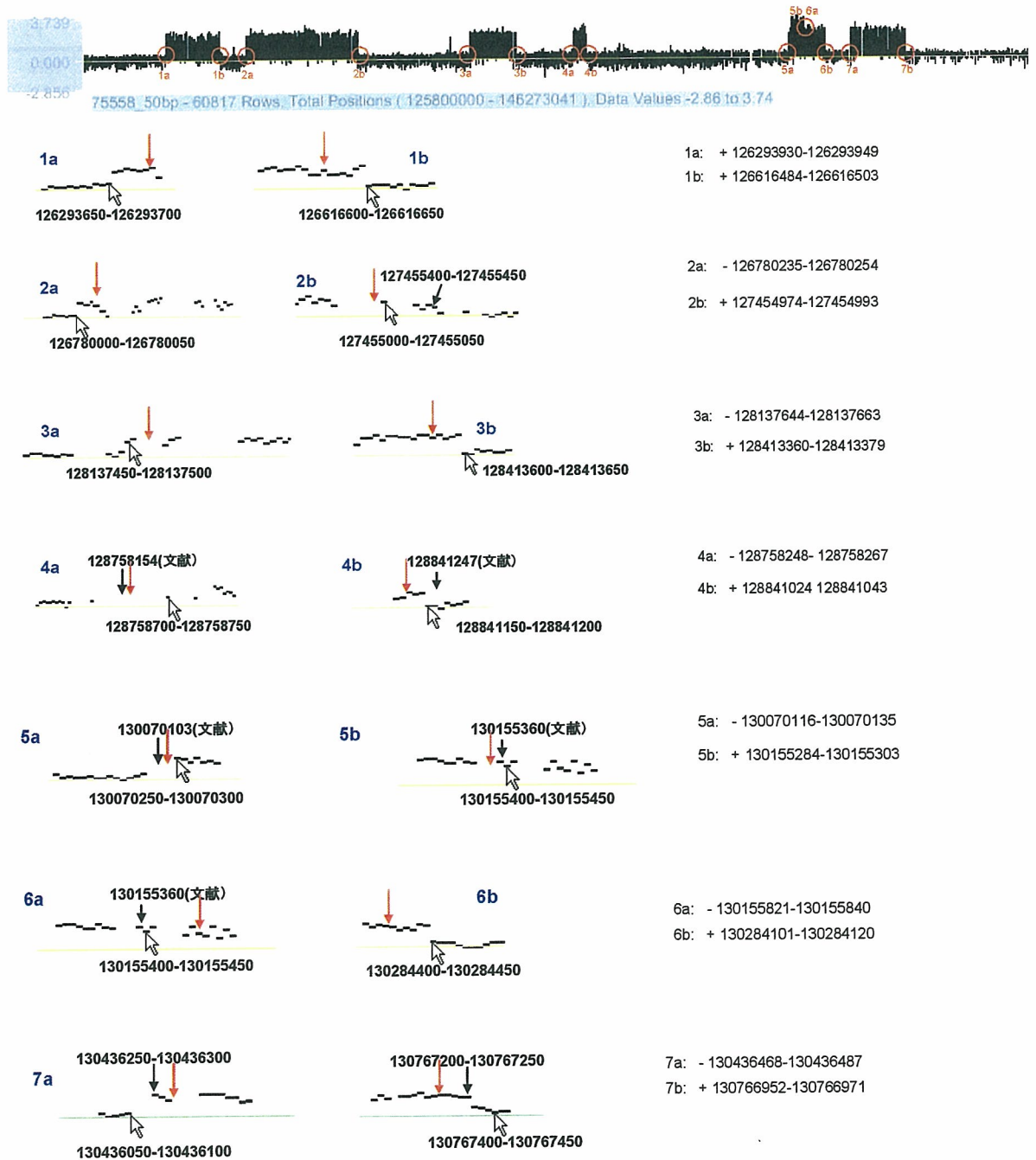
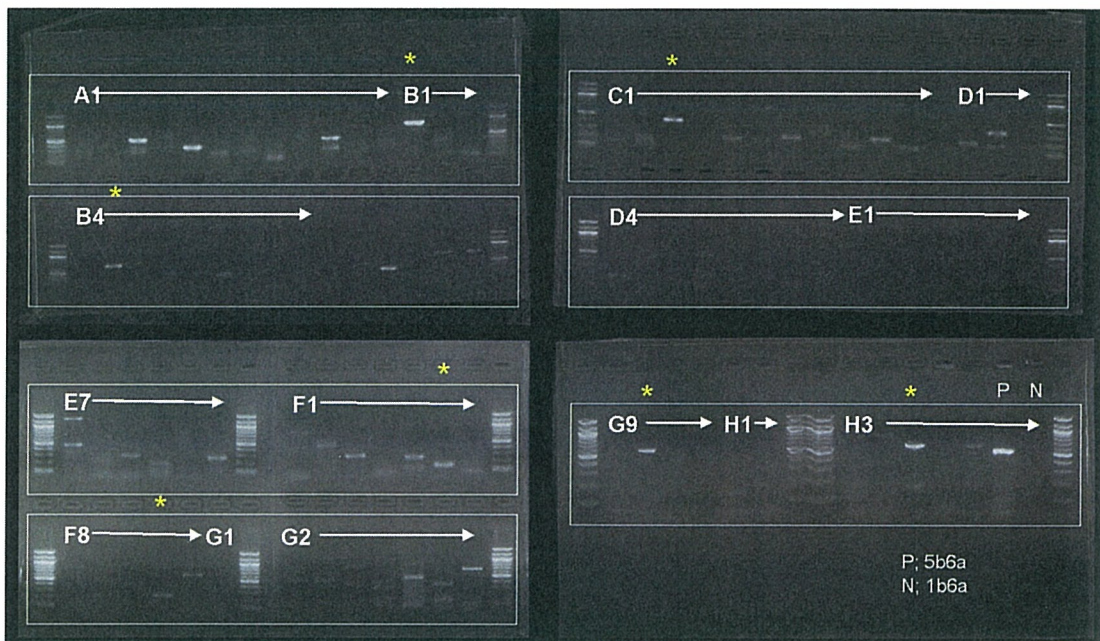


Fig.8 総当たり PCR

	1	2	3	4	5	6	7	8	9	10	11	12
A	1a1b	1a2a	1a2b	1a3a	1a3b	1a4a	1a4b	1a5a	1a5b	1a6a	1a6b	1a7a
B	1a7b	1b2a	1b2b	1b3a	1b3b	1b4a	1b4b	1b5a	1b5b	1b6a	1b6b	1b7a
C	1b7b	2a2b	2a3a	2a3b	2a4a	2a4b	2a5a	2a5b	2a6a	2a6b	2a7a	2a7b
D	2b3a	2b3b	2b4a	2b4b	2b5a	2b5b	2b6a	2b6b	2b7a	2b7b	3a3b	3a4a
E	3a4b	3a5a	3a5b	3a6a	3a6b	3a7a	3a7b	3b4a	3b4b	3b5a	3b5b	3b6a
F	3b6b	3b7a	3b7b	4a4b	4a5a	4a5b	4a6a	4a6b	4a7a	4a7b	4b5a	4b5b
G	4b6a	4b6b	4b7a	4b7b	5a5b	5a6a	5a6b	5a7a	5a7b	5b6a	5b6b	5b7a
H	5b7b	6a6b	6a7a	6a7b	7a7b							



	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b
1a	-													
1b	-	-												
2a	-	-	-											
2b	-	-	-	-										
3a	-	-	-	-	-									
3b	-	-	-	-	-	-								
4a	-	-	-	-	-	-	-							
4b	-	-	-	-	-	-	-	-						
5a	-	-	-	-	-	-	-	-	-					
5b	-	-	-	-	-	-	-	-	-	-				
6a	-	-	-	-	-	-	-	-	-	-	-			
6b	-	-	-	-	-	-	-	-	-	-	-	-		
7a	-	-	-	-	-	-	-	-	-	-	-	-	-	
7b	-	-	-	-	-	-	-	-	-	-	-	-	-	-

シークエンス解析の結果、正しい増幅が認められたプライマーペア

Fig.9 HL60 細胞における増幅単位の構造

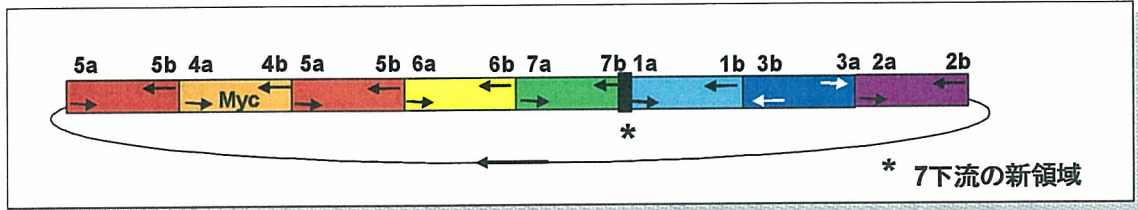


Fig.10 各増幅単位のつながり

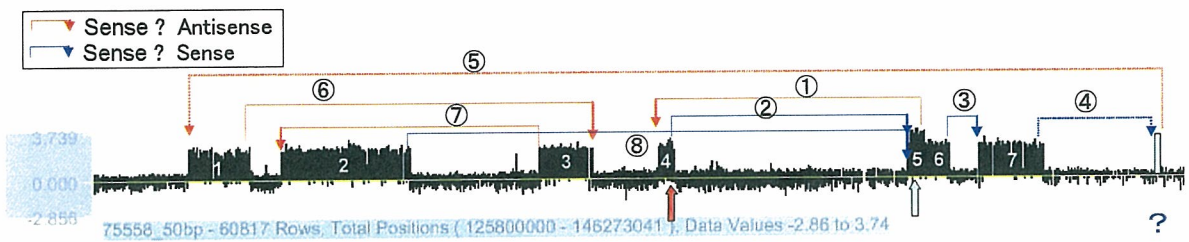
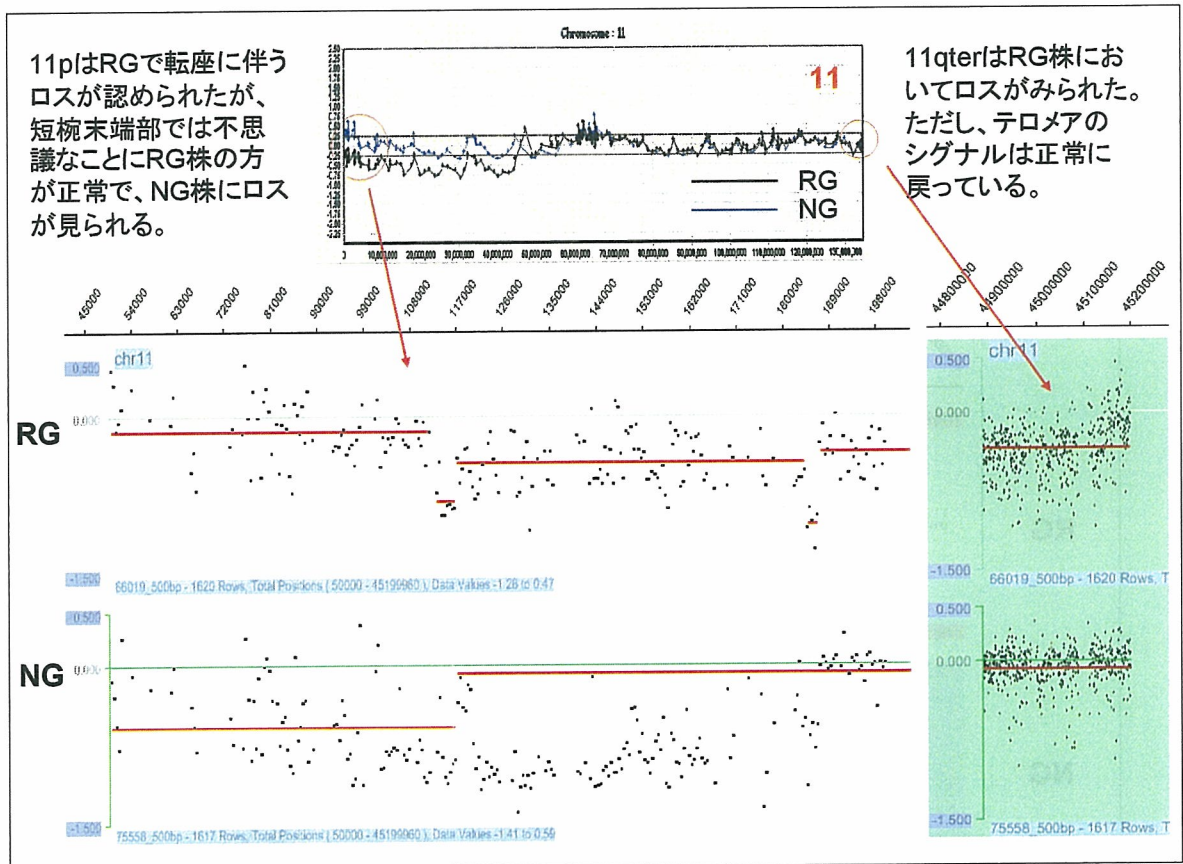
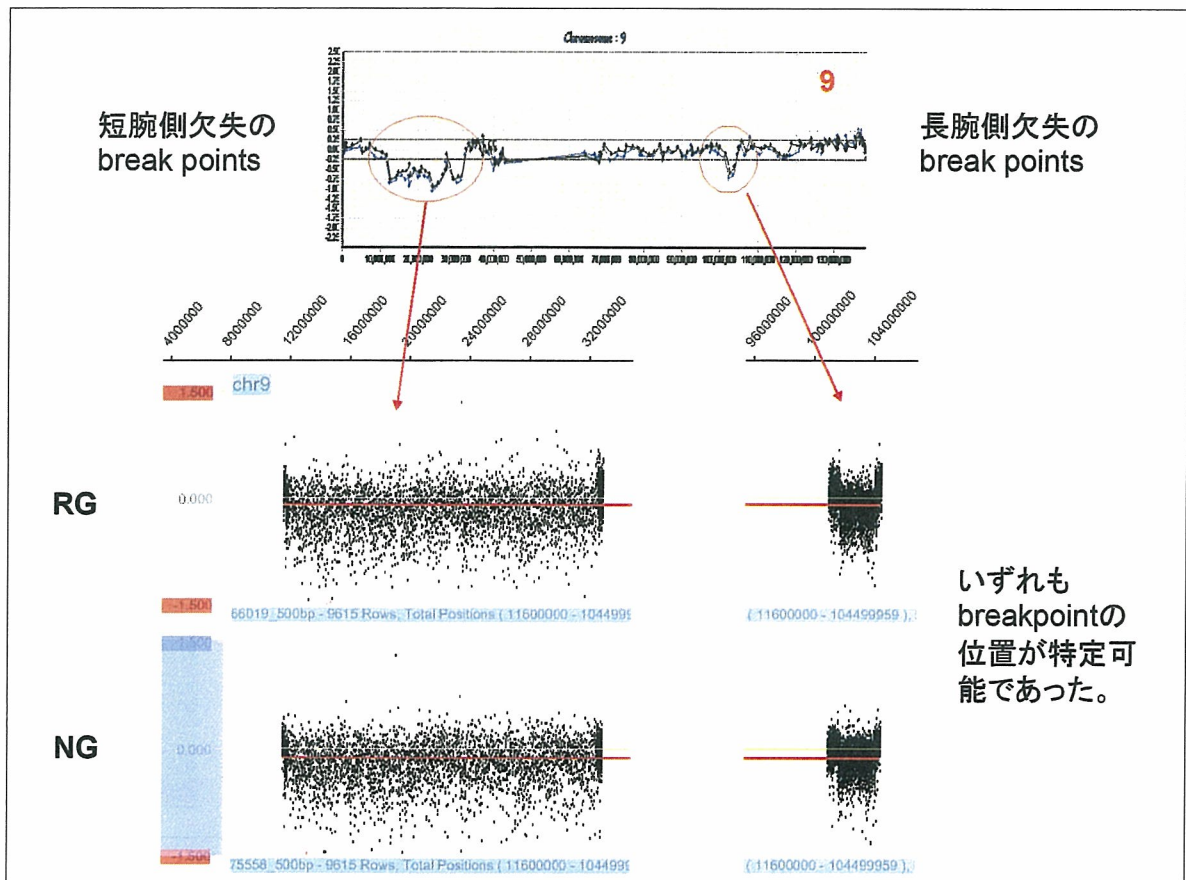
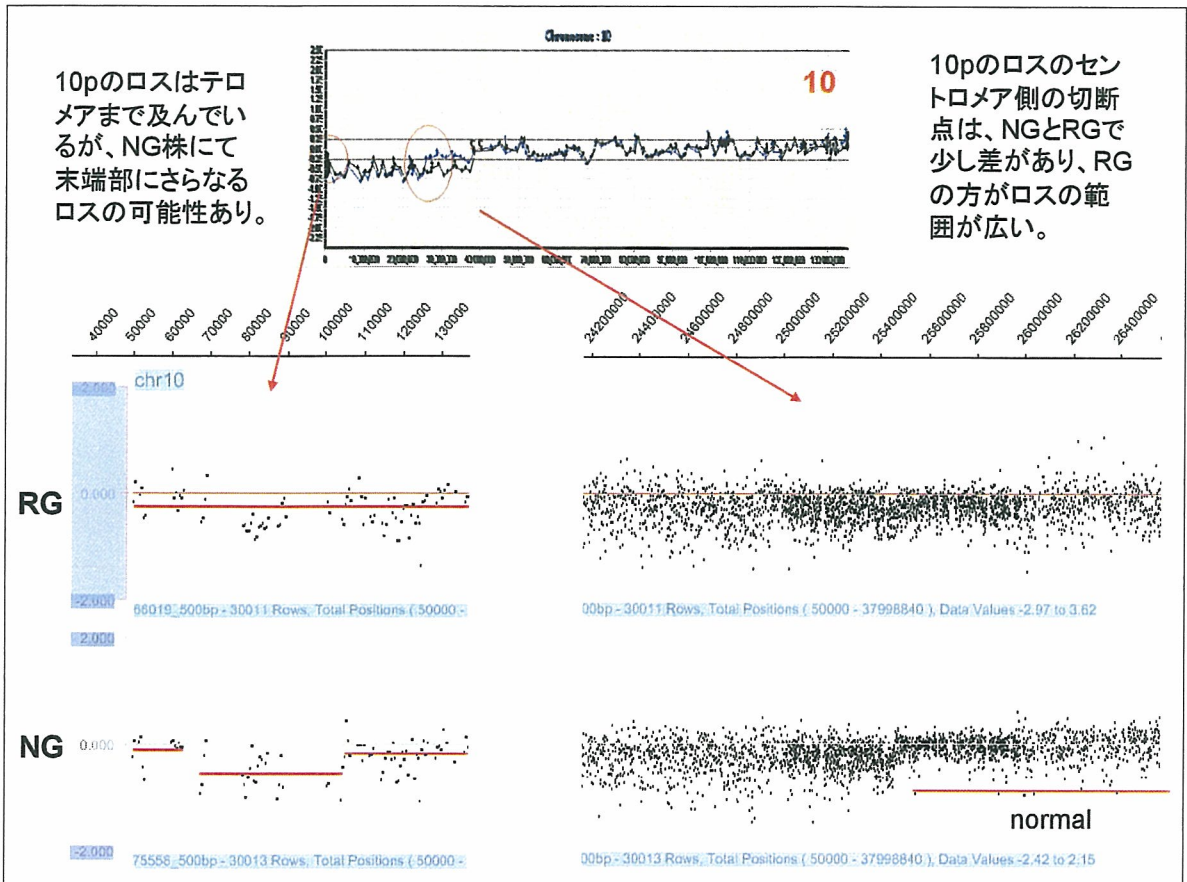


Fig.11 oligoCGHによるHL60細胞とHL60-RG株の比較





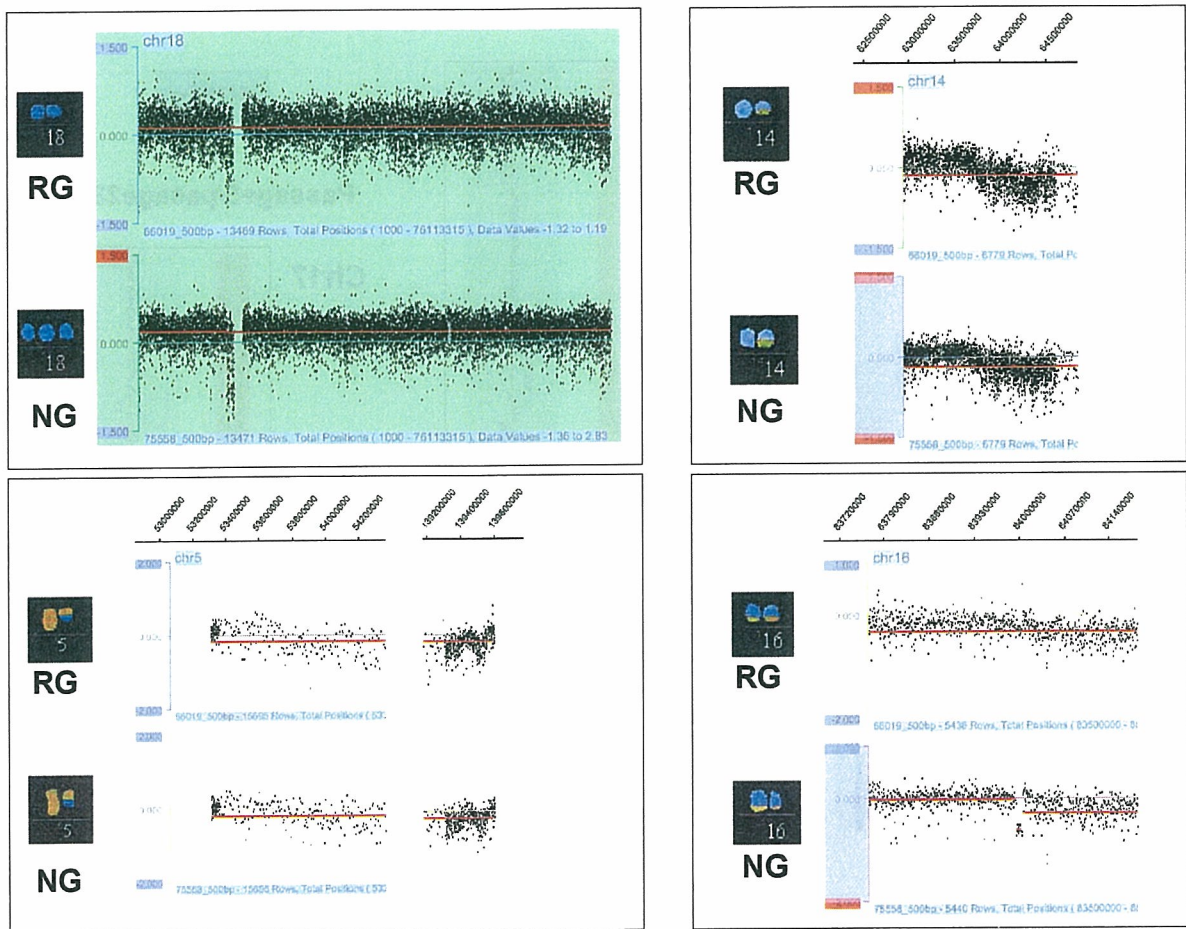
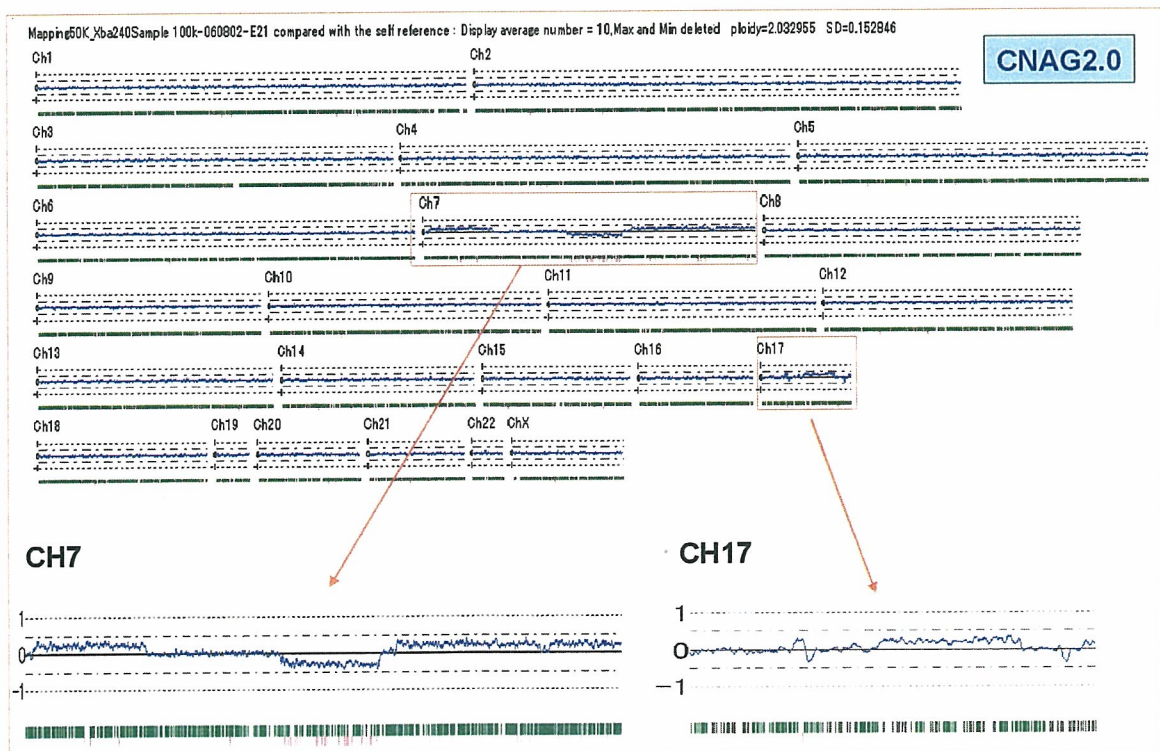


Fig.12 ヒト間葉系幹細胞の 50K SNP チップによる CGH 解析



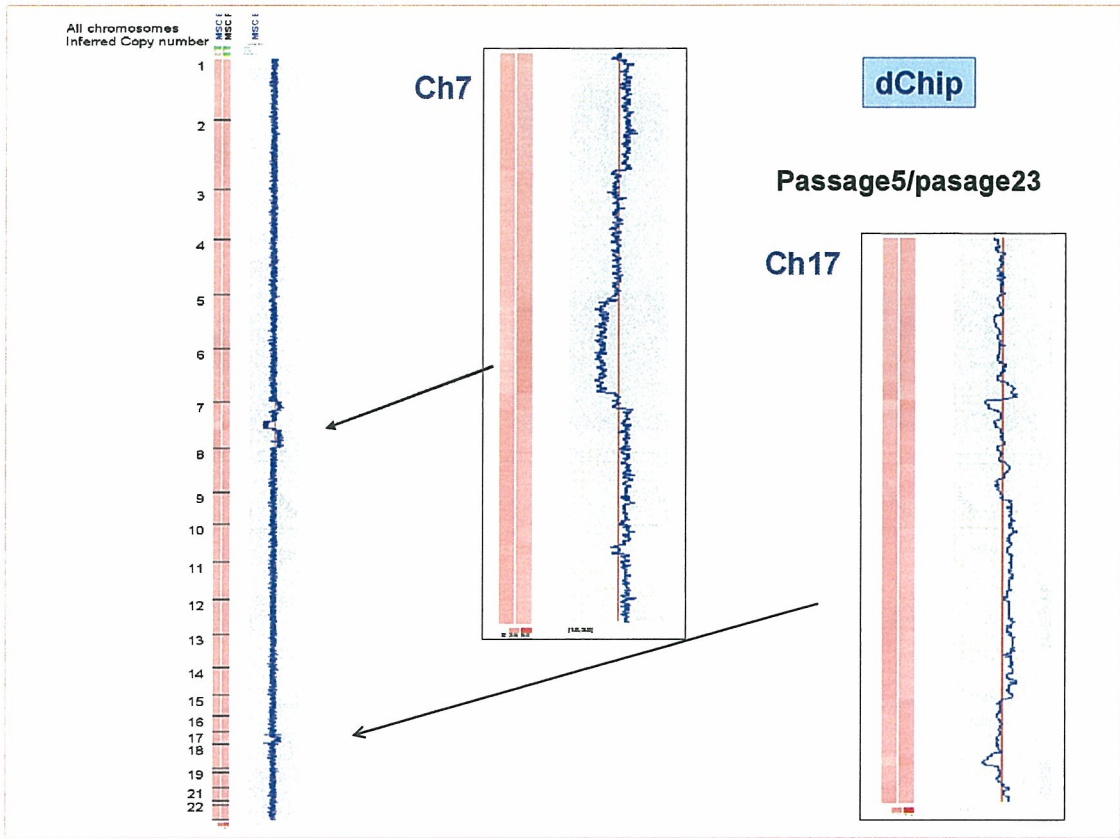


Fig.13 ヒト間葉系幹細胞の 50K SNP チップによる LOH 解析

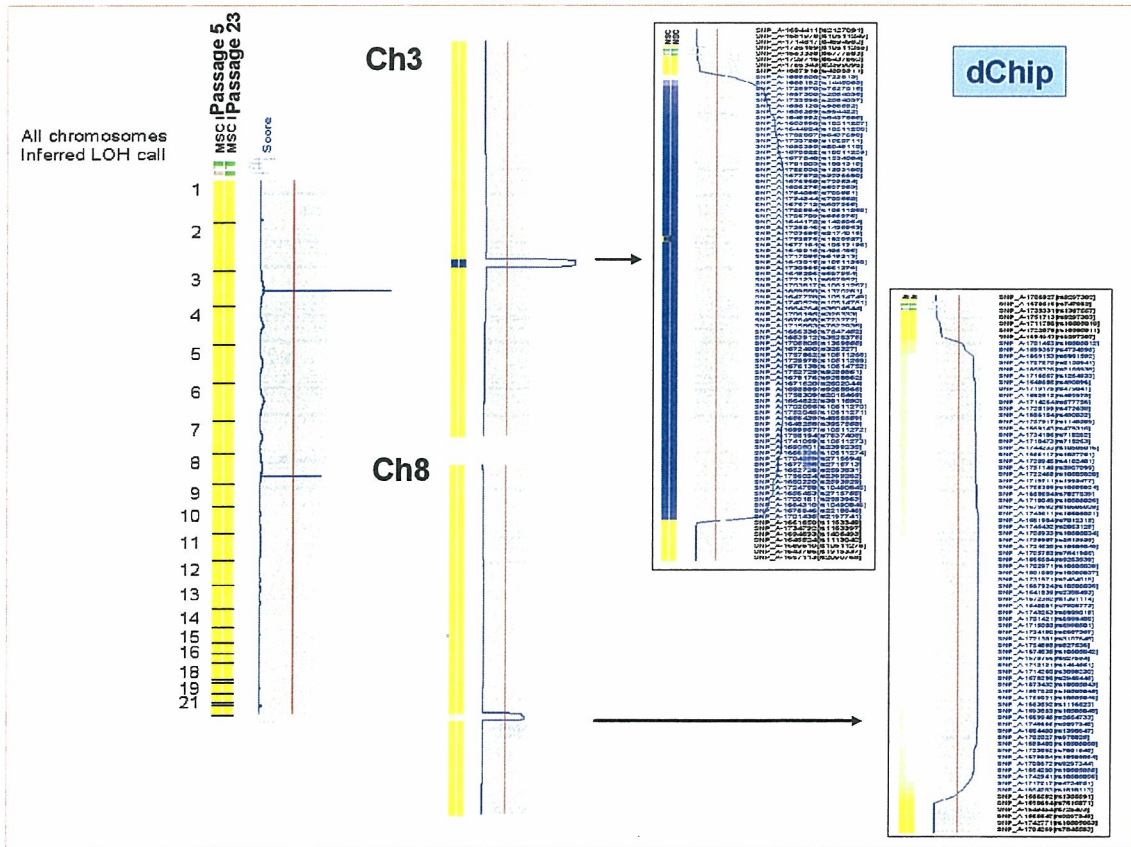
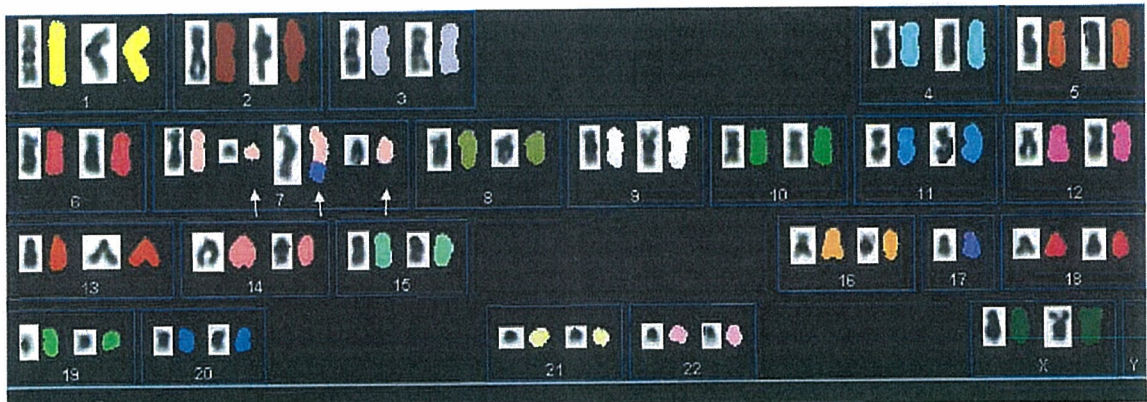
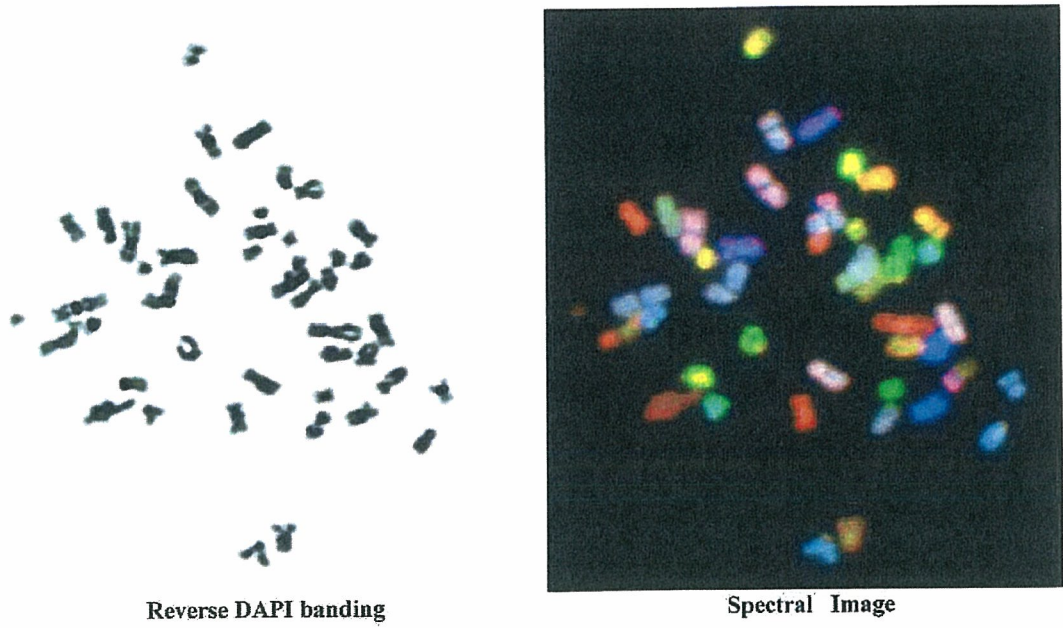


Fig.14 25 継代目 hMSC 細胞の Spectro Karyotyping 解析結果



Comprehensive Karyotyping (left side: reverse DAPI; right side: SKY)

(同一継代6 継代目の正常核型を示した細胞)

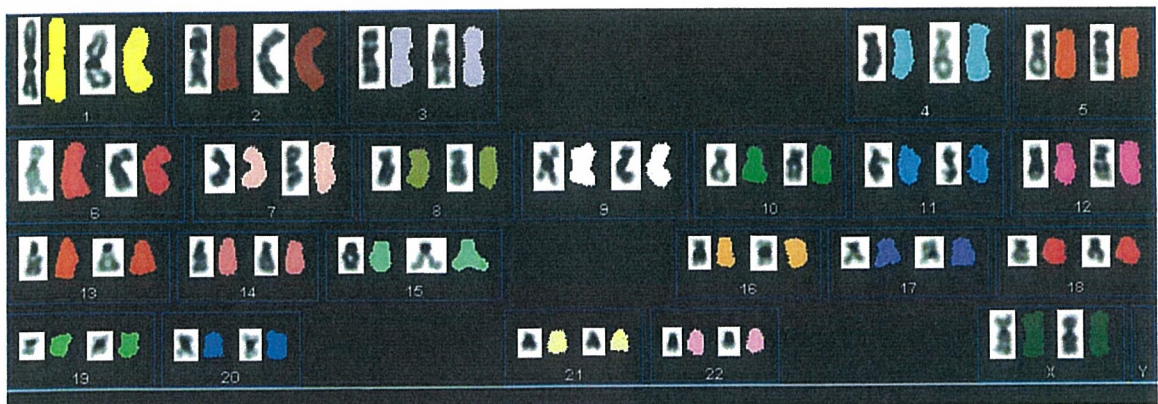


Fig.15 核型解析の結果と CGH 解析結果の比較

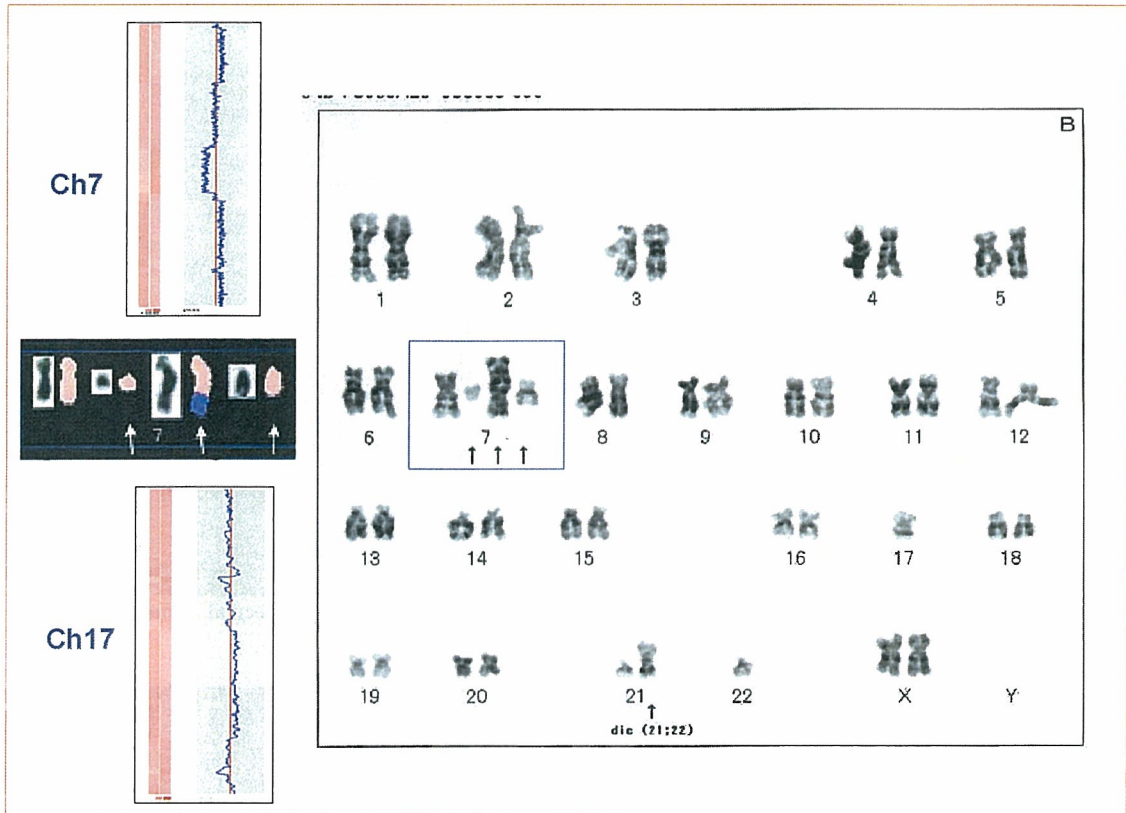


Fig.16 オンラインナノ LC-MS/MS システム

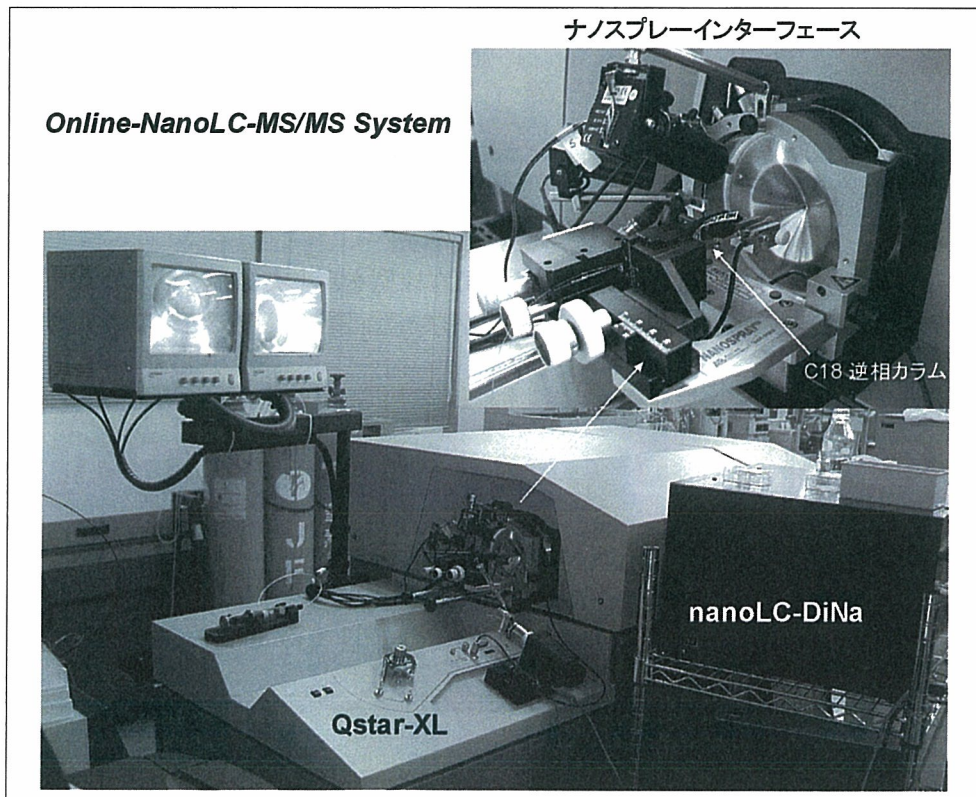
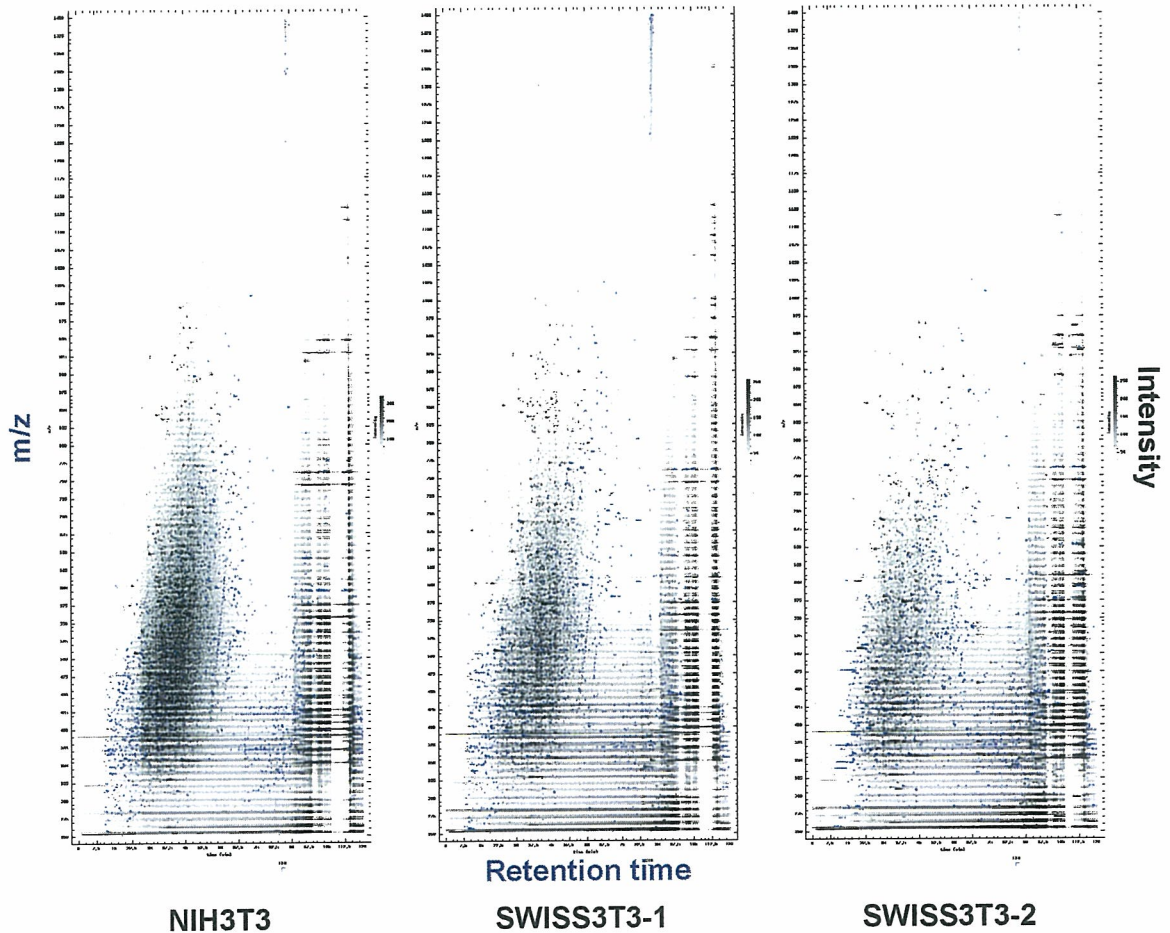


Fig.17 トリプシン処理細胞上清を用いたペプチドーム解析結果の3次元グラフ化



gi|1405933 M2-type pyruvate kinase [Mus musc
 gi|123681 Heat shock protein HSP 90-beta (HSF
 gi|194027 heat-shock protein hsp84
 gi|309317 84 kD heat shock protein
 gi|74178273 unnamed protein product [Mus musc
 gi|74213524 unnamed protein product [Mus musc
 gi|74177708 unnamed protein product [Mus musc
 gi|74223027 unnamed protein product [Mus musc
 gi|49868 put. beta-actin (aa 27-375) [Mus musc
 gi|31560030 tropomyosin 1, alpha [Mus musculus]
 gi|74181043 unnamed protein product [Mus musc
 gi|6752952 actin, gamma 2, smooth muscle, enter
 gi|94392330 PREDICTED: similar to Actin, cytopla
 gi|55291 unnamed protein product [Mus musc
 gi|54912 unnamed protein product [Mus musc
 gi|20178336 Tropomyosin alpha-3 chain (Tropomy
 gi|6681273 eukaryotic translation elongation fact
 gi|6794399 peptidylprolyl isomerase A [Mus musc
 gi|74197865 unnamed protein product [Mus musc
 gi|37675525 AHNAK [Mus musculus]
 gi|61743961 AHNAK nucleoprotein isoform 1 [Mus
 gi|30425250 hypothetical protein LOC238880 [Mus
 gi|29437296 Actg2 protein [Mus musculus]
 gi|13529413 Fbln2 protein [Mus musculus]
 gi|12846159 unnamed protein product [Mus musc
 gi|118600845 Unknown (protein for IMAGE:534593)
 gi|14165437 heterogeneous nuclear ribonucleoprot
 gi|11875203 tropomyosin 2, beta [Mus musculus]
 gi|6647752 Heterogeneous nuclear ribonucleoprot
 gi|33563250 desmin [Mus musculus]
 gi|695638 M-TAXREB107 [Mus musculus]
 gi|220474 lamin A [Mus musculus]
 gi|12963491 enolase 1, alpha non-neuron [Mus mu
 gi|50815 unnamed protein product [Mus musc
 gi|26331354 unnamed protein product [Mus musc
 gi|28173554 histone H2B [Homo sapiens]
 gi|200785 ribosomal protein L7
 gi|94390118 PREDICTED: similar to ribosomal prot
 gi|49753 unnamed protein product [Mus musc
 gi|83745120 ribosomal protein, large P2 [Mus musc
 gi|13430890 histone 1, H1e [Mus musculus]
 gi|51304 unnamed protein product [Mus musc
 gi|94370417 PREDICTED: similar to H2A histone f
 gi|74195793 unnamed protein product [Mus musc
 gi|6755392 S100 calcium binding protein A6 (calc
 gi|47847498 mFLJ00279 protein [Mus musculus]
 gi|28972888 mKAAA3005 protein [Mus musculus]
 gi|6755350 ribosomal protein L10A [Mus musculus]
 gi|33659624 S100 calcium binding protein A4 [Mus
 gi|191765 alpha-fetoprotein
 gi|4507729 tubulin, beta 2 [Homo sapiens]
 gi|74211311 unnamed protein product [Mus musc

gi|74178273 unnamed protein product [Mus musc
 gi|74213524 unnamed protein product [Mus musc
 gi|49868 put. beta-actin (aa 27-375) [Mus musc
 gi|12652068 unnamed protein product [Mus musc
 gi|1181242 fibronectin [Mus musculus]
 gi|94392330 PREDICTED: similar to Actin, cytopla
 gi|29437296 Actg2 protein [Mus musculus]
 gi|30425250 hypothetical protein LOC238880 [Mus
 gi|50797 unnamed protein product [Mus musc
 gi|55291 unnamed protein product [Mus musc
 gi|13529413 Fbln2 protein [Mus musculus]
 gi|202210 alpha-tubulin isotype M-alpha-2
 gi|191765 alpha-fetoprotein
 gi|117167791 Coda112 protein [Mus musculus]
 gi|94404435 PREDICTED: hypothetical protein [M
 gi|484531 H3.3 like histone MH321 - mouse
 gi|12847552 unnamed protein product [Mus musc
 gi|28892871 SET and MYND domain containing 4 [M
 gi|309317 84 kD heat shock protein
 gi|40556608 heat shock protein 1, beta [Mus musc
 gi|6680177 hemoglobin Y, beta-like embryonic chi
 gi|12833514 unnamed protein product [Mus musc

gi|387082 skeletal muscle alpha-actin
 gi|94366797 PREDICTED: similar to Actin, alpha ca
 gi|387090 alpha-cardiac actin
 gi|74213524 unnamed protein product [Mus musc
 gi|49868 put. beta-actin (aa 27-375) [Mus musc
 gi|74178273 unnamed protein product [Mus musc
 gi|94392330 eukaryotic translation elongation fact
 gi|6681273 PREDICTED: similar to Actin, cytoplas
 gi|1181242 fibronectin [Mus musculus]
 gi|94404435 PREDICTED: hypothetical protein [Mu
 gi|202210 alpha-tubulin isotype M-alpha-2
 gi|12833514 unnamed protein product [Mus musc
 gi|123681 Heat shock protein HSP 90-beta (HSP
 gi|6754254 heat shock protein 1, alpha [Mus musc
 gi|6680177 hemoglobin Y, beta-like embryonic cha
 gi|50511223 mKAAA1991 protein [Mus musculus]
 gi|74197965 unnamed protein product [Mus musc
 gi|34147288 hypothetical protein LOC226118 [Mus
 gi|51304 unnamed protein product [Mus muscu
 gi|13529413 Fbln2 protein [Mus musculus]
 gi|553925 beta-globin [Mus musculus]
 gi|12963491 enolase 1, alpha non-neuron [Mus mus
 gi|9910228 FK506 binding protein 12-rapamycin at
 gi|82894062 PREDICTED: hypothetical protein [Mu

MS/MS 測定データより MASCOT 検索にて
Significant hit として同定されたタンパク

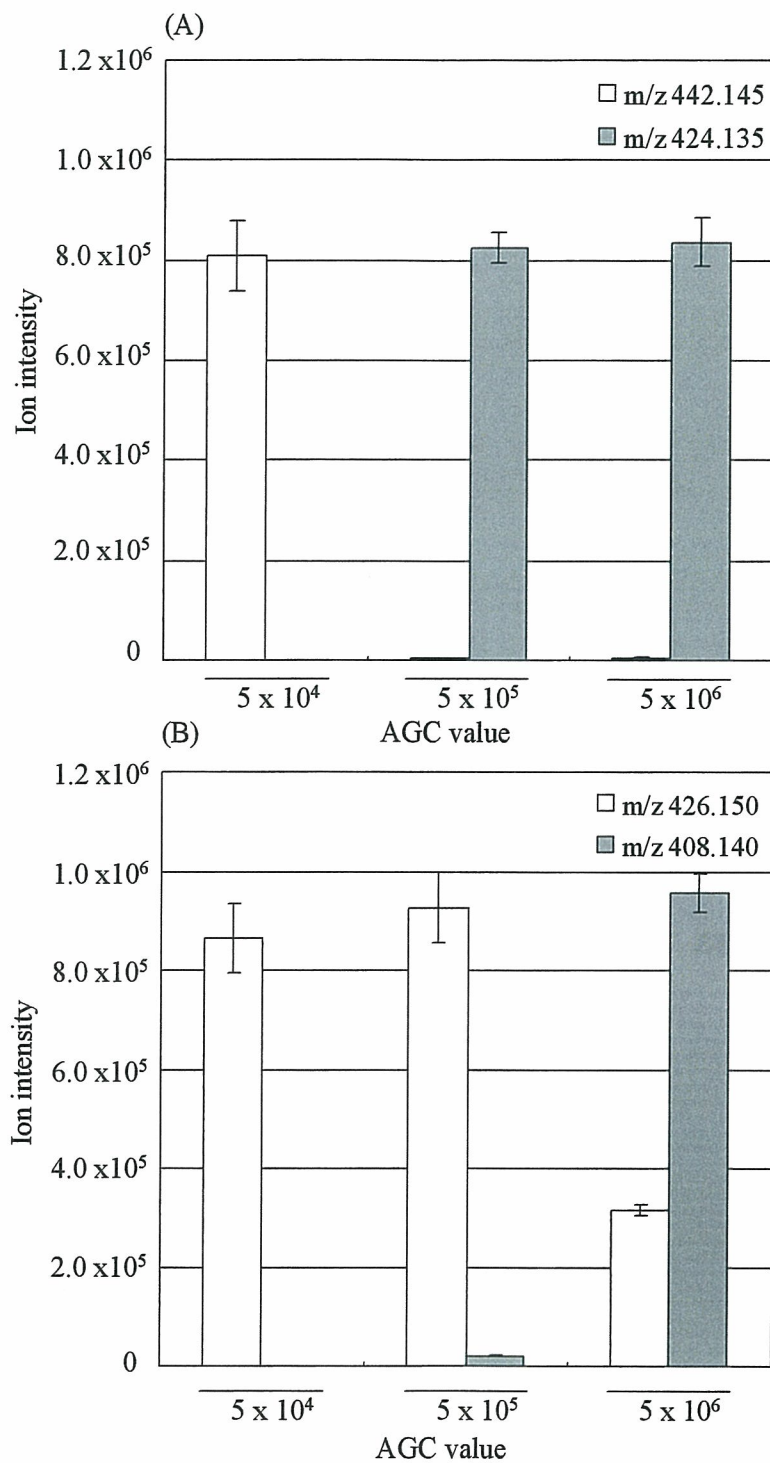


Fig.18 分子イオン及び脱水イオンのイオン強度に対する最大イオン取り込み量 (AGC 値) の影響. 数値 (イオン強度) は各測定条件における3回分析のイオン強度平均値 \pm S.D. として表す. (A) [DMB-NeuGc + H]⁺ (*m/z* 442.145) 及び [DMB-NeuGc - H₂O + H]⁺ (*m/z* 424.135). (B) [DMB-NeuAc + H]⁺ (*m/z* 426.150) 及び [DMB - NeuAc-H₂O + H]⁺ (*m/z* 408.140)

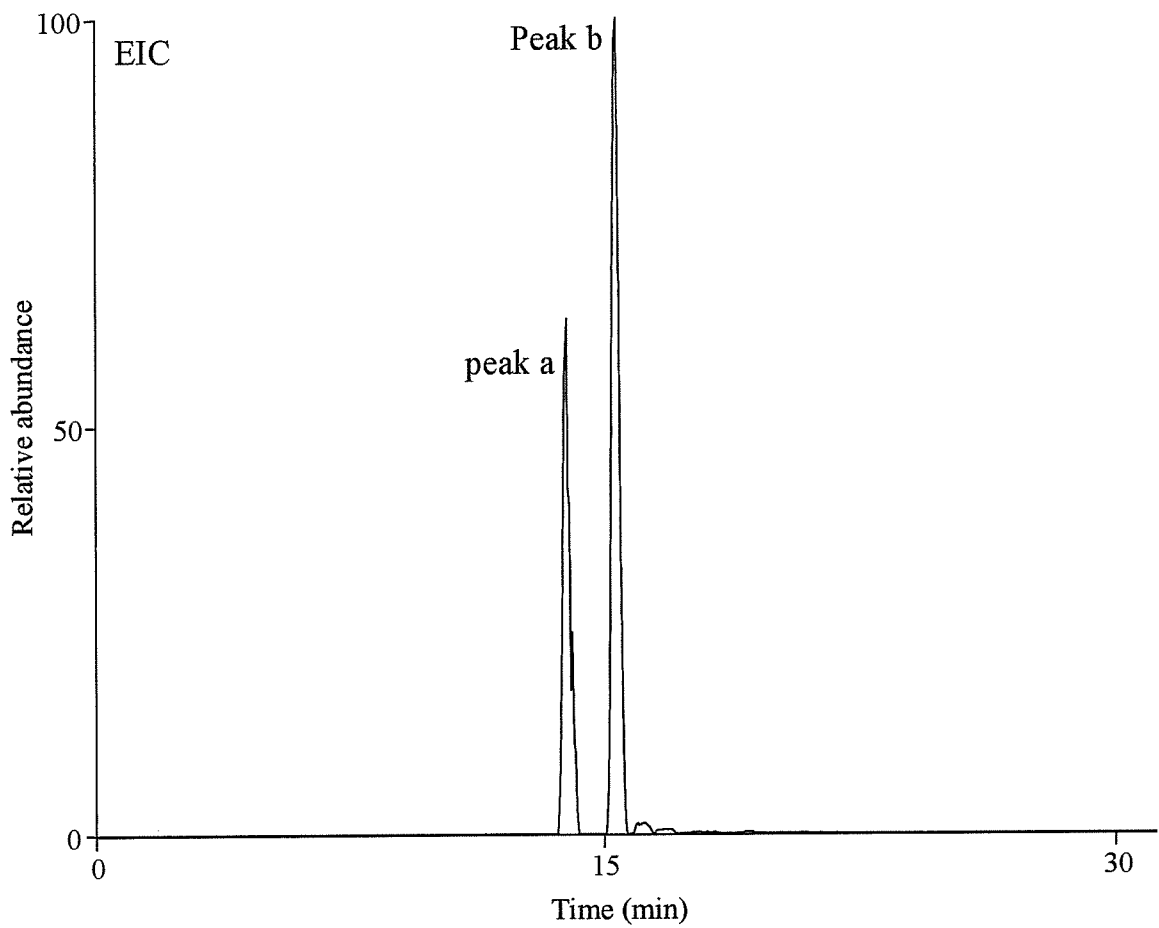


Fig.19 DMB-NeuGc 及び DMB-NeuAc の SIM (m/z 400-450, ポジティブイオンモード) により得られた m/z 426.13-426.17 及び m/z 442.12-442.16 の EIC.

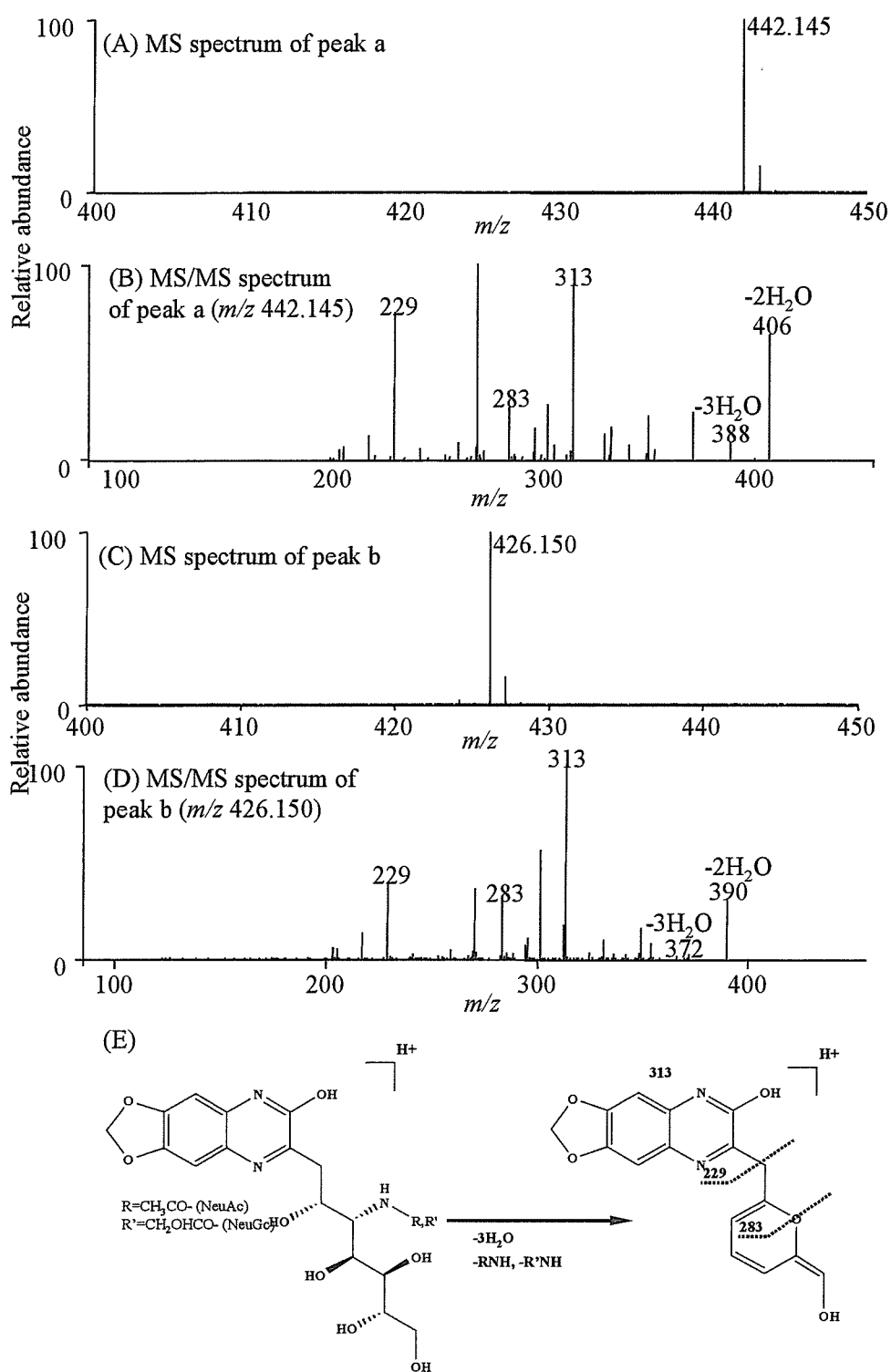


Fig.20 (A) peak a のマススペクトル, (B) peak a で検出された $[M + H]^+$ (m/z 442.145) のMS/MS スペクトル, (C) peak b のマススペクトル, (D) peak b で検出された $[M + H]^+$ (m/z 426.150) のMS/MS スペクトル, (E) DMB-NeuGc 及び DMB-NeuAc のフラグメンテーション.