

Fig. 29 Agilent CGHアレイを用いた17番染色体異常の確認

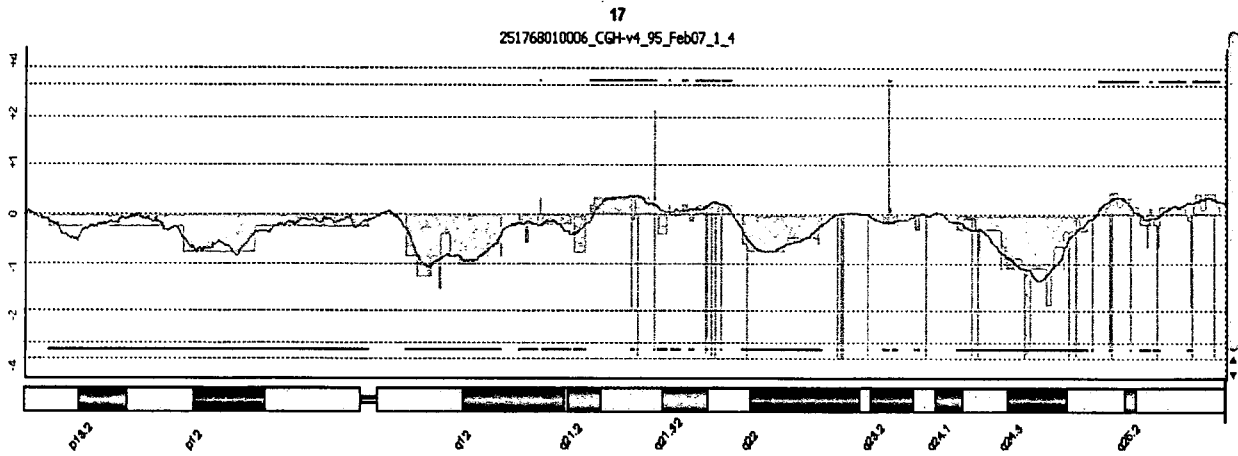


Fig.30 HSMM細胞株の50KSNPチップによるCGH解析結果

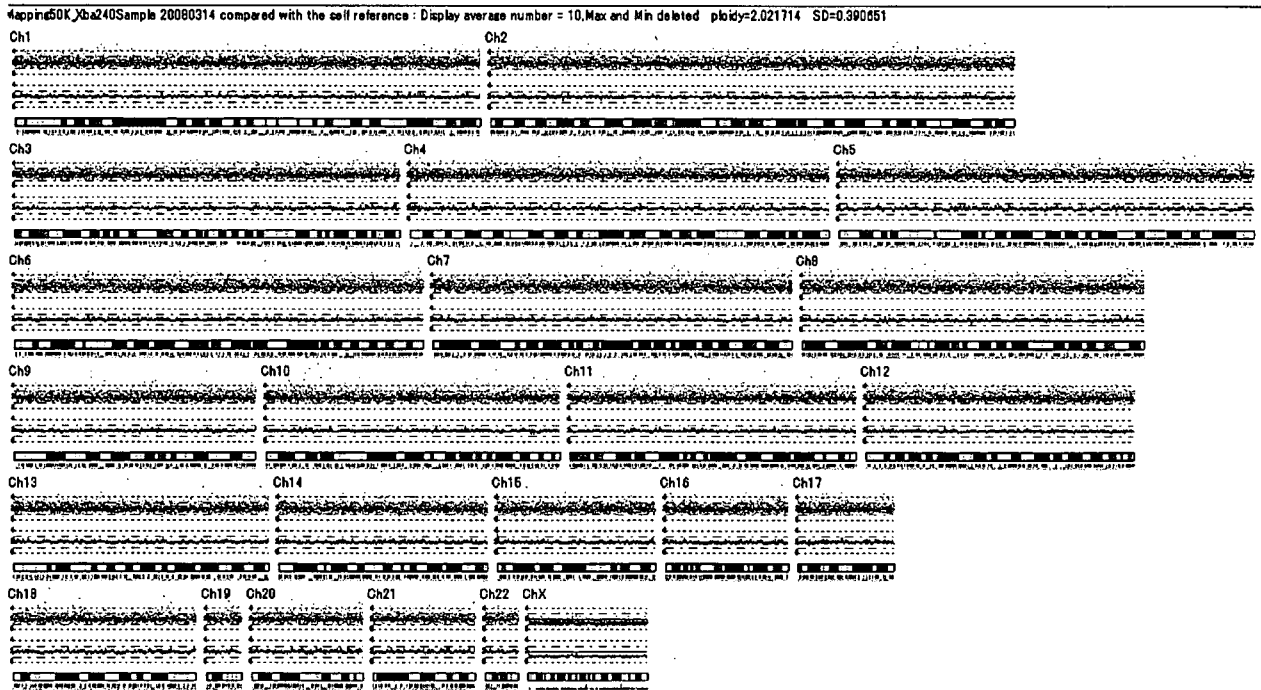
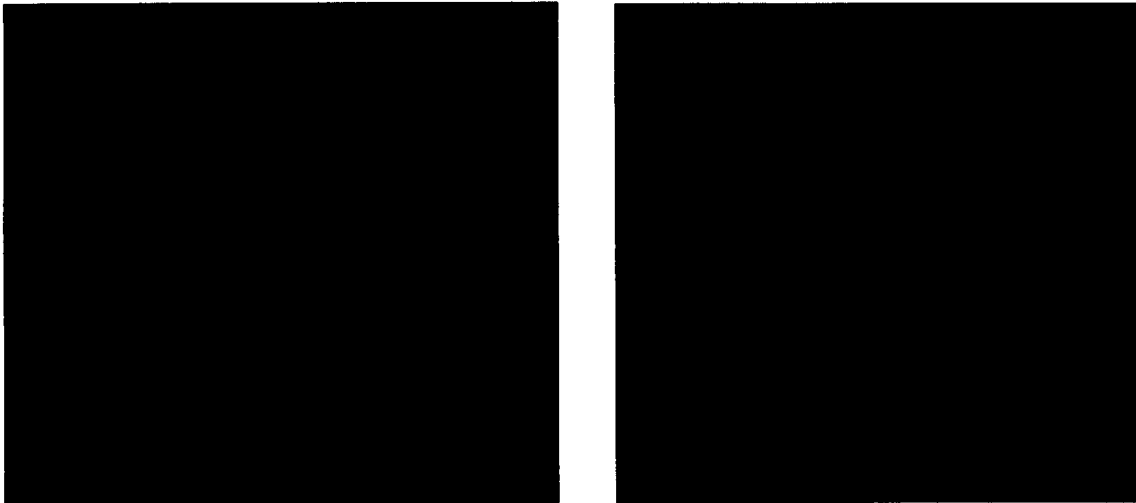


Fig. 31 セントロメアFISHプローブによる異常hMSC細胞(25継代)の間期核染色



25継代細胞における7番(赤)8番染色体(緑) セントロメアプローブによる2重染色FISH
25継代細胞における17番染色体セントロメアプローブFISH

Fig.32 21継代細胞の8番および17番染色体セントロメアプローブによる2重染色FISH

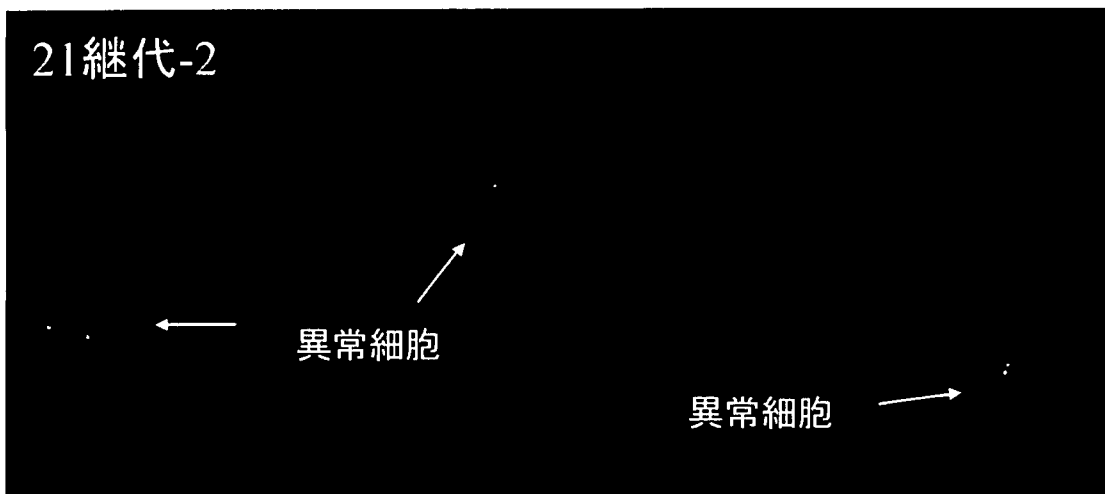
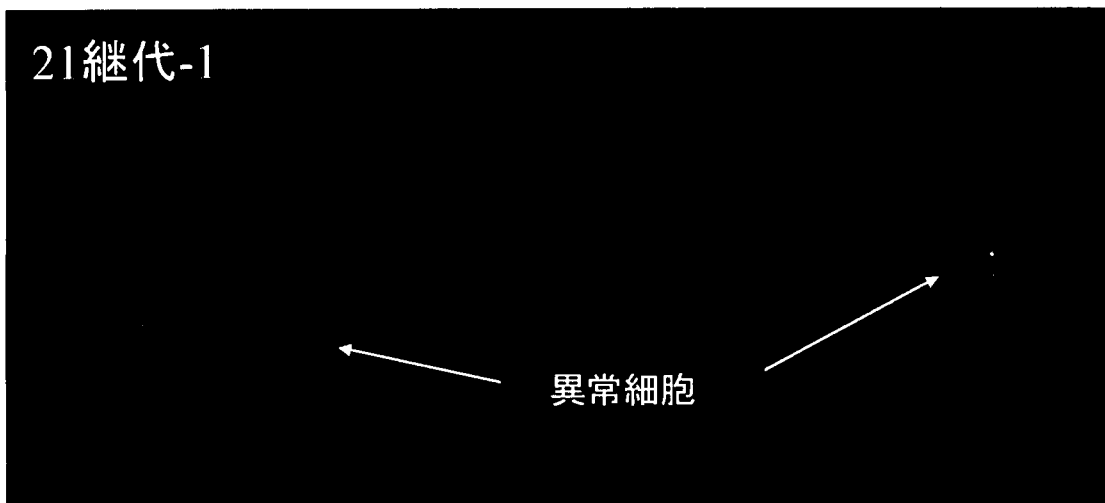


Fig.33 12継代細胞の8番および17番染色体セントロメアプローブによる2重染色FISH

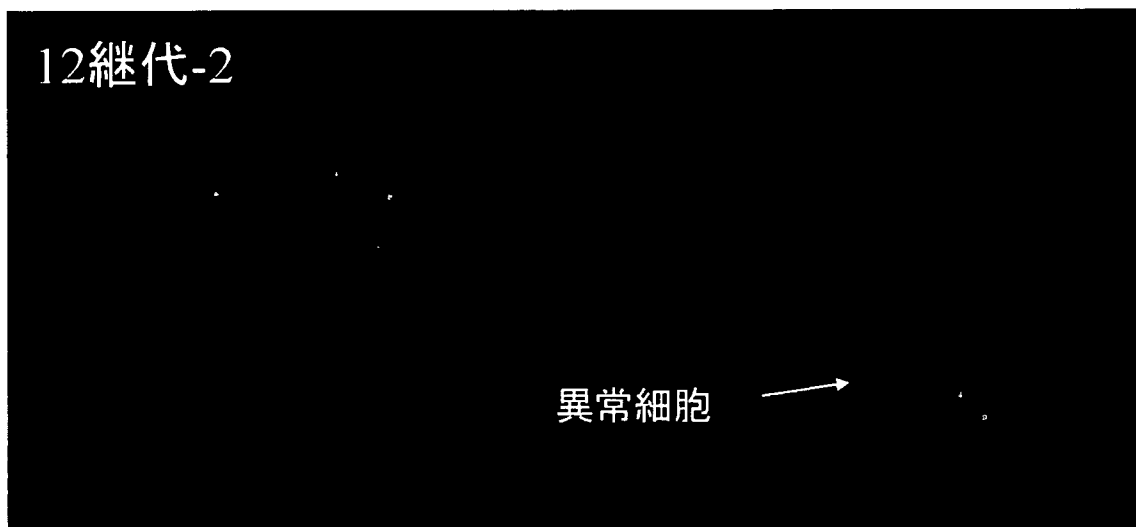
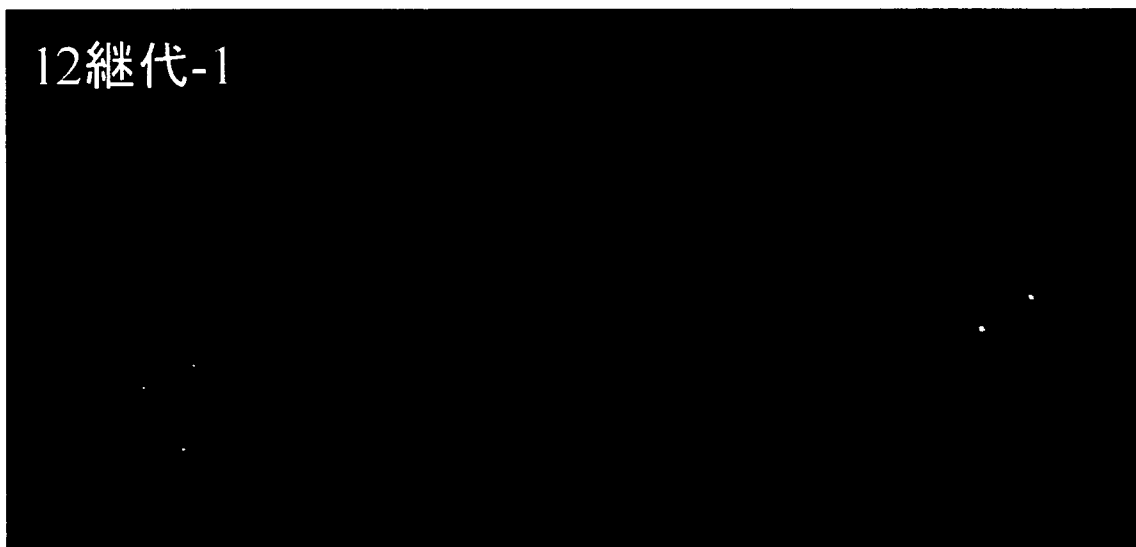


Fig. 34 hMSC細胞各ロットの増殖曲線および異常ロットの異常出現頻度

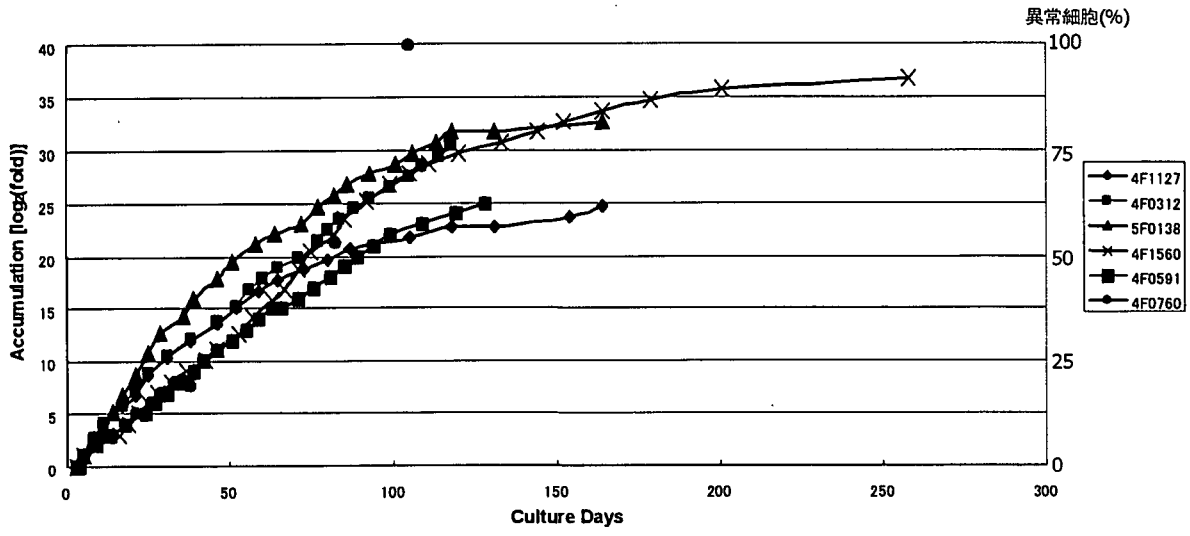


Table 6 カスタムolgo-CGHアレイのプロローブデザイン

| | 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 | 3635 | 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:333951 | | | | |

*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、5kb のインターバルにて追加設計しました。

Table 7 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 |

Table 8 hMSCのLOH領域に存在する遺伝子群

| SLNo. | Gene Name | Description | SLNo. | Gene Name | Description |
|-----------------------|-----------|--|-------------------------|-----------|--|
| Ch 3 LOH region genes | | | Ch 8 - LOH region genes | | |
| 1 | NXD-SP17 | testes development-related NXD-SP17 | 1 | BC068633 | TFOP2L3 protein |
| 2 | CD47 | CD47 antigen (Rb-related antigen, integrin-associated signal transducer) | 2 | TFOP2L3 | transcription factor OP2-like 3 |
| 3 | ESRRB1 | estrogen-related receptor beta like 1 | 3 | NCALD | neurocalcin delta |
| 4 | HHLA2 | HERV-HLTR-associating 2 | 4 | AB036063 | Hypothetical protein DKFZ668M05248 |
| 5 | AB040957 | Hypothetical protein FLJ12850 | 5 | FRM2B | ribonucleotide reductase M2 B (TP53 inducible) |
| 6 | KIAA1524 | p90 autotigen | 6 | AB163437 | P53-inducible ribonucleotide reductase small subunit 2 short form gamma |
| 7 | AY227654 | Splice isoform 2 of Q86Y13 | 7 | AB166669 | P53-inducible ribonucleotide reductase small subunit 2 short form beta |
| 8 | REINLB | colon and small intestine-specific cysteine-rich | 8 | EDD | progesterin-induced protein |
| 9 | DZIP3 | zinc finger DAZ interacting protein 3 | 9 | ODF1 | outer dense fiber of sperm tails 1 |
| 10 | AF278948 | Hypothetical protein FLJ27117 | 10 | KLF10 | Kruppel-like factor 10 |
| 11 | AF454941 | Hypothetical protein FLJ2291 | 11 | BC085399 | Transforming growth factor-beta-inducible early growth response protein 1 (TGF-beta-inducible early growth response protein 1) (XIEG-1) (Kruppel-like factor 10) (EGFbeta) |
| 12 | BEX | HMGBOX transcription factor BEX | 12 | AZIN1 | ornithine decarboxylase antizyme inhibitor |
| 13 | AK130843 | Hypothetical protein FLJ26833 | 13 | ATP6V1C1 | ATPase, H+ transporting, lysosomal 42kDa, V1 |
| 14 | AK130627 | Hypothetical protein FLJ27117 | 14 | BC029562 | MGC39526 protein |
| 15 | TRAF1 | T-cell receptor interacting molecule | 15 | BC005038 | BAALC isoform 1-8 (BAALC protein) (BAALC 1-8) |
| 16 | GUCA1C | guanylate cyclase activator 1C | 16 | AK125733 | Hypothetical protein FLJ43745 |
| 17 | MCRC | microorchidia homolog | 17 | AB209734 | Putative G-protein coupled receptor |
| | | | 18 | CTHFC1 | collagen triple helix repeat containing 1 |
| | | | 19 | AK027799 | Hypothetical protein FLJ11012 |
| | | | 20 | VDSCF1 | hypothetical protein LOC25879 |
| | | | 21 | AF161549 | HSPC064 |
| | | | 22 | FZD6 | frizzled 6 |
| | | | 23 | MFTC | mitochondrial folate transporter/carrier |
| | | | 24 | RIMS2 | regulating synaptic membrane exocytosis 2 |
| | | | 25 | TM/SF4 | dendritic cell-specific transmembrane protein |
| | | | 26 | AF090801 | PF00195 |
| | | | 27 | DPYS | dihydropyrimidinase |
| | | | 28 | AY203960 | LP220a |
| | | | 29 | BC017381 | LFP12 protein |

Table 9 hMSC細胞の培養過程における染色体変化の普遍性

| Cell | hMSC | | | | | | | | HSMM |
|--------------------|-----------|-----------|-----------|----------|-----------|----------|-----------|----------|------|
| | Lot # | 4F1127 | | | 4F0312 | | 5F0138 | | |
| <i>Passage #</i> | <i>11</i> | <i>11</i> | <i>23</i> | <i>7</i> | <i>23</i> | <i>5</i> | <i>23</i> | <i>7</i> | |
| LOH | - | - | - | + | + | - | - | - | |
| Copy Number Change | - | - | - | - | + | - | - | - | |

Table 10 12および21継代細胞におけるFISHシグナル数と異常頻度

| シグナル個数 | | 判定 | 細胞数 | |
|--------|------|----|-------|-------|
| Ch8 | Ch17 | | 12 継代 | 21 継代 |
| 1 | 2 | 正常 | 2 | 0 |
| | 3 | 異常 | 1 | 1 |
| 2 | 1 | 正常 | 1 | 0 |
| | 2 | 正常 | 75 | 41 |
| | 3 | 異常 | 11 | 33 |
| | 4 | 異常 | 5 | 13 |
| | 5 | 異常 | 0 | 4 |
| | 6 | 異常 | 0 | 1 |
| 3 | 2 | 正常 | 3 | 1 |
| | 3 | 正常 | 1 | 2 |
| | 4 | 正常 | 0 | 3 |
| 4 | 4 | 正常 | 1 | 0 |
| | 7 | 異常 | 0 | 1 |
| 合計 | | 正常 | 83 | 47 |
| | | 異常 | 17 | 53 |

Table 11 各種染色体解析手法とその応用性に関するまとめ

| 解析手法 | 構造異常 (量的変化) | 染色体の欠失増幅 大きなサイズ 小さなサイズ | | 転座 | 複製型 LOH | 数の異常 | 点突然変異 | 個人識別 | コスト | 難易度 | 信頼性 |
|-----------------------|----------------|---------------------------|-----|----|------------|------|-------|------|-----|-----|-----|
| G-banding | ○ | ○ | △ | △ | x | ○ | x | x | L | H | L |
| multicolor-FISH | ○ | ○ | △ | ○ | x | ○ | x | x | H | H | M |
| microsatellite marker | x | x | x | x | △ | △ | x | ○ | M | L | H |
| metaphase CGH | x | ○ | △/x | x | x | ○ | x | x | H | H | M |
| CGH array | x | ○ | △ | x | x | ○ | x | x | H | M | H |
| SNP array | x | ○ | ○ | x | ○ | ○ | △ | ○ | H | L | H |
| sequencing | x | x | ○ | x | △ | x | ○ | ○ | M | L | H |

○ 可能 H high
 △ 一部可能 M middle
 x 不可能 L low
 [] 最適とされる方法

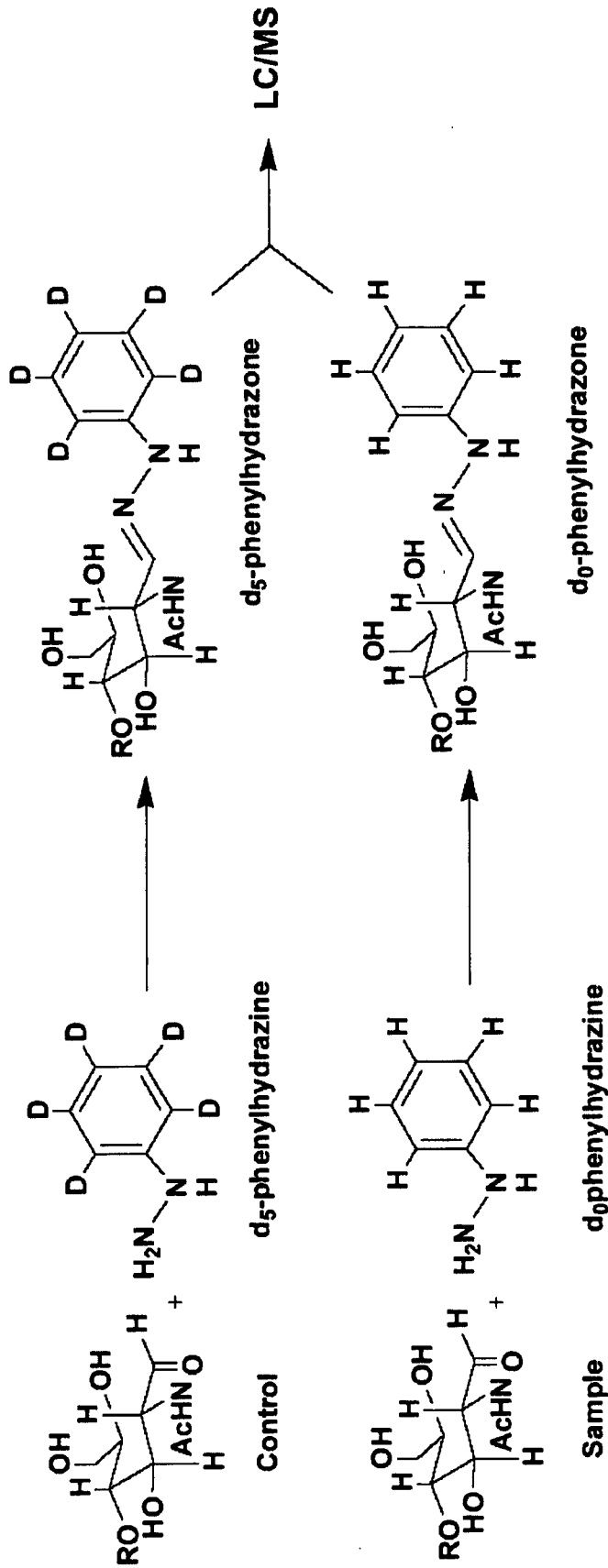
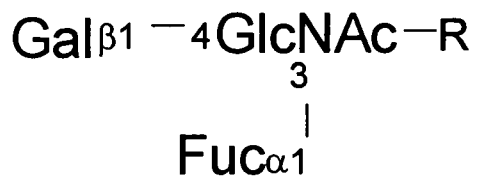
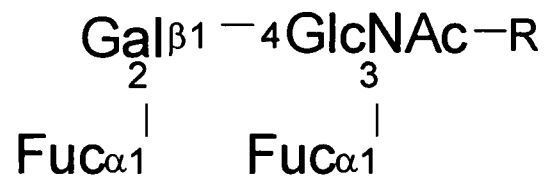


Fig.35 d_5 重水素置換フェニルヒドラゾン糖鎖を内部標準とした定量的糖鎖プロファイリング法

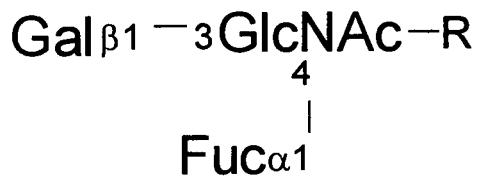
Lewis x (Le^x)



Lewis y (Le^y)



Lewis a (Le^a)



Lewis b (Le^b)

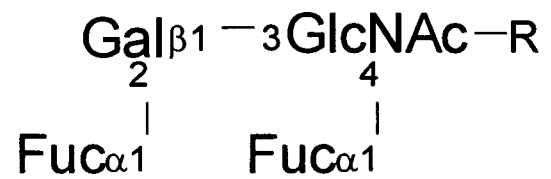
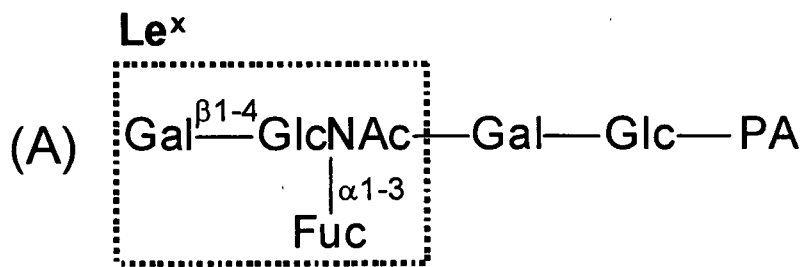
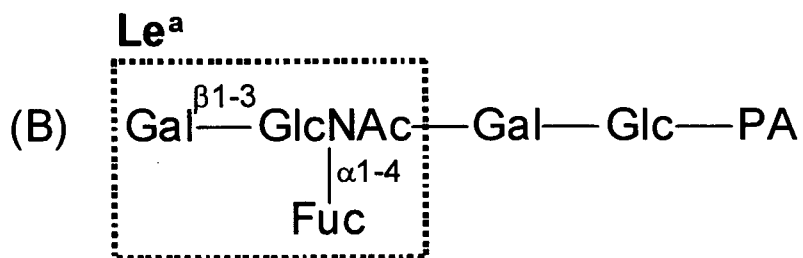


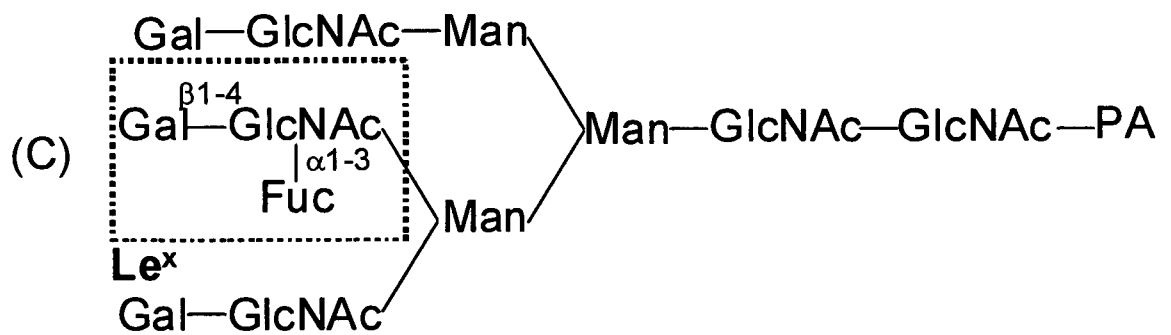
Fig.36 Le^x , Le^y , Le^a 及び Le^b 糖鎖の構造



oligosaccharide I



oligosaccharide II



oligosaccharide III

Fig.37 Le^x 及び Le^a モデル糖鎖

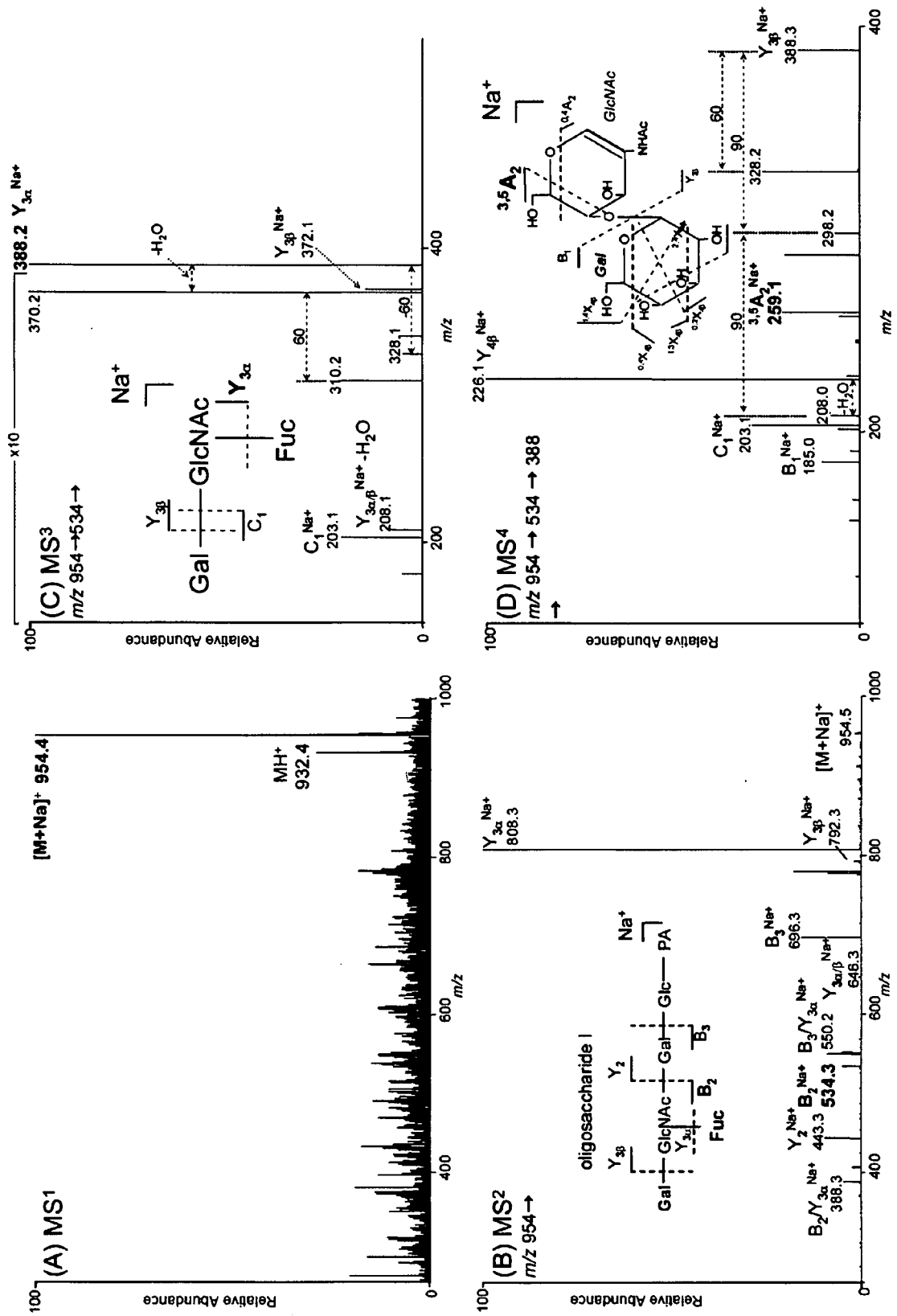


Fig.38 Le^xモデル(糖鎖!)のMS¹⁻⁴スペクトル

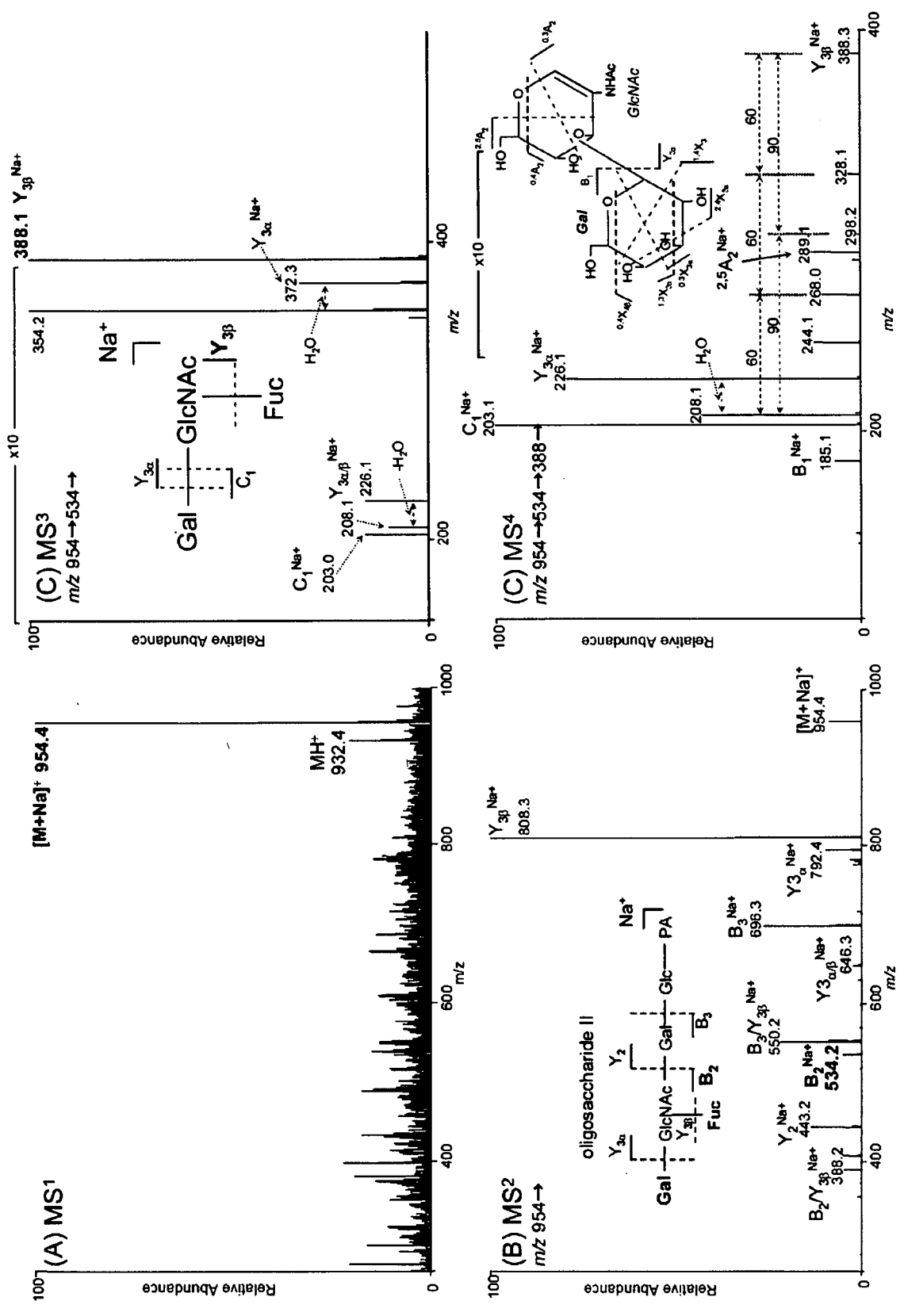


Fig.39 Leaモデル(糖鎖II)のMS¹⁻⁴スペクトル

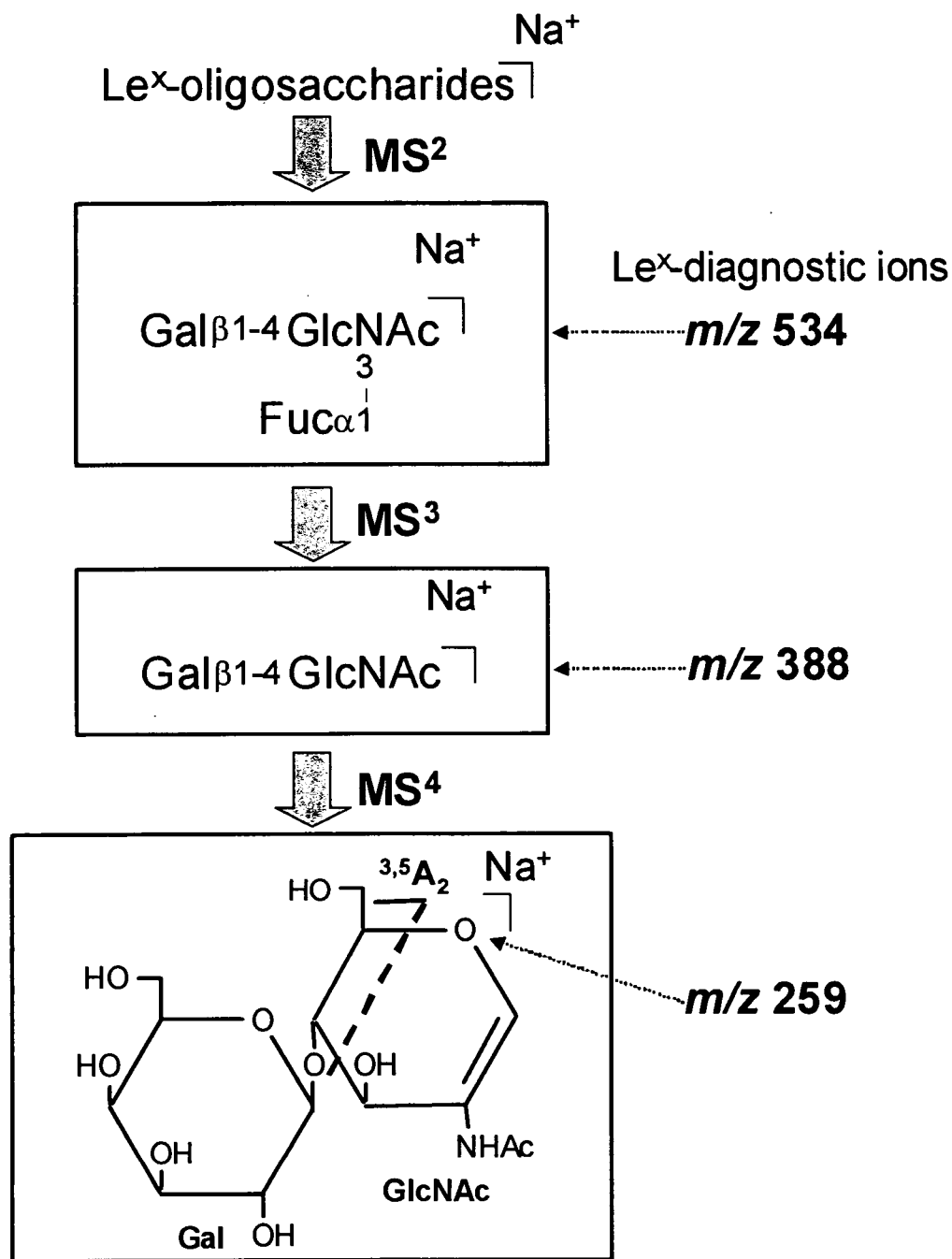


Fig.40 Le^x糖鎖の開裂とLe^x診断イオン

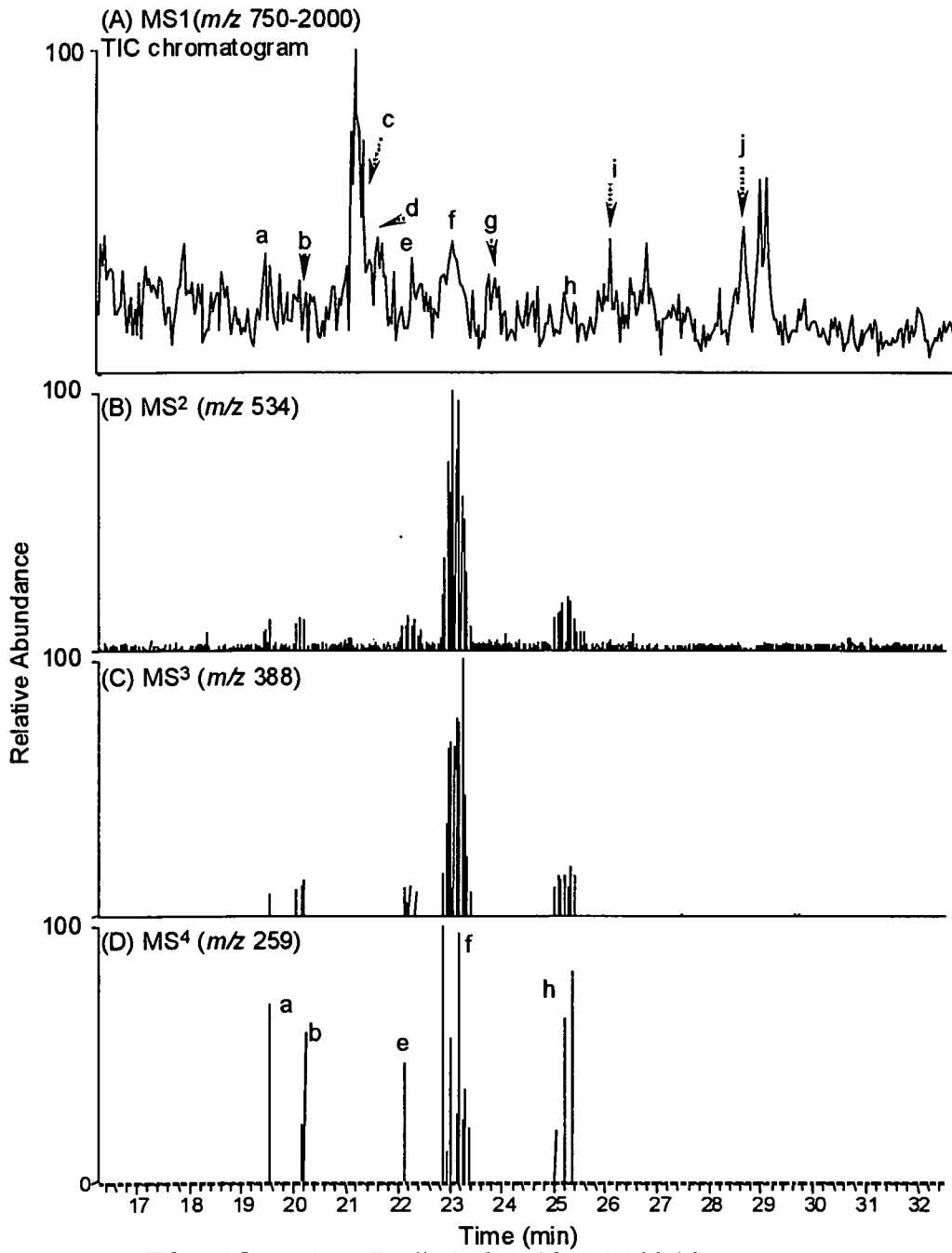


Fig.42 マウス腎臓由来N結合型糖鎖のLC/MS¹⁻⁴

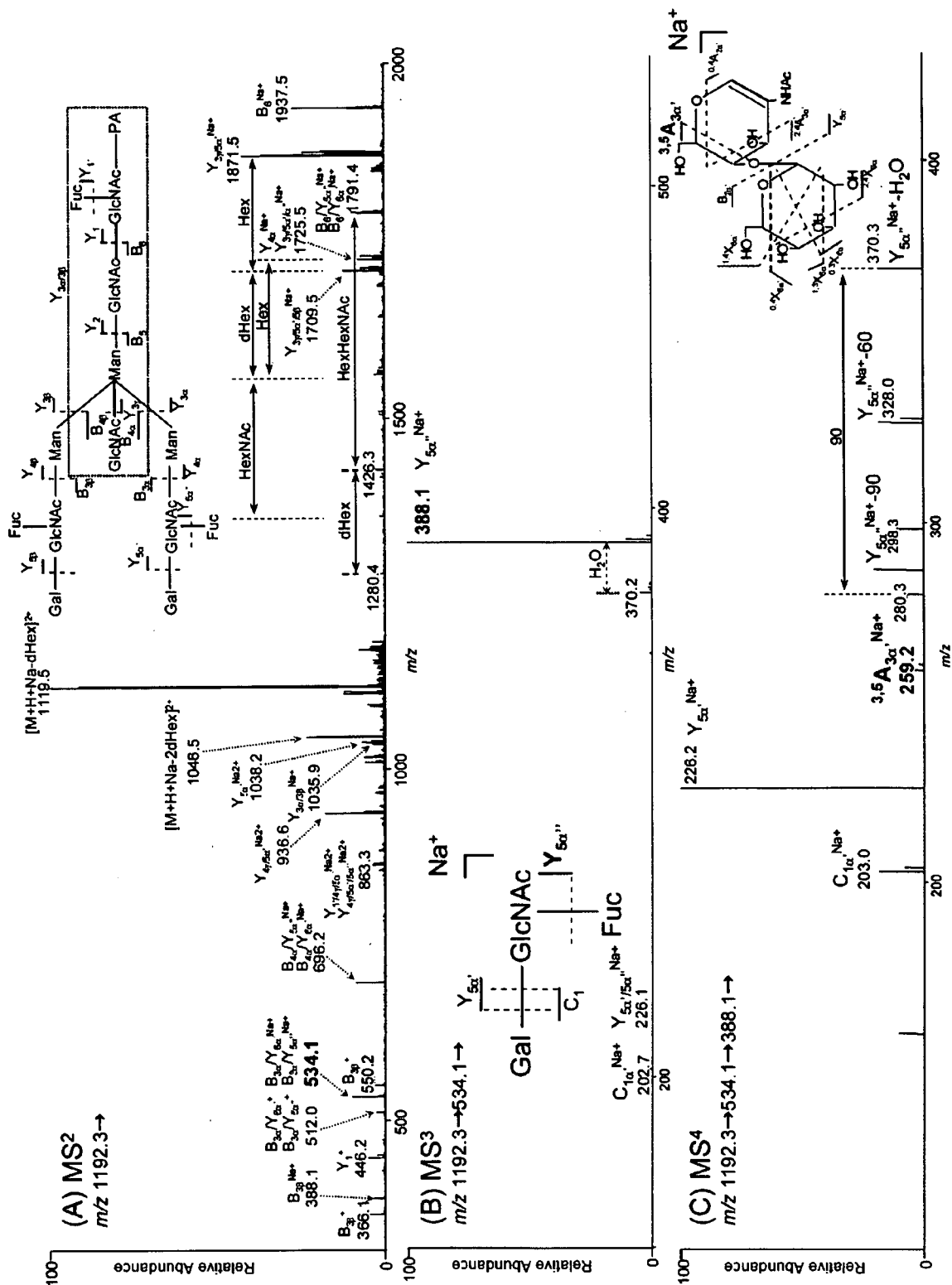


Fig.43 マウス腎臓由来糖鎖(ピーク)のMS²⁻⁴スペクトル

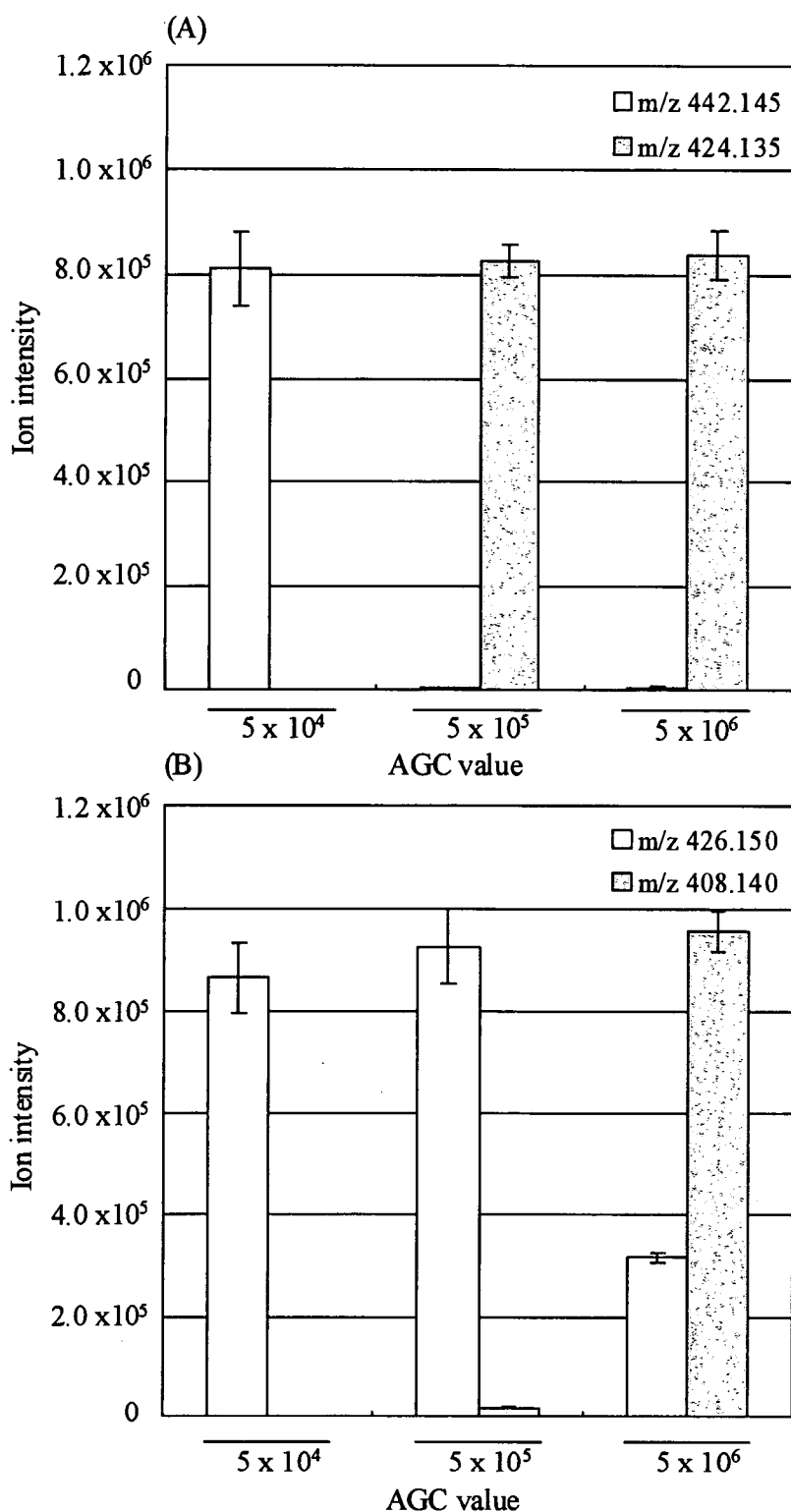


Fig.44 分子イオン及び脱水イオンのイオン強度に対する最大イオン取り込み量 (AGC 値) の影響. 数値 (イオン強度) は各測定条件における3回分析のイオン強度平均値 \pm S.D. として表す. (A) [DMB-NeuGc + H]⁺ (m/z 442.145) 及び [DMB-NeuGc - H₂O + H]⁺ (m/z 424.135). (B) [DMB-NeuAc + H]⁺ (m/z 426.150) 及び [DMB - NeuAc-H₂O + H]⁺ (m/z 408.140)

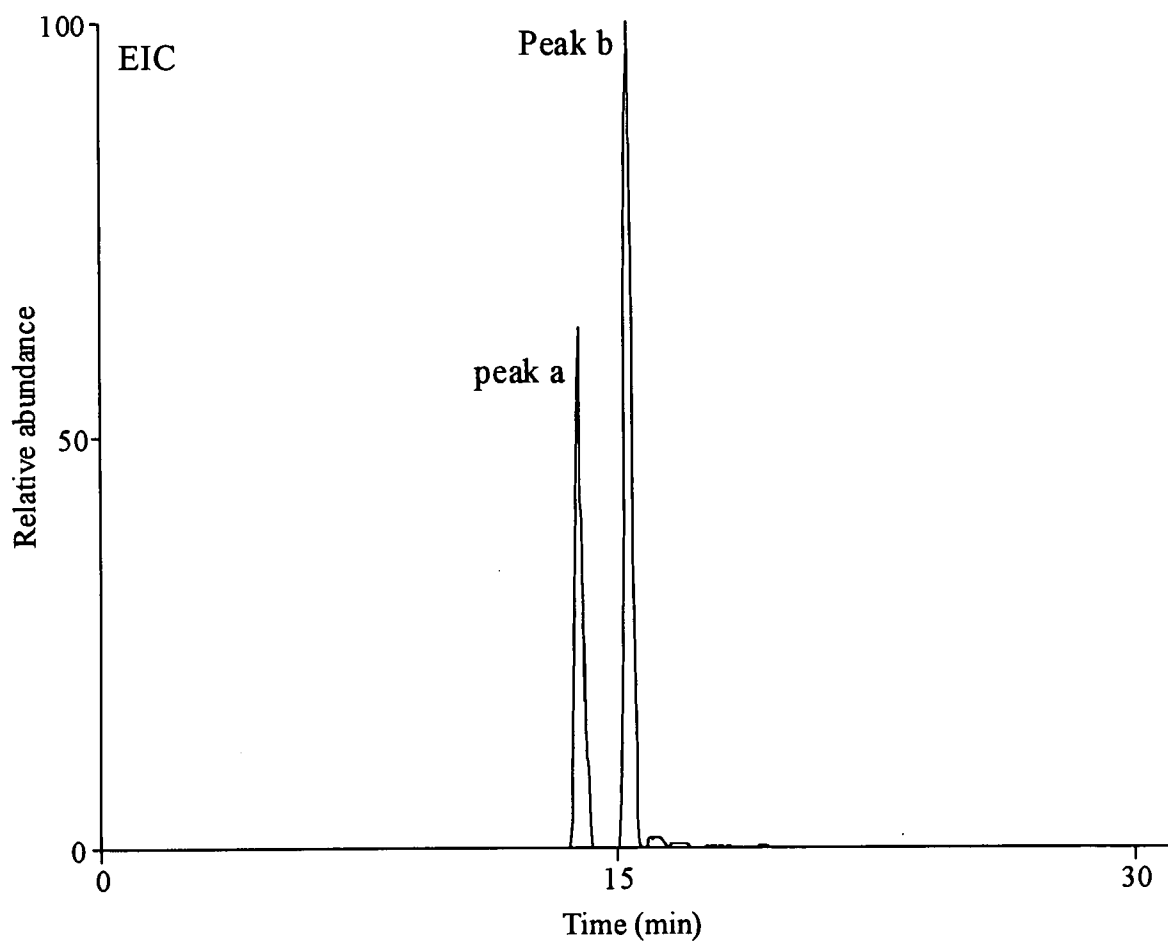


Fig.45 DMB-NeuGc 及び DMB-NeuAc の SIM (m/z 400-450, ポジティブイオンモード) により得られた m/z 426.13-426.17 及び m/z 442.12-442.16 のマスクロマトグラム.

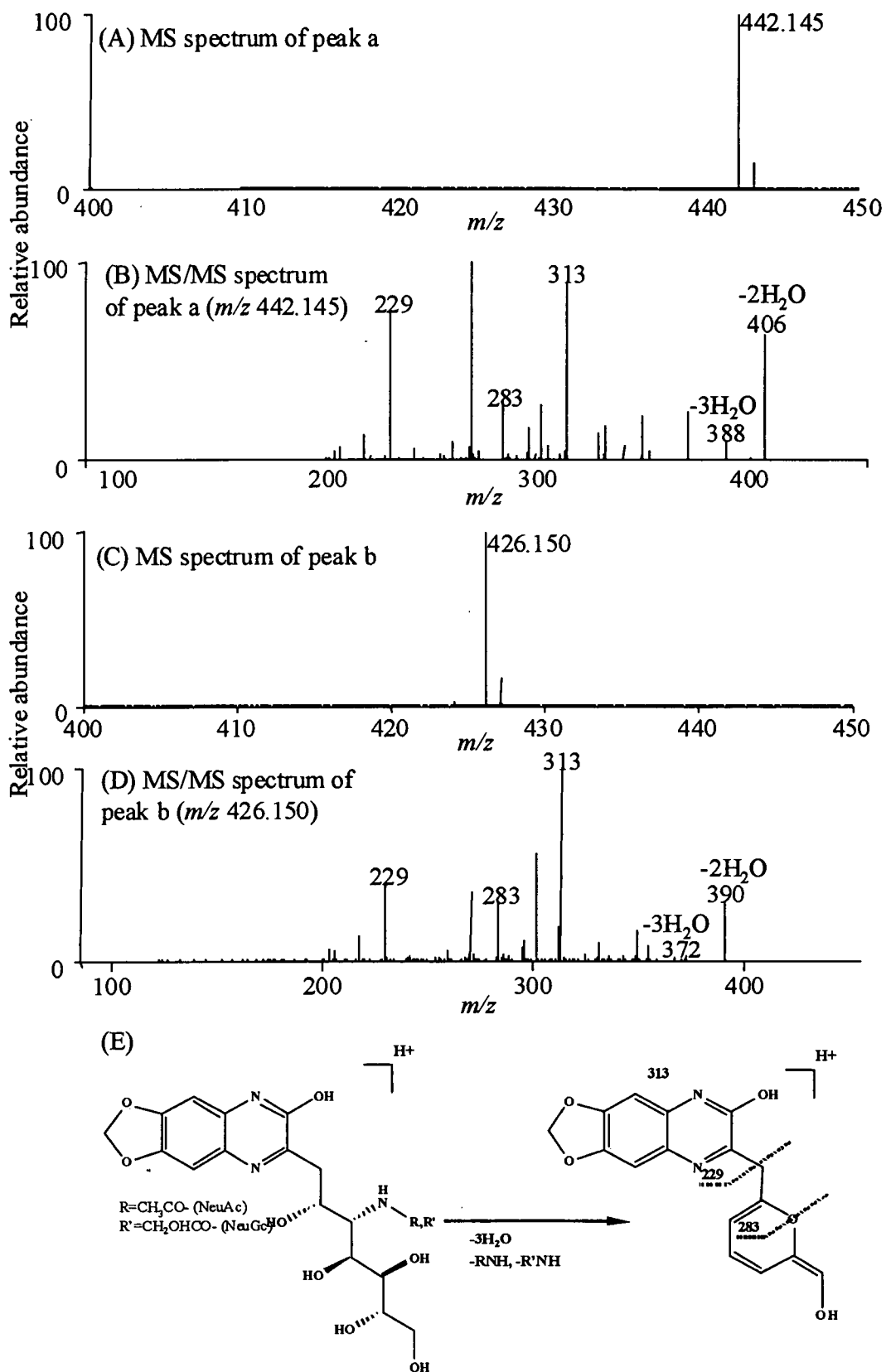


Fig.46 (A) peak a のマススペクトル, (B) peak a で検出された $[\text{M} + \text{H}]^+$ (m/z 442.145) のMS/MS スペクトル, (C) peak b のマススペクトル, (D) peak b で検出された $[\text{M} + \text{H}]^+$ (m/z 426.150) のMS/MS スペクトル, (E) DMB-NeuGc 及び DMB-NeuAc のフラグメンテーション.

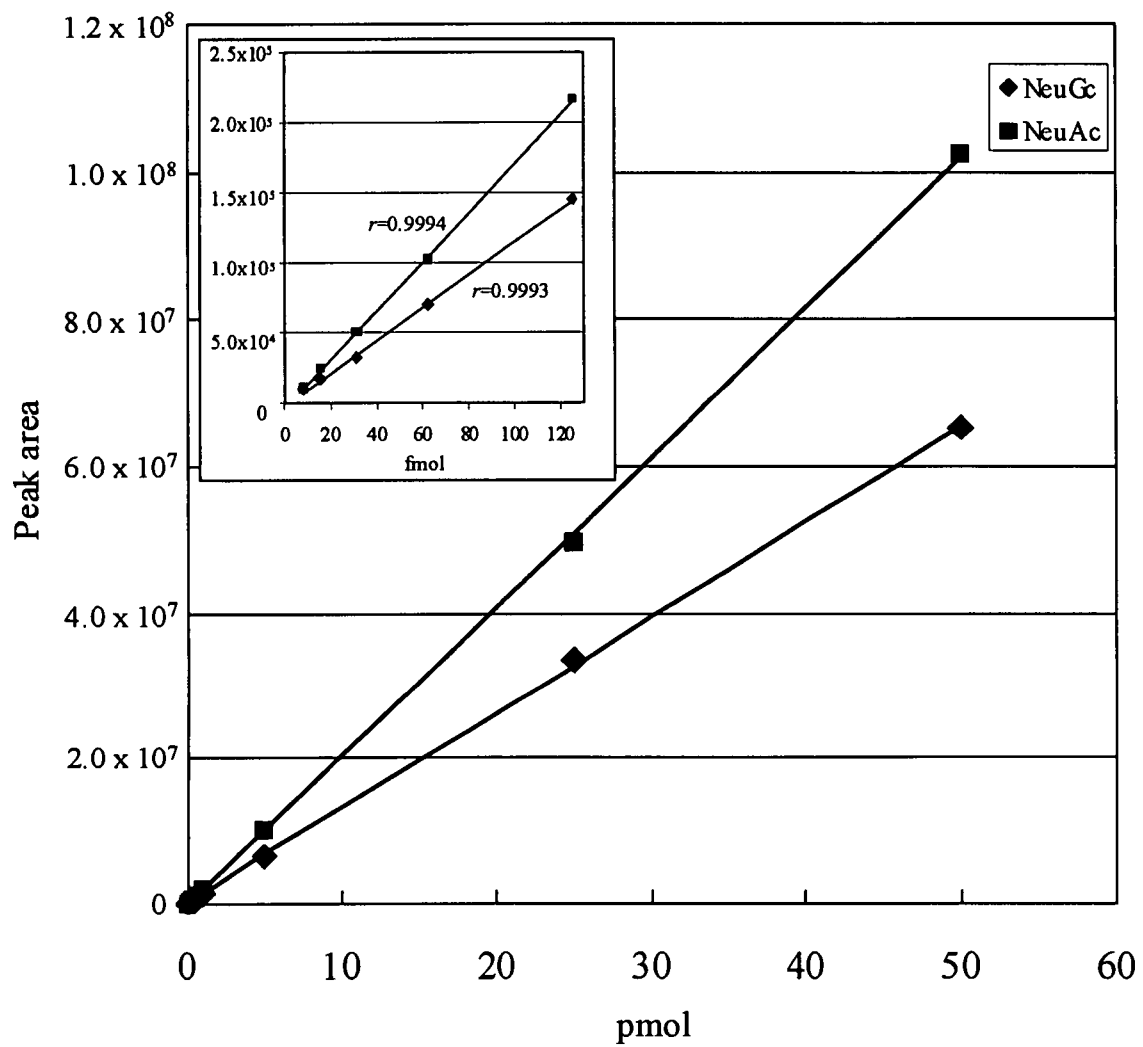


Fig.47 DMB-NeuGc ($r=0.9998$) 及び DMB-NeuAc ($r=0.9995$) の回帰直線.