Differentiation Propensity. International Society for Stem Cell Research 12 th Annual Meeting, Vancouver （2014年6月18-21日）

24. Tano K, <u>Yasuda S</u>, <u>Umezawa A</u>, <u>Sato Y</u>. A highly efficient culture method for growth and detection of undifferentiated human pluripotent stem cells present as impurities in cell-processed therapeutic products. 20 th International Society for Cellular Therapy, Paris （2014年4月23-26日）

H．知的財産権の出願・登録状況
H-1．特許取得
発明者　草川森士，<u>安田智</u>，<u>佐藤陽治</u>
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　　　　【名称】公益財団法人　ヒューマンサイエンス振興財団
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特許出願日　　平成26年9月1日

H-2．実用新案登録
なし

H-3.その他
特記事項なし

Nude mice

1973 Introduced from Friis, Denmark.
1977~ BALB/cA-nu, BALB/cA-Dh, nu,
1982 CBA/N-nu, C57BL/6N-nu,
C3H/HeN-nu, NFS/N-nu, NIH(S)-
nu were established.

SCID mice

1985 Introduced from FCCC, USA.
1989~1995 BALB/cA-scid, BALB/cA-
scid, nu, BALB/cA-bg, scid,
BALB/cA-Dh, scid, B6-scid,
AKR-scid were established.

IL-2Rg KO mice

1997 Introduced IL-2Rg KO mice from
Dr. Sugamura, Tohoku Univ. and
start back-cross mating to B6 or
BALB/cA mice.
1998 N=8

NOD/Shi mice

1983 Introduced F35 generation from
Shionogi Pharmaceuticals Inc.

NOD/Shi-scid mice

1990 Started Cross-intercross
mating between NOD and
SCID mice to examine the
mechanisms of non-obese
diabetis.
1995 N=9

RAG2 KO mice

1995 Introduced from Dr. Alt
1998 BALB/cA- RAG2 KO and B6
RAG2 KO mice were
established.

NOG mice

1998 Started backcross mating IL-
2Rg KO to NOD/Shi-scid
2000 N=8

**RAG2, IL-2Rg dKO mice
(BRG)**

1998 Started backcross mating IL-
2Rg KO to BALB/cA- or B6
RAG2 KO mice
2000 N=8

**RAG2, IL-2Rg dKO – nude Mice
(BRG-nu)**

2007 Started mating to BALB/cA-nude mice

**RAG2, IL-2Rg dKO – hairless Mice
(BRG-hr)**

2007 Started mating to BALB/cA- hairless mice

Fig. 1 BRG, BRG-nu および BRG-hr マウスの開発過程

Table 1 移植後 16 週における TPD$_{50}$, 対ヌードマウス比およびマトリゲル増強効果

| 背景遺伝子系統 移植細胞 / 項目	BALB/c										
	Nude	BRG			BRG-nu			BRG-hr[a]		
	TPD50	TPD50	vs. Nude	マトリゲル増強効果	TPD50	vs. Nude	マトリゲル増強効果	TPD50	vs. Nude	マトリゲル増強効果
HeLa細胞	$0.68 \sim 4.21 \times 10^5$	1.00×10^4	1/7	x 1	1.78×10^3	1/83	x 1	3.17×10^4	1/10	x 1
HeLa細胞+マトリゲル	–	3.2×10^1	1/2157	x 317	6.8×10^1	1/2163	x 26	4.64×10^2	1/682	x 68

a: 移植後10週におけるデータ
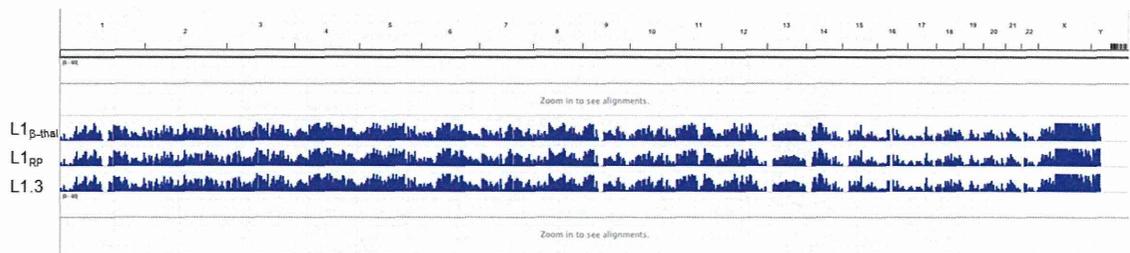
Fig. 2. ヒトゲノム中の転移可能な LINE-1s 配列の BLAST 検索
上段の数字は染色体番号．青棒はその領域に予測されたそれぞれの LINE-1s の数を示す．
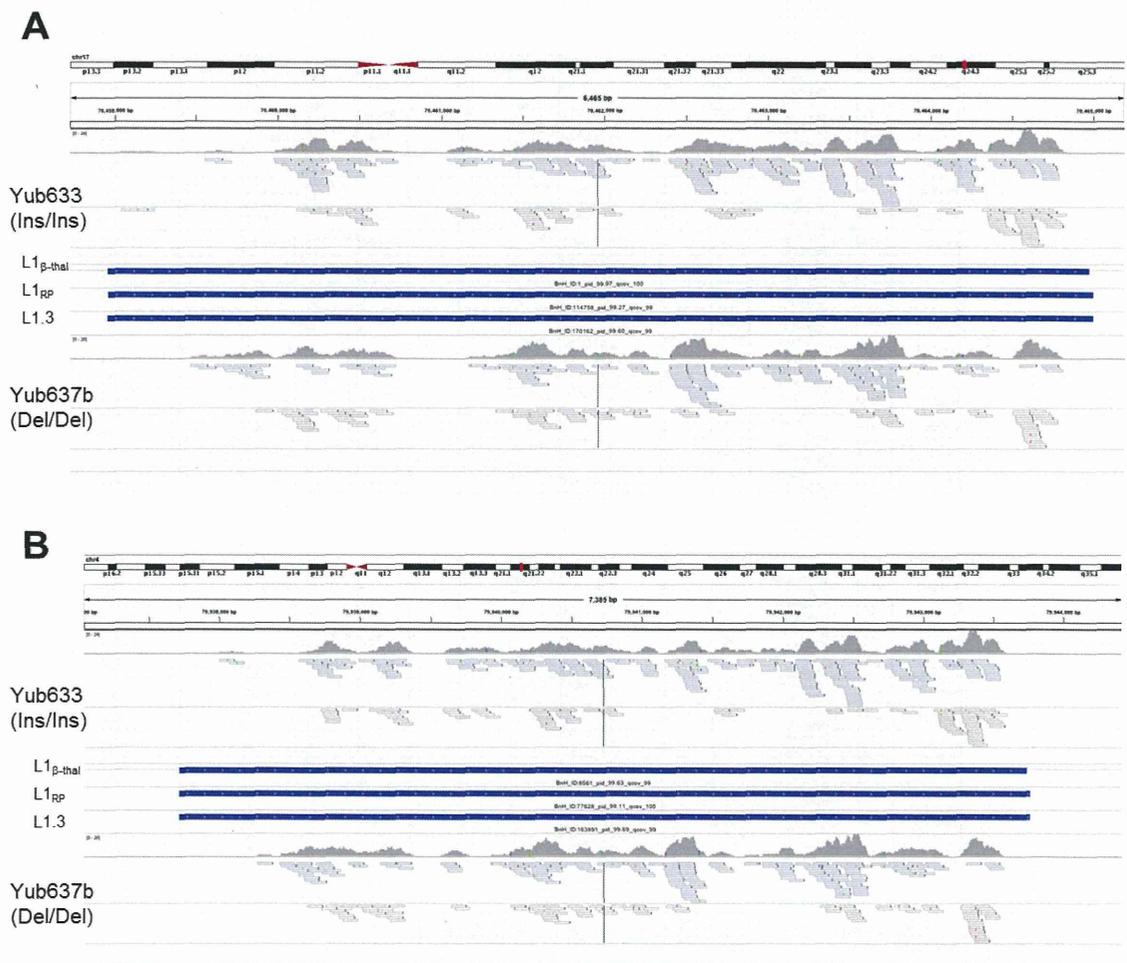
Fig. 3. L1$_{β-thal}$, L1$_{RP}$ 及び L1.3 様配列にマッピングされたリードの例
(A)17 番染色体 q24.3 70458956-70464974 におけるマッピング
(B)4 番染色体 q21.22 19077884-19083928 におけるマッピング

Fig. 4. L1$_{\beta\text{-thal}}$ 様配列にマッピングされた RNA の発現解析（一部）

Ir941;Yub633(Ins/Ins), Ir942;Yub637b(Del/Del), pid;BLAST の結果得られた L1$_{\beta\text{-thal}}$ との一致度(%), qcov; L1$_{\beta\text{-thal}}$ 全長に占める該当領域の割合, locus;該当領域の位置情報, length;該当領域の長さ, read_count;当該領域にアライメントされている全リード数, unique_read_count;当該領域にアライメントされているリードの中で, 一箇所のみアライメントされているリードの数, estimate_read_count;各リードのマルチマップ数を考慮して補正を行った, 当該領域に含まれる予想リード数, norm_read_count;各検体間の総取得リード数を考慮して補正を行った検体間比較用の予想リード数

tracking_id	pid	qcov	locus	length	Ir941_no_mismatch				Ir942_no_mismatch				Ir942_no_mismatch Ir941_no_mismatch log2(fold change)	MAX unique read_count (x_mismatch)	MAX unique read_count (no_mismatch)	for IGV
					read_count	unique_read_count	estimate_read_count	norm_read_count	read_count	unique_read_count	estimate_read_count	norm_read_count				LOCUS
BrH:ID:77823	99.9	99	4:19077884-19083928	6044	108	2	4.17805	0.0894927	158	4	4.74653	0.102799	0.19998432	4	4	Link
BrH:ID:77824	99.7	99	4:74717542-74723588	6044	182	0	2.08308	0.0442548	181	0	0.893922	0.0150912	-1.552119894	0	0	Link
BrH:ID:77825	99.28	99	4:138293503-138299545	6042	172	0	0.542865	0.0116293	134	0	0.442255	0.0096903	-0.27810297	0	0	Link
BrH:ID:77826	99.24	99	4:21159394-21165430	6036	148	0	0.458502	0.0097828	124	0	0.449125	0.0097393	-0.006423418	0	0	Link
BrH:ID:77827	99.17	99	4:90875713-90881754	6041	152	0	0.847428	0.0181804	118	0	0.33787	0.0073289	-1.309530994	0	0	Link
BrH:ID:77828	99.11	100	4:79937713-79943782	6047	138	0	0.505784	0.0108389	132	0	0.52277	0.0113383	0.064758945	0	0	Link
BrH:ID:77829	99.12	100	4:87347109-87353147	6038	180	0	0.470935	0.0100921	128	0	0.39971	0.0088885	-0.21983012	0	0	Link
BrH:ID:77830	99.07	99	4:78347983-78354029	6048	192	0	1.37805	0.029531	184	0	0.478419	0.0103312	-1.51525186	0	0	Link
BrH:ID:77831	99.09	99	4:81939923-81945961	6038	180	0	0.523068	0.0112097	110	0	0.326553	0.0069512	-0.689401258	0	0	Link
BrH:ID:77832	99.11	99	4:15841550-15847581	6031	184	0	0.878992	0.0188388	134	0	0.412355	0.0089419	-1.074890903	0	0	Link
BrH:ID:77833	99.09	99	4:79966910-79972939	6029	108	0	0.534324	0.0114508	128	0	0.424596	0.0092074	-0.314549833	0	0	Link
BrH:ID:77834	99.07	99	4:189515501-189521529	6028	104	0	0.497923	0.0106705	148	0	0.400072	0.0088621	-0.297503527	0	0	Link
BrH:ID:77835	99.02	99	4:93838302-93844335	6033	148	0	0.530187	0.0113819	100	0	0.388913	0.0084338	-0.429973344	0	0	Link
BrH:ID:77836	99.02	99	4:186589978-186578011	6033	134	0	0.384457	0.0078100	102	0	0.310228	0.0067273	-0.215347927	0	0	Link
BrH:ID:77837	99.07	99	4:186755900-186761922	6022	158	0	0.578122	0.0123891	128	0	0.392924	0.0085206	-0.54003784	0	0	Link
BrH:ID:77838	98.96	99	4:78105735-78111774	6039	142	0	0.557115	0.011909	106	0	0.321429	0.0069701	-0.77841211	0	0	Link
BrH:ID:77839	98.96	99	4:91978213-91984251	6038	122	0	0.491812	0.0105393	90	0	0.320701	0.0069544	-0.59979395	0	0	Link
BrH:ID:77840	98.96	99	4:119948720-119954737	6037	172	0	0.524492	0.0112399	108	0	0.813573	0.0178425	0.65042581	0	0	Link
BrH:ID:77841	98.96	99	4:59079848-59084883	6035	130	0	0.392939	0.0084206	94	0	0.351803	0.0076232	-0.143153995	0	0	Link
BrH:ID:77842	98.91	99	4:79704550-79710597	6044	120	0	0.842892	0.0180832	100	0	0.28748	0.0062342	-1.534772525	0	0	Link
BrH:ID:77843	98.92	99	4:111894800-111900838	6035	122	0	0.470741	0.010088	98	0	0.418971	0.0090421	-0.157910403	0	0	Link
BrH:ID:77844	98.91	99	4:189137088-189143119	6033	120	0	0.498329	0.0106383	98	0	0.314974	0.0068302	-0.838979748	0	0	Link
BrH:ID:77845	98.84	100	4:52816456-52822503	6047	122	0	0.475823	0.0101989	114	0	0.770923	0.0187155	0.713055891	0	0	Link
BrH:ID:77846	98.87	99	4:96392422-96398480	6038	108	0	0.294401	0.0063090	80	0	0.183694	0.0040289	-0.847779235	1	0	Link
BrH:ID:77847	98.87	99	4:14755118-14761149	6033	110	0	0.444090	0.0095169	88	0	0.25148	0.0054529	-0.8004538555	0	0	Link
BrH:ID:77848	98.79	99	4:23814773-23820620	6047	120	0	0.529781	0.0113528	78	0	0.848283	0.0140682	0.308383724	0	0	Link
BrH:ID:77849	98.77	99	4:33819155-33825191	6038	120	0	0.38003	0.0081440	112	0	0.348339	0.0075538	-0.106542061	0	0	Link
BrH:ID:77850	98.73	99	4:27375872-27381718	6044	110	0	0.351908	0.0075413	104	0	0.445587	0.0096928	0.35739601	0	0	Link
BrH:ID:77851	98.89	100	4:143100259-143106307	6048	128	0	1.3841	0.0296812	100	0	0.331242	0.0071830	-2.043908500	0	0	Link
BrH:ID:77852	98.89	99	4:52538473-52544514	6041	180	0	0.879308	0.0145578	122	0	0.32741	0.0071000	-1.035885344	0	0	Link
BrH:ID:77853	98.89	99	4:57582309-57588348	6037	94	0	0.255702	0.0054796	88	0	0.153772	0.0033345	-0.718389217	0	0	Link
BrH:ID:77854	98.81	99	4:108571027-108577071	6044	42	0	0.143385	0.0030723	44	0	0.143553	0.0031580	0.038933398	0	0	Link
BrH:ID:77855	98.89	99	4:48051955-48057983	6028	144	0	0.605534	0.0129788	120	0	0.384851	0.0083412	-0.837579838	0	0	Link
BrH:ID:77856	98.48	99	4:128213790-128219823	6030	128	4	5.02184	0.107814	134	6	1.88883	0.0365749	-1.558939952	4	4	Link
BrH:ID:77857	98.39	99	4:82728935-82732904	6029	94	0	0.277777	0.0059527	92	0	0.381144	0.0082851	0.473489255	0	0	Link
BrH:ID:77858	98.31	99	4:93808297-93814335	6038	88	0	0.538123	0.0114891	94	0	0.203010	0.0044023	-1.383910204	0	0	Link
BrH:ID:77859	98.24	99	4:100000525-100006553	6028	42	0	0.186384	0.0038080	30	0	0.12938	0.0028052	-0.3831117	0	0	Link
BrH:ID:77860	98.18	99	4:148350022-148356052	6030	38	0	0.120553	0.0025834	24	0	0.077891	0.0018890	-0.813052781	0	0	Link
BrH:ID:77861	98.02	99	4:102204931-102210955	6024	38	0	0.801805	0.017179	70	0	0.192045	0.0041845	-2.044417004	0	0	Link
BrH:ID:77862	97.98	99	4:128928259-128934291	6032	38	0	0.138318	0.0029641	48	0	0.121733	0.0028396	-0.187182501	0	0	Link
BrH:ID:77863	97.78	99	4:84859122-84865188	6048	38	0	0.157377	0.0033786	92	0	1.43977	0.0312217	3.208790547	0	0	Link
BrH:ID:77864	97.77	99	4:132544410-132550449	6039	68	0	0.234421	0.0050236	82	0	0.111701	0.0024222	-1.052379354	0	0	Link
BrH:ID:77865	97.74	99	4:181408885-181414912	6027	82	0	0.188843	0.0038140	38	0	0.085599	0.0018581	-0.98128737	0	0	Link
BrH:ID:77866	97.68	99	4:184553484-184559520	6036	88	0	0.178915	0.0037912	72	0	0.129911	0.0028171	-0.428452432	0	0	Link
BrH:ID:77867	98.04	99	4:127026571-127026528	5955	38	0	0.143322	0.0030713	42	0	0.093343	0.0021107	-0.541103341	2	0	Link
BrH:ID:77868	97.6	99	4:119274085-119280124	6039	38	0	0.234411	0.0050234	54	0	0.198306	0.0043003	-0.224232109	0	0	Link
BrH:ID:77869	97.68	99	4:87177043-87183058	6015	44	0	0.133913	0.0028897	34	0	0.149319	0.0032380	0.17418238	0	0	Link
BrH:ID:77870	97.47	99	4:184350731-184358793	6042	38	0	0.224894	0.0048194	48	0	0.738277	0.0159683	1.728080824	0	0	Link
BrH:ID:77871	97.73	99	4:145389389-145375389	5960	54	0	0.188802	0.0038174	60	0	0.189562	0.0041108	0.184415478	0	0	Link
BrH:ID:77872	97.51	99	4:158084231-158090283	6032	52	0	0.300824	0.0064486	82	2	2.09552	0.0454418	2.817390181	2	2	Link

Fig. 5. L1$_{RP}$様配列にマッピングされた RNA の発現解析（一部）

Ir941;Yub633(Ins/Ins), Ir942;Yub637b(Del/Del), pid;BLAST の結果得られた L1$_{RP}$ との一致度(%), qcov; L1$_{RP}$ 全長に占める該当領域の割合, locus;該当領域の位置情報, length;該当領域の長さ, read_count;当該領域にアライメントされている全リード数, unique_read_count;当該領域にアライメントされているリードの中で，一箇所のみアライメントされているリードの数, estimate_read_count;各リードのマルチマップ数を考慮して補正を行った，当該領域に含まれる予想リード数, norm_read_count;各検体間の総取得リード数を考慮して補正を行った検体間比較用の予想リード数

Fig. 6. L1.3 様配列にマッピングされた RNA の発現解析（一部）

Ir941;Yub633(Ins/Ins), Ir942;Yub637b(Del/Del), pid;BLAST の結果得られた L1.3 との一致度(%), qcov; L1.3 全長に占める該当領域の割合, locus;該当領域の位置情報, length;該当領域の長さ, read_count;当該領域にアライメントされている全リード数, unique_read_count;当該領域にアライメントされているリードの中で, 一箇所のみアライメントされているリードの数, estimate_read_count;各リードのマルチマップ数を考慮して補正を行った, 当該領域に含まれる予想リード数, norm_read_count;各検体間の総取得リード数を考慮して補正を行った検体間比較用の予想リード数
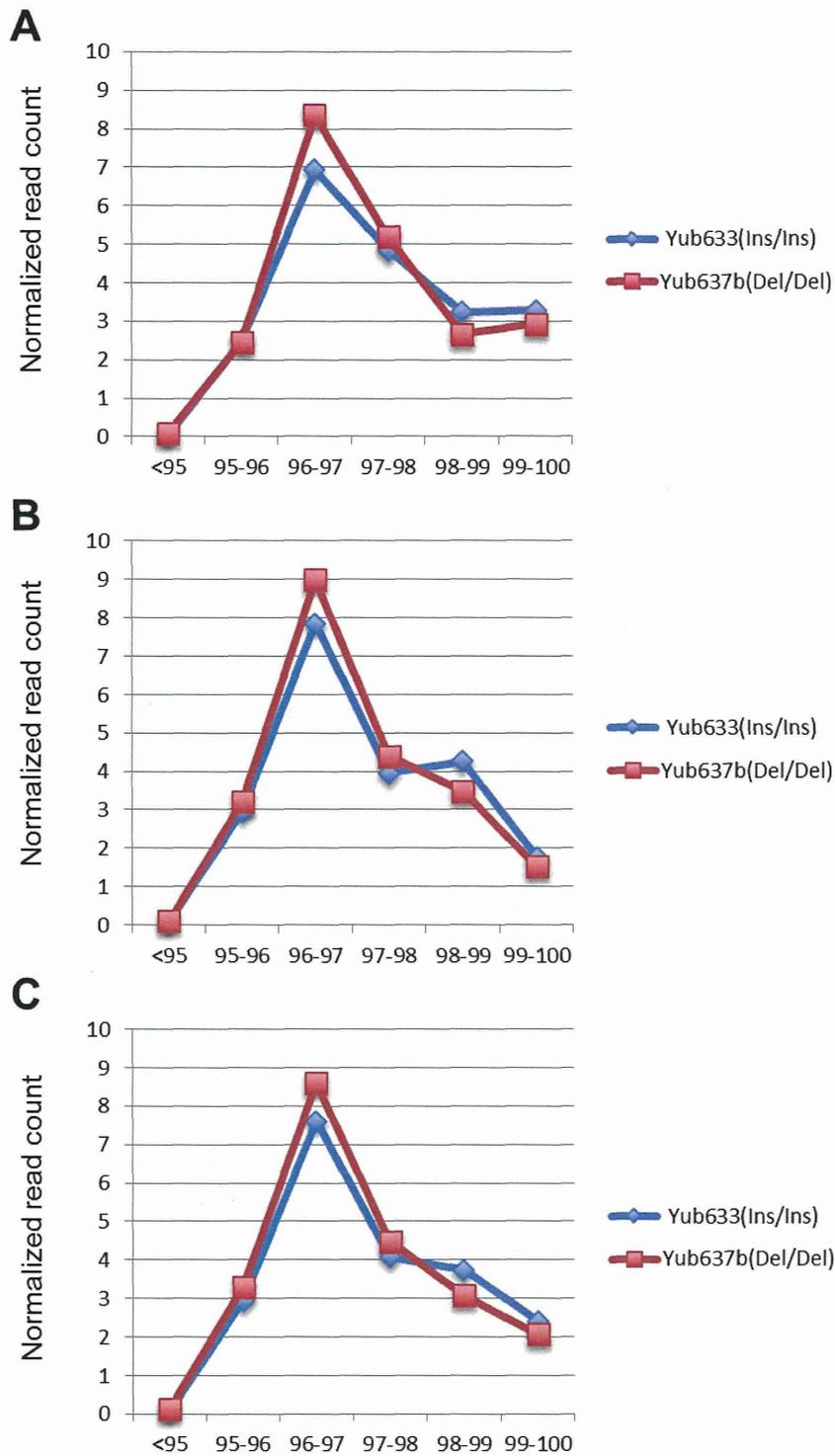
Fig. 7. LINE-1 配列との各一致度における normalized read count 数
(A) L1$_{\beta\text{-thal}}$ (B) L1$_{RP}$ (C) L1.3 全長に占める割合が 99%以上の LINE-1s 様配列を抽出し，各塩基配列の一致度におけるリード数を合計した．
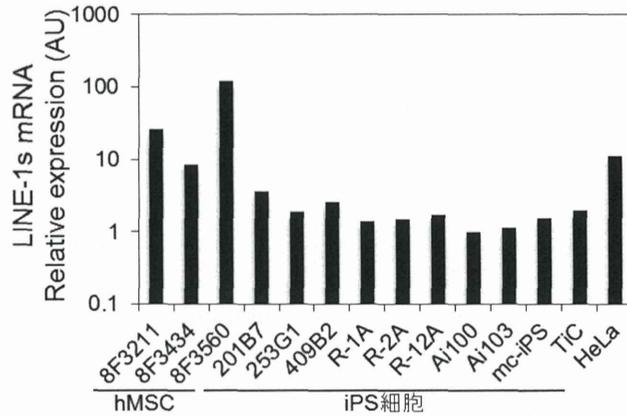
Fig. 8.　hMSCs 及び iPS 細胞における LINE-1s mRNA の発現解析
Ai103 の発現量を 1 とする.
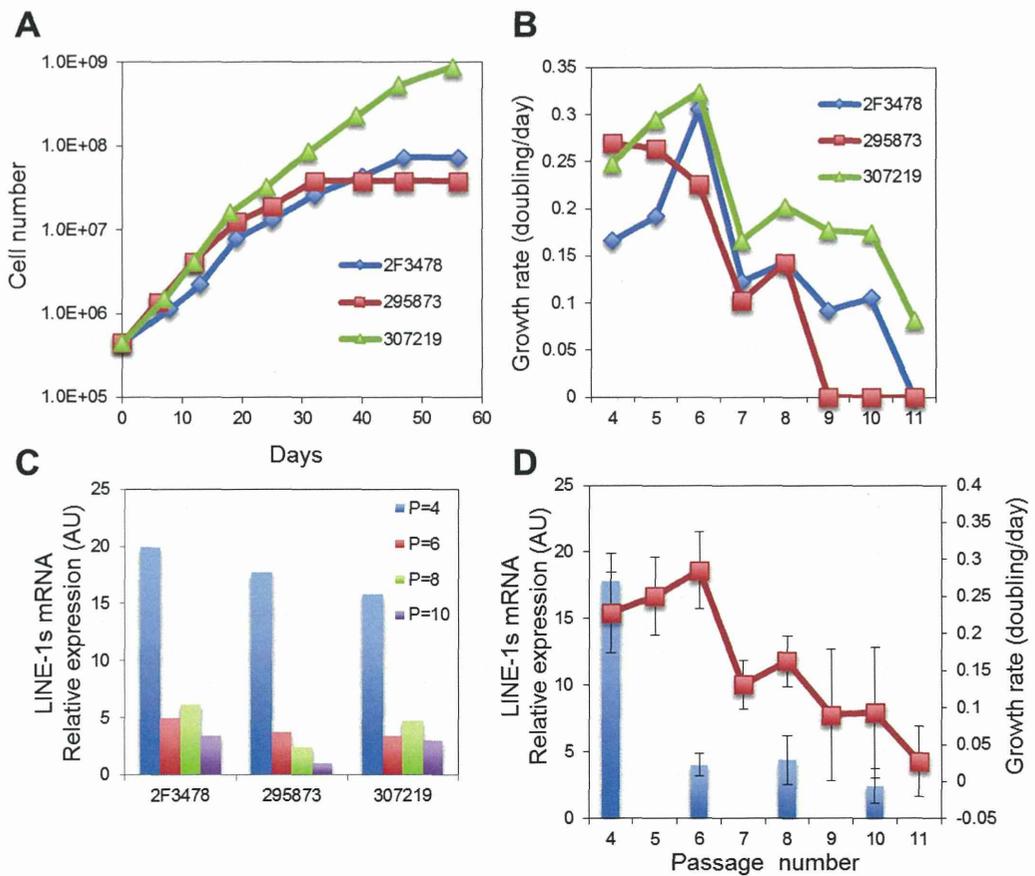
Fig. 9.　hMSCs の増殖及び LINE-1s の発現解析
(A) hMSCs 3 ロットの増殖曲線. (B) 各継代間での増殖速度. (C) 各継代数における LINE-1s の発現比較 (295873 P=10 の発現量を 1 とする). (D) (B)及び(C)の平均値

A
脂肪分化培地　　　　　　　増殖培地

2F3478
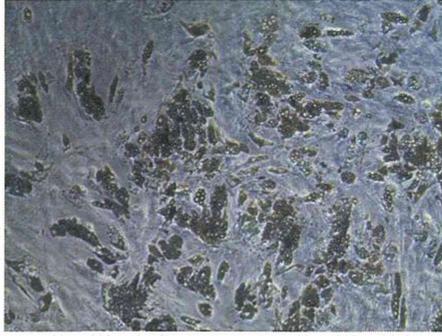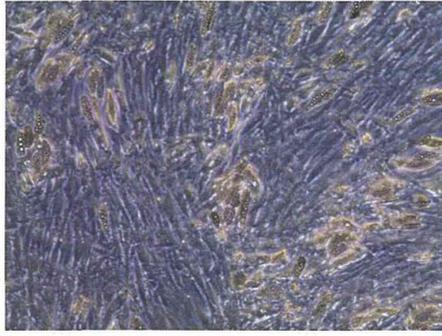
295873

307219
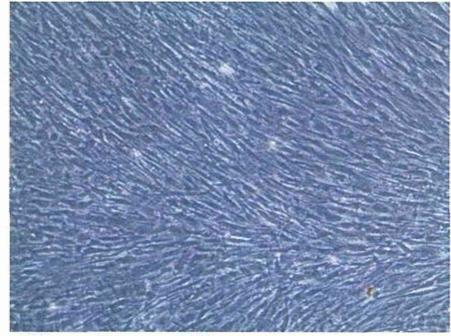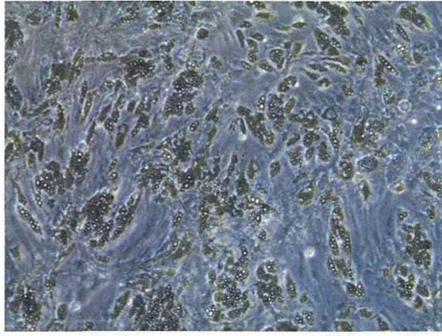
B
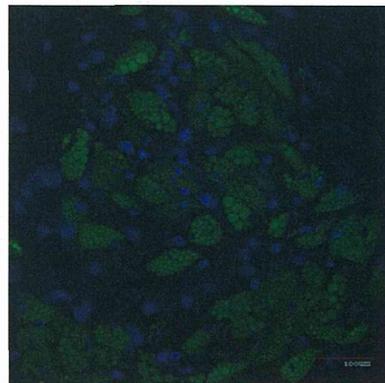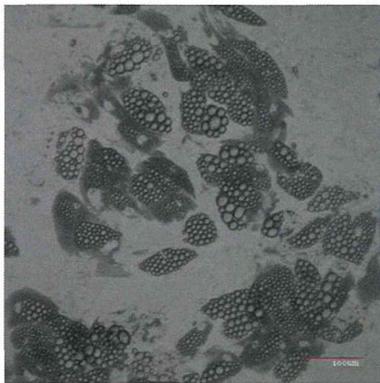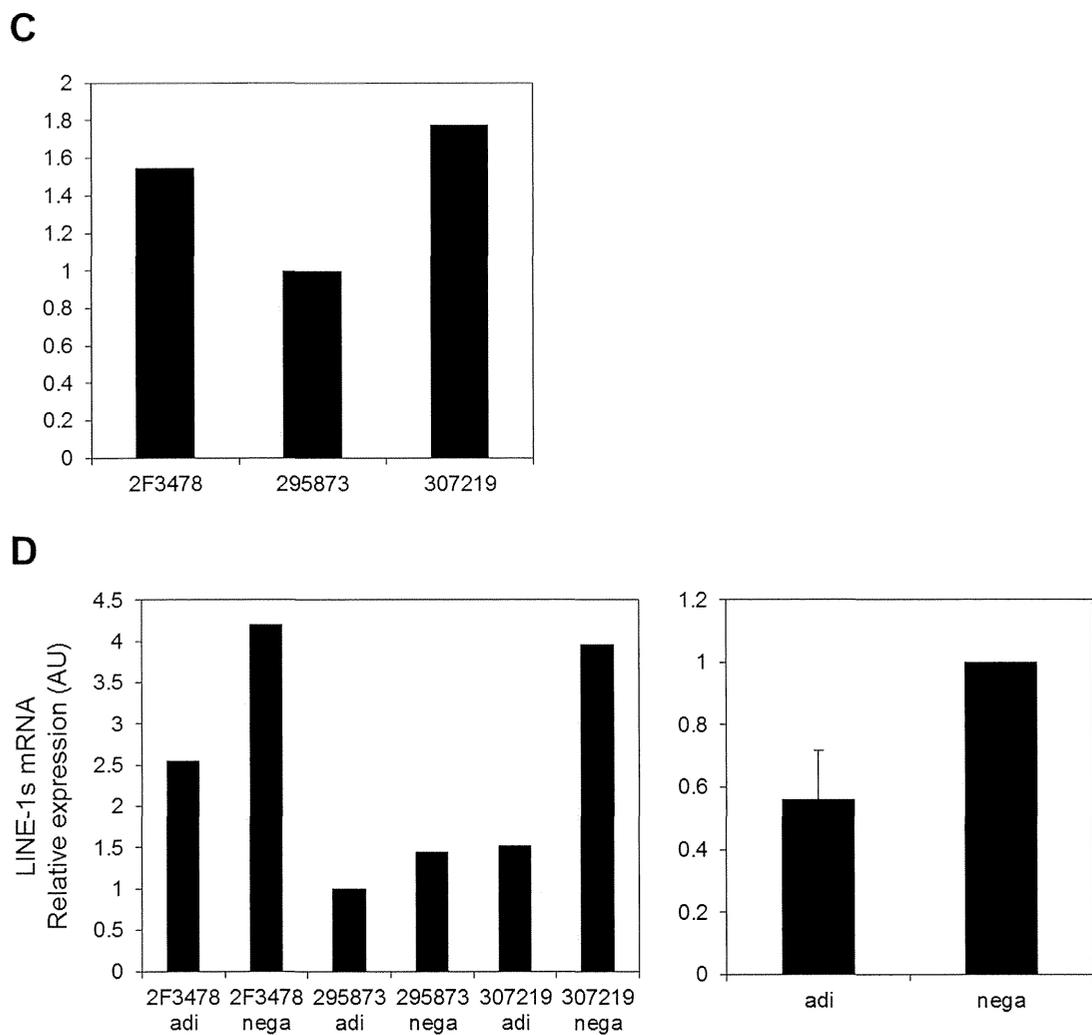
Fig. 10. hMSCs の脂肪分化と LINE-1s の発現解析

(A) 脂肪分化培地及び増殖培地で培養した hMSCs 3 ロットの顕微鏡写真. (B) 脂肪分化培地で培養した hMSCs の脂肪球蛍光染色観察写真. 緑；脂肪球. 青；核(C) hMSCs 各ロットの脂肪球が染色された面積の比較（295873 の面積を 1 とする）. (D) 脂肪分化培地 (adi)及び増殖培地(nega)で培養した hMSCs 3 ロットの LINE-1 発現比較（脂肪分化 295873 を 1 とする）（左パネル）とそれぞれの増殖培地（nega）を 1 とした時の脂肪分化培地 hMSCs における LINE-1s 発現の平均値（右パネル）.

Table 2　HL60-RG 細胞における c-myc 増幅領域のジャンクションの配列

| Junction | breakpoint position（hg19） | | Junction sequence | | inserted sequence |
	left segment	right segment	left end	right start	
6b–4a	130086178	128689007	CCTCAGGGTC**TC**	CTGTTCCTGA	None
4b–5a	128772037	130000919	CTTCCTCC**CA**	GAGAAGCCTG	None
5b–7a	130215269	130367023	ACACACTTG**T**	AGAGGGTGGG	None
7b–8	130698147	136580808	CATTC**CAACAC**	TCTTAACTCC(r)	None
8–1a	136580616	126224548	ATG AATTTCG(r)	GAGACGTCTC	None
1b–3b	126547448	128344474	CACCT AATTA	AAGGCAGCAG(r)	ATAACTTG
3a–2a	128068264	126710881	ATGTGCCCCT(r)	GGAGGCTCTG	AAAACATA
2b–6a	not analyzed				

赤字：重複配列　　　　　（r）：逆向き配列

Table 3　TK6 細胞 14-20 番染色体転座切断点候補領域の検索

| chr 14 55,382,000-55,38,2999 | | | | 10箇所 | | | | | | | | |
# read_name	read_num	hit	strand	start	end	unique	read_num	hit	strand	start	end	unique
MG00HS05:361:C3W91ACXX:4:2115:3017:28497	2	chr14	+	55382209	55382310	○	1	chr20	+	49,282,334	49282434	○
MG00HS05:361:C3W91ACXX:5:2205:14160:62557	2	chr14	−	55382209	55382309	○	1	chr20	−	49282957	49283057	○
MG00HS05:361:C3W91ACXX:6:2210:21129:96634	1	chr14	−	55382211	55382311	○	2	chr20	−	49282966	49283063	○
MG00HS14:443:C3YEDACXX:7:2104:14959:59907	1	chr14	−	55382213	55382311	○	2	chr20	−	49283436	49283493	○
MG00HS05:361:C3W91ACXX:4:1202:10455:11484	1	chr14	+	55382214	55382314	○	2	chr20	+	49282510	49282610	○
MG00HS05:361:C3W91ACXX:6:2303:16054:77158	1	chr14	+	55382214	55382305	○	2	chr20	+	49282514	49282598	○
MG00HS05:361:C3W91ACXX:7:2201:17991:62643	2	chr14	+	55382214	55382314	○	1	chr20	+	49282450	49282550	○
MG00HS05:361:C3W91ACXX:5:1215:17129:3600	1	chr14	+	55382215	55382315	○	2	chr20	+	49282417	49282517	○
MG00HS14:443:C3YEDACXX:7:2111:10449:86501	2	chr14	+	55382215	55382315	○	1	chr20	+	49282508	49282608	○
MG00HS05:361:C3W91ACXX:6:2315:17139:90283	2	chr14	+	55382217	55382317	○	1	chr20	+	49282361	49282461	○

Table 4 TK6 細胞の変異原処理による tk 遺伝子突然変異試験

Table3
Mutation
frequency

検体名	Positive well no.			Total well no.	Negative well no.	PE3	Mut. freq. (×10⁻⁶)			%SG
	N	S	Total				N-MF	S-MF	T-MF	
control	2	5	7	384	377	0.9	0.1	0.4	0.5	71.6
MMS (6ug/ml)	25	8	33	192	159	0.2	14.3	5.0	19.3	26.0
ENU (12ug/ml)	139	16	155	192	37	0.8	38.3	10.7	49.0	21.8
γ-ray 2Gy	2	82	84	192	108	0.5	0.6	30.7	31.3	98.2

Comments:

PE: Plating efficiency

RS: Relative survival

RSG: Relative suspension growth

RTG: Relative total growth

MF: Mutation frequency; N: Normally growing colony; S: Slowly growing colony; T: Total

%SG: Ratio of S-MF to T-MF

Table 5 PacBio シークエンサーにて検出された mtDNA 変異

Sample	Mapped Read	Mapped bp	Mutations
TK6_cont_1st	9258	6,685,859	31
TK6_cont_2nd	6,647	5,102,372	33
TK6_ENU	14,018	12,469,403	32
TK6_MMS	1,272	1,089,609	28
TK6_γ −ray	2,361	2,409,918	36
HL60_RG	292	2,999,400	17

Table 6　TK6/BLM 細胞で確認された突然変異の例

Location	Ref seq		TK6	hom/hetero	TK6/BLM	hom/hetero
chr17	T	::	G	hom	A	hom
15420960		call	(G37/C32)	G/C hetero	A27/G 25	A/G hetero
	周辺seq		ATCAC（G/C）TGCTT		ATCAC（A/G）TGCTT	
	変異		(G/C)から(A/G)に変化　C to A mutation			

Table 7　TK6/BLM 細胞にて発現変化を示したタンパク

Accession	Peptide c	Unique peptides	Confidence	Anova (p)	Max fold	Highest	Lowest	Description
CD20	8	7	446.44	0.0106	3.20	BLM	TK6	B-lymphocyte antigen CD20
BRWD3	2	1	32.15	0.0158	2.06	TK6	BLM	Bromodomain and WD repeat-containing protein 3
CCL3	1	1	17.37	0.0253	4.32	BLM	TK6	C-C motif chemokine 3
CHD3	9	1	226.66	0.0273	2.06	TK6	BLM	Chromodomain-helicase-DNA-binding protein 3
IGKC	8	8	433.31	0.0056	3.28	TK6	BLM	Ig kappa chain C region
KV402 and KV401	4	4	210.12	0.0305	3.18	TK6	BLM	Ig kappa chain V-IV region Len
IGHM;MUCB	15	13	630.79	0.0158	7.29	TK6	BLM	Ig mu chain C region
NUCB1	6	2	164.57	0.0204	2.42	TK6	BLM	Nucleobindin-1
SCAM1	2	2	54.25	0.0068	3.20	TK6	BLM	Secretory carrier-associated membrane protein 1
SPTB1	13	1	309.44	0.0485	2.03	TK6	BLM	Spectrin beta chain, erythrocytic
PUR2	3	2	56.74	0.0457	2.16	TK6	BLM	Trifunctional purine bi
CN166	3	2	118.78	0.0025	2.05	BLM	TK6	UPF0568 protein C14orf166

Protein Candidates after removing peptides with sore < 10

Fig. 11　HL60-RG 細胞 c−myc 増幅領域の概略
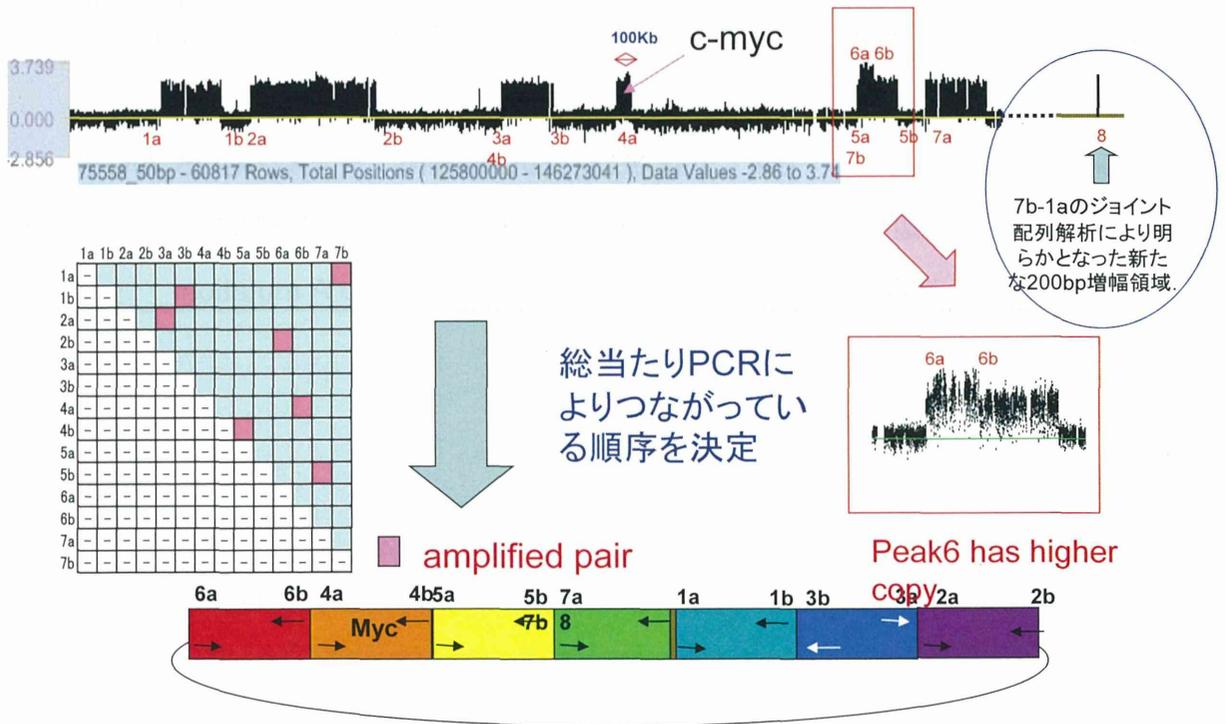
Fig. 12　HL60-RG 細胞 c−myc 増幅領域の融合点を含む配列の検出
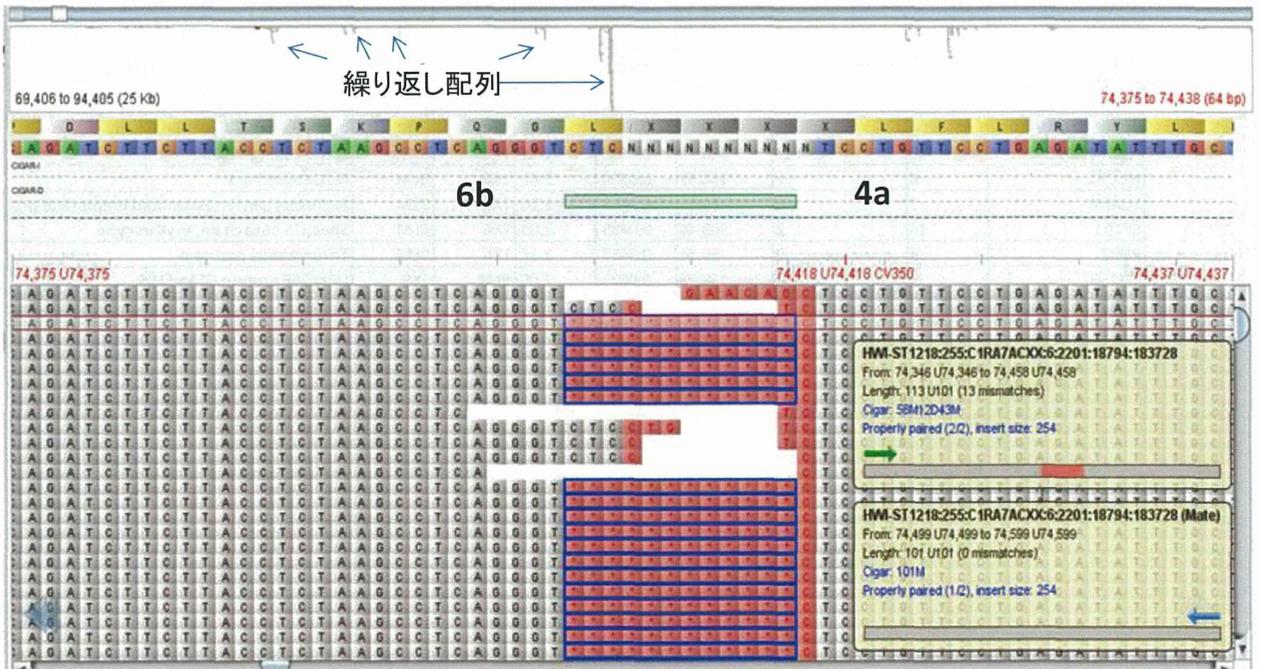
Fig. 13　TK6 細胞の染色体解析
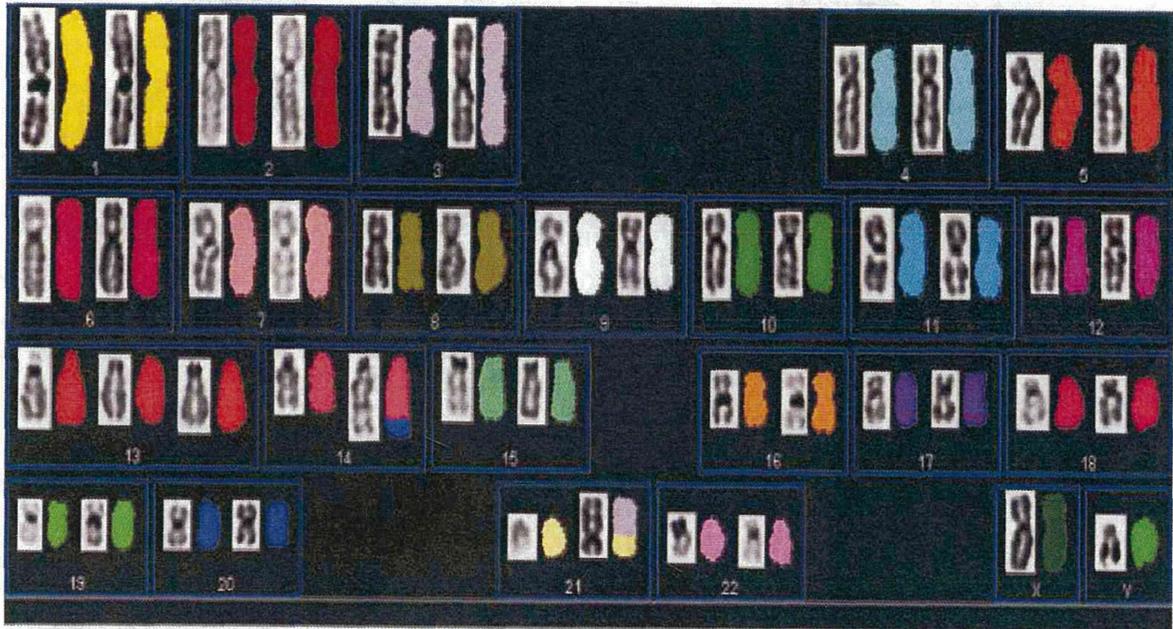
Fig. 14　抽出した mtDNA の品質チェック
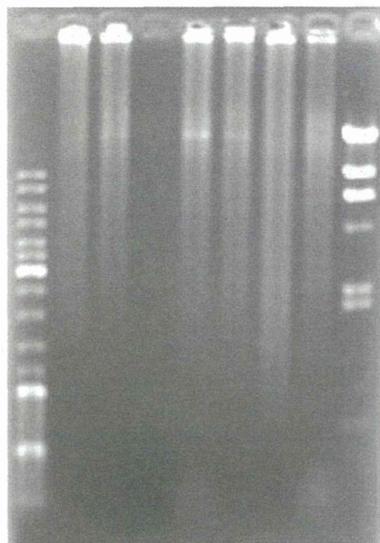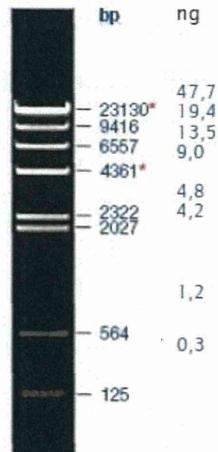
lane	sample name	μl
1	Gene Ruler DNA Ladder Mix	5
2	TK6_cont	2
3	TK6_ENU	3
4	TK6_MMS	5
5	TK6_MMS_2	3
6	TK6_RAY	2
7	HL60_RG	4
8	HL60_RG_2	2
9	Lambda Hind III	5
10		

Sample No.	Sample Name	Conc. [ng/μl]	Total volume[μl]
1	TK6_cont	11,4	100
2	TK6_ENU	7,13	100
3	TK6_MMS	2,23	100
4	TK6_MMS_2	6,06	100
5	TK6_RAY	11,2	100
6	HL60_RG	5,24	100
7	HL60_RG_2	13,7	100

Fig. 15 PacBioRS シークエンサーによる解析パフォーマンス
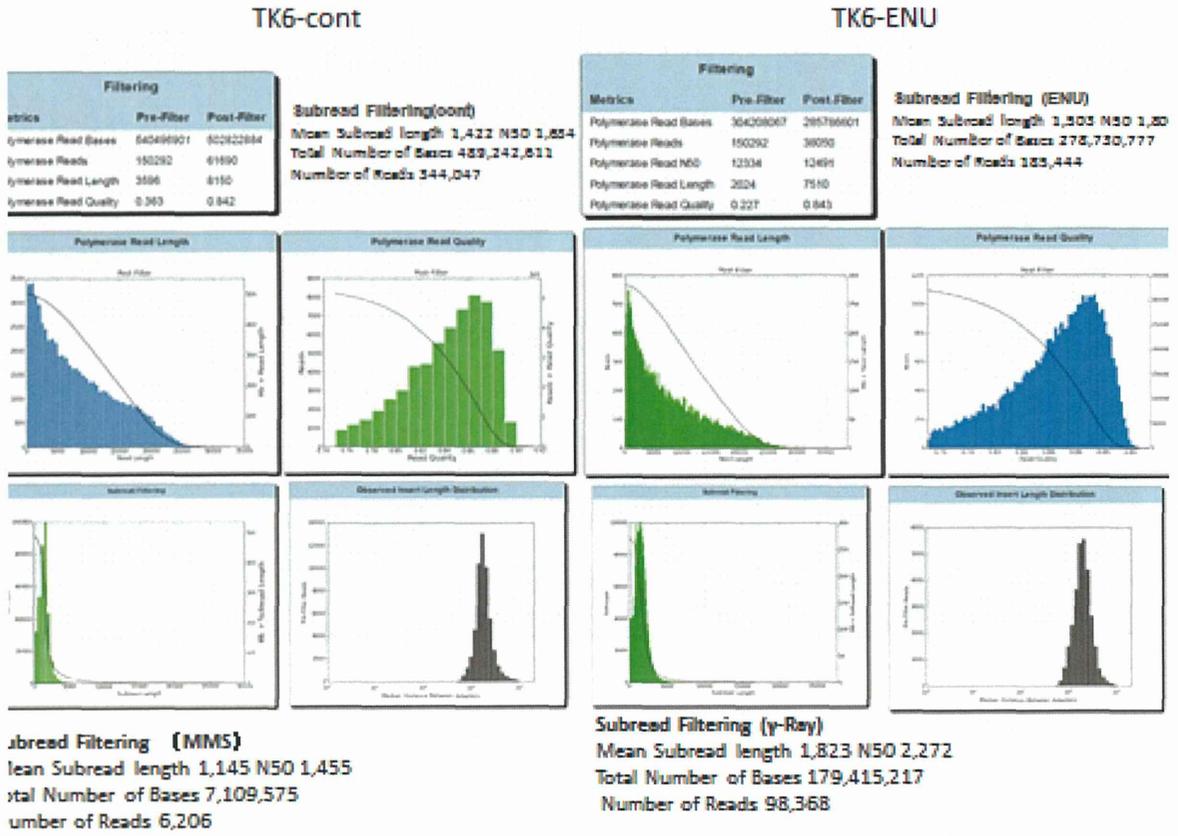
Fig. 16 Tablet 上での PacBio シークエンサーデータの確認
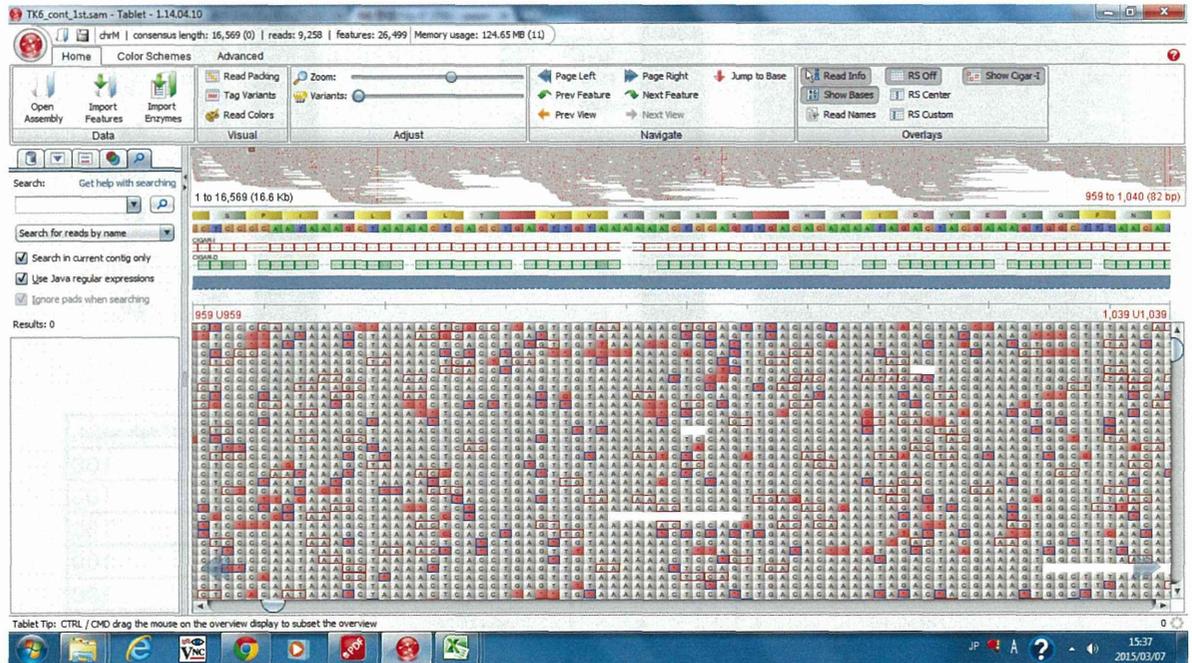
Fig. 17　ProteoMap Online ソフトウエアによるプロテオームデータ公開

1)　ProteoMap Online ソフトウエア概略

ProteoMap Online 1.0

1. ProteoMap Online 1.0 is a web based tool for sharing basic LC-MS/MS data such as 2D image of the LC-MS data, MS/MS spectrum and list of identified proteins and its peptides.
2. User can upload the 2D image of LC-MS, MS/MS spectrum as .mgf file format and list of identified proteins as .xml file format (Desktop version of ProteoMap required).
3. ProteoMap Online will display the MS/MS spectrum and identified proteins on the 2D image.
4. The location of MS/MS data with and without a peptide/protein are shown in two different colours blue and red respectively.
5. User can choose to display only the identified proteins or only the MS/MS spectrum of unidentified proteins or both.
6. User can select a protein to display only the peptides of the selected protein.
7. Clicking on the blue cross the identified peptides sequence, its assigned protein and their score are displayed along with its MS/MS spectrum.

2)　ProteoMap Online 操作画面

1. ProteoMap Online 1.0 is a web based tool for sharing basic LC-MS/MS data such as 2D image of the LC-MS data, MS/MS spectrum and list of identified proteins and its peptides.
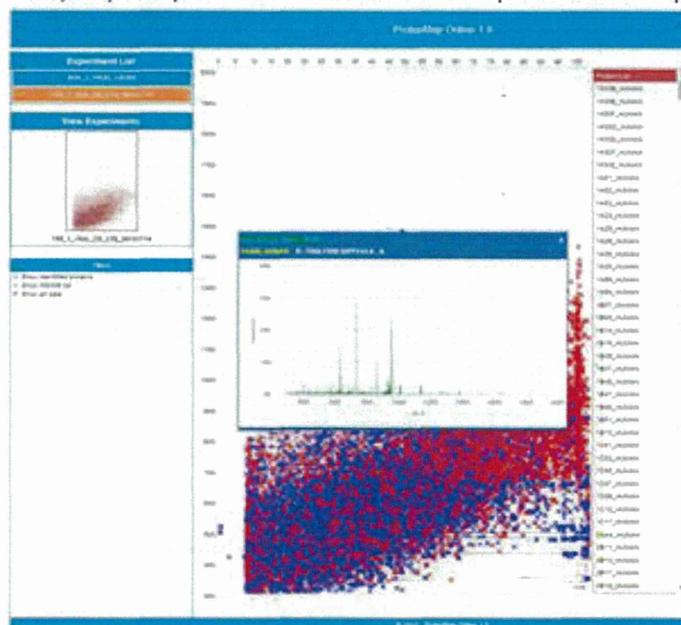
2. User can upload the 2D image of LC-MS, MS/MS spectrum as a .mgf file format and list of identified proteins as .XML file format (Desktop version of ProteoMap required).
3. ProteoMap Online will display the MS/MS spectrum and identified proteins on the 2D image.

4. The location of MS/MS data with and without a peptide/protein hit are shown in two different colours, blue and red respectively.
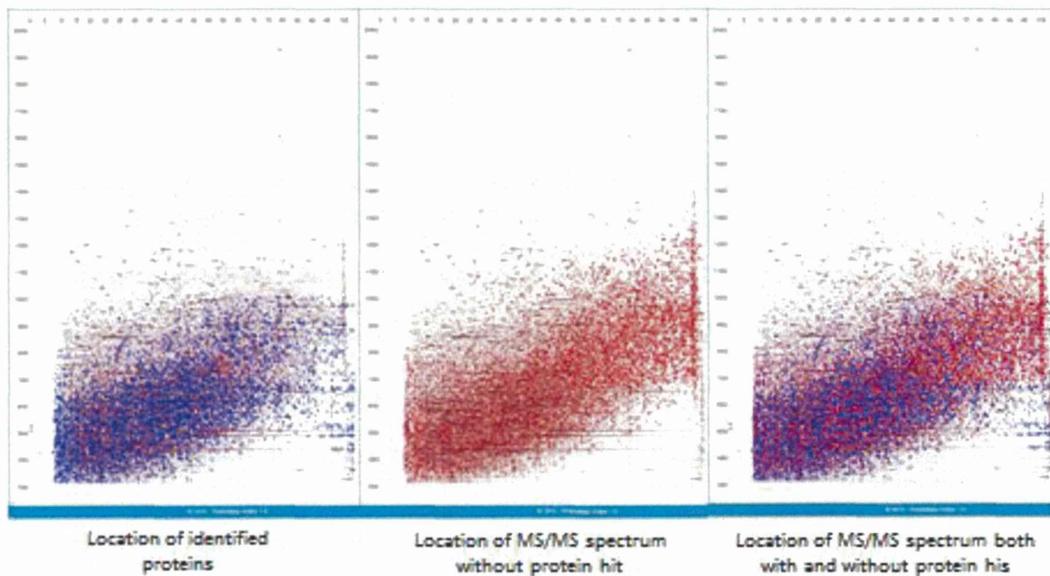
Location of identified proteins   Location of MS/MS spectrum without protein hit   Location of MS/MS spectrum both with and without protein his

5. User can choose to display only the identified proteins or only the MS/MS spectrum of unidentified proteins or both.

6. User can select a protein to display only the peptides of the selected protein.

View of the location of peptides for the selected protein

7. Clicking on the blue cross the identified peptides sequence, its assigned protein and their score are displayed along with its MS/MS spectrum.

Table 8　標準ゲノムDNAのアレル頻度

Gene	Chromosome Number	Reference Base	Observed Base	Amino Acid Change	Allelic Frequency	Codon Change	Exon ID	Validated by Digital PCR	Validated by NGS	Class of Mutation
BRAF	chr7	A	T	V600E	10.5	GTG/GAG	Exon 7 140453075_140453193	Yes	Yes	Missense
KIT	chr4	A	T	D816V	10.0	GAC/GTC	Exon 17 55599236_55599358	Yes	No	Missense
EGFR	chr7	AAGGAATTAAGAGAAGCA	AA	I E746 − A750	2.0	N/A	Exon 19 55174772_55174870	Yes	No	Deletion
EGFR	chr7	T	G	L858R	3.0	CTG/CCG	Exon 21 55191719_55191874	Yes	No	Missense
EGFR	chr7	C	T	T790M	1.0	ACG/ATG	Exon 20 55181293_55181478	Yes	No	Missense
EGFR	chr7	G	A	G719S	24.5	GGC/AGC	Exon 18 55241614_55241736	Yes	Yes	Missense
KRAS	chr12	C	T	G13D	15.0	GGC/GAC	Exon 12 25398208_25398329	Yes	Yes	Missense
KRAS	chr12	G	A	G12D	6.0	GGT/GAT	Exon 2 2539869_2539748	Yes	No	Missense
NRAS	chr1	C	A	Q61K	12.5	CAA/AAA	Exon 3 115256599_115256777	Yes	No	Missense
PIK3CA	chr3	G	A	E545K	9.0	GAG/AAG	Exon 10 178935998−178936122	Yes	No	Missense
PIK3CA	chr3	A	G	H1047R	17.5	CAT/CGT	Exon 3 178951882_178957881	Yes	Yes	Missense
CDX2	chr13	AC	A	V306fs	41.5	N/A	N/A	Yes	Yes	Deletion
ARID1A	chr1	GC	G	P1562fs	33.5	N/A	N/A	Yes	Yes	Deletion
CCND2	chr12	AT	A	N/A	32.5	N/A	N/A	Yes	No	Deletion
BRCA2	chr13	CA	C	A1689fs	33.0	N/A	N/A	Yes	Yes	Deletion
ALK	chr2	G	A	P1543S	33.0	CCT/TCT	Exon 2 29415640_29416788	Yes	Yes	Missense
CTNNB1	chr3	C	A	S33Y	32.5	TCT/TAT	Exon 3 41266017_41266202	Yes	Yes	Missense
FBXW7	chr4	TC	T	G667fs	33.5	N/A	N/A	Yes	Yes	Deletion
PDGFRA	chr4	G	A	G426D	33.5	GGC/GAC	Exon 4 55138561_55138687	Yes	Yes	Missense
APC	chr5	C	T	R2714C	33.0	CGT/TGT	Exon 5 112173250_112181936	Yes	Yes	Missense
NOTCH1	chr9	G	A	P668S	31.5	CCG/TCG	Exon 9 139409742_139409852	Yes	Yes	Missense
FLT3	chr13	GGA	G	S985fs	10.5	N/A	N/A	Yes	Yes	Deletion
FLT3	chr13	A	G	V197A	11.5	GTG/GCG	Exon 13 28626682_28626811	Yes	Yes	Missense
IDH1	chr2	G	A	S261L	10.0	TCA/TTA	Exon 2 209106718_209106869	Yes	Yes	Missense
CTNNB1	chr3	CCTT	C	S45del	10.0	N/A	N/A	Yes	Yes	Deletion
MET	chr7	GT	G	V237fs	6.5	N/A	N/A	Yes	Yes	Deletion
SH2D2A/NTRK1*	chr1	AC	A	N/A	8.5	N/A	N/A	Yes	Yes	Deletion
ABL2	chr1	TG	T	P986fs	8.0	N/A	N/A	Yes	Yes	Deletion
CDH1	chr16	A	G	N/A	7.5	N/A	N/A	Yes	No	None
FANCA	chr16	ACT	A	E345fs	7.5	N/A	N/A	Yes	Yes	Deletion
NF1	chr17	CT	C	L626fs	7.5	N/A	N/A	Yes	Yes	Deletion
NF2	chr22	AC	A	P275fs	8.0	N/A	N/A	Yes	Yes	Deletion
EP300	chr22	CA	C	K291fs	8.0	N/A	N/A	Yes	Yes	Deletion
MLH1	chr3	C	A	L187M**	8.5	CTG/ATG	Exon 3 37061801_37061954	Yes	Yes	Missense
FGFR1	chr8	G	A	P124L***	8.5	CCC/CTC	Exon 8 38285439_38285611	Yes	Yes	Missense

* This chromosome location was annotated to both SH2D2A and NTRK1 using hg19 and GRCH37.
** L187M is the correct annotation with reference to ENST00000383761. It may be observed as L323M in other transcripts.
** P124L is the correct annotation with reference to ENST00000335922. It may be observed as P150L in other transcripts.

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