

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

24. Tano K, Yasuda S, Umezawa A, Sato Y. 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, 安田智, 佐藤陽治

出願人【識別番号】803000056

【名称】公益財団法人 ヒューマンサイエンス振興財団

特許出願番号 特願2014-176861

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

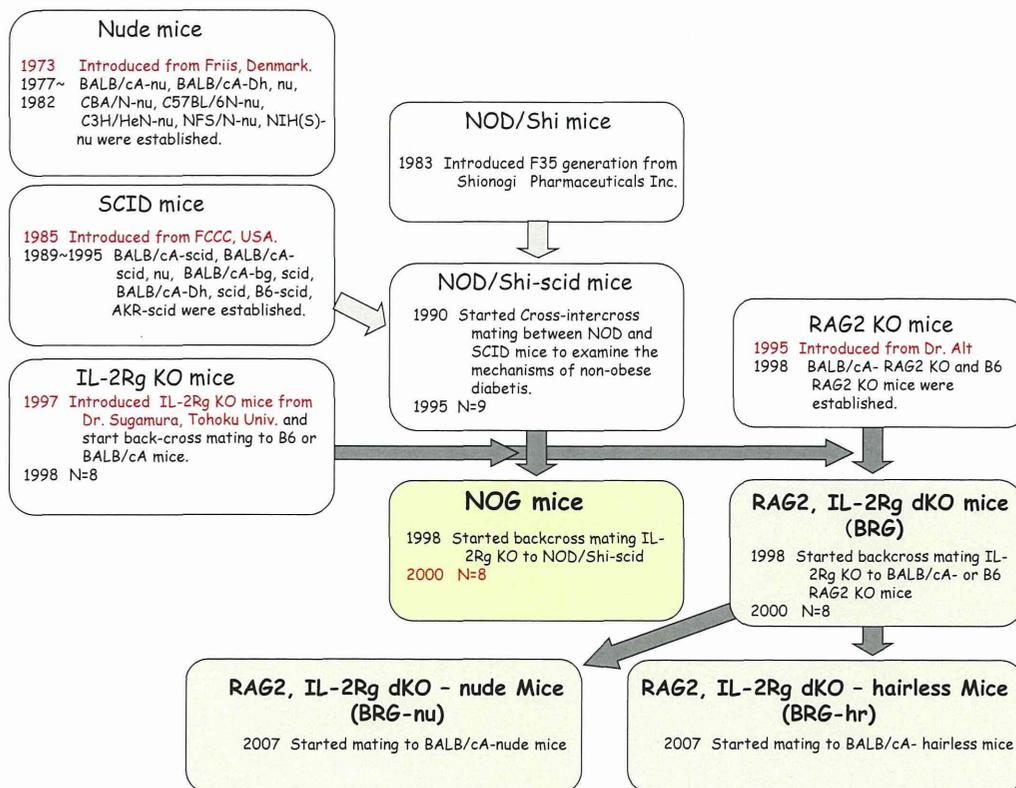


Fig. 1 BRG, BRG-nu および BRG-hr マウスの開発過程

Table 1 移植後 16 週における TPD₅₀, 対ヌードマウス比およびマトリゲル増強効果

移植細胞	背景遺伝子 系統 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	0.68~4.21 x 10 ⁵	1.00 x 10 ¹	1/7	x 1	1.78 x 10 ³	1/83	x 1	3.17 x 10 ¹	1/10	x 1			
HeLa細胞+マトリゲル	-	3.2 x 10 ¹	1/2157	x 317	6.8 x 10 ¹	1/2163	x 26	4.64 x 10 ²	1/682	x 68			

a: 移植後10週におけるデータ

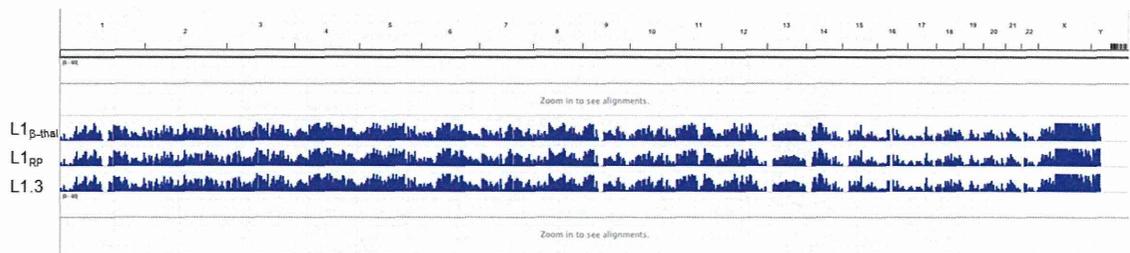


Fig. 2. ヒトゲノム中の転移可能な LINE-1s 配列の BLAST 検索
 上段の数字は染色体番号, 青棒はその領域に予測されたそれぞれの LINE-1s の数を示す。

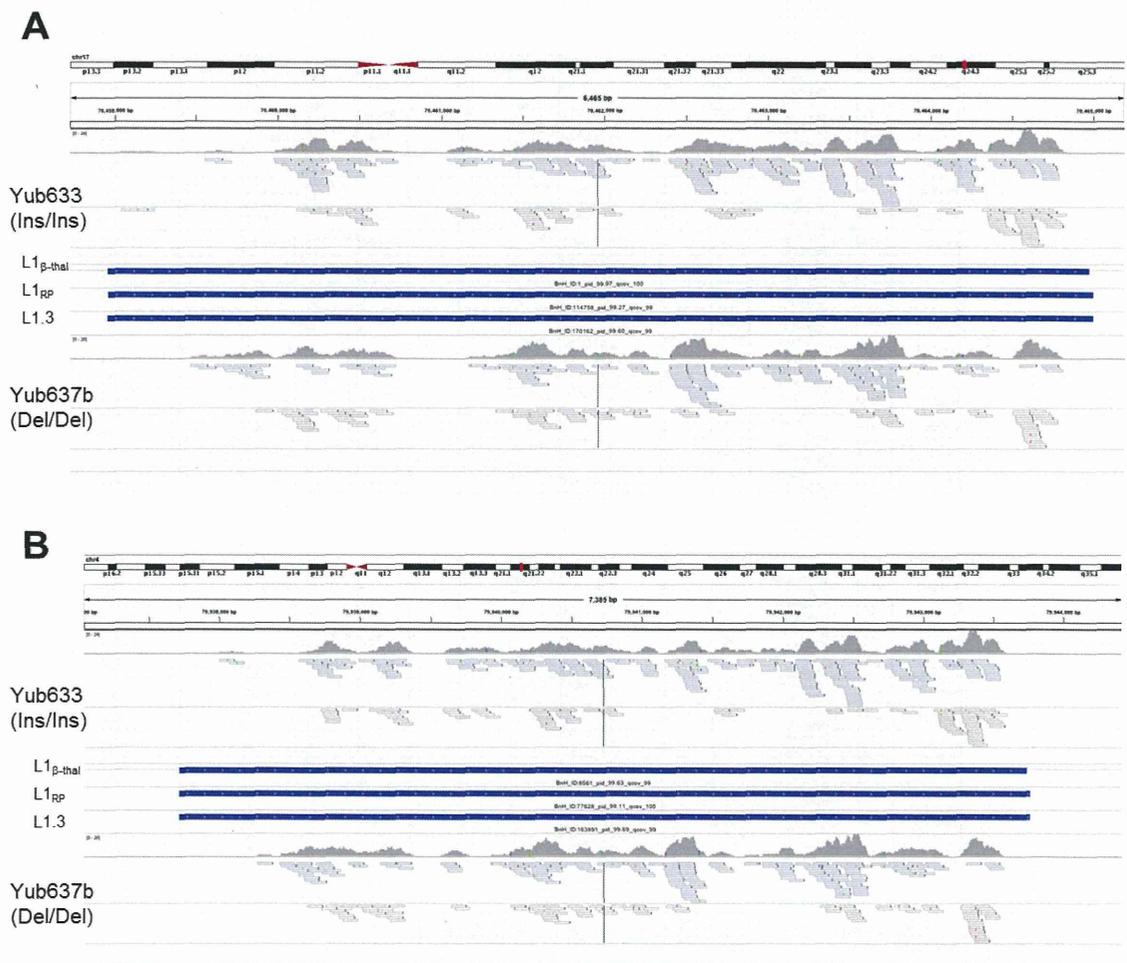


Fig. 3. L1_{β-thal}, L1_{RP} 及び L1.3 様配列にマッピングされたリードの例
 (A)17 番染色体 q24.3 70458956-70464974 におけるマッピング
 (B)4 番染色体 q21.22 19077884-19083928 におけるマッピング

trackingId	pid	qcov	locus	length	Ir941_no_mismatch				Ir942_no_mismatch				Ir942_no_mismatch log2(Ratio change)	MAX unique read_count (Ir941_mismatch)	MAX unique read_count (Ir942_mismatch)	For EGV
					read_count	unique_read_count	estimate_read_count	norm_read_count	read_count	unique_read_count	estimate_read_count	norm_read_count				
BrF-UD-1	99.97	100	17_70459308-70460974	8018	182	0	0.514499	0.0110257	130	0	0.412897	0.00693078	-0.300304699	0	0	0
BrF-UD-2	99.33	99	17_70444323-70530841	8018	188	0	0.208419	0.0108328	150	0	0.402200	0.0087219	-0.315328362	0	0	0
BrF-UD-3	99.17	100	17_9611965-9620004	8019	114	0	0.383209	0.00821218	110	2	2.27411	0.0493148	2.88818119	2	2	2
BrF-UD-4	99.00	99	17_98598593-98602392	9999	142	0	0.370087	0.00793098	142	0	0.283221	0.00618308	-0.338703231	0	0	0
BrF-UD-5	98.45	99	17_12445918-12455932	8018	134	0	0.411008	0.0088079	130	0	0.357942	0.0077802	-0.182880383	0	0	0
BrF-UD-6	97.98	99	17_32887138-32893131	8013	88	0	0.218188	0.00483288	98	0	0.702490	0.0153237	1.717291396	0	0	0
BrF-UD-7	97.89	99	17_80881453-80892474	8021	52	2	2.84792	0.0087448	84	0	0.841947	0.0183278	-1.835977799	2	2	2
BrF-UD-8	97.88	99	17_55001717-55007728	8011	82	0	0.280482	0.00601073	48	0	0.223384	0.00484803	-0.310141359	0	0	0
BrF-UD-9	97.86	99	17_32878271-32884272	8001	30	2	2.25514	0.0483278	42	0	0.128881	0.00279047	-4.114287308	2	2	2
BrF-UD-10	97.72	99	17_34292484-34294452	9988	80	0	0.270717	0.00380148	86	0	0.298881	0.00338618	-0.059728432	0	0	0
BrF-UD-11	97.34	99	17_41200880-41208898	8018	38	0	0.132448	0.00283832	42	0	0.113745	0.00248993	-0.202825172	0	0	0
BrF-UD-12	97.31	100	17_81110229-81118241	8012	44	0	0.289603	0.0128059	40	0	0.272431	0.00590777	-1.098855086	0	0	0
BrF-UD-13	97.49	99	17_3178631-3182544	8013	38	0	0.274902	0.0038811	50	0	0.125778	0.00272752	-1.110939312	0	0	0
BrF-UD-14	96.74	99	17_52403880-52409888	8003	34	0	0.280069	0.00359808	38	0	0.127148	0.00273732	-1.019853182	0	0	0
BrF-UD-15	96.69	99	17_88408137-88414142	8000	30	2	2.17938	0.0486827	34	2	2.13942	0.0483303	-0.006955986	2	2	2
BrF-UD-16	96.83	99	17_98599139-98600148	8007	34	0	0.121088	0.00288449	34	0	0.11938	0.0023931	-0.0000782005	0	0	0
BrF-UD-17	96.18	99	17_3147380-3153409	8029	44	0	0.224008	0.0048004	38	0	0.102282	0.00212732	-1.11420028	0	0	0
BrF-UD-18	96.09	99	17_27099175-27100178	8001	4	0	0.0003890	0.00011934	4	0	0.00479453	0.000103972	-0.198930979	0	0	0
BrF-UD-19	96	100	17_3330389-33309943	9994	34	0	0.343193	0.00739748	22	0	0.0275488	0.000397402	-0.8300259918	0	0	0
BrF-UD-20	96.7	99	17_71315973-71327498	5325	18	0	0.0732578	0.00181277	32	0	0.087878	0.00190132	0.237486808	0	0	0
BrF-UD-21	96.51	81	17_11881040-11889285	3235	42	0	0.124778	0.00287393	50	0	0.108343	0.00233337	-0.184014387	0	0	0
BrF-UD-22	96.4	81	17_34063033-34088289	3234	28	0	0.039183	0.0008397	34	0	0.0308328	0.00011803	0.488884004	0	0	0
BrF-UD-23	95.71	81	17_37039879-37045518	3234	10	0	0.029794	0.00055278	18	0	0.123392	0.00287578	2.275221328	0	0	0
BrF-UD-24	96.88	86	17_3319389-3324807	4833	8	0	0.0153282	0.00002843	14	0	0.0212477	0.000480781	0.4881999	0	0	0
BrF-UD-25	93.98	81	17_3319389-3324807	3218	12	0	0.028604	0.00035728	8	0	0.0407441	0.000888344	0.884938437	0	0	0
BrF-UD-26	93.88	81	17_8802495-8807718	3221	4	0	0.0118883	0.000254788	14	0	0.0147480	0.000019777	0.327890422	0	0	0
BrF-UD-27	93.84	81	17_33878023-33883244	3221	4	0	0.0188079	0.000035907	2	0	0.013988	0.000030289	-0.208601134	0	0	0
BrF-UD-28	93.84	81	17_34031277-34038332	3245	8	0	0.00041014	0.000115839	6	0	0.00255717	3.50E-05	-1.084033733	0	0	0
BrF-UD-29	93.44	81	17_33039172-33044408	3238	4	0	0.0148417	0.000318057	4	0	0.00720705	0.000118629	-1.024988021	0	0	0
BrF-UD-30	93.29	81	17_15555983-15580852	3242	0	0	0	0	2	0	0.00815385	0.000133448	0	0	0	0
BrF-UD-31	93.15	81	17_31781297-31788332	3235	2	0	0.00117823	2.32869E-05	0	0	0	0	0	0	0	0
BrF-UD-32	92.94	81	17_28738817-28741844	3227	0	0	0	0	0	0	0	0	0	0	0	0
BrF-UD-33	92.78	81	17_834809-835883	3234	2	0	0.00003778	0.00010798	2	0	0.00815385	0.000133448	0.305780802	0	0	0
BrF-UD-34	92.95	81	17_32840949-32848132	5183	2	0	1	0.02143	1	0	0.333333	0.0072284	-1.387883802	0	0	0
BrF-UD-35	98.28	78	17_98004020-98008069	4589	18	0	0.0002299	0.000847828	8	0	0.00533734	0.000120078	-2.431834518	0	0	0
BrF-UD-36	92.5	81	17_34209782-34214959	3237	2	0	0.0183488	0.0003932	4	0	0.0378337	0.0008183	1.004202347	2	2	2
BrF-UD-37	94.17	83	17_54181124-54188007	4903	4	0	0.00047753	3.30947E-05	6	0	0.147325	0.00319911	3.972988933	0	0	0
BrF-UD-38	92.25	81	17_43792510-43797741	3231	8	2	2.04902	0.0439103	0	0	0	0	0	2	2	2
BrF-UD-39	91.93	81	17_38848953-38851529	3248	8	0	0.0122813	0.000283192	2	0	0.0010598	2.30E-03	-3.17432883	0	0	0
BrF-UD-40	91.84	81	17_31884928-31891173	3247	2	0	0.181818	0.00389838	8	8	8	0.150111	3.081472147	8	8	8
BrF-UD-41	91.84	81	17_22469688-22470188	3220	0	0	0	0	0	0	0	0	0	0	0	0
BrF-UD-42	91.79	81	17_41340801-41348003	3202	0	0	0	0	4	0	0.12381	0.0028848	0	0	0	0
BrF-UD-43	93.77	79	17_98382090-98388471	4381	8	0	0.0497459	0.00108803	20	14	14.0448	0.30458	8.19830798	18	14	14
BrF-UD-44	90.91	86	17_27250183-27255342	5179	2	0	0.00005778	0.00010798	2	0	0.00815385	0.000133448	0.305780802	0	0	0
BrF-UD-45	90.88	86	17_3337288-3342472	5184	2	0	0.00021343	8.89E-03	0	0	0	0	0	0	0	0
BrF-UD-46	90.32	86	17_80781228-80788398	5170	0	0	0	0	0	0	0	0	0	0	0	0
BrF-UD-47	89.83	86	17_34840151-34845341	5190	0	0	0	0	2	0	0.00501803	8.54E-03	0	0	0	0
BrF-UD-48	89.81	86	17_34821812-34828790	5178	0	0	0	0	2	0	0.0222222	0.000481930	0	0	0	0
BrF-UD-49	94.93	89	17_29897110-29901278	4188	8	0	0.0227038	0.000488338	2	0	0.08	0.0017348	1.834181588	0	0	0
BrF-UD-50	94.73	89	17_33914727-33918894	4187	8	0	0.00894735	0.000191742	2	0	0.00212993	4.82E-03	-2.033378404	0	0	0

Fig. 4. L1 _{β -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 _{β -thal} との一致度(%), qcov; L1 _{β -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

trackingId	pid	qcov	locus	length	Ir941_no_mismatch				Ir942_no_mismatch				Ir942_no_mismatch		MAX unique read_count (no_mismatch)	MAX unique read_count (no_mismatch)	For GCV
					read_count	unique_read_count	estimate_read_count	norm_read_count	read_count	unique_read_count	estimate_read_count	norm_read_count	log2(RA) change				
													Ir941_no_mismatch	Ir941_no_mismatch			
IrH_ID: 77823	99.9	99	4:19077834-19083928	8044	186	2	4.17905	0.0694927	156	4	4.74033	0.102759	0.15999432	4	0	0	0
IrH_ID: 77824	99.7	99	4:74717542-74720288	8044	182	0	2.08908	0.0442548	181	0	0.895922	0.0150912	-1.532119894	0	0	0	0
IrH_ID: 77825	99.28	99	4:138293303-138299545	8042	172	0	0.542883	0.0118293	134	0	0.442255	0.0092903	-0.27810287	0	0	0	0
IrH_ID: 77826	99.24	99	4:211159394-211164430	8038	148	0	0.458302	0.0097823	124	0	0.449125	0.0097330	-0.006420419	0	0	0	0
IrH_ID: 77827	99.17	99	4:50875773-50881254	8041	152	0	0.847428	0.0181804	118	0	0.337873	0.0073289	-1.308300994	0	0	0	0
IrH_ID: 77828	99.11	100	4:79937715-79943782	8047	158	0	0.803974	0.0106389	132	0	0.522777	0.0113385	0.084736845	0	0	0	0
IrH_ID: 77829	99.12	100	4:87347109-87353147	8048	190	0	0.470935	0.0100921	128	0	0.399671	0.0088899	-0.21983012	0	0	0	0
IrH_ID: 77830	99.07	99	4:76347963-76354029	8048	192	0	1.37803	0.0293318	184	0	0.478419	0.0103312	-1.515231883	0	0	0	0
IrH_ID: 77831	99.05	99	4:81939923-81945981	8038	180	0	0.523088	0.0112697	110	0	0.320353	0.0069312	-0.869401298	0	0	0	0
IrH_ID: 77832	99.11	99	4:13841530-13847381	8031	184	0	0.878992	0.0188388	134	0	0.412333	0.0084419	-1.074830603	0	0	0	0
IrH_ID: 77833	99.05	99	4:7996910-79972939	8029	188	0	0.543234	0.0114308	128	0	0.424296	0.0092074	-0.314549833	0	0	0	0
IrH_ID: 77834	99.07	99	4:189315301-189321329	8028	184	0	0.497923	0.0108703	146	0	0.400372	0.0088821	-0.297303327	0	0	0	0
IrH_ID: 77835	99.02	99	4:50838302-50844360	8033	148	0	0.530187	0.0113819	100	0	0.388913	0.0084338	-0.429973344	0	0	0	0
IrH_ID: 77836	99.02	99	4:198382978-198389111	8033	134	0	0.384457	0.0078103	102	0	0.310228	0.0087273	-0.215347927	0	0	0	0
IrH_ID: 77837	99.07	99	4:188753900-188759222	8022	158	0	0.578122	0.0123891	128	0	0.392924	0.0083208	-0.540037884	0	0	0	0
IrH_ID: 77838	98.98	99	4:78105735-78111793	8039	142	0	0.557115	0.011939	108	0	0.321428	0.0069701	-0.778412110	0	0	0	0
IrH_ID: 77839	98.98	99	4:91378215-91384273	8038	122	0	0.491812	0.0103330	90	0	0.320701	0.0065444	-0.39979339	0	0	0	0
IrH_ID: 77840	98.98	99	4:119948720-119954757	8037	172	0	0.526492	0.0112399	108	0	0.813370	0.0178423	0.85042381	0	0	0	0
IrH_ID: 77841	98.98	99	4:59078848-59084893	8035	130	0	0.392939	0.0084208	94	0	0.351833	0.0078232	-0.143133999	0	0	0	0
IrH_ID: 77842	98.91	99	4:79704533-79710597	8044	120	0	0.842892	0.0193832	100	0	0.287487	0.0062342	-1.534772523	0	0	0	0
IrH_ID: 77843	98.92	99	4:111894803-111900838	8033	122	0	0.470741	0.010069	98	0	0.418971	0.0096421	-0.157910403	0	0	0	0
IrH_ID: 77844	98.91	99	4:189137088-189143119	8033	120	0	0.496329	0.0108383	98	0	0.314974	0.0088302	-0.838979748	0	0	0	0
IrH_ID: 77845	98.84	100	4:82818458-82824513	8047	122	0	0.475823	0.0101989	114	0	0.770823	0.0187153	0.713035891	0	0	0	0
IrH_ID: 77846	98.87	99	4:58392422-58398480	8038	108	0	0.294401	0.0083090	80	0	0.183894	0.0040289	-0.847778233	1	0	0	0
IrH_ID: 77847	98.87	99	4:14353118-14359145	8033	118	0	0.444093	0.0095189	88	0	0.251488	0.0054329	-0.803433833	0	0	0	0
IrH_ID: 77848	98.79	99	4:23814773-23820820	8047	120	0	0.529781	0.0113328	78	0	0.848283	0.0140882	0.308383724	0	0	0	0
IrH_ID: 77849	98.77	99	4:53819153-53825191	8038	120	0	0.38003	0.0081440	112	0	0.348339	0.0075338	-0.108542081	0	0	0	0
IrH_ID: 77850	98.73	99	4:27317875-27323922	8044	118	0	0.351908	0.0075413	104	0	0.445587	0.0098928	0.33579801	0	0	0	0
IrH_ID: 77851	98.69	100	4:143100258-143106307	8048	128	0	1.3841	0.0298812	100	0	0.331242	0.0071830	-2.043968303	0	0	0	0
IrH_ID: 77852	98.69	99	4:53238473-53244511	8041	188	0	0.879008	0.0145578	122	0	0.327418	0.0071000	-1.033883344	0	0	0	0
IrH_ID: 77853	98.69	99	4:57923029-57929076	8037	94	0	0.255702	0.0047598	88	0	0.153772	0.0033343	-0.718389217	0	0	0	0
IrH_ID: 77854	98.81	99	4:108571027-108577071	8044	42	0	0.143383	0.0030723	44	0	0.145353	0.0031283	0.038933398	0	0	0	0
IrH_ID: 77855	98.69	99	4:48031935-48037983	8028	144	0	0.803334	0.0129788	120	0	0.384821	0.0083412	-0.837378838	0	0	0	0
IrH_ID: 77856	98.48	99	4:128213793-12827437	8030	128	4	5.02184	0.107814	134	0	1.88883	0.0383749	1.538939932	4	0	0	0
IrH_ID: 77857	98.39	99	4:82728935-82734984	8029	94	0	0.277777	0.0059237	92	0	0.381144	0.0062851	0.473489233	0	0	0	0
IrH_ID: 77858	98.31	99	4:59838297-59844353	8038	88	0	0.538123	0.0114891	94	0	0.203013	0.0044023	-1.383910204	0	0	0	0
IrH_ID: 77859	98.24	99	4:100000325-100006333	8028	42	0	0.188384	0.0038090	30	0	0.12938	0.0028932	-0.3831117	0	0	0	0
IrH_ID: 77860	98.18	99	4:148330022-148336032	8030	38	0	0.120353	0.0028343	24	0	0.0778915	0.0018890	-0.813032781	0	0	0	0
IrH_ID: 77861	98.02	99	4:102204931-102210943	8024	28	0	0.801833	0.017179	70	0	0.192045	0.0041845	-2.044417004	0	0	0	0
IrH_ID: 77862	97.98	99	4:128928258-128934291	8032	54	0	0.138318	0.0029841	48	0	0.121753	0.0028398	-0.187182501	0	0	0	0
IrH_ID: 77863	97.79	99	4:84839122-84845185	8048	98	0	0.157377	0.0033789	92	0	1.43977	0.0312217	3.208705047	0	0	0	0
IrH_ID: 77864	97.77	99	4:132844410-132850449	8039	88	0	0.234421	0.0050238	82	0	0.111701	0.0024229	-1.032379334	0	0	0	0
IrH_ID: 77865	97.74	99	4:181408883-181414912	8027	82	0	0.188843	0.0038140	38	0	0.0833988	0.0018381	-0.98128787	0	0	0	0
IrH_ID: 77866	97.88	99	4:184333484-184339520	8038	88	0	0.178915	0.0037912	72	0	0.129911	0.0028171	-0.428452432	0	0	0	0
IrH_ID: 77867	98.04	99	4:127020371-127026398	2935	28	0	0.143322	0.0030173	42	0	0.0973343	0.0021707	-0.341183341	2	0	0	0
IrH_ID: 77868	97.8	99	4:119274083-119280124	8039	38	0	0.234411	0.0030234	34	0	0.198308	0.0034300	-0.234232109	0	0	0	0
IrH_ID: 77869	97.88	99	4:87177043-8718308	8015	44	0	0.133913	0.0028897	34	0	0.149019	0.0032380	0.17418238	0	0	0	0
IrH_ID: 77870	97.47	99	4:184330731-184336793	8042	44	0	0.224894	0.0048194	48	0	0.738277	0.0139883	1.728080824	0	0	0	0
IrH_ID: 77871	97.78	99	4:143389388-143395489	3980	54	0	0.188802	0.0038174	60	0	0.189382	0.0041108	0.184413478	0	0	0	0
IrH_ID: 77872	97.51	99	4:138084231-138090283	8032	32	0	0.300824	0.0084488	82	2	2.09532	0.0454418	2.817398181	2	0	0	0

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; 該当領域にアライメントされている全リード数, unique_read_count; 該当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

trackingId	pid	qcov	locus	length	Ir941_no_mismatch			Ir942_no_mismatch			Ir941_no_mismatch read_count	Ir942_no_mismatch read_count	Ir941_no_mismatch norm_read_count	Ir942_no_mismatch norm_read_count	For IGV
					read_count	unique_read_count	estimate_read_count	read_count	unique_read_count	estimate_read_count					
Ir941:15487 2	99.92	99	R:72875559- 72881368	8049	178	0	1.1423	0.0244795	108	0	0.383282	0.0078778	-1.853700239	0	0 Link
Ir941:15487 3	99.99	99	R:128452965- 128459018	8053	180	0	0.493128	0.0100877	118	0	0.390029	0.0084578	-0.321298531	0	0 Link
Ir941:15487 4	99.5	99	R:134070720- 134078773	8053	154	0	0.428983	0.0091927	114	0	0.333201	0.0072103	-0.350422507	0	0 Link
Ir941:15487 5	99.8	99	R:125082887- 125088859	8002	178	0	0.517095	0.0110813	150	0	0.393813	0.0083553	-0.378888047	0	0 Link
Ir941:15487 6	99.21	99	R:27113819- 27119853	8054	144	0	0.412833	0.0088479	132	0	0.310407	0.0087312	-0.394317857	0	0 Link
Ir941:15487 7	99.16	99	R:91522080- 91528121	8041	120	0	0.33052	0.0075073	98	0	0.410294	0.0088973	-0.345085475	0	0 Link
Ir941:15487 8	99.02	99	R:138438075- 138444102	8027	92	0	0.342895	0.0073482	88	0	0.312183	0.0067897	-0.118298189	0	0 Link
Ir941:15487 9	98.98	99	R:88914860- 88920745	8055	138	0	0.978244	0.0209838	104	0	0.329842	0.0071528	-1.551342429	0	0 Link
Ir941:15488 0	98.94	99	R:88885709- 88891780	8051	128	0	0.333205	0.0071834	92	0	0.328977	0.0070894	-0.192089535	0	0 Link
Ir941:15488 1	98.78	100	R:40452213- 40458221	8058	130	0	0.880199	0.0184341	98	0	0.280187	0.0084422	-1.708044714	0	0 Link
Ir941:15488 2	98.81	99	R:133875883- 133881907	8044	108	0	0.359507	0.0077042	104	0	0.328484	0.0071228	-1.113204287	0	0 Link
Ir941:15488 3	98.78	99	R:83040813- 830411885	8052	188	0	0.992728	0.0212742	118	0	0.38411	0.0083293	-1.352801348	0	0 Link
Ir941:15488 4	98.79	99	R:5475962- 5476508	8044	198	0	0.409883	0.0087837	138	0	0.398322	0.0127142	0.533582878	0	0 Link
Ir941:15488 5	98.78	99	R:91508889- 91514708	8057	124	0	0.30823	0.0080053	102	0	0.229987	0.0049888	-0.400501889	0	0 Link
Ir941:15488 6	98.7	99	R:104739813- 104745874	8043	88	0	0.388298	0.0083211	52	0	0.155539	0.0033883	-1.304881813	0	0 Link
Ir941:15488 7	98.71	99	R:83030223- 830308285	8042	92	0	0.482517	0.0099117	82	0	0.298784	0.0084787	-0.813419170	0	0 Link
Ir941:15488 8	98.85	99	R:79821304- 79827355	8051	128	0	0.459781	0.0098351	112	0	0.49748	0.0107875	0.170375855	0	0 Link
Ir941:15488 9	98.18	99	R:97290803- 97301854	8051	98	0	0.300473	0.0084339	74	0	0.193231	0.0042338	-0.804961795	4	0 Link
Ir941:15489 0	98	99	R:8814848- 88154893	8048	88	0	0.398578	0.0084888	52	0	0.0878895	0.0019011	-2.180371409	0	0 Link
Ir941:15489 1	97.9	99	R:83797385- 83805437	8052	54	0	0.428053	0.0091303	38	0	0.144397	0.0031312	-1.545917845	0	0 Link
Ir941:15489 2	97.95	99	R:133259102- 133265142	8040	82	0	0.292912	0.0062771	80	0	0.211503	0.0045884	-0.452707898	0	0 Link
Ir941:15489 3	97.98	99	R:129132123- 129138150	8025	80	0	0.979851	0.0209148	78	0	0.147178	0.0031915	-2.71218317	0	0 Link
Ir941:15489 4	97.77	99	R:71408383- 71414415	8052	92	0	0.349183	0.0074830	78	0	0.312211	0.0067703	-0.144390011	0	0 Link
Ir941:15489 5	97.89	99	R:123839985- 123846007	8022	82	0	0.273127	0.0083831	53	0	0.0909887	0.0019728	-1.869630312	1	0 Link
Ir941:15489 6	98.02	99	R:1807578- 1813570	3992	82	0	0.231011	0.0049208	58	0	0.370842	0.0080417	0.899924933	0	0 Link
Ir941:15489 7	97.87	100	R:98814448- 98820504	8058	84	0	0.738869	0.0157853	74	0	0.240303	0.0032110	-1.588982281	0	0 Link
Ir941:15489 8	97.77	99	R:98388373- 98394858	8055	40	0	0.487232	0.0104414	38	0	0.200008	0.0049883	-1.083871889	0	0 Link
Ir941:15489 9	97.7	99	R:72147433- 72153484	8051	48	0	0.269013	0.0044838	40	0	0.849912	0.0146933	1.851808225	0	0 Link
Ir941:15490 0	97.88	99	R:35328040- 35334071	8051	78	0	0.358752	0.0078451	78	0	0.483243	0.010494	0.452810183	0	0 Link
Ir941:15490 1	97.59	99	R:87321783- 87327829	8044	34	0	0.180138	0.0039803	28	0	0.045298	0.0009822	-1.974483509	0	0 Link
Ir941:15490 2	97.34	100	R:35730318- 35736354	8038	48	0	0.524432	0.0112388	52	0	0.248903	0.0035344	-1.089735381	2	0 Link
Ir941:15490 3	97.5	99	R:98280278- 98286310	8054	70	2	2.17187	0.0485432	48	0	0.184588	0.0033890	-3.70404828	2	2 Link
Ir941:15490 4	97.51	99	R:84415044- 84421075	8029	24	0	0.184374	0.0035223	44	0	0.39304	0.0129101	1.873818277	0	0 Link
Ir941:15490 5	97.45	99	R:128891489- 128897519	8050	42	0	2.80555	0.0598389	34	0	0.208049	0.0044882	-3.843448723	0	0 Link
Ir941:15490 6	97.32	99	R:74942831- 74948878	8045	42	0	0.181357	0.0034578	22	0	0.0388288	0.0008419	-2.038032209	0	0 Link
Ir941:15490 7	97.45	99	R:119120385- 119182410	8015	84	0	0.197371	0.0042288	54	0	0.18275	0.0039861	-0.09390851	0	0 Link
Ir941:15490 8	97.28	99	R:15578281- 15584333	8042	38	0	0.157388	0.0033723	28	0	0.15213	0.0032989	-0.031753728	0	0 Link
Ir941:15490 9	97.29	99	R:88946957- 88952919	8022	42	0	0.308393	0.0088688	44	0	0.183042	0.0035348	-0.90244837	0	0 Link
Ir941:15491 0	97.53	99	R:131770920- 131776924	3974	48	0	0.172547	0.0037082	38	0	0.107727	0.0023380	-0.883870519	0	0 Link
Ir941:15491 1	97.11	99	R:18943989- 18950025	8038	38	0	0.177577	0.0038054	38	0	0.483121	0.0104788	1.481024033	0	0 Link
Ir941:15491 2	97.28	99	R:73479441- 73485485	8022	24	0	0.31902	0.0087508	28	0	0.144188	0.0031283	-1.110814734	0	0 Link
Ir941:15491 3	97.14	99	R:35171429- 35177508	8049	44	0	0.132431	0.0028379	48	0	0.130849	0.0028531	-0.002482519	0	0 Link
Ir941:15491 4	97.25	99	R:83447145- 83453189	8034	54	0	0.108827	0.0022893	58	0	0.133108	0.0033201	0.538837544	0	0 Link
Ir941:15491 5	97.21	99	R:120844182- 120850187	8025	52	0	0.283855	0.0089229	88	0	0.198394	0.0042851	-0.417254081	0	0 Link
Ir941:15491 6	97.18	99	R:128759818- 128800844	8024	44	0	0.381939	0.0120423	40	0	0.0209224	0.0011042	-3.448933032	0	0 Link
Ir941:15491 7	97.17	99	R:128759818- 128800844	8029	40	0	0.121510	0.0028045	38	0	0.18979	0.0008819	0.459893389	0	0 Link
Ir941:15491 8	97.09	99	R:48793901- 48799950	8029	20	0	0.0287119	0.0008152	42	0	0.0957738	0.0020788	1.750088398	0	0 Link
Ir941:15491 9	98.99	99	R:85254220- 85260271	8051	38	0	0.242851	0.0032000	38	0	0.217278	0.0047118	-0.142272838	0	0 Link
Ir941:15492 0	97.07	99	R:84484152- 84490184	8052	48	0	0.832989	0.0135849	58	0	0.184815	0.0040077	-1.75901399	0	0 Link
Ir941:15492 1	97.12	99	R:74888322- 74894345	8023	38	0	0.0727181	0.0015883	88	0	0.0921005	0.0019972	0.357973982	0	0 Link

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

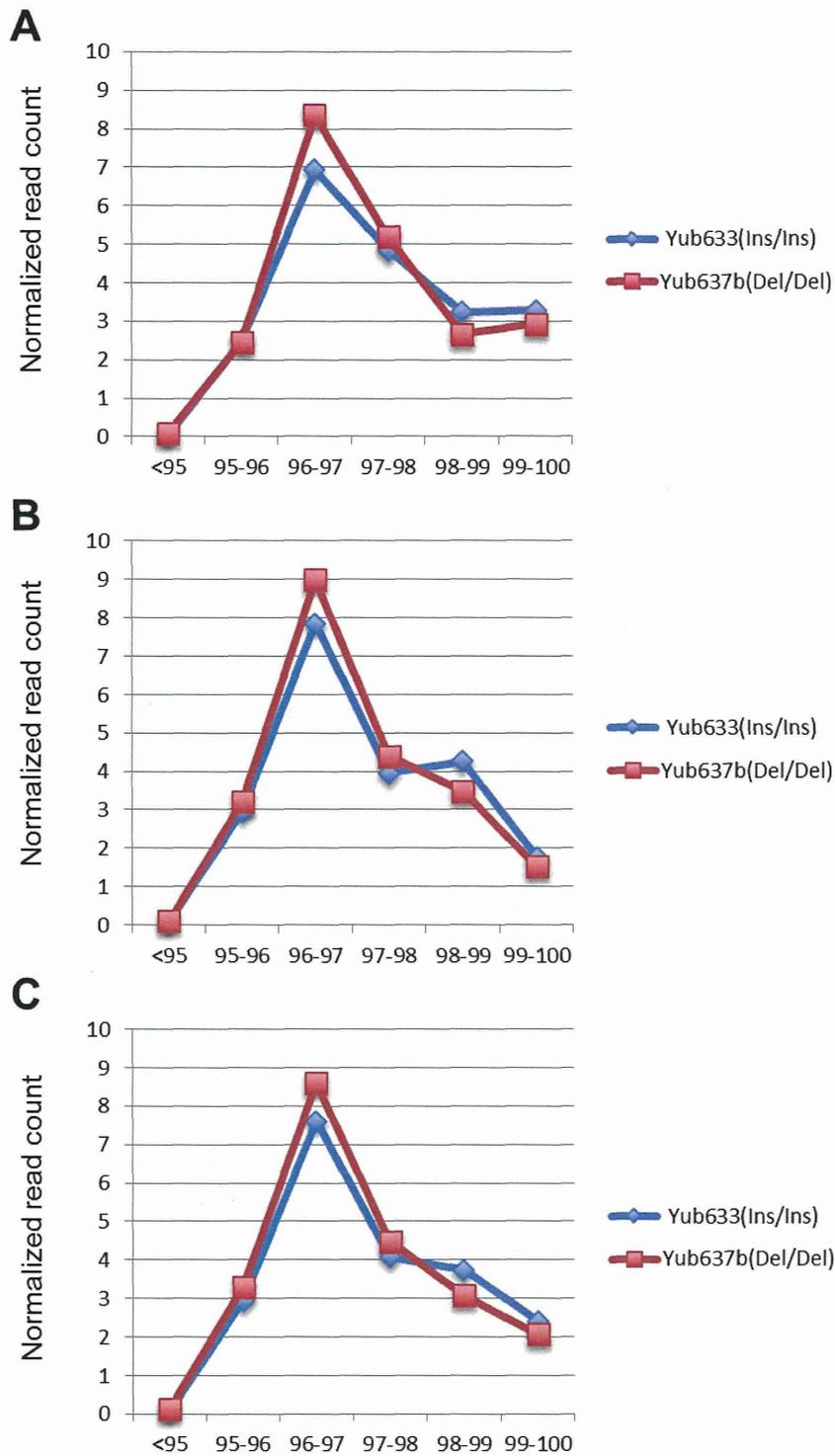


Fig. 7. LINE-1 配列との各一致度における normalized read count 数
 (A) $L1_{\beta\text{-thal}}$ (B) $L1_{RP}$ (C) $L1.3$ 全長に占める割合が 99%以上の LINE-1s 様配列を抽出し、各塩基配列の一致度におけるリード数を合計した。

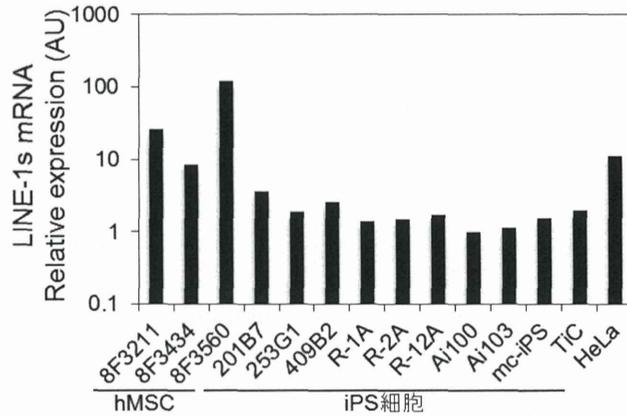


Fig. 8. hMSCs 及び iPS 細胞における LINE-1s mRNA の発現解析
Ai103 の発現量を 1 とする.

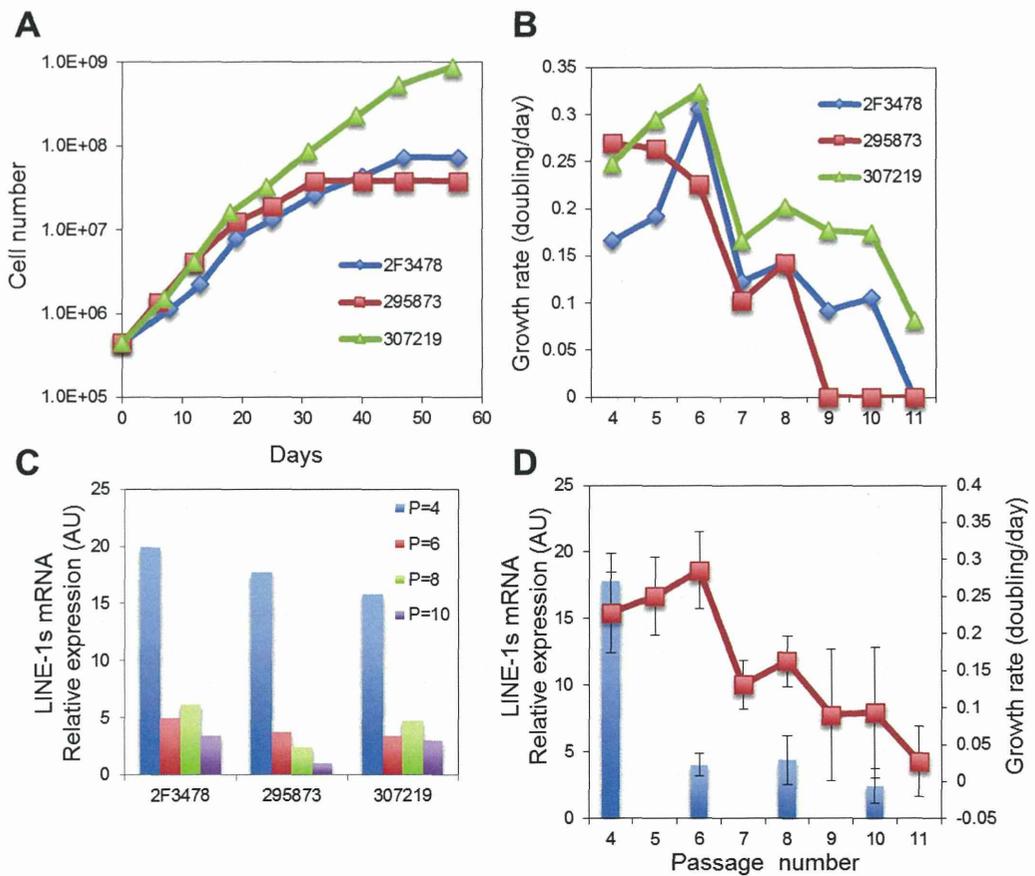


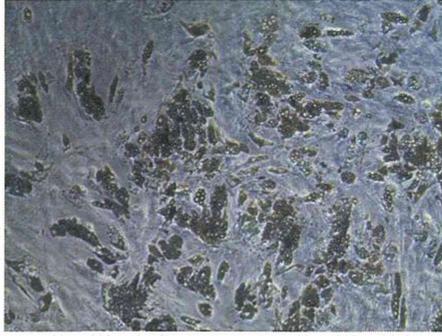
Fig. 9. hMSCs の増殖及び LINE-1s の発現解析
(A) hMSCs 3 ロットの増殖曲線. (B) 各継代間での増殖速度. (C) 各継代数における LINE-1s の発現比較 (295873 P=10 の発現量を 1 とする). (D) (B)及び(C)の平均値

A

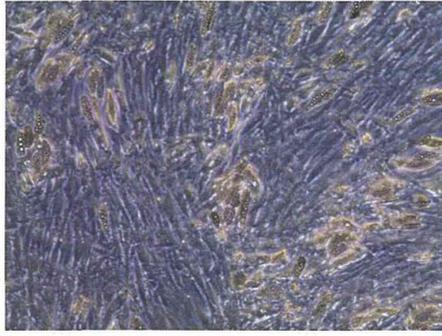
脂肪分化培地

增殖培地

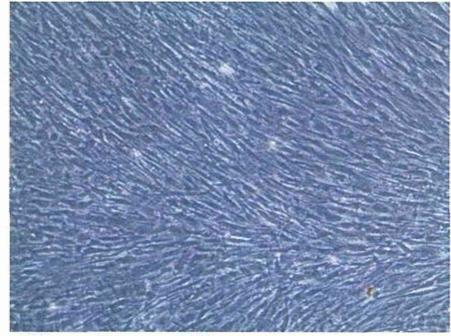
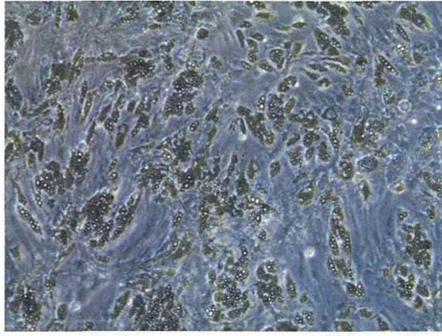
2F3478



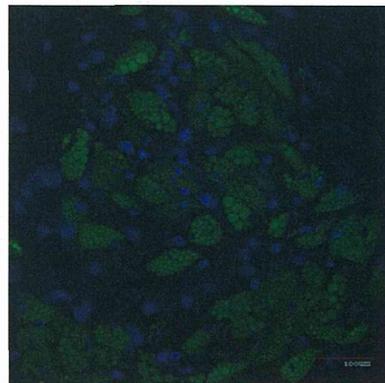
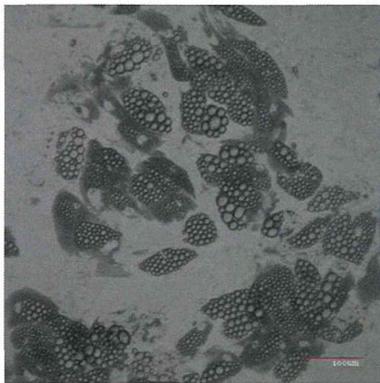
295873



307219



B



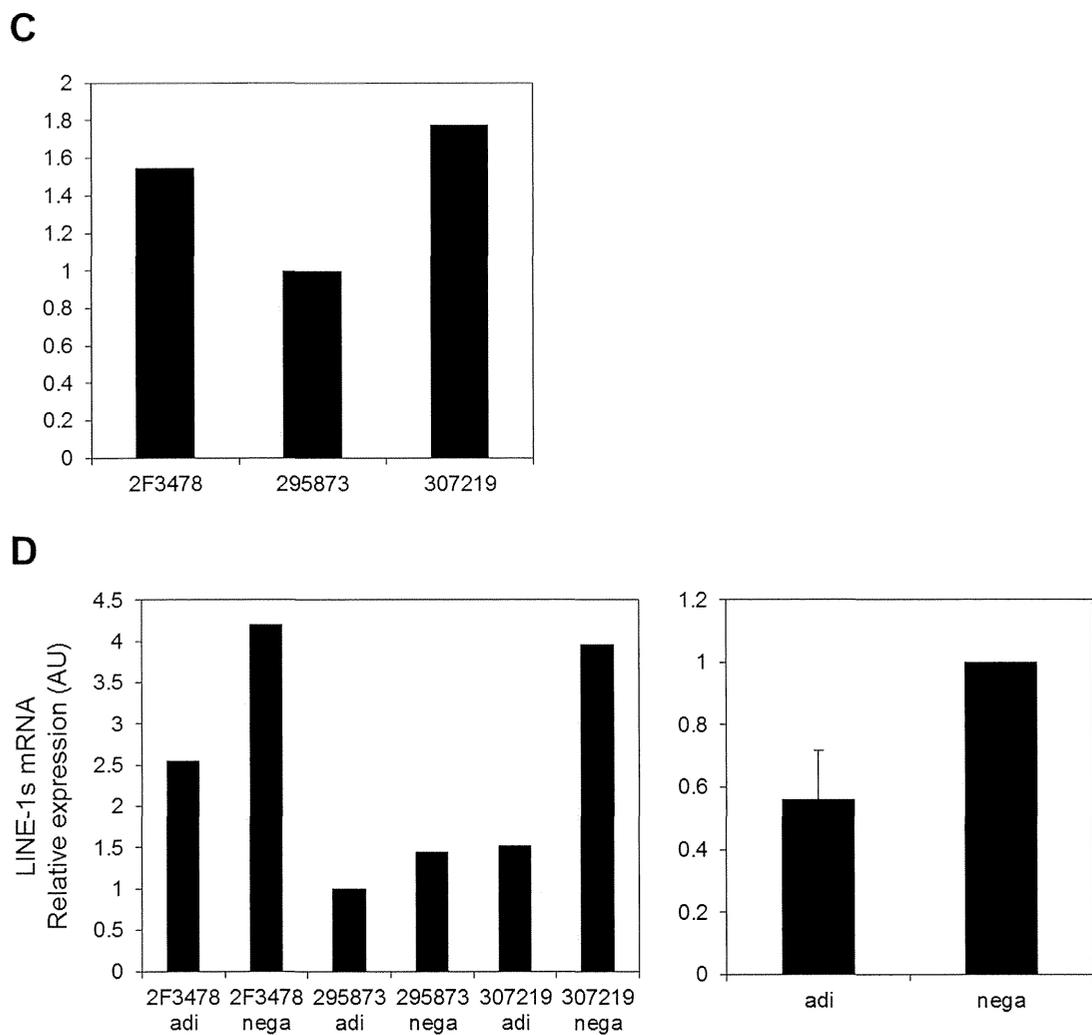


Fig. 10. hMSCs の脂肪分化と LINE-1s の発現解析

(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	CCTCAGGGT CTC	CTGTTCTGA	None
4b-5a	128772037	130000919	CTTCCT CCA	GAGAAGCCTG	None
5b-7a	130215269	130367023	ACACACTT GT	AGAGGGTGGG	None
7b-8	130698147	136580808	CATT CAACAC	TCTTA ACTCC (r)	None
8-1a	136580616	126224548	ATG AATTT CG (r)	GAGAC GTCTC	None
1b-3b	126547448	128344474	CACCT A ATTA	AAGGCAG CAG (r)	ATA ACTTG
3a-2a	128068264	126710881	ATGTG CCCCT (r)	GGAG GCTCTG	AAA CATA
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	+	49282334	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.7:2201:17991:62843	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. ($\times 10^{-6}$)			%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

Protein Candidates after removing peptides with score < 10								
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

Fig. 11 HL60-RG 細胞 c-myc 増幅領域の概略

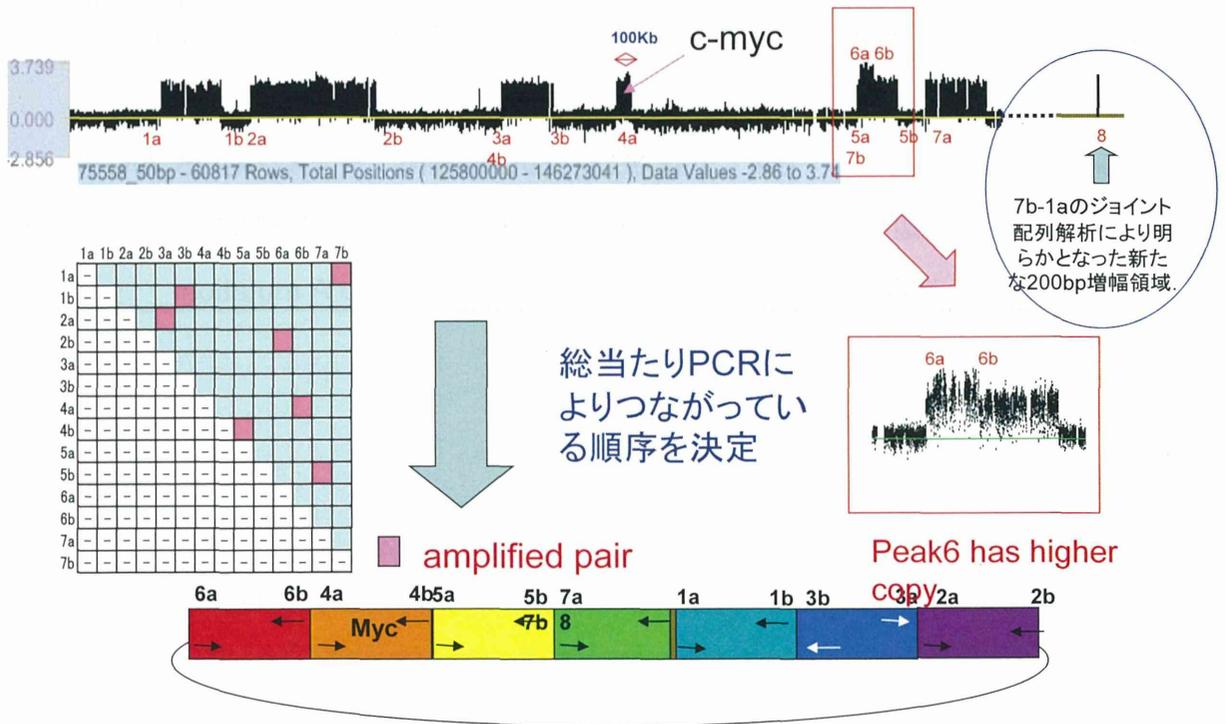


Fig. 12 HL60-RG 細胞 c-myc 増幅領域の融合点を含む配列の検出

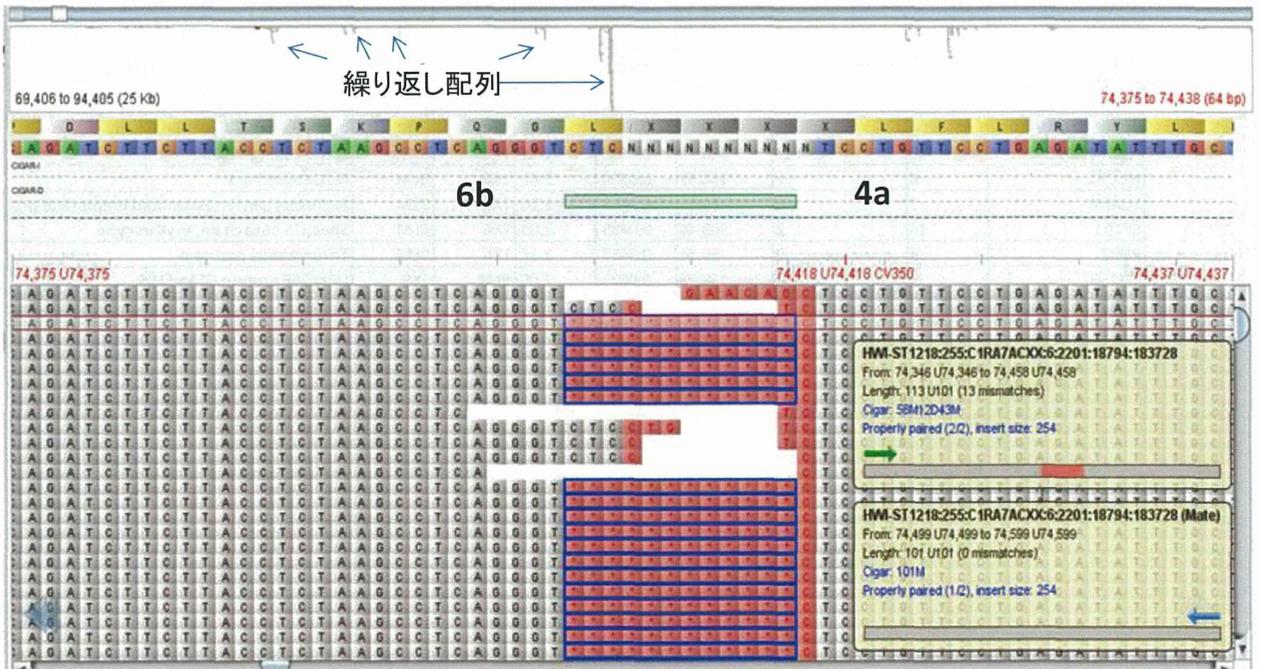


Fig. 13 TK6 細胞の染色体解析

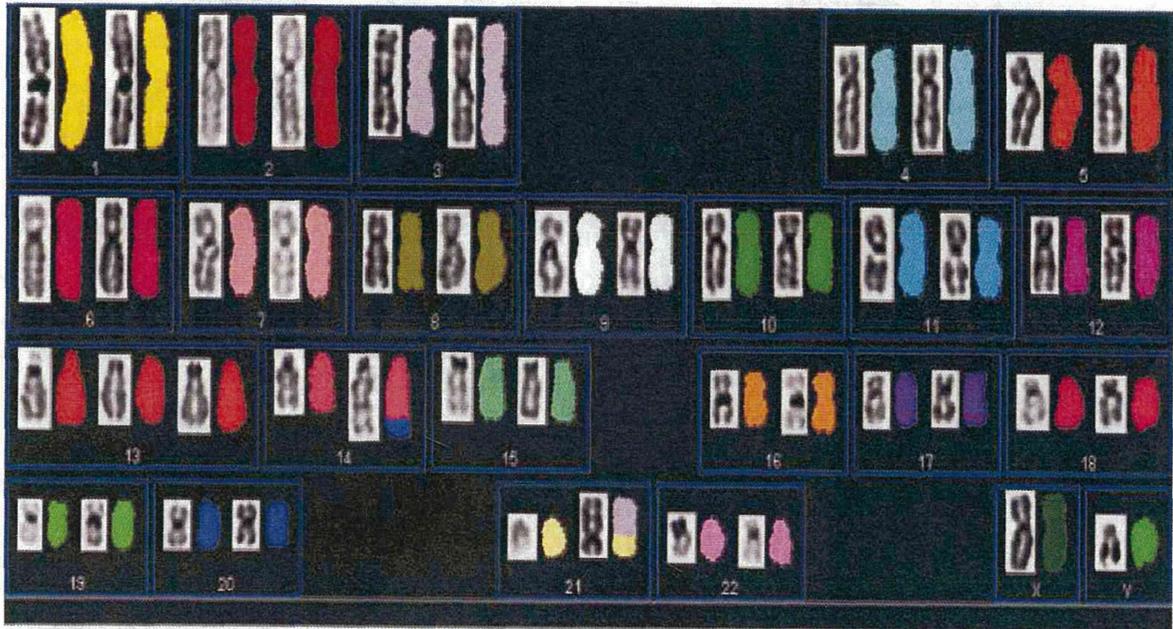
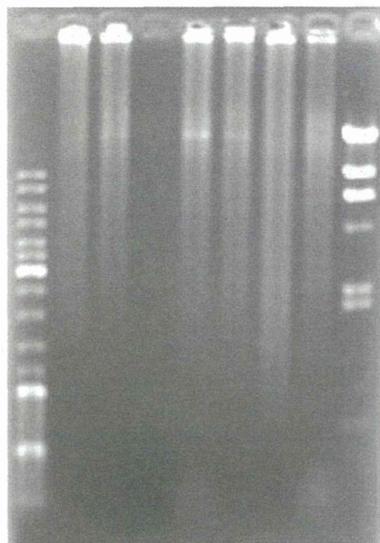
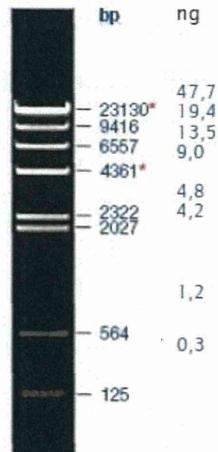


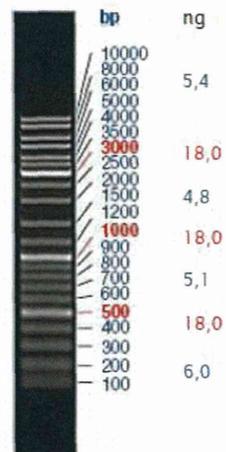
Fig. 14 抽出した mtDNA の品質チェック



Lambda DNA/HindIII Marker
5 μ l; 20ng / μ l



GeneRuler DNA Ladder Mix
5 μ l; 20ng / μ l



lane	sample name	μ l
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/ μ l]	Total volume[μ l]
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

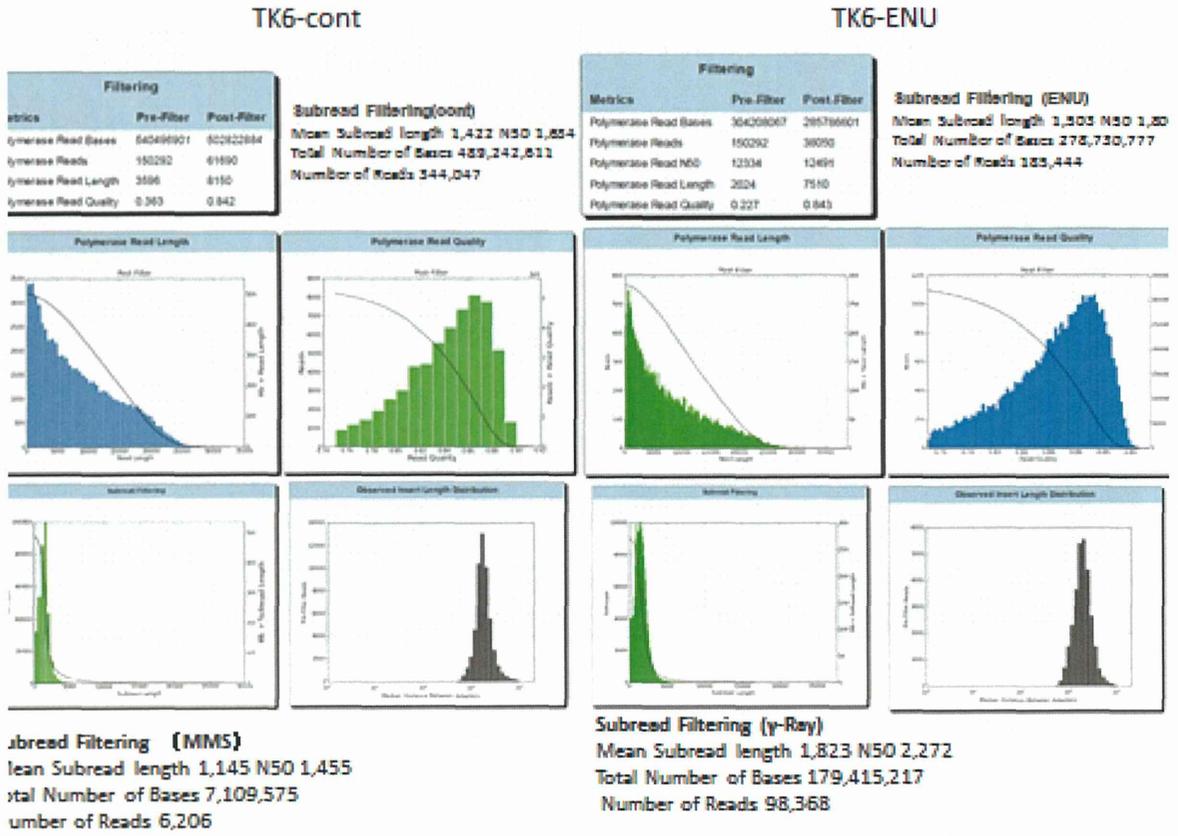


Fig. 16 Tablet 上での PacBio シークエンサーデータの確認

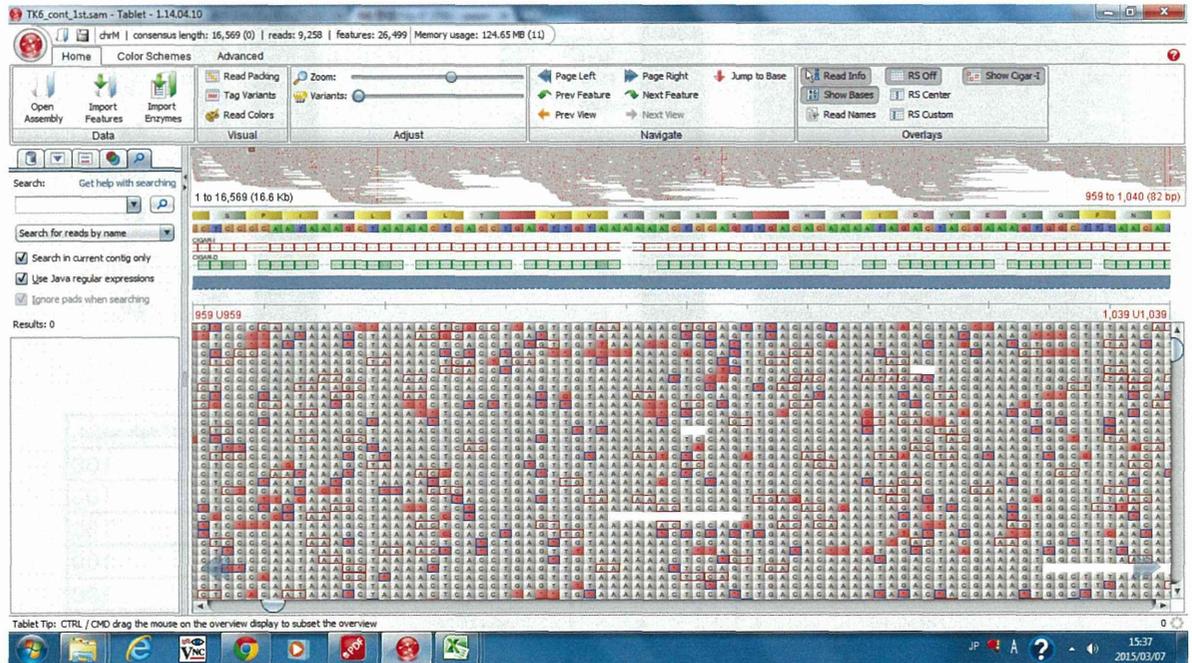


Fig. 17 ProteoMap Online ソフトウェアによるプロテオームデータ公開

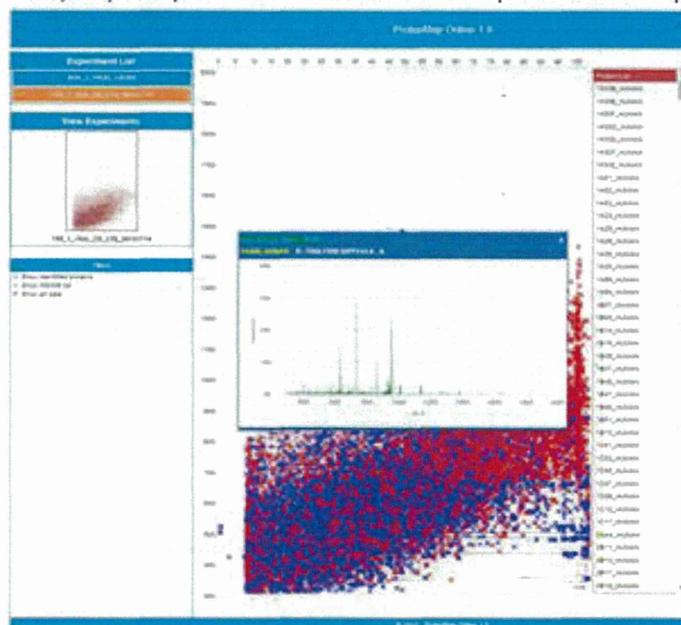
1) ProteoMap Online ソフトウェア概略

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.

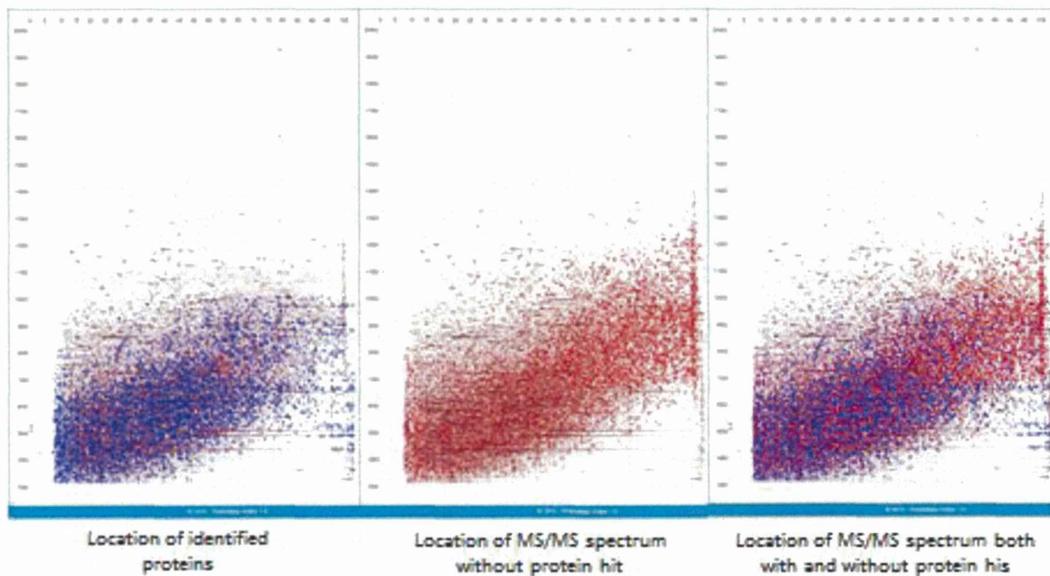
2) ProteoMap Online 操作画面

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.



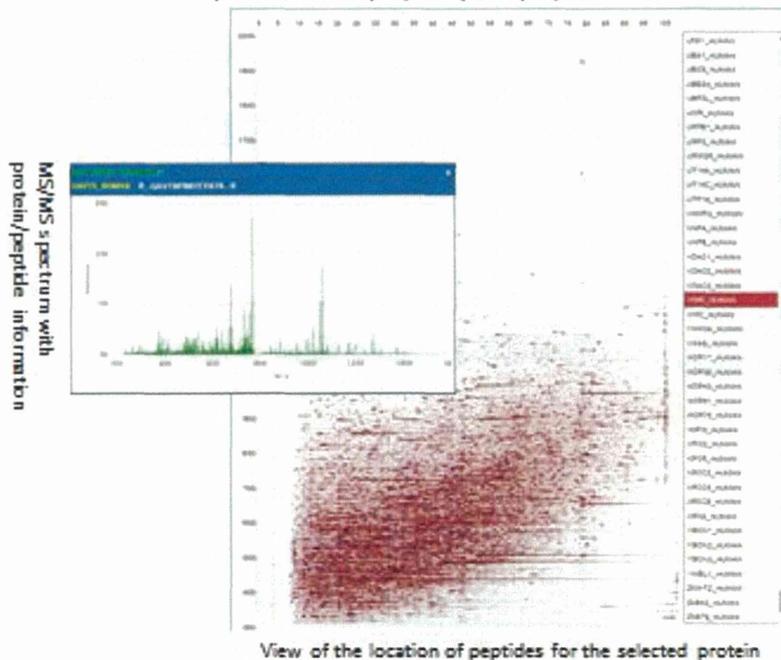
2. User can upload the 2D image of LC-MS, MS/MS spectrum as a .mgf file format and list of identified proteins as .XML file format (Desktop version of ProteoMap required).
3. ProteoMap Online will display the MS/MS spectrum and identified proteins on the 2D image.

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



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.

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	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	GQC/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	G	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	CGC/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.