

図 1-16 iPS 細胞-SNL78/7 細胞, SNL76/7 細胞への FCV の感染：細胞内のウイルス RNA の產生（1）

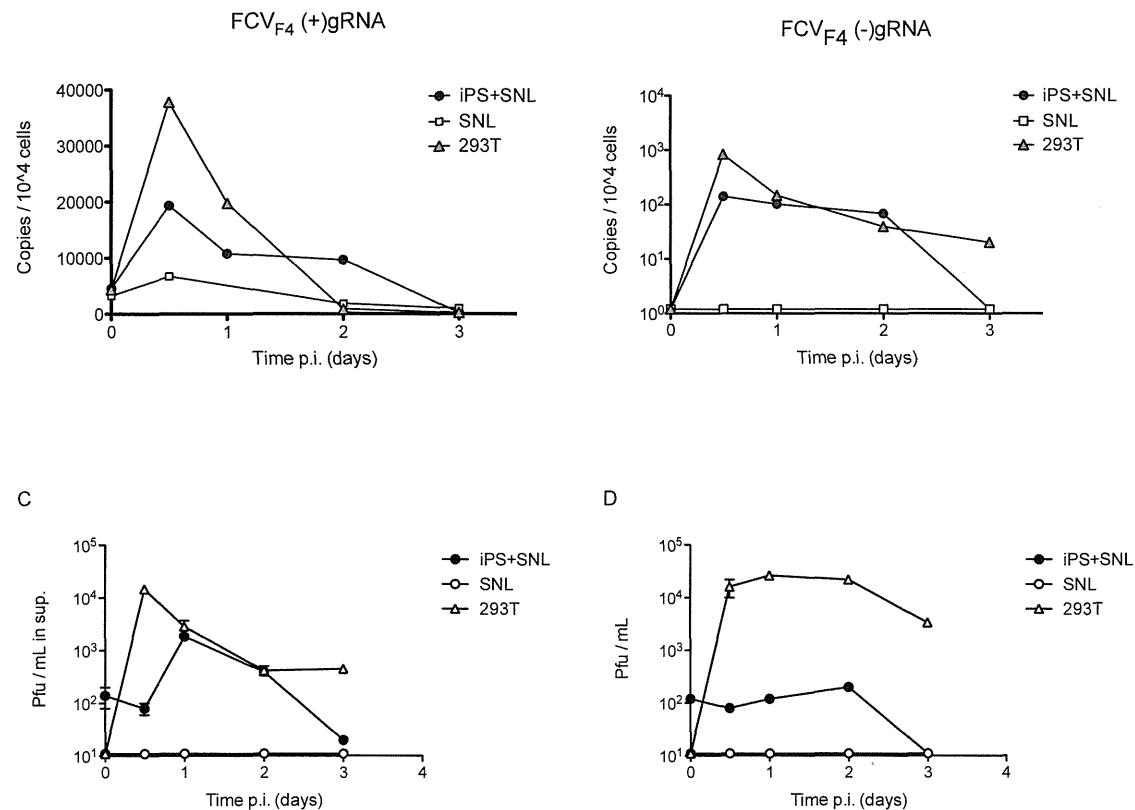


図 1-17 iPS 細胞-SNL78/7 細胞, SNL76/7 細胞への FCV の感染：細胞内のウイルス RNA の產生（2）

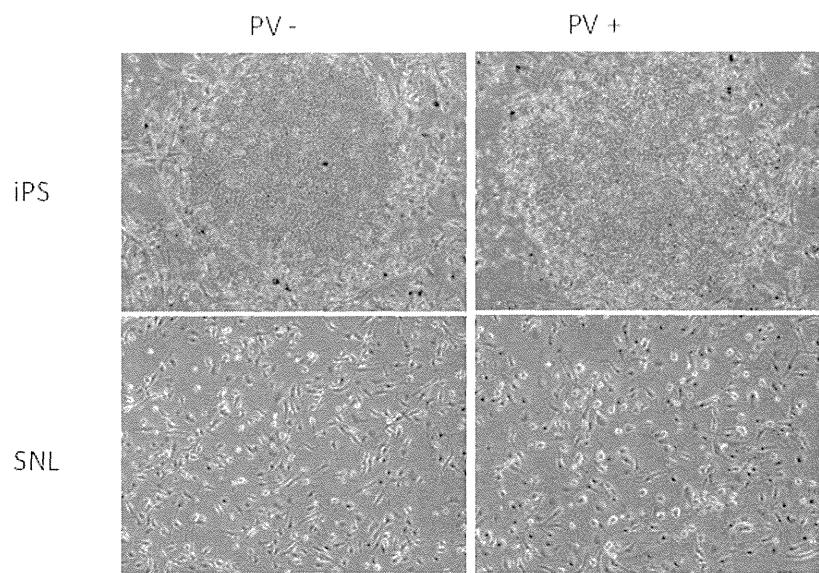


図 1-18 iPS 細胞-SNL78/7 細胞, SNL76/7 細胞へのポリオウイルスの感染

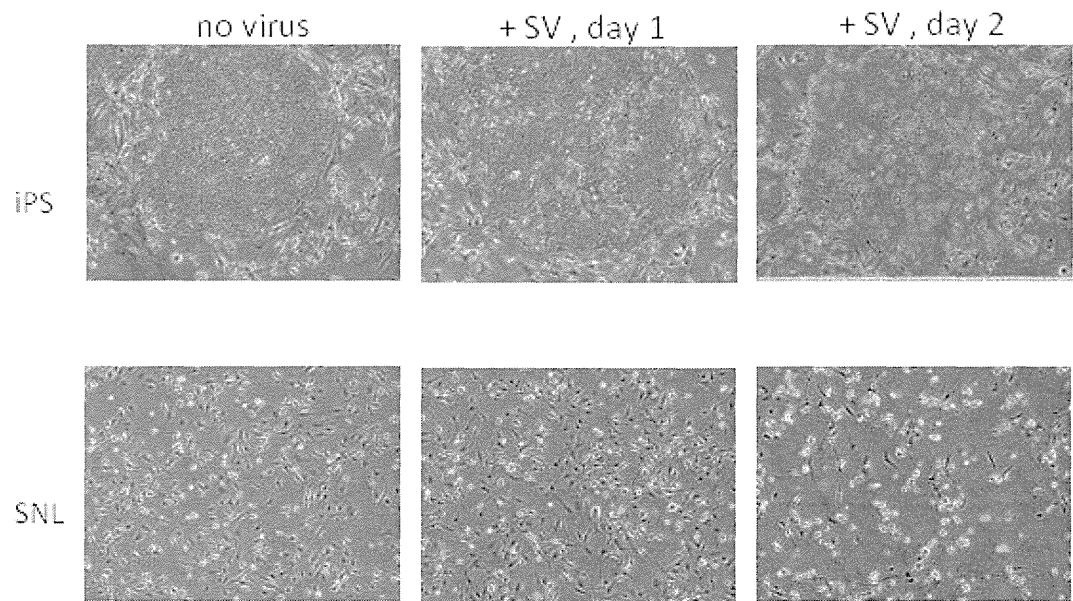


図 1-19 iPS 細胞-SNL78/7 細胞, SNL76/7 細胞へのシンドビスウイルスの感染

**細胞**  
(細胞ストック, フィーダー細胞, 中間製品, 最終製品等)

▼  
**RNA 5 µg (2~5×10<sup>5</sup> cells)**

▼  
**NGSによる解析**

▼  
**RNA-seq data 解析**

▼  
**virus seqs?**

**Yes or No**

図 1-20 次世代シークエンサー(NGS)を使ったウイルス検出法

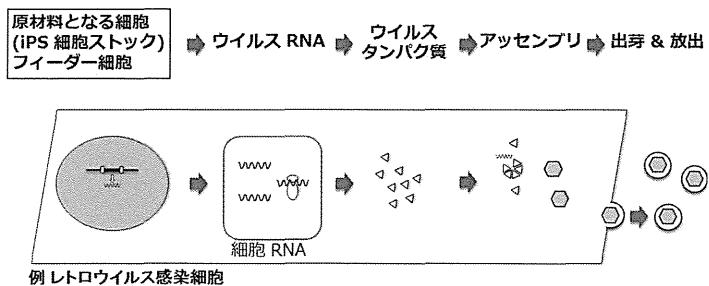


図 1-21 次世代シークエンサー(NGS)を使ったウイルス検出法

Samples	No. of reads	length/read (nt)	Bases (%>=30)
HEK293	23M	194	2.310G (88.47)
HEK293 + FCV 12 h	33M	195	3.316G (88.72)
HEK293 + FCV 24 h	31M	194	30.96G (88.35)

表 1-2 HEK293 細胞, FCV 感染 HEK293 細胞 RNA-seq データ  
(Illumina HiSeq 2500 使用)

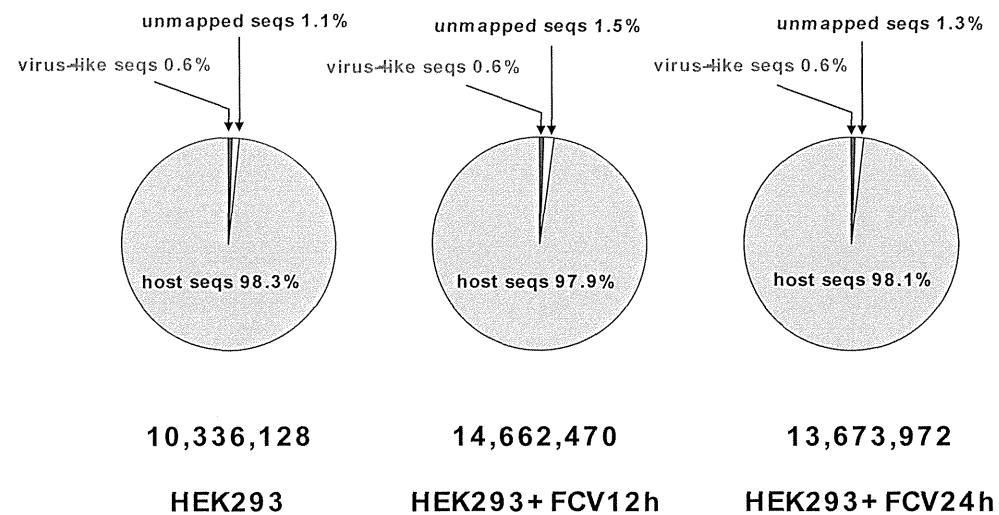


図 1-22 HEK293 細胞, FCV 感染 HEK293 細胞の RNA-seq データに含まれるウイルス様シークエンス

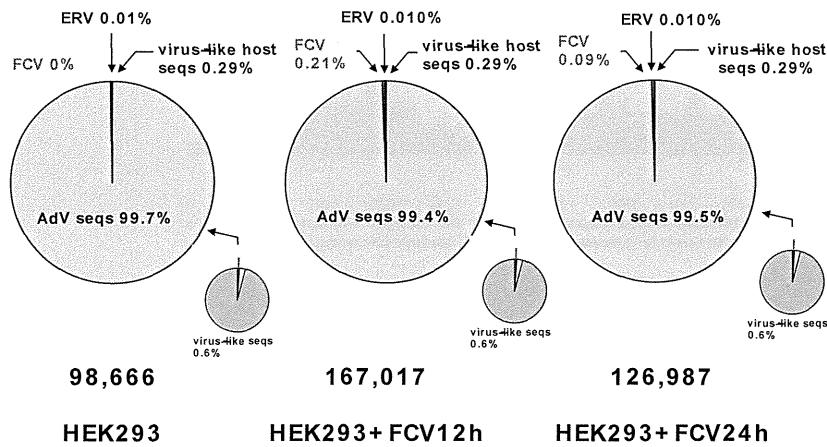


図 1-23 HEK293 細胞, FCV 感染 HEK293 細胞に含まれるウイルス様シークエンスの内訳 (1)

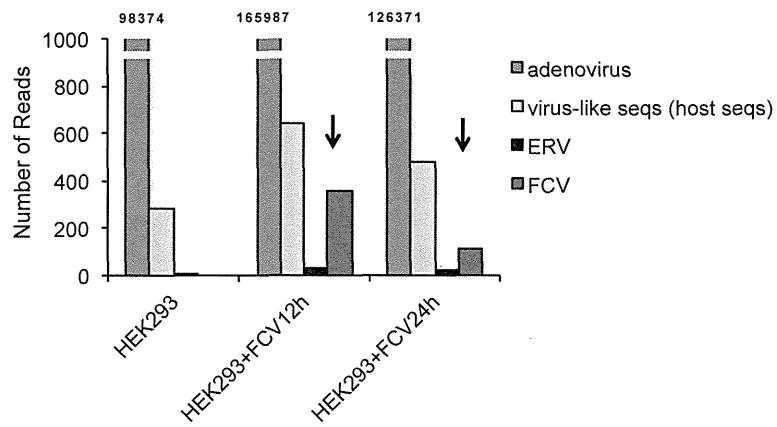


図 1-24 HEK293 細胞, FCV 感染 HEK293 細胞に含まれるウイルス様シークエンスの内訳 (2)

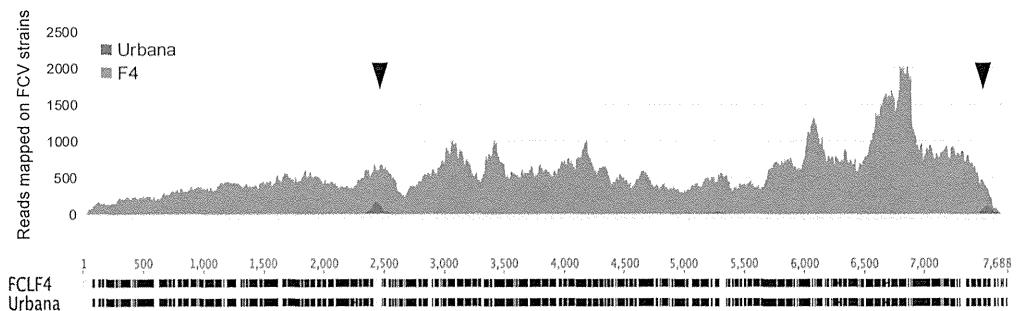


図 1-25 RNA ウィルスは多様なシークエンスバリエントから構成されているので検出に使うウィルスデータベースによって検出感度が変わる

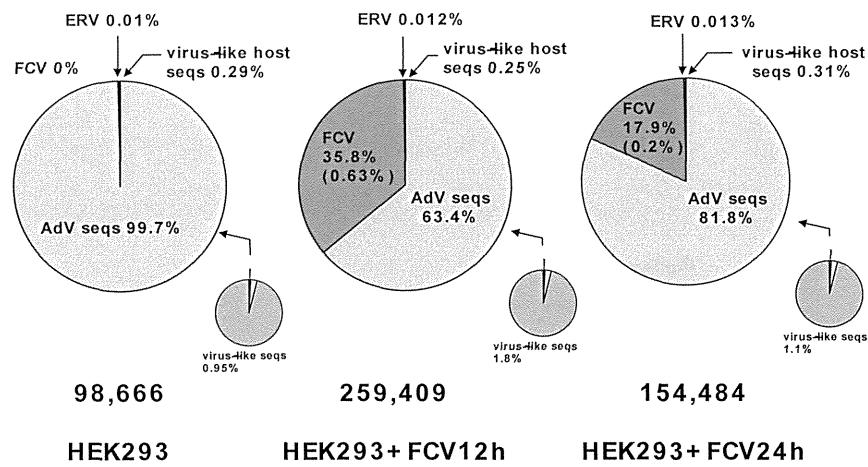


図 1-26 拡張ウィルスデータベースを使ったウイルス様シークエンスの内訳 (2)

## NGS raw data from RNA-seq

- ↓ trimmomatic-0.2.32
- ↓ mapping, star (or bowtie, bowtie2, tophat2) with hg38
- ↓ mapping, star with viral.1.1
- ↓ counting seq frequency, samtools

## virus-like seqs

- ↓ identification of back ground seqs
- ↓ mapping of short reads on virus genome
- ↓ confirmation with extended virus database
- ↓ de novo assembly

## virus seqs

図 1-27 NGS を用いた細胞のウイルス感染を検出するための  
パイプライン

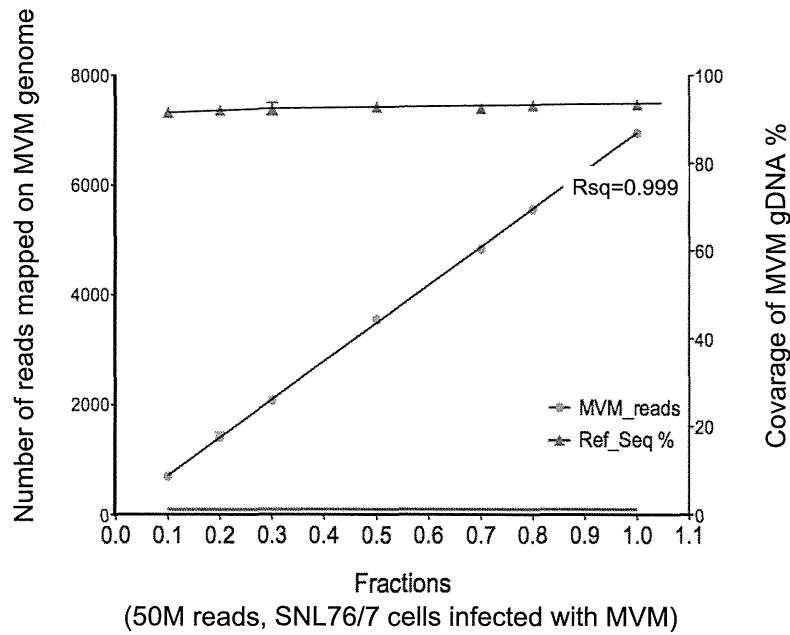


図 1-28 感染ウイルス検出に必要なサイズの RNA-seq データ

	<b>Number of MVM reads</b>	<b>Time for analysis</b>
<b>bowtie1</b>	<b>472</b>	<b>8 min</b>
<b>bowtie2</b>	<b>6819</b>	<b>10 min</b>
<b>Bwa</b>	<b>6928</b>	<b>15 min</b>
<b>tophat2</b>	<b>6636</b>	<b>66 h</b>
<b>star</b>	<b>6952</b>	<b>3 min</b>

表 1-3 異なる解析プログラムによる RNA-seq データの解析にかかる時間と検出されるウイルス様シーケンスの数

試薬・解析	価格	1 サンプル	時間
RNA 精製 (RNeasy plus universal mini kit)	44500円 (50 sample用)	890円	2 時間
鏡型調製用キット (TruSeq RNA Sample Prep Kit v2 - Set A)	628000円 (48 sample用)	13083円 (=628000/48)	2日 (サンプル調製)
フローセル (TruSeq SR Cluster Kit v3 - cBot - HS)	666000円/ラン (RNA-seq, 20Mリード; 約9サン プル/1レーン, 8レーン×9×8=7 2サンプル/ラン)	9250円 (=666000/72)	3日 (シーケンシング)
反応試薬 (TruSeq SBS Kit v3 - HS, 50 Cycle)	391000円/ラン	5430円 (=391000/72)	
RNA-seq ウィルス検 出データ解析		? 円	2 日
合計		(28653+α)円	~1週間

表 1-4 NGS を使ったウイルス検出法のコストと検出時間について  
(例: HiSeq 2500, Illumina)

	Number of reads	length/read (nt)	Bases (%>=30)
SNL76/7	54M	125	6.791G (93.6/90.2)
SNL76/7 Irradiated	58M	125	7.246G (93.6/90.5)
SNL76/7 + MVM	50M	125	6.271G (93.7/90.9)

表 1-5 SNL76/7 細胞の RNA-seq data (Illumina HiSeq 2500)

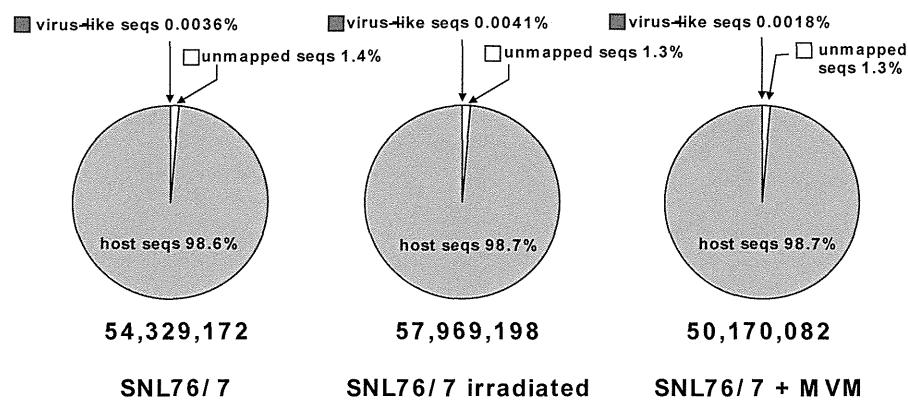


図 1-29 SNL76/7 細胞, 放射線照射済 76/7 細胞, MVM 感染 SNL76/7 細胞 RNA-seq に含まれるウイルス様シークエンス

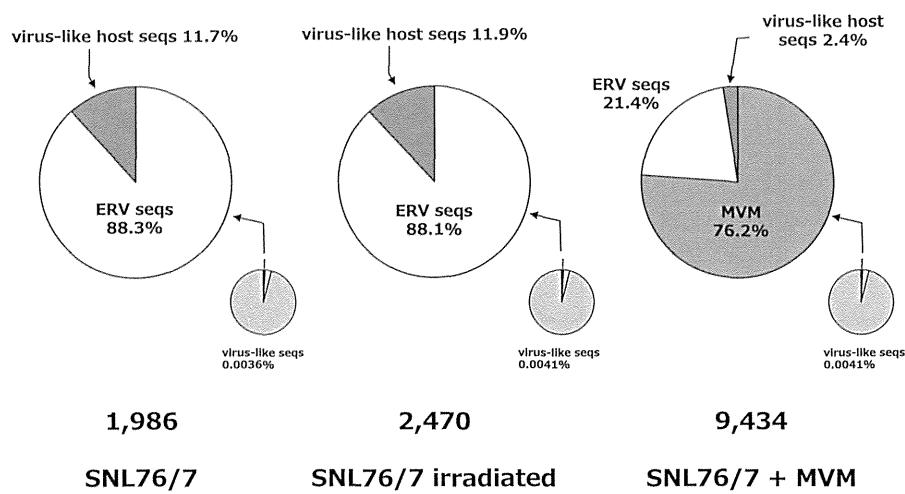


図 1-30 SNL76/7 細胞, 放射線照射済 76/7 細胞, MVM 感染 SNL76/7 細胞 RNA-seq のウイルス様シークエンス内訳

表 1-6. ウイルス受容体分子と、それらを利用することができるウイルス 1

Receptor	Virus	Ref
Neuropilin-1 (Vascular endothelial cell growth factor 165 receptor, CD antigen CD304)	Human T cell leukemia virus 1	10
NPC1 (Niemann-Pick C1 protein)	Ebola virus	10
claudin-1 (Senescence-associated epithelial membrane protein), claudin-6	HCV	10, 11
LDLR (Low-density lipoprotein receptor)	Rhinovirus (minor group)	10
	Human immunodeficiency virus type 1	1
	Human immunodeficiency virus type 2	1
	Simian immunodeficiency virus	1
	Human herpesvirus 7	1
CD4 (T-cell surface glycoprotein CD4)	New World arenaviruses	10
	Mouse mammary tumor virus	1
Icam-1 (CD54, Intercellular adhesion molecule 1)	Coxsackievirus A13, A18, A21	1
	Major group rhinovirus (91 serotypes)	1, 10
MHC I	Simian virus 40	1
MHC II	Adenovirus type 5 (subgroup C)	1
	Epstein-Barr virus	10
Hsp70	Japanese encephalitis virus	10
Low-affinity nerve growth factor receptor(CD271, Tumor necrosis factor receptor superfamily member 16)	Rabies virus	1
	Coxsackievirus A21	1
	Coxsackievirus B1, B3, B5	1
DAF (Decay-accelerating factor, CD55)	Coxsackievirus B	10
	Echovirus 3, 6, 7, 11 to 13, 20, 21, 24, 29, 33	1
	Echovirus 70	1
GLUT-1 (Solute carrier family 2, facilitated glucose transporter member 1, Glucose transporter type 1, erythrocyte/brain, HepG2 glucose transporter)	Human T cell leukemia virus 1	10
N-CAM-1 (CD56, Neural cell adhesion molecule)	Rabies virus	1
Carcinoembryonic antigen-related cell adhesion molecule 1, (CD66a, Bgp, biliary glycoprotein)	Mouse hepatitis virus	1
Aminopeptidase N (CD13)	Human coronavirus 229E	1
	Transmissible gastroenteritis virus	1
Poliovirus receptor (Pvr, NECL-5, CD155)	Poliovirus types 1-3	1
	Poliovirus	10
CD46 (membrane cofactor protein, trophoblast leukocyte common antigen, TLX)	Adenovirus (B1, B2, D)	3
	Measles virus	1
VCAM-1 (Vascular cell adhesion protein 1, INCAM-100, CD106)	Encephalomyocarditis virus	1
nucleolin (protein C23)	Adenovirus (C)	3
	RSV (respiratory syncytial virus)	4
CD21 (Complement receptor type 2, Cr2, Complement C3d receptor, Epstein-Barr virus receptor, EBV receptor)	Epstein-Barr virus	1, 10
Pit1 (Pituitary-specific positive transcription factor 1)	Gibbon ape leukemia virus	1
	Feline leukemia virus T	1
	Feline leukemia virus B	1
CD80 (activation B7-1 antigen, BB1, CTLA-4 counter-receptor B7.1, B7)	Adenovirus (B1, B2)	3
Laminin receptor(40S ribosomal protein SA, 37 kDa laminin receptor precursor, 37LRP, 37/67 kDa laminin receptor, LRP/LR, 67 kDa laminin receptor, 67LR, Laminin receptor 1, LamR, Laminin-binding protein precursor p40, LBP/p40)	Sindbis virus	1, 10
CD86 (activation B7-2 antigen, B70, BU63, CTLA-4 counter-receptor B7.2, FUN-1)	Adenovirus (B1, B2)	3
CCR5 (C-C chemokine receptor type 5, C-C CKR-5, CC-CKR-5, CCR-5, CHEMR13, HIV-1 fusion coreceptor, CD antigen CD195)	HIV	5, 10
Ephrin-B2 (EPH-related receptor tyrosine kinase ligand 5, LERK-5, HTK ligand, HTK-L)	Henipavirus	8, 10
Asialoglycoprotein receptor GP-2	Sendai virus	1
CD81 (26 kDa cell surface protein TAPA-1, Target of the antiproliferative antibody 1, Tetraspanin-28, Tspan-28)	HCV	10

表 1-6. ウイルス受容体分子と、それらを利用することができるウイルス 2

Receptor	Virus	Ref
CXCr4 (C-X-C chemokine receptor type 4)	HIV	5, 10
	Human immunodeficiency virus type 2	1
	Feline immunodeficiency virus	1
Car (coxsackievirus-adenovirus receptor)	Adenovirus 2	10
	Adenovirus subgroups A, C, D, E, F	1
	Coxsackievirus B1 to B6	1
Par-1 (proteinase-activated receptor 1)	Porcine endogenous retrovirus A	1
Par-2 (proteinase-activated receptor 2)	Porcine endogenous retrovirus A	1
Pit2	Feline leukemia virus B	1
	Amphotropic murine leukemia virus	1
SLAM (Signaling lymphocytic activation molecule, CD150)	Measles virus	1, 10
Lysosome membrane protein 2 (85 kDa lysosomal membrane sialoglycoprotein, LGP85, CD36 antigen-like 2, Lysosome membrane protein II, LIMP II, Scavenger receptor class B member 2, CD antigen CD36)	Enterovirus 71	10
α-Dystroglycan (Dystrophin-associated glycoprotein 1 [Cleaved into: Alpha-dystroglycan (Alpha-DG); Beta-dystroglycan (Beta-DG)]]	Old World arenaviruses	10
desmoglein-2 (DSG2, cadherin family member 5, HDGC)	Adenovirus (B1, B2)	3
PSGL-1 (P-selectin glycoprotein ligand 1)	Enterovirus 71	10
Nectin-1 (Poliovirus receptor-related protein 1, Herpes virus entry mediator C, Herpesvirus entry mediator C, HveC, Herpesvirus Ig-like receptor, HlgR, Nectin-1, CD antigen CD111)	Herpes simplex virus 1/2	10
RDr	RD114	1
Ephrin-B3 (EPH-related receptor transmembrane ligand ELK-L3, EPH-related receptor tyrosine kinase ligand 8, LERK-8)	henipavirus	8
occludin	Coxsackievirus B (HCV)	10 10
EGFR (epidermal growth factor receptor)	Vaccinia virus	1
Scavenger receptor class B member 1 (SR-B1, CD36, CD36 and LIMP II analogous 1, Collagen type I receptor, thrombospondin receptor-like 1)	HCV	2, 10
Nectin-2 (Poliovirus receptor-related protein 2, Herpes virus entry mediator B, Herpesvirus entry mediator B, HveB, Nectin-2, CD antigen CD112)	Herpes simplex virus 1/2	10
HVEM (Tumor necrosis factor receptors superfamily member 14, Herpes virus entry mediator A, Herpesvirus entry mediator A, HveA, Tumor necrosis factor receptor-like 2, TR2, CD270)	Herpes simplex virus 1/2	10
Hepatitis A virus cellular receptor 1 (HAVcr-1, kidney injury molecule 1, KIM-1, T-cell immunoglobulin and mucin domain-containing protein 1, TIMD-1, T-cell immunoglobulin mucin receptor 1, TIM-1, T-cell membrane protein 1)	Hepatitis A virus Ebolavirus (EBOV) Marburgvirus (MARV) Hepatitis A virus Ebola virus	1 7 7 10 10
Nectin-4 (Poliovirus receptor-related protein 4, Ig superfamily receptor LNIR, Nectin-4 [Cleaved into: Processed poliovirus receptor-related protein 4])	Measles virus	6, 10
ACE2 (angiotensin converting enzyme 2)	SARS coronavirus	9, 10
L-SIGN (C-type lectin domain family 4 member M, CD209 antigen-like protein 1, DC-SIGN-related protein, DC-SIGNR, Dendritic cell-specific ICAM-3-grabbing non-integrin 2, DC-SIGN2, Liver/lymph node-specific ICAM-3-grabbing non-integrin, CD antigen CD299)	SARS coronavirus	10
DC-SIGN (CD209, C-type lectin domain family 4 member L, Dendritic cell-specific ICAM-3-grabbing non-integrin 1, DC-SIGN1)	Dengue virus HCV CMV KSV (HHV8) HIV HTLV Bunyavirus Ebola virus	2 2 2 2 2 2 10 2

表 1-6. ウイルス受容体分子と、それらを利用することができるウイルス 3

Receptor	Virus	Ref
CXCr4 (C-X-C chemokine receptor type 4)	HIV	5, 10
XPr1	Xenotropic and polytropic murine leukemia virus	1
scavenger receptor class A member 2, macrophage receptor MARCO, macrophage receptor with collagenous structure)	Adenovirus (C)	3
Langerin (CD207, C-type lectin domain family 4 member K)	HIV	2
JAM (Junction adhesion molecule)	Reovirus	10
	Orthoreheovirus	1
Fivcr	Feline leukemia virus C	1
$\alpha 2\beta 1$ (Vla-2, CD49b)	Echovirus 1 and 8	1
$\alpha 2\beta 1, \alpha 4\beta 1$	Human rotavirus	1
$\alpha 2\beta 1, \alpha V\beta 3$	Echovirus	2
$\alpha 3\beta 1, \alpha 2\beta 1$	Human herpesvirus 8	1
$\alpha M\beta 2$	Adenovirus type 2 (subgroup C)	1
$\alpha V$ integrins	Adenovirus type 9 (subgroup C)	1
	Adenovirus 2	10
$\alpha V\beta 3$ (vitronectin receptor)	Foot and mouth disease virus (FMDV)	2
	Coxsackievirus A9	1
	Echovirus 22	1
$\beta 3$ integrins	Hantavirus	1
integrins	Rotavirus	10
Chondroitin sulfate proteoglycans	Vaccinia virus	2
	Circovirus	2
	HSV1 and 3	2
GM1	SV40 polyomavirus	10
Histo-blood group antigens	Norovirus	10
Heparan sulfate proteoglycans	Vaccinia virus, Adenovirus, Norovirus, Circovirus, Dengue virus, HCV, CMV, HSV1 and 2, KSV (HHV9), RSV, Rhinovirus89, Foot and mouth disease virus (FMDV), HIV, HTLV	2
Lactoseries tetrasaccharide c	John Cunningham polyomavirus	10
	Adenovirus	2
sialic acid	Rhinovirus87	2
	Rotavirus	10
	Influenza A	10
$\alpha 2,3$ sialic acid	Sendai virus	2
$\alpha 2,6$ or $\alpha 2,3$ sialic acid	Influenza A virus (avian)	2
$\alpha 2,6$ sialic acid	Rotavirus	2
	Influenza A virus (human)	2

## References

- "Principles of virology" Flint SJ *et al.* (2nd ed. chapter 5, Table 5.1)
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- "SARS-CoV and Emergent Coronaviruses: Viral Determinants of Interspecies Transmission" Bolles M *et al.*, *Current Opinion in Virology* 2011 1(6): 624-634.
- "The cell biology of receptor-mediated virus entry" Grove J and Marsh M, *The Journal of Cell Biology* 2011 195(7):1071-1082.
- "The tight junction proteins claudin-1, -6, and -9 are entry cofactors for hepatitis C virus ", *J Virol* 2008 82(7): 3555-60.

表 1-7. 同定されたウイルス受容体タンパク質と代表的なペプチド

Entry	Accession	position	Sequence	MH <sup>+</sup> [Da]	z	m/z	RT [min]	detection		
								whole	Tris	guanidine
intercellular adhesion molecule 1 [Cricetulus griseus]	350539683	290-300	cVLELADQILK	1302.70	2	651.86	124.26	n	y	y
		210-222	QLWTFDLPVTEPK	1573.83	2	787.42	123.14	n	y	y
low-density lipoprotein receptor [Cricetulus griseus]	350540068	335-352	IGYEcLcPNGFQLVDOHR	2207.99	3	736.67	106.59	n	n	y
		429-441	NVVALDTEVANNR	1414.73	2	707.87	64.95	n	y	y
Shared peptide among MHC1 Hm1-C2, Hm1-C4, and Hm1-C5		244-280	EEEEQTQDMELVETRPSGDGTQK	2783.23	3	928.42	74.64	n	y	y
MHC class I antigen Hm1-C4 [Cricetulus griseus]	345842479	156-169	TWTAADAAQITQR	1533.77	2	767.39	77.79	n	y	y
		79-89	EGPEYWEETR	1424.60	2	712.80	68.21	n	y	y
MHC class I antigen Hm1-C5 [Cricetulus griseus]	345842477	268-280	WAIAVVPSGEEQK	1399.73	2	700.37	71.39	n	n	y
		79-89	EGPEYWEGQTR	1351.60	2	676.30	61.68	n	y	y
PREDICTED: neural cell adhesion molecule 1 [Cricetulus griseus]	354472688	60-70	FFLcQVAGEAK	1270.62	2	635.81	97.56	n	y	y
PREDICTED: nucleolin [Cricetulus griseus]	354502803	449-470	NLSFNITEDDELKEVFEDALEIR	2624.32	3	875.44	177.63	n	y	y
40S ribosomal protein SA [Cricetulus griseus]	345842370	129-155	ADHQPLTEASYVNLPITALcNTDSPL R	2997.49	3	999.83	109.89	y	y	n
		64-80	ANAIENPADVSVISSR	1740.96	2	870.99	82.50	y	y	n
PREDICTED: dystroglycan [Cricetulus griseus]	354476371	795-823	GVPIIFADELDDSKPPPSSSMPLILQ EEK	3152.62	3	1051.54	128.69	n	n	y
		518-533	IPSDTFYDNEDETTDK	1861.80	2	931.40	64.72	n	n	y
PREDICTED: chondroitin sulfate proteoglycan 4 [Cricetulus griseus]	354471469	234-247	SQQAPLAFAQAGDQR	1516.75	2	758.88	62.02	n	y	y
		715-734	QGAGGVEGTEWWETLAFHQR	2259.07	3	753.69	120.02	n	y	y
		1759-1771	GQLLVSEEPLHAR	1448.79	2	724.90	69.25	n	y	y
PREDICTED: sulfated glycoprotein 1 [Cricetulus griseus]	354494438	171-185	EAVDSYLPVILDMIK	1705.92	2	853.46	161.51	n	y	y

各条件でMS/MSによる同定が可能な程度に検出されたものをyで、検出されなかつたかまたは量が不十分だったものをnで示す。

表 1-8 同定されたウイルス受容体関連タンパク質 1

Accession	Description	Score	Coverage	# AAs	MW [kDa]					関連ウイルス
P08865	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 - [RSSA_HUMAN]	29.43	21.36	295	32.8					Sindbis virus
	Sequence	# PSMs	Modifications	ΔCn	Xcorr	Charge	MH+ [Da]	ΔM [ppm]		
	FLAAGTHLGGTNLDQFQMEQYIYK	3		0.0000	5.36	3	2617.27732	0.64		
	ADHQPLTEASYVNLPTIALcNTDSPLR	2	C20(Carboxymethyl)	0.0000	4.66	3	2997.45536	-2.33		
	FAAATGATPIAGR	2		0.0000	3.44	2	1203.64653	-1.32		
	Sequence	# PSMs	Modifications	ΔCn	Xcorr	Charge	MH+ [Da]	ΔM [ppm]		
	QQMENYPK	1		0.0000	1.48	2	1037.47173	-0.35		
P60033	CD81 antigen OS=Homo sapiens GN=CD81 PE=1 SV=1 - [CD81_HUMAN]	3.30	8.47	236	25.8					HCV
	Sequence	# PSMs	Modifications	ΔCn	Xcorr	Charge	MH+ [Da]	ΔM [ppm]		
	QFYDQALQQAVVDDANNAK	1		0.0000	3.30	3	2253.04374	0.81		
P56747	Claudin-6 OS=Homo sapiens GN=CLDN6 PE=1 SV=2 - [CLDN6_HUMAN]	11.02	9.09	220	23.3					HCV
	Sequence	# PSMs	Modifications	ΔCn	Xcorr	Charge	MH+ [Da]	ΔM [ppm]		
	DFYNPLVAEAQK	2		0.0000	3.40	2	1394.69206	-2.18		
	GPSEYPTK	2		0.0000	2.42	2	878.42461	-0.96		
P78310	Coxsackievirus and adenovirus receptor OS=Homo sapiens GN=CXADR PE=1 SV=1 - [CXAR_HUMAN]	15.49	12.60	365	40.0					Adenovirus 2 Adenovirus subgroups A, C, D, E, F Coxsackievirus B1 to B6
	Sequence	# PSMs	Modifications	ΔCn	Xcorr	Charge	MH+ [Da]	ΔM [ppm]		
	FTLSPEDQGPLDIEWLSPADNQK	2		0.0000	5.01	3	2713.33994	1.57		
	TQYNQVPSEDFER	1		0.0000	3.39	2	1612.72576	1.19		
	VGSDQcLLR	1	C6(Carboxymethyl)	0.0000	2.55	2	1048.50798	-1.18		
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]	157.19	36.51	641	70.0					Japanese encephalitis virus
	Sequence	# PSMs	Modifications	ΔCn	Xcorr	Charge	MH+ [Da]	ΔM [ppm]		
	TLSSSTQASLEIDSFEGIDFYTSITR	3		0.0000	5.70	3	2981.45304	-3.24		
	ELEQVNPNPISGLYQGAGGPGPGFFGAQGPK	2	C6(Carboxymethyl)	0.0000	5.46	3	3056.47574	-0.86		
	SINPDEAVAYGAAVQAAILMGDK	1		0.0000	5.25	3	2304.15891	2.06		
	IIINEPTAAAIAYGLDR	1		0.0000	4.01	2	1687.89690	-2.69		
	NQVALNPQNTVFDAK	2		0.0000	3.58	2	1658.84636	-2.03		
	TTPSYVAFTDTER	4		0.0000	3.58	2	1487.70391	1.74		
	VEI1ANDQGNR	4		0.0000	3.41	2	1228.52663	-1.19		
	ATAGDTLGGEDFDNR	2		0.0000	3.37	3	1675.72959	-0.69		
	NALESYAFNMK	1		0.0000	2.92	2	1287.60393	0.06		
	YKAEDDEVQR	2		0.0000	2.50	2	1137.55229	-1.08		
	cQEVIISWLDAANTLAEKDEFEHK	2	C1(Carboxymethyl)	0.0000	2.46	4	2663.22988	0.15		
	AAAIGIDLGTITYScGVVFQHGK	1	C14(Carboxymethyl)	0.0000	2.41	3	2266.13047	5.79		
	MVOEAEK	31		0.0000	2.02	2	834.40227	-0.43		
	FGDPVVQSDMK	1		0.0000	2.59	2	1222.57488	-2.00		

表 1-8 同定されたウイルス受容体関連タンパク質 2

Accession	Description	Score	Coverage	# AAs	MW [kDa]	関連ウイルス			
P34932	Heat shock 70 kDa protein 4 OS=Homo sapiens GN=HSPA4 PE=1 SV=4 - [HSP74_HUMAN]	264.36	61.19	840	94.3				Japanese encephalitis virus
	Sequence	# PSMs	Modifications	ΔCn	XCorr	Charge	MH+ [Da]	ΔM [ppm]	
	SNLAYDIVQLPTGLTGK	5		0.0000	5.20	2	1903.05278	-0.42	
	LEDTENWLYEDGEDQPK	1		0.0000	5.80	2	2080.89849	0.10	
	MQVDQEEPHVEEQQQQTPAENK	3		0.0000	5.58	3	2622.17997	2.37	
	他 32本	2		0.0000	5.33	3	3301.59702	0.90	
P05556	Integrin beta-1 OS=Homo sapiens GN=ITGB1 PE=1 SV=2 - [ITB1_HUMAN]	6.92	2.51	798	88.4				Echovirus 1 and 8 Human rotavirus Human herpesvirus 8
	Sequence	# PSMs	Modifications	ΔCn	XCorr	Charge	MH+ [Da]	ΔM [ppm]	
	SAVTTVNPK	2		0.0000	2.29	2	1015.57250	-5.70	
	DNTNEIYSGK	1		0.0000	2.60	2	1140.51543	-1.19	
Q9Y624	Junctional adhesion molecule A OS=Homo sapiens GN=F11R PE=1 SV=1 - [JAM1_HUMAN]	22.84	22.07	299	32.6				Reovirus Orthoreovirus
	Sequence	# PSMs	Modifications	ΔCn	XCorr	Charge	MH+ [Da]	ΔM [ppm]	
	LIVLVPPSKPTVNIPSSATIGNR	3		0.0000	3.09	3	2373.38340	-1.32	
	VTFLPTGIFK	2		0.0000	2.73	2	1223.70268	-0.66	
	FDQGDTR	2		0.0000	2.43	2	939.41484	-1.97	
	ITASYEDR	1		0.0000	2.17	2	954.45110	-1.71	
	VIYSQPSAR	1		0.0000	1.87	2	1020.54448	-2.76	
	LVcYNNK	1	C3(Carboxymethyl)	0.0000	1.56	2	911.42888	-0.30	
Q14108	Lysosome membrane protein 2 OS=Homo sapiens GN=SCARB2 PE=1 SV=2 - [SCRB2_HUMAN]	0.00	6.28	478	54.3				Enterovirus 71
	Sequence	# PSMs	Modifications	ΔCn	XCorr	Charge	MH+ [Da]	ΔM [ppm]	
	FVSAIEGMHPNQEDHETFVDINPLTGILK	2		0.0000	2.31	4	3364.67885	-2.83	
P19338	Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3 - [NUCL_HUMAN]	14.79	9.72	710	76.6				RSV (respiratory syncytial virus)
	Sequence	# PSMs	Modifications	ΔCn	XCorr	Charge	MH+ [Da]	ΔM [ppm]	
	GYAFIEFASFEDAK	1		0.0000	4.23	2	1594.74248	0.00	
	GLSEDTTEETLKG	1		0.0000	2.90	2	1322.63054	-1.29	
	TLVLSNLSSATAEETLQEVFEK	1		0.0000	2.51	3	2501.26792	0.83	
	VTQDELK	1		0.0000	2.10	2	832.43938	-2.08	
	FGYVDFESAEDEK	1		0.0000	3.05	2	1648.75591	10.99	
P11166	Solute carrier family 2, facilitated glucose transporter member 1 OS=Homo sapiens GN=SLC2A1 PE=1 SV=2 - [GTR1_HUMAN]	11.24	8.13	492	54.0				Human T cell leukemia virus 1
	Sequence	# PSMs	Modifications	ΔCn	XCorr	Charge	MH+ [Da]	ΔM [ppm]	
	TPEELFHPLGADSQV	1		0.0000	3.68	2	1639.79875	1.50	
	TFDEIASGFR	1		0.0000	3.06	2	1142.54656	-1.02	
	QGGASQSDKTPPELFHPLGADSQV	1		0.0000	2.89	3	2498.17710	-0.95	
	FLLINR	2		0.0000	1.61	2	775.48125	-1.64	

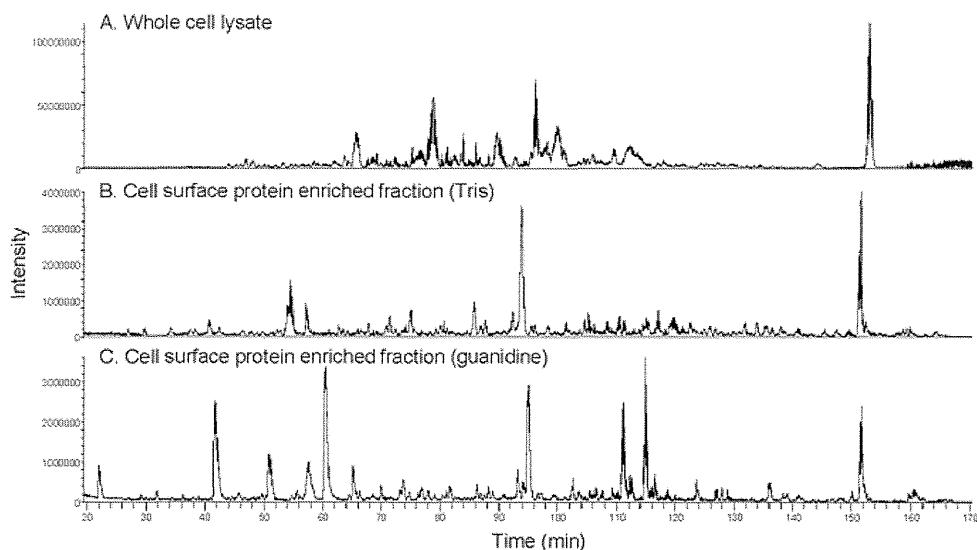


図 1-31 CHO 細胞 RIPA buffer 抽出物のベースピーククロマトグラム

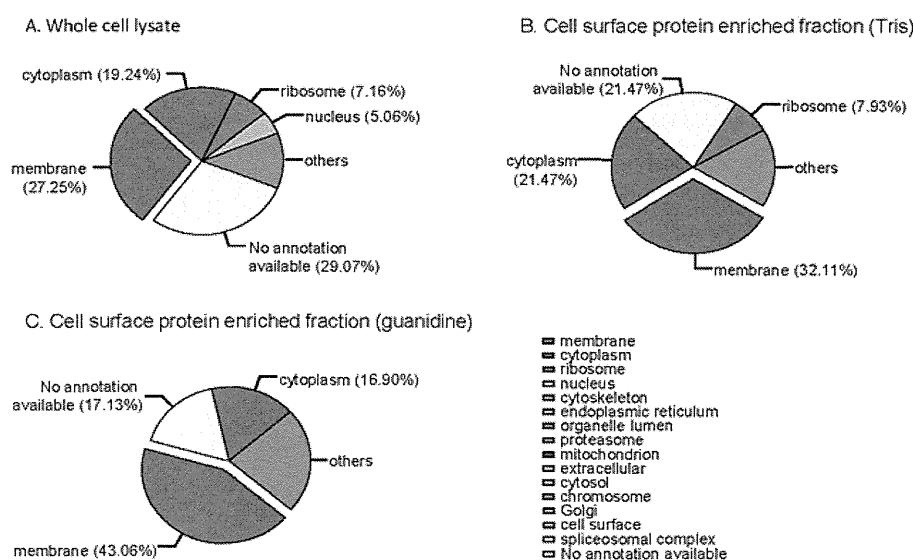
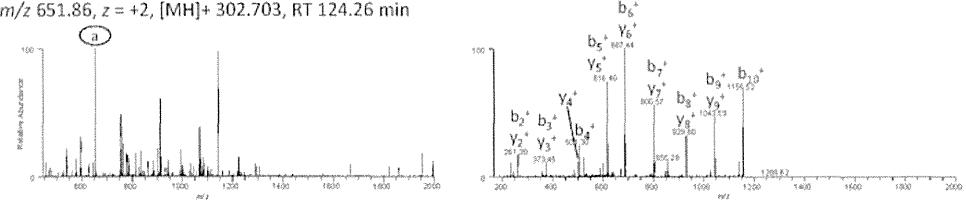


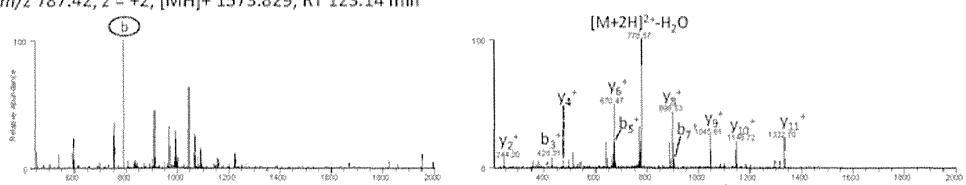
図2. 同定された CHO細胞由来タンパク質の細胞内局在に基づく分類。

図 1-32 同定された CHO 細胞由来タンパク質の細胞内局在に基づく分類

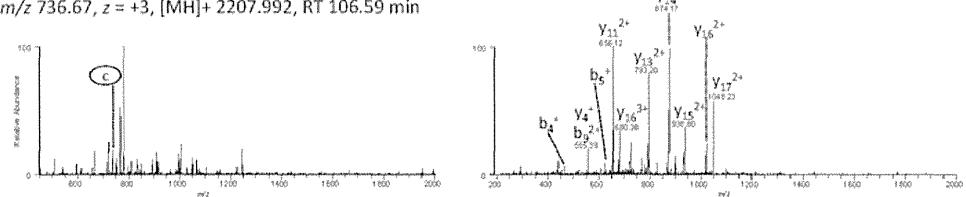
A. intercellular adhesion molecule 1, 290-300, cVLELADQILK,  
 $m/z$  651.86,  $z = +2$ , [MH]<sup>+</sup> 302.703, RT 124.26 min



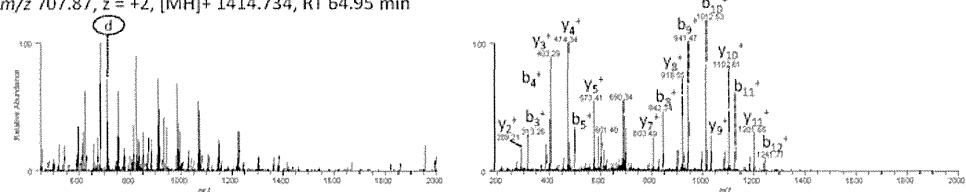
B. intercellular adhesion molecule 1, 210-222, QLWTFDLPVTEPK,  
 $m/z$  787.42,  $z = +2$ , [MH]<sup>+</sup> 1573.829, RT 123.14 min



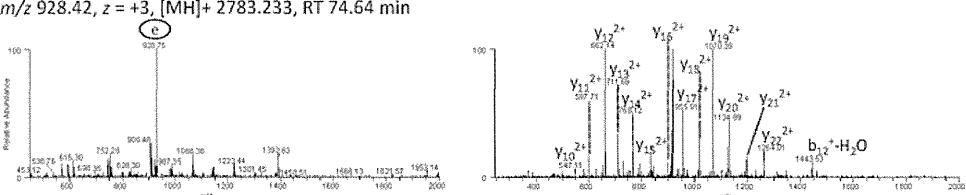
C. low-density lipoprotein receptor, 335-352, IGYEcLcPNGFQLVDQHR,  
 $m/z$  736.67,  $z = +3$ , [MH]<sup>+</sup> 2207.992, RT 106.59 min



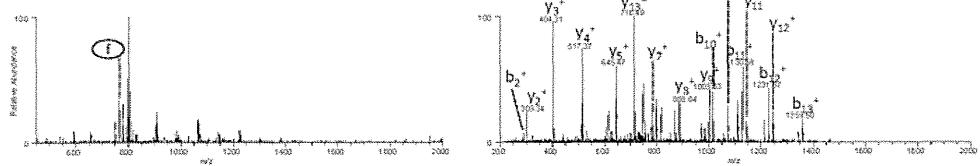
D. low-density lipoprotein receptor, 429-441, NVVALDTEVANNR,  
 $m/z$  707.87,  $z = +2$ , [MH]<sup>+</sup> 1414.734, RT 64.95 min



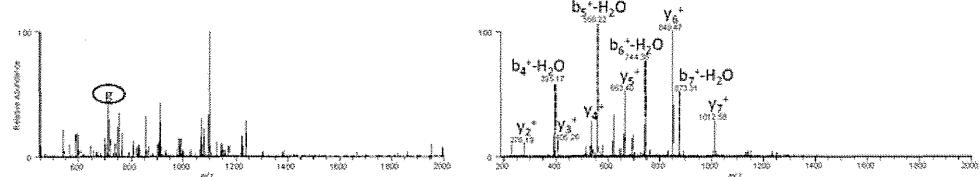
E. Shared peptide among Hm1-C4, Hm1-C5, and Hm1-C2, 244-280, EEEEQTQDMELVETRPSGDGTQK,  
 $m/z$  928.42,  $z = +3$ , [MH]<sup>+</sup> 2783.233, RT 74.64 min



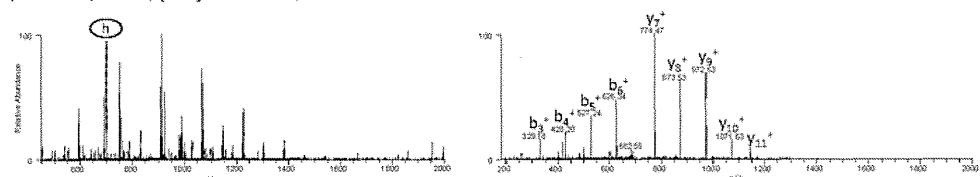
F. MHC class I Hm1-C4, 156-169, TWTAADTAQITQR,  
 $m/z$  767.39,  $z = +2$ , [MH]<sup>+</sup> 1533.771, RT 77.79 min



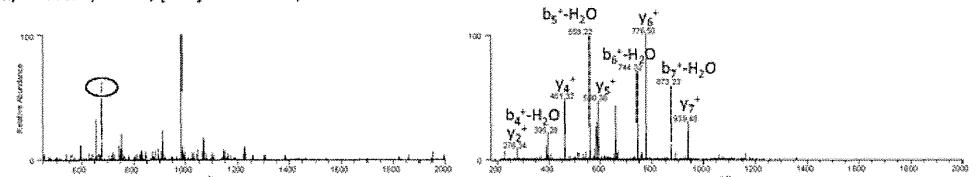
G. MHC class I Hm1-C4, 79-89, EGPEYWEEETR,  
 $m/z$  712.80,  $z = +2$ , [MH]<sup>+</sup> 1424.600, RT 68.21 min



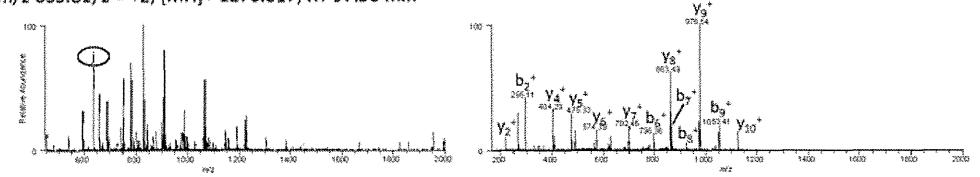
H. MHC class I Hm1-C5, 268-280, WAAVVVPSGEEQK,  
 $m/z$  700.37,  $z = +2$ , [MH]<sup>+</sup> 1399.728, RT 71.39 min



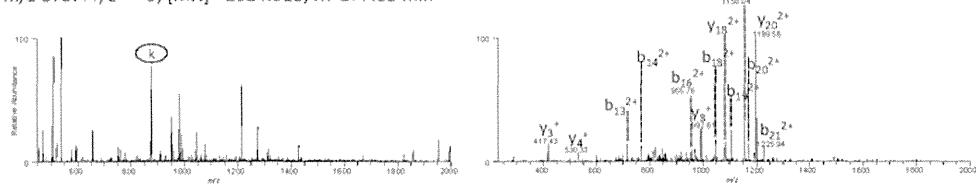
I. MHC class I Hm1-C5, 79-89, EGPEYWEGQTR,  
 $m/z$  676.30,  $z = +2$ , [MH]<sup>+</sup> 1351.596, RT 61.68 min



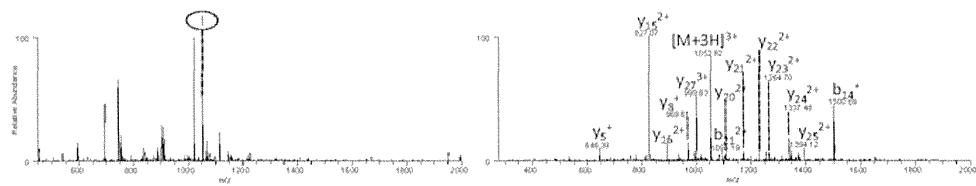
J. neural cell adhesion molecule 1, 60-70, FFLcQVAGEAK,  
 $m/z$  635.81,  $z = +2$ , [MH]<sup>+</sup> 1270.617, RT 97.56 min



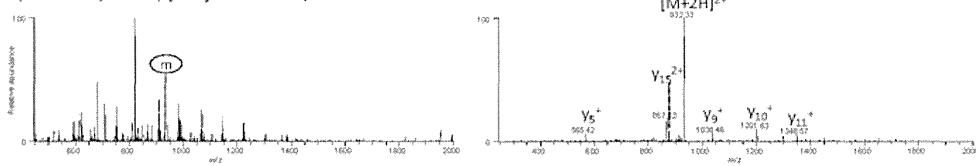
K. nucleolin, 449-470, NLSFNITEDELKEVFEDALEIR,  
*m/z* 875.44, *z* = +3, [MH]<sup>+</sup> 2624.318, RT 177.63 min



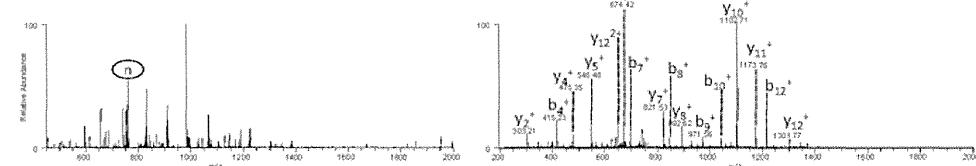
L. dystroglycan, 795-823, GVPPIFADELDDSKPPPSSSMPLILQEEK,  
*m/z* 1051.54, *z* = +3, [MH]<sup>+</sup> 3152.619, RT 128.69 min



M. dystroglycan, 518-533, IPSDTFYDNEDETTDK,  
*m/z* 931.40, *z* = +2, [MH]<sup>+</sup> 1861.800, RT 64.72 min



N. chondroitin sulfate proteoglycan 4, 234-247, SQQAPLAFAQAGDQR, m/z 758.88, z = +2, [MH]<sup>+</sup> 1516.755, RT 62.02 min



O. chondroitin sulfate proteoglycan 4, 715-734, QGAGGVEGTEWWETLAFHQ, m/z 753.69, z = +3, [MH]<sup>+</sup> 2259.066, RT 120.02 min

