Latvijas Biomedicīnas
pētījumu un studiju centrs
biomedicīnas pētījumi un izglītība no gēniem līdz cilvēkam

Alfavīrusu vektori kā gēnu piegādes līdzekļi *in vivo*

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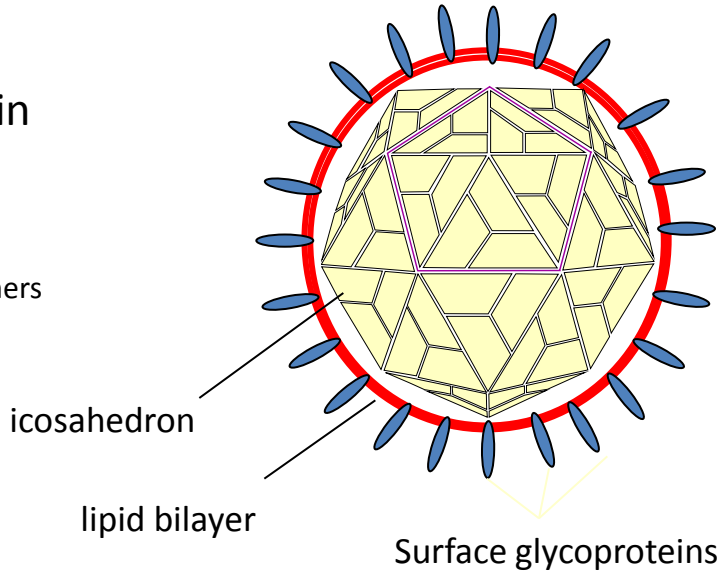
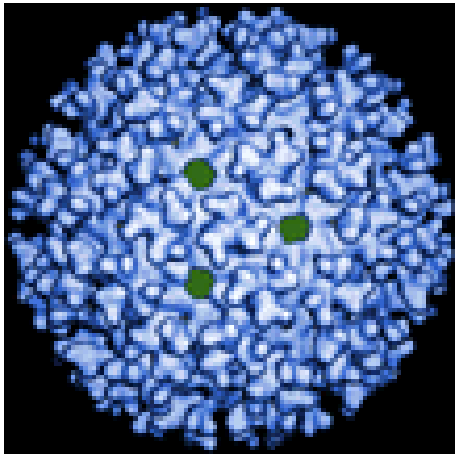
Alphaviruses

Enveloped virus
Icosahedron : 240 copies of 1 protein
Spherical : 65-70nm
Envelope : 80 trimer spikes

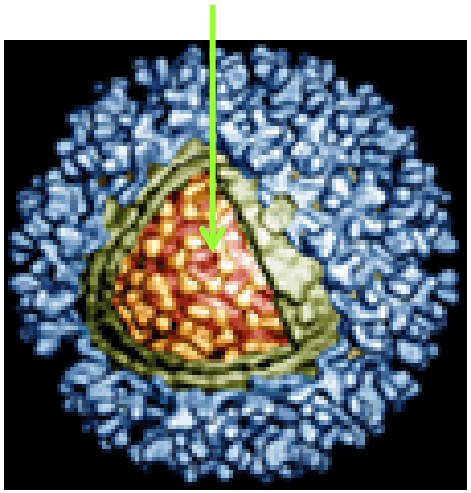
each spike = 3 x E1/E2 heterodimers



(blue = surface glycoprotein)

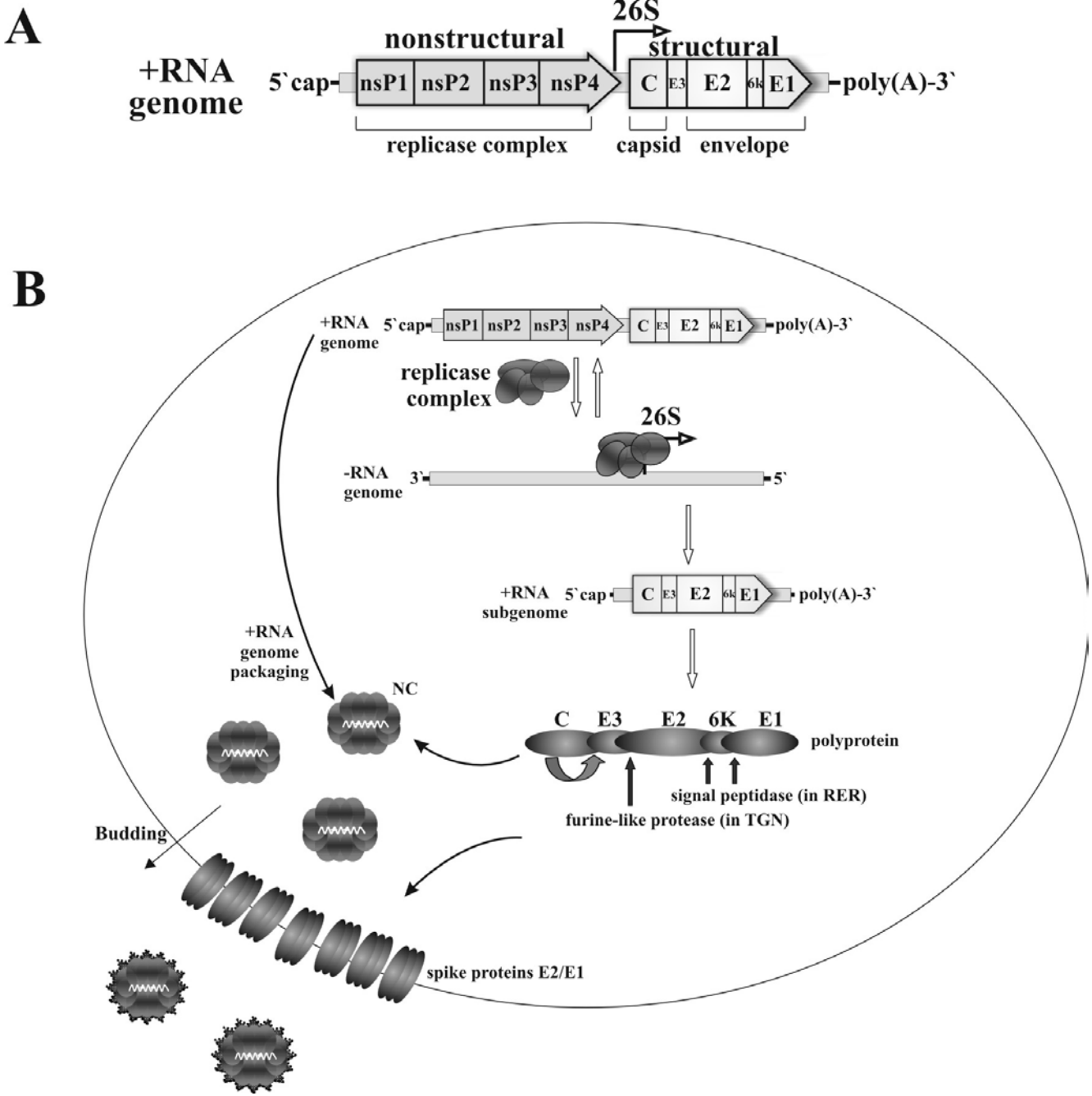


icosahedron

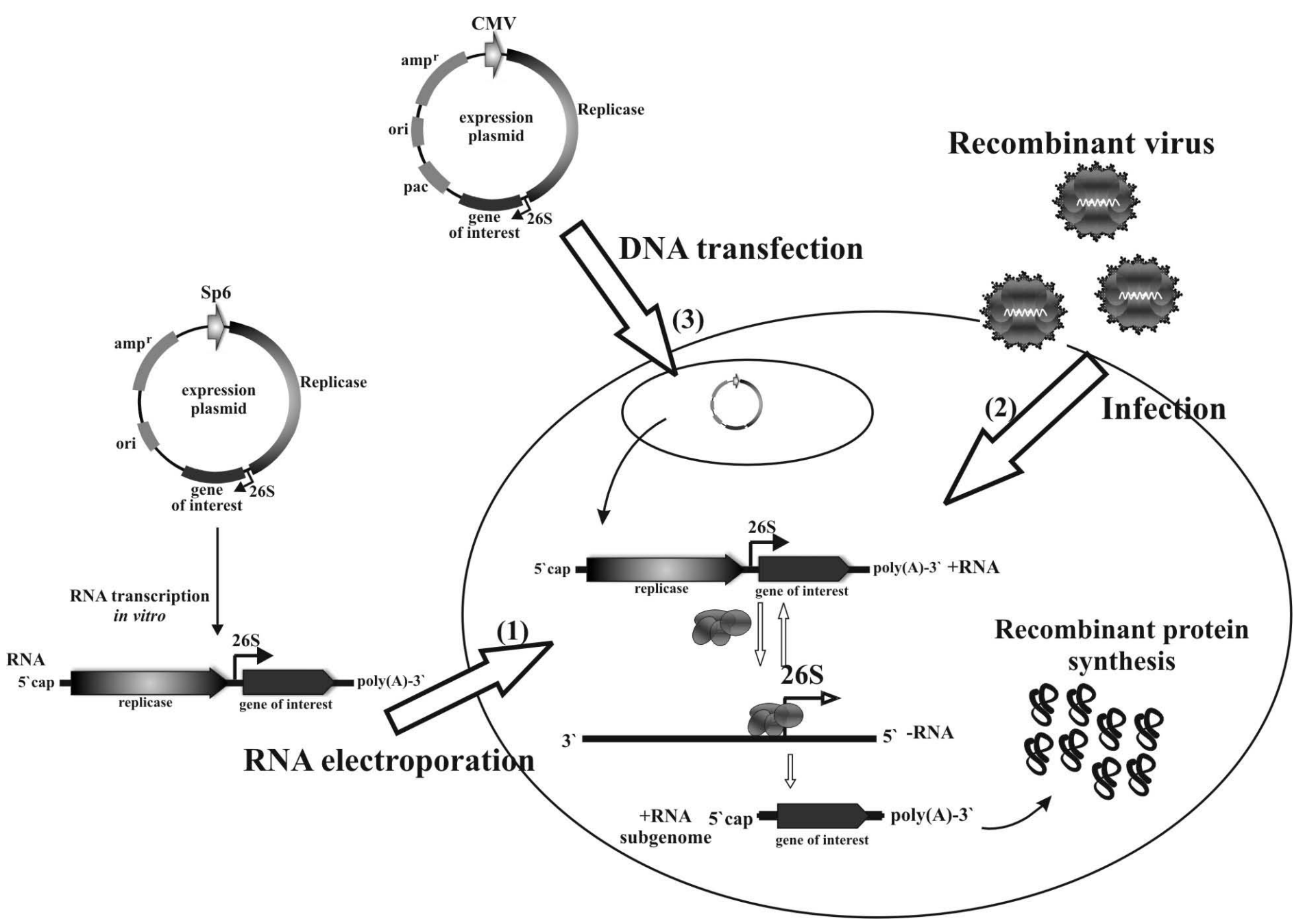


(green = lipid bilayer)

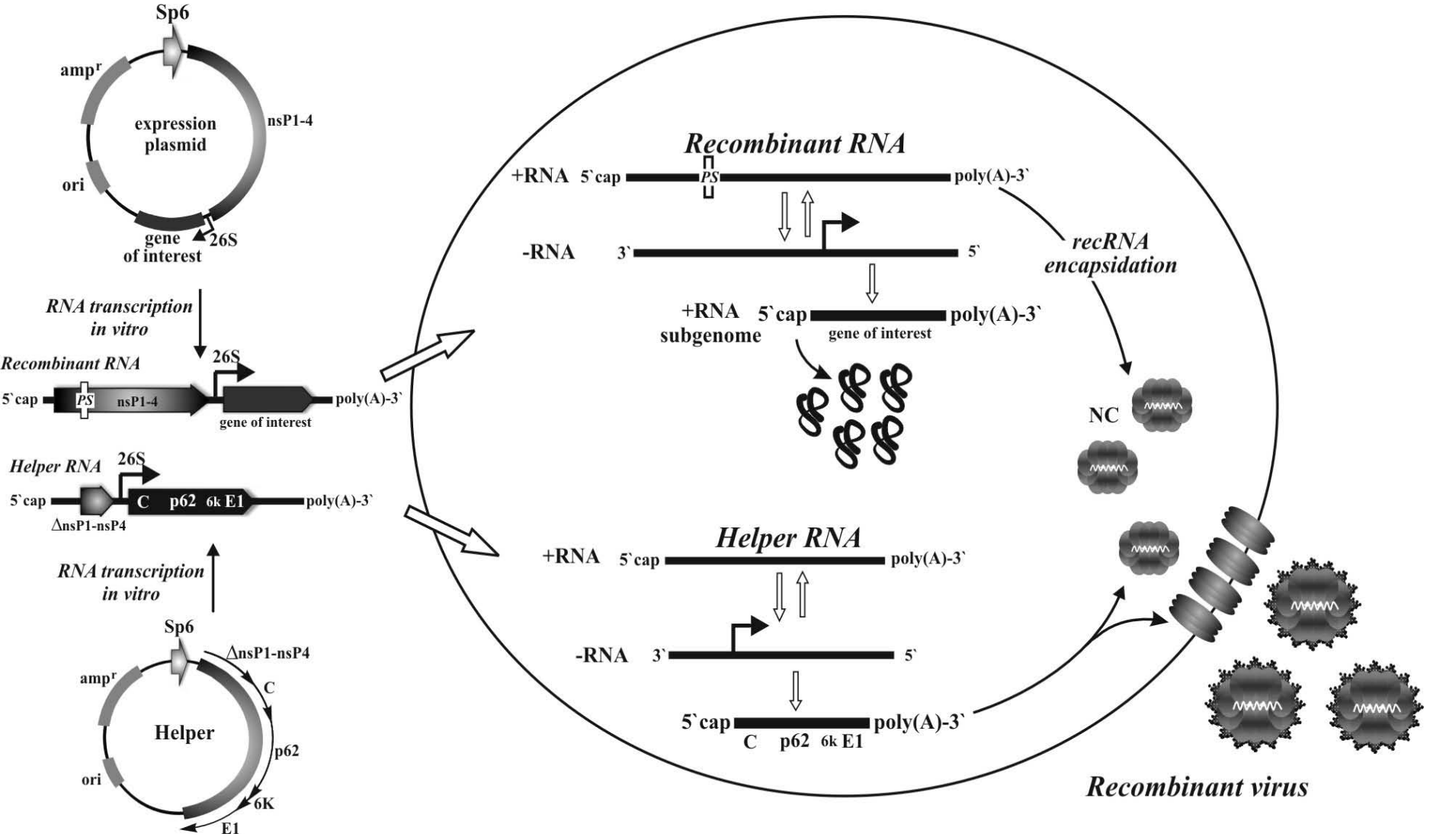
Mancini *et* al, 2000



(A) Genome structure and (B) replication cycle of alphaviruses (Zajakina *et* al, 2009)



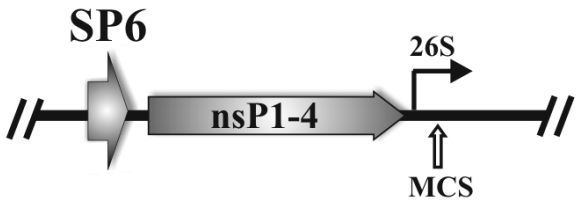
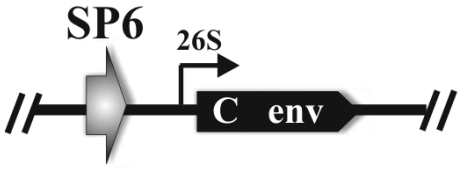
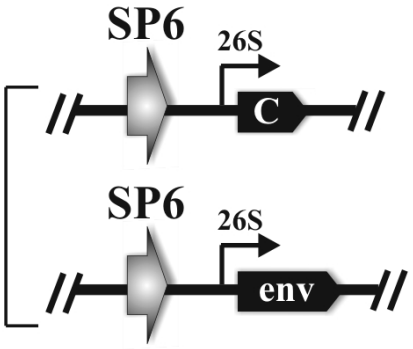
Three ways of the expression of gene of interest by alphaviruses



Recombinant alphavirus production.

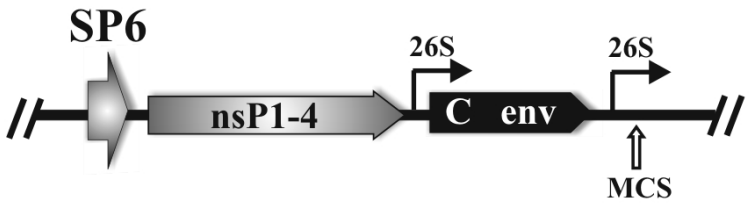
RNA vectors:

Examples:

	SFV	SIN
Replication-deficient		
	***Cytopathic***	
	pSFV-1 (Liljestrom and Garoff, 1991)[31]	**pSinRep5** (Xiong et al., 1989)[32]
	pSFV-3 (Liljestrom and Garoff, 1991)[31]	
	Non-cytopathic	
	SFV(PD) (Lundstrom et al., 2003)[51]	**pSINrep19** (Agapov et al., 1998)[50]
	SFV(PD713P) (Lundstrom et al., 2003)[51]	
Helper		
	**Helper-1** (Liljestrom and Garoff, 1991)[31]	**DH-EB(5'SIN)**
	Helper-2 (Berglund et al., 1993)[41]	**DH-EB**
		DH-BB(5'SIN)
		DH-BB
		DH(26S) (Bredenbeek et al., 1993)[39]
Split-Helper		
	**SFV-helper-C**	**DH-BB-Csin**
	SFV-helper-S	**DH-BB-Crrv**
	(Smerdou and Liljestrom, 1999)[45]	(Frolov et al., 1997)[47]

Schematic diagram of recombinant constructs developed on the basis of alphaviruses.

Replication-competent

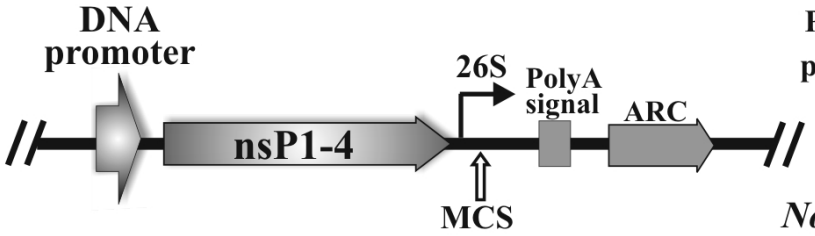


VA7-EGFP
(Vaha-Koskela et al., 2003)[37]

dsSIN (Hahn et al., 1992)[36]

DNA vectors:

Suicidal



PBK-SFV (Berglund et al., 1998)[57]

pSCAβ (DiCiommo and Bremner, 1998)[38]

pDCMVSIN, pDLTRSIN
(Dubensky, Jr. et al., 1996)[56]

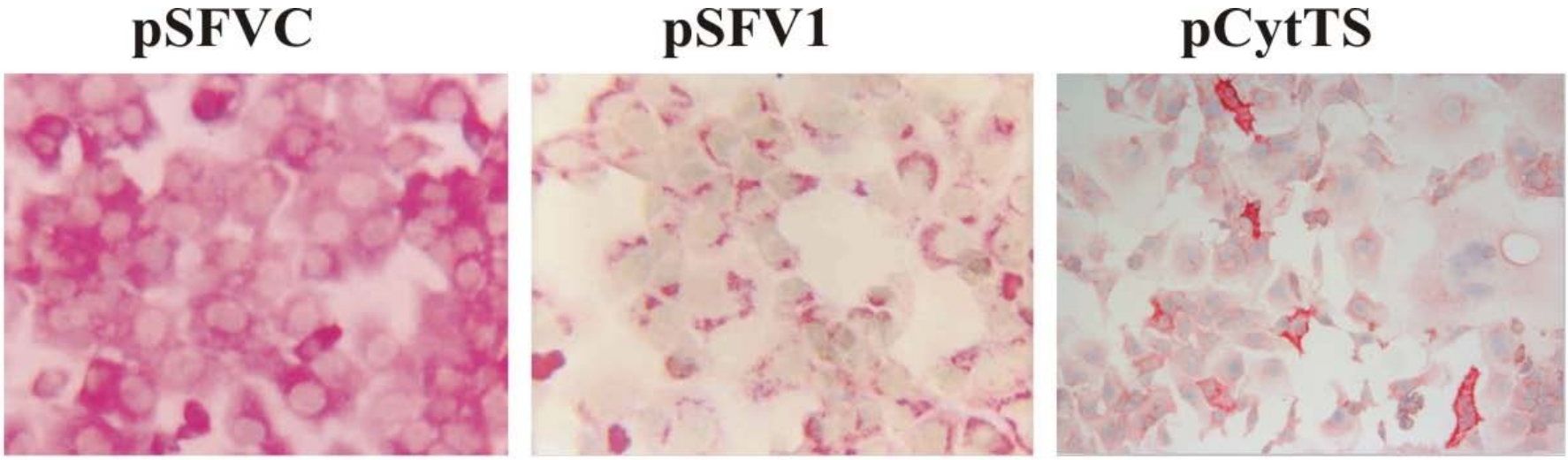
pSin-SV40-HDV-SV40pA
(Yamanaka and Xanthopoulos, 2004)[59]

Non-cytopathic

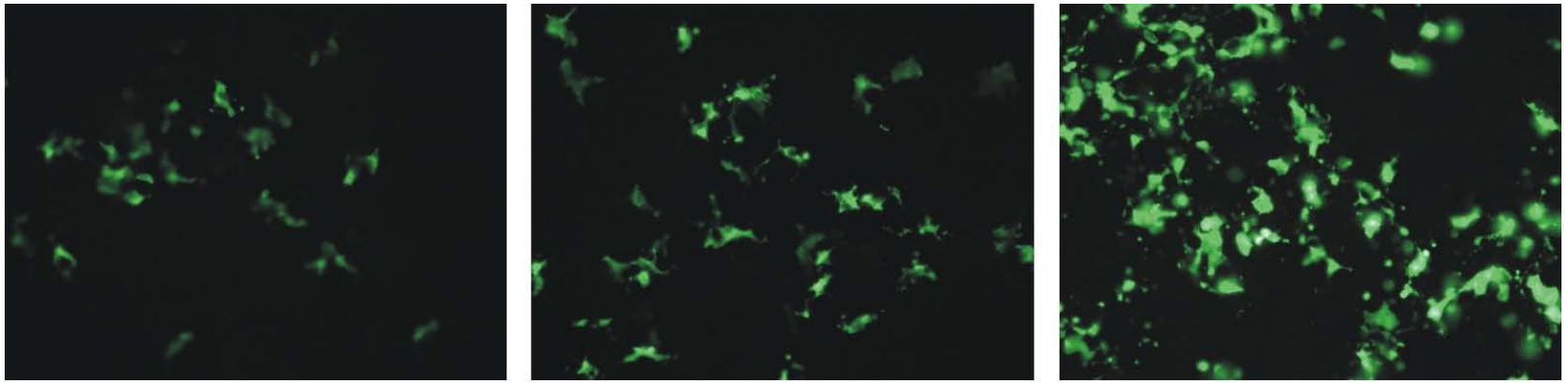
pSINrep21 (Agapov et al., 1998)[50]

pCytTS (Boorsma et al., 2000)[54]

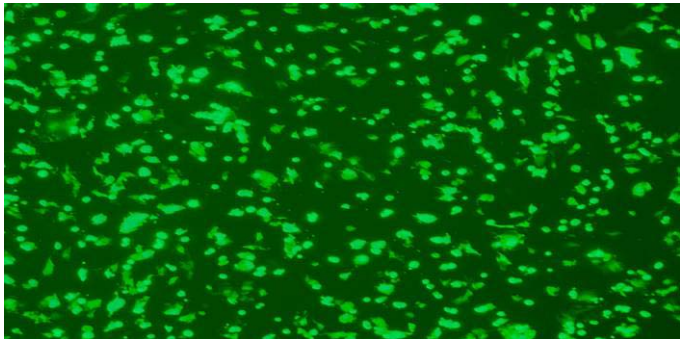
Schematic diagram of recombinant constructs developed on the basis of alphaviruses.



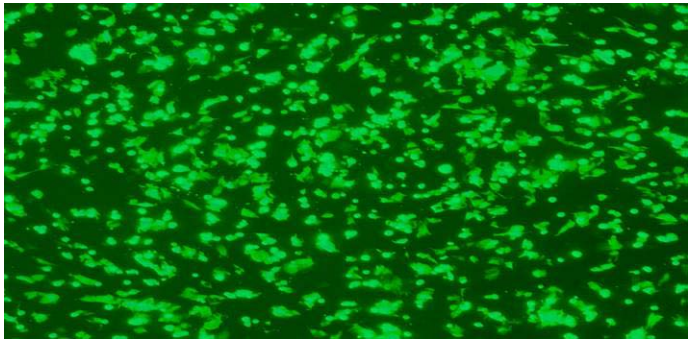
Comparison of recombinant hepatitis B virus core (HBc) gene expression by three vectors: pSFVC, pSFV1, pCytTS. (Zajakina *et* al, 2009)



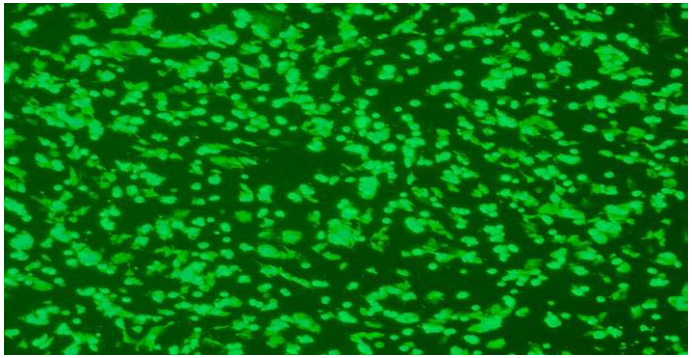
Kinetic of GFP protein expression in BHK cells transfected with pCytTS/EGFP. Induction time is indicated (3, 6, and 9 days), unpublished.



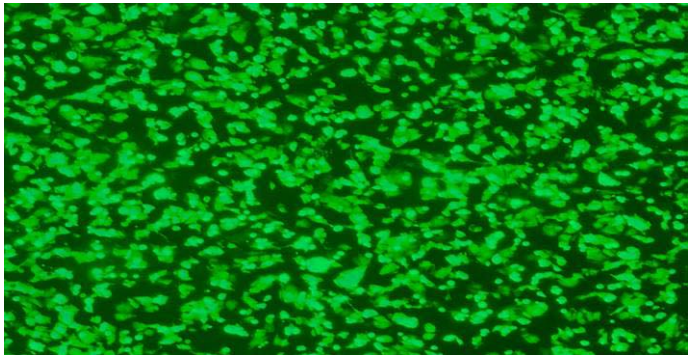
2 min



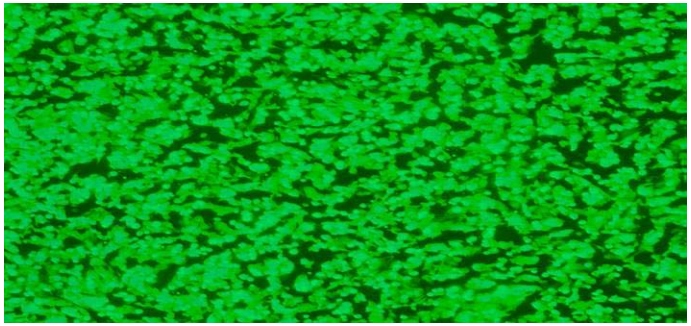
5 min



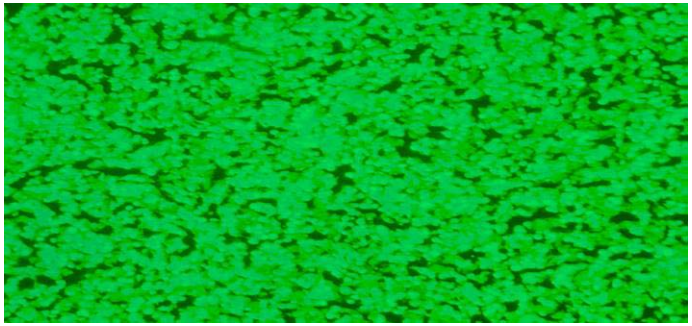
15 min



30 min

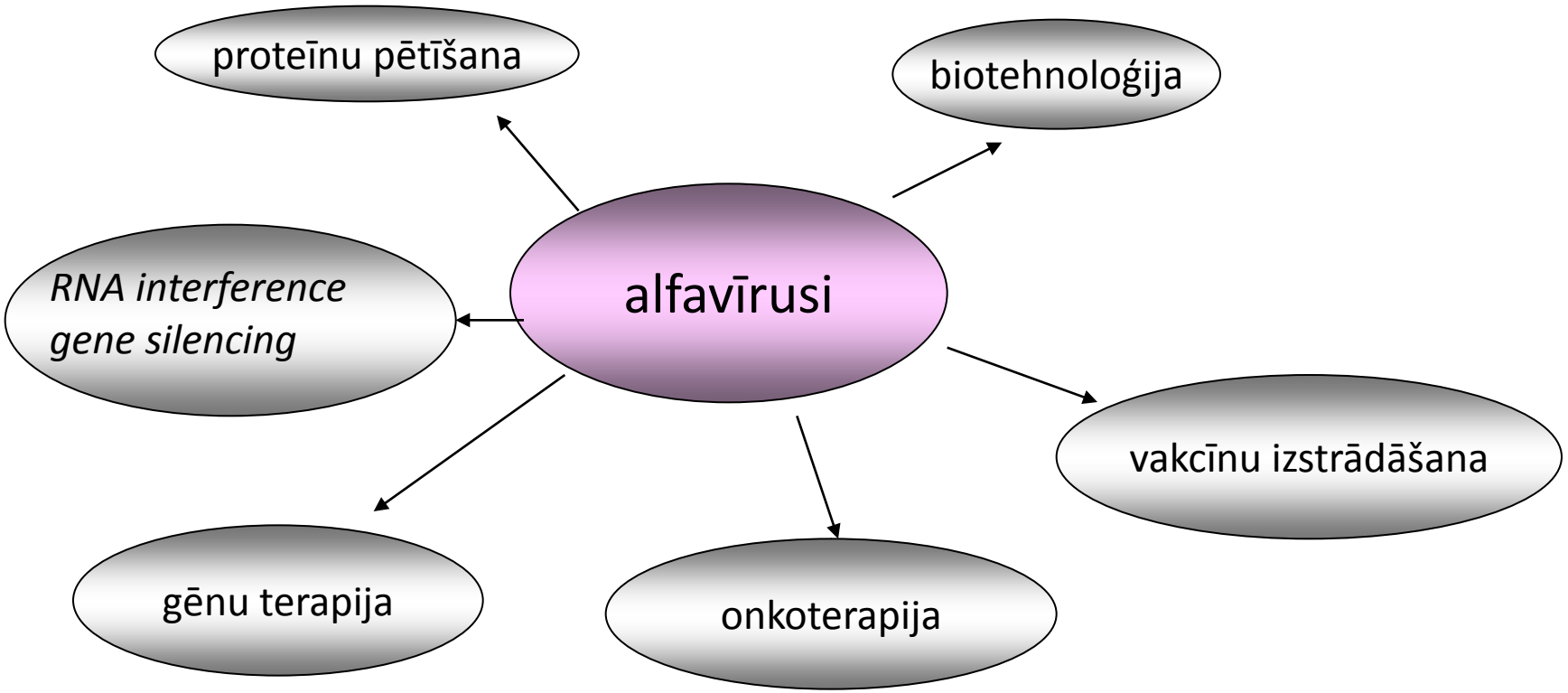


60 min



120 min

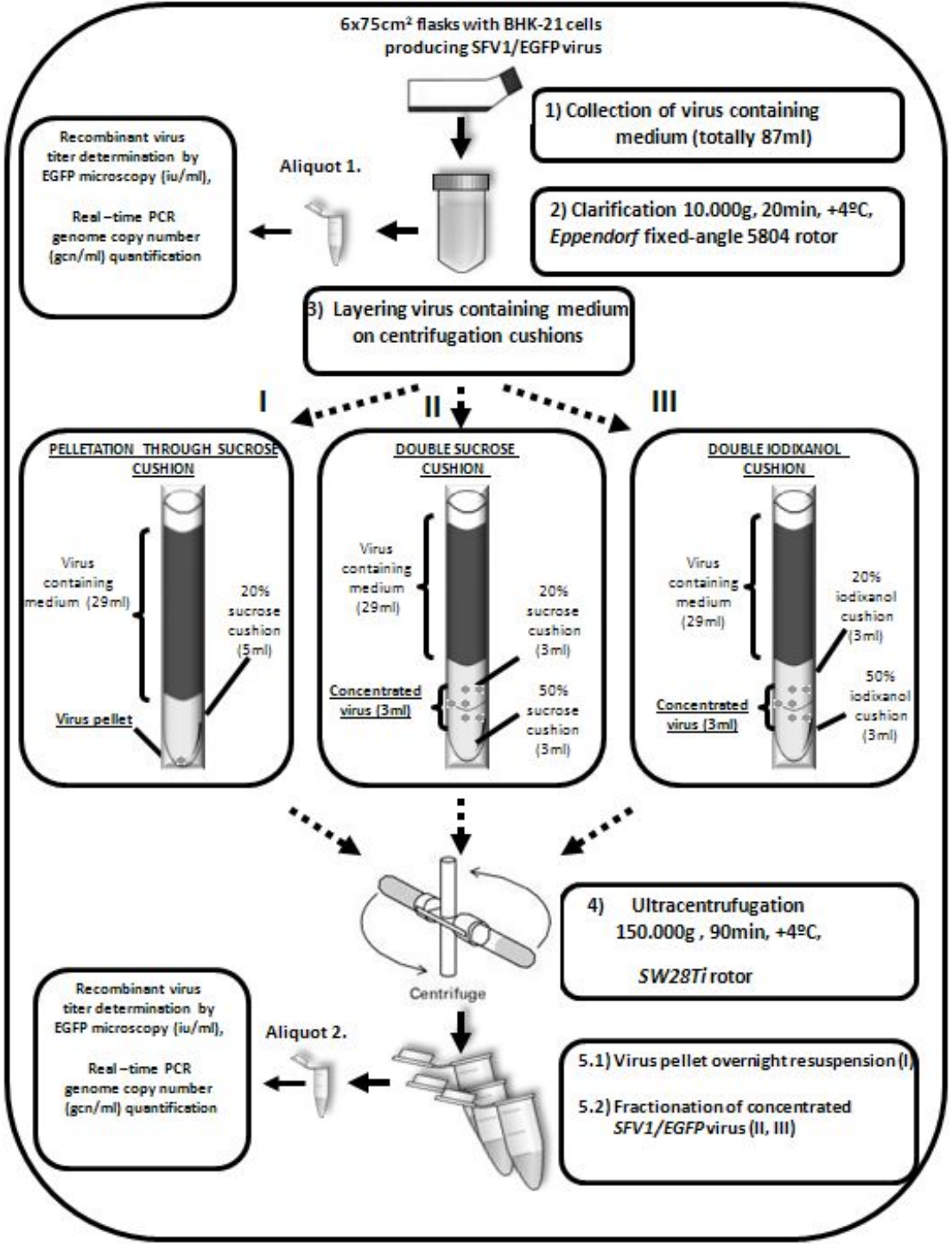
SFV-GFP Infection Kinetics in BHK Cells. (Lundstrom, 2002)



- ✓ Semliki Forest virus (SFV)
- ✓ Sindbis virus (SIN)
- ✓ Venezuelan Equine Encephalitis virus (VEE)

Alfavīrusu vektoru **priekšrocības** gēnu terapijas mērķiem

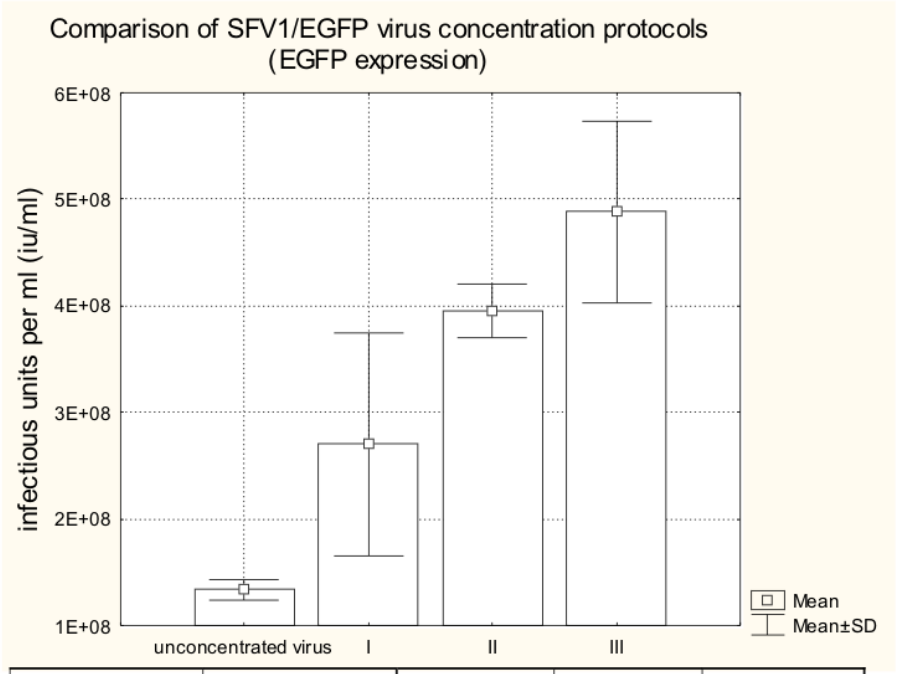
- ✓augsts transgēna produkcijas līmenis
- ✓plašs šūnu tropisms
- ✓pietiekoša vektora kapacitāte
- ✓biodrošs cilvēkiem
- ✓citopatiskais efekts
- ✓nav vektora pre-imunitātes
- ✓ir iespējams iegūt augstu vīrusa titru



Comparison of ultracentrifugation methods for concentration of recombinant alphaviruses: iodixanol and sucrose cushions

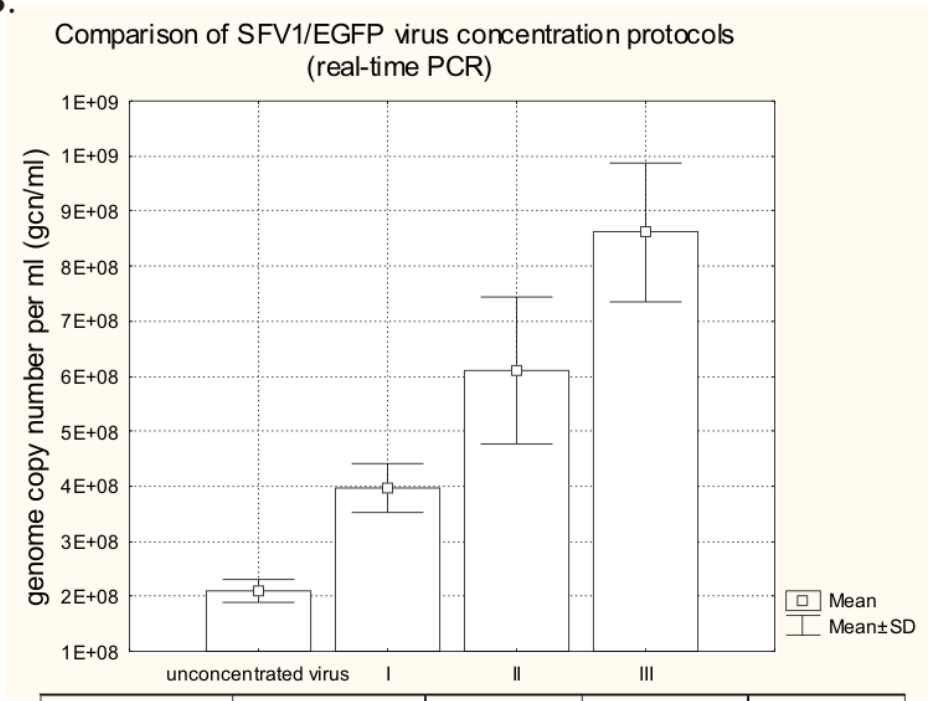
(Hutornojs et al, 2011, submitted at *Biol. Pocedures on Line*)

A.



	unconcentrated virus	I (pelletation)	II (double sucrose cushion)	III (double iodixanol cushion)
concentration factor (times)	× 1	× 2.02	× 2.95	× 3.64
SD of *iu*/ml values	+/- 6.57 %	+/-34.46 %	+/-5.86 %	+/-15.59 %
recovery yield	-	6.9 %	30.5 %	37.7 %

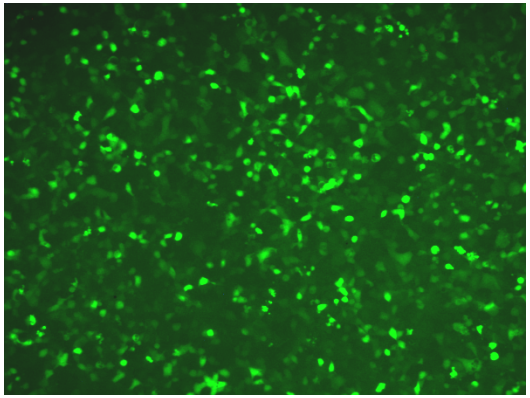
B.



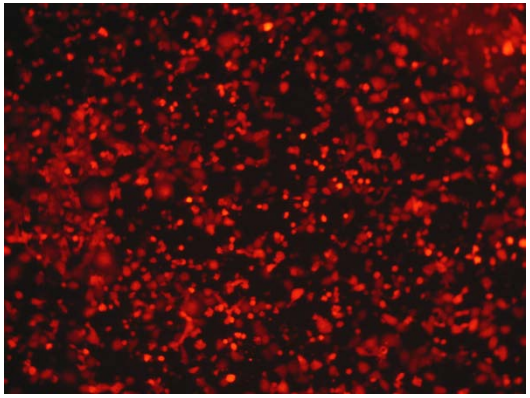
	unconcentrated virus	I (pelletation)	II (double sucrose cushion)	III (double iodixanol cushion)
concentration factor (times)	× 1	× 1.88	× 2.91	× 4.10
SD of *gcn*/ml values	+/- 8.43 %	+/-9.19 %	+/-17.83 %	+/-12.78 %
recovery yield	-	6.5 %	30.1 %	42.5 %

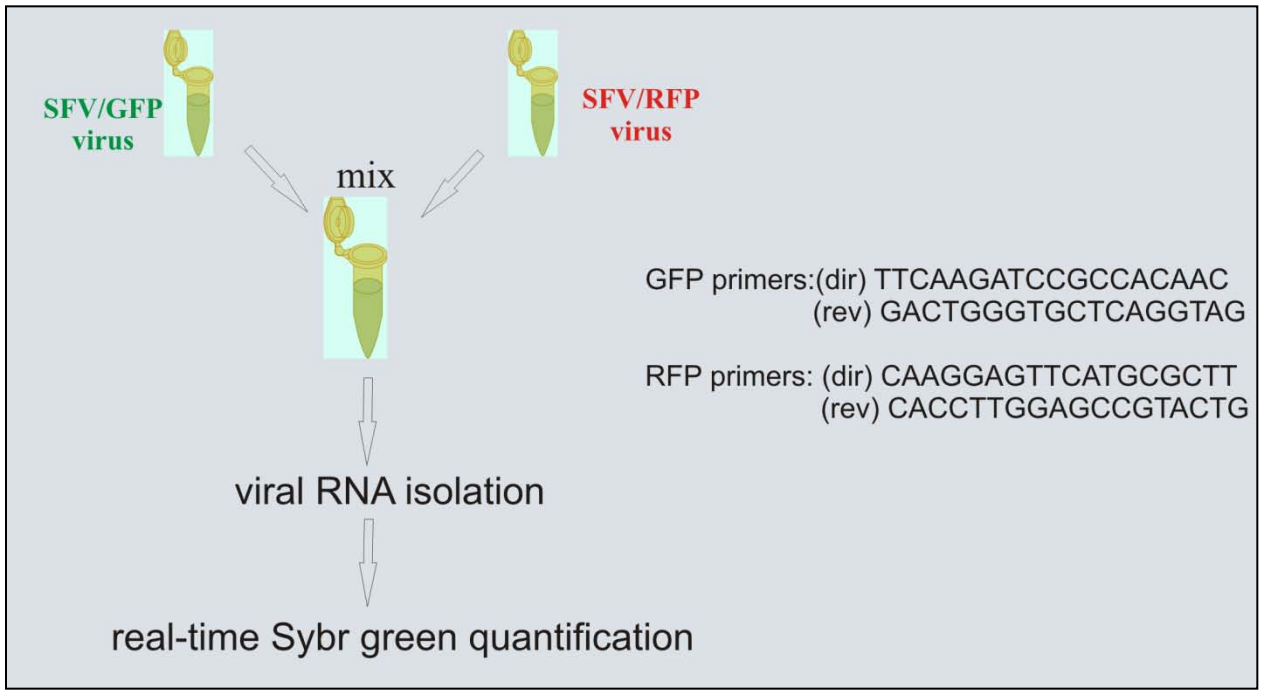
Comparison of ultracentrifugation methods for concentration of recombinant alphaviruses: iodixanol and sucrose cushions
(Hutornojs *et* al, 2011, submitted at *Biol. Pocedures on Line*)

SFV1/GFP



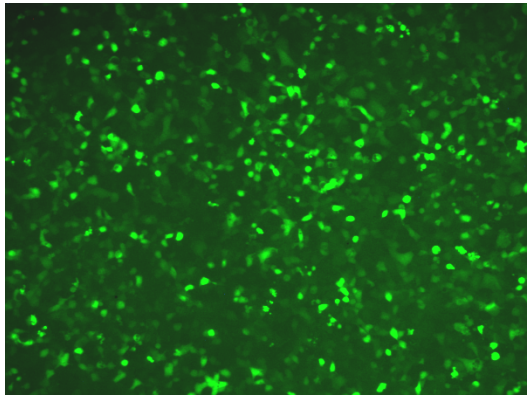
SFV1/Red





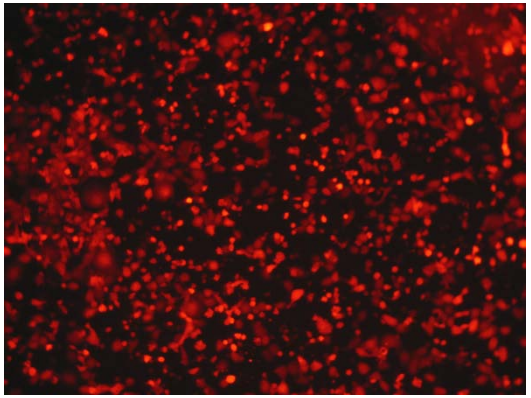
Reference virus as an internal standard for Semliki Forest virus real-time PCR quantification (Zajakina *et* al, 2011, *Current Opinion in Biotechnology*, Vol: 22: S113-114)

SFV1/GFP

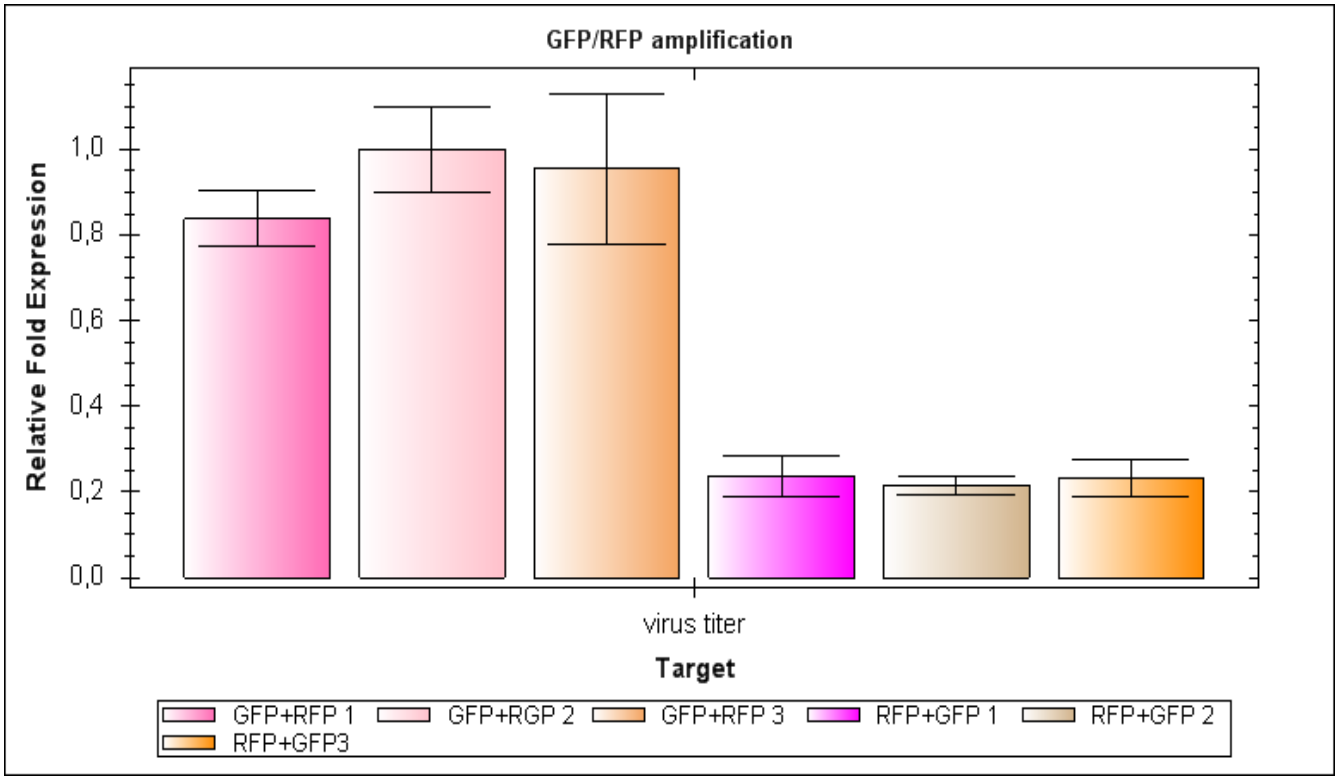


2,60 x 10^7 i.u./ml

SFV1/Red



0,56 x 10^7 i.u./ml



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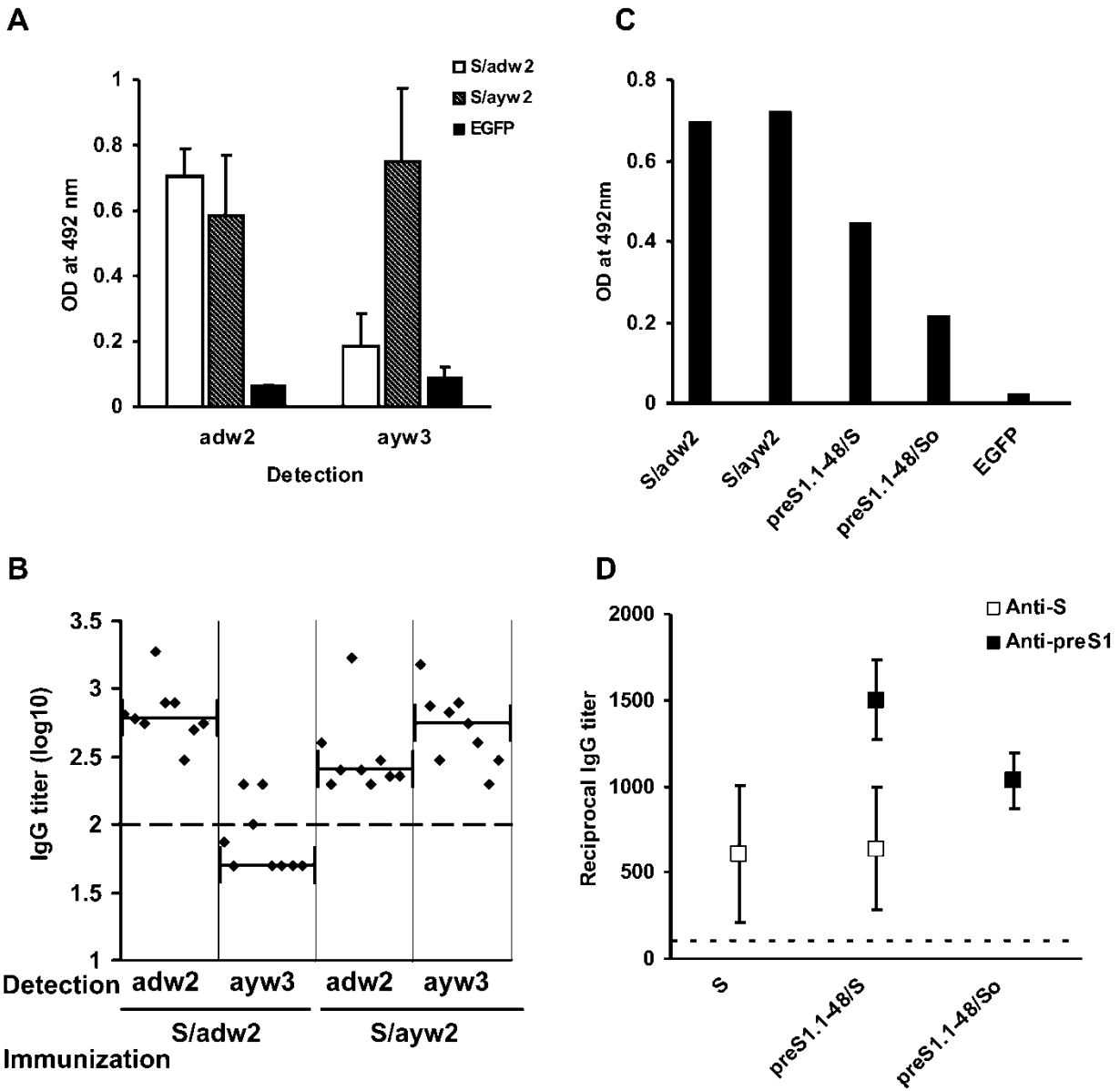
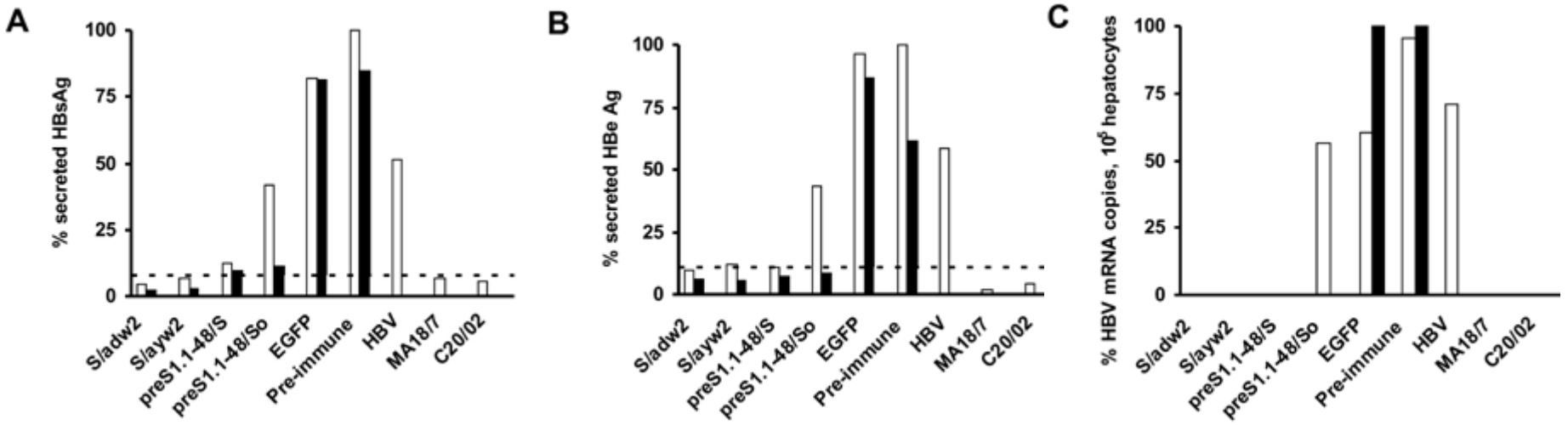


ELISA of HBV antibodies induced in BALB/c mice by immunization with rSFV vectors. (Niedre-Ottomere, *et* al 2011, accepted for *J. Viral Hepatitis*)



Markers of HBV infection in PTH cultures after inoculation with virus/antibody mixtures. (Niedre-Ottomere, *et* al 2011, accepted for *J. Viral Hepatitis*)

Table 1. Application of alphavirus vectors for generation of tumor vaccines.

Target	Gene	Vector/Delivery	Response	Ref
Brain tumor	IL-12	SFV/particles	Immunogenicity	[19]
Cervical cancer	HPV E6-E7	SFV/particles	Tumor protection	[20]
Glioma	B16, 203	SFV/particles	Tumor protection	[28]
Tumor	β-gal	SFV/RNA	Tumor protection	[26]
Tumor	HPV E7	VEE/particles	Tumor protection	[27]
Tumor	HPVE7-VP22	SIN/particles	$CD8^+$ T-cell response	[21]
Tumor	P815A	SFV/particles	Tumor protection	[22]
Tumor antigen	MHC Class II	SFV/particles, DNA	Immunogenicity	[23]
Tumor antigen	P185	SFV/particles	CTL, tumor protection	[25]
Tumor antigen	Tyr-related prot-1	SIN/DNA	Antitumor activity	[24]
Melanoma	MUC18	SIN/DNA	Tumor protection	[29]
Tumor	Neu	VEE/particles	Tumor protection	[30]
Prostate cancer	PSMA	VEE/particles	Immunogenicity	[31]

β-gal, β-galactosidase; CTL, Cytotoxic T-lymphocyte activity; HPV, human papilloma virus; IL, interleukin; MHC, major histocompatibility complex; MCAM, melanoma cell adhesion molecule; PSMA, prostate-specific membrane antigen; SFV, Semliki Forest virus; SIN, Sindbis virus; VEE, Venezuelan equine encephalitis virus.

Lundstrom, 2009

Alphavirus vectors

induce protective and therapeutic immune responses
against many tumor associated antigens

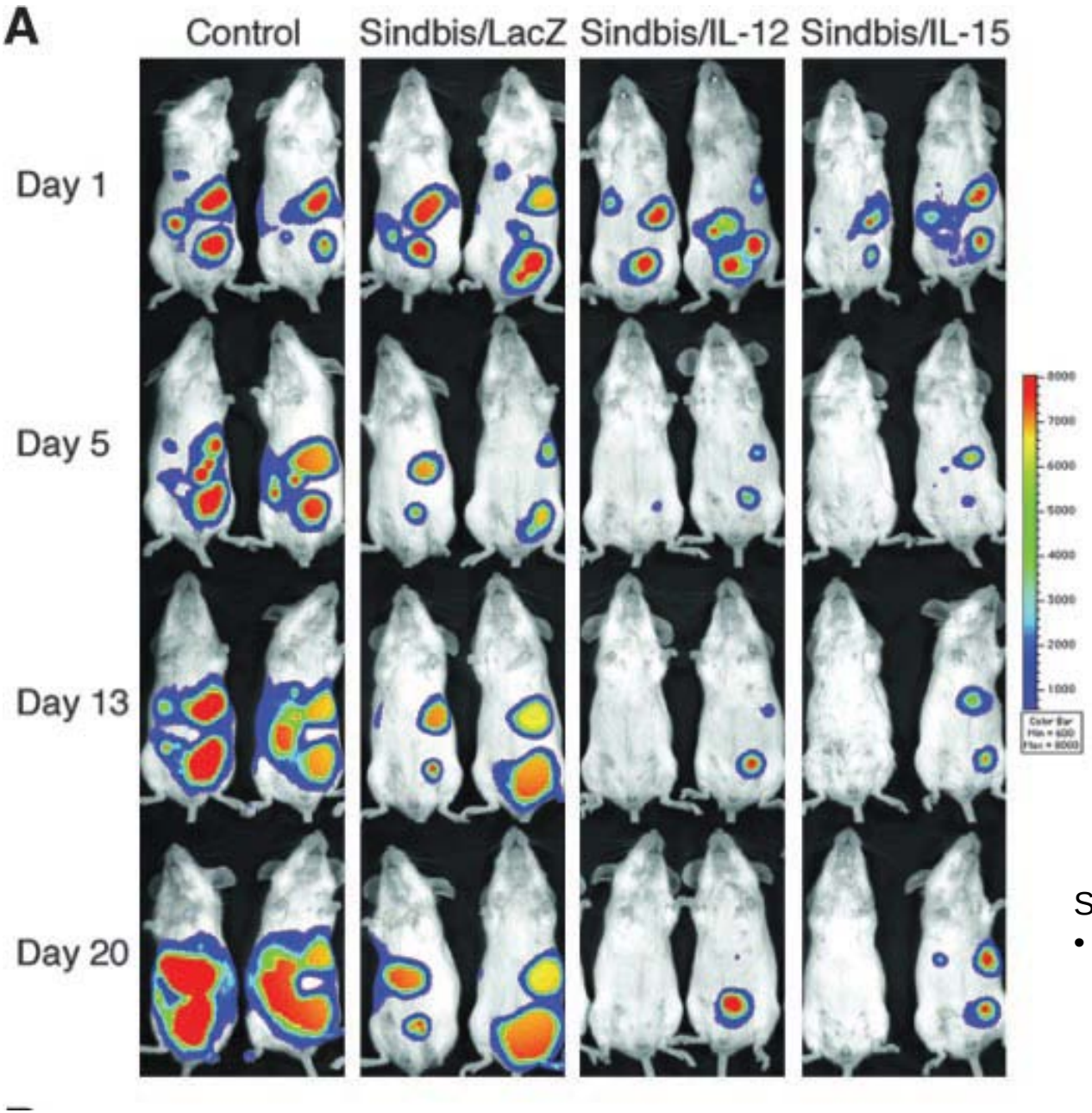
A phase I/II clinical trial for a CEA
(Carcinoembryonic Antigen) tumor vaccine

Colorectal cancer
Colorectal liver metastases
Breast cancer
Lung cancer
Skeletal metastases
Nonmalignant liver disease
Pancreatic disease
Smoking
Ageing
Atherosclerosis

antitumoral efficacy by expressing
antitumoral molecules in tumor cells:
cytokines, antiangiogenic factors
or toxic proteins

LipoVIL12 are in Phase I/II Clinical trials
in Europe against seven major
human malignancies
(lung, breast, prostate, pancreatic, head & neck,
melanoma and kidney carcinomas).

«Natural» tumor targeting



Sindbis Virus

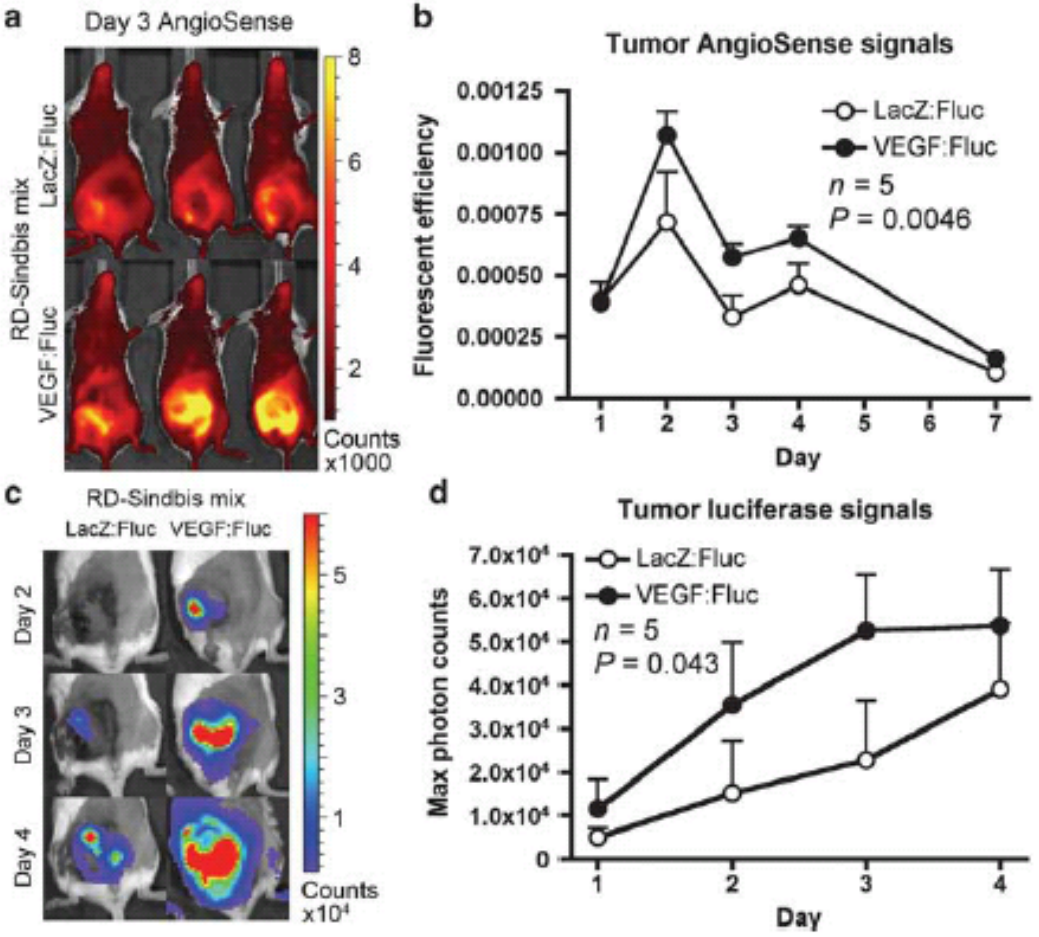
- Tumor targeting of naked replication-deficient virus (Tseng et al., Nat. Biotech 2004)

Cancer Gene Therapy (2010) 17, 244–255
 0929-1903/10 $32.00
www.nature.com/cgt

ORIGINAL ARTICLE

Enhanced specific delivery and targeting of oncolytic Sindbis viral vectors by modulating vascular leakiness in tumor

J-C Tseng[1], T Granot[1], V DiGiacomo[1], B Levin[1] and D Meruelo[1]



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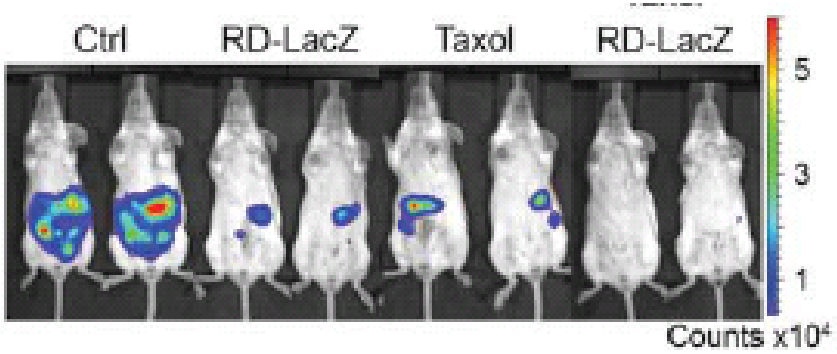

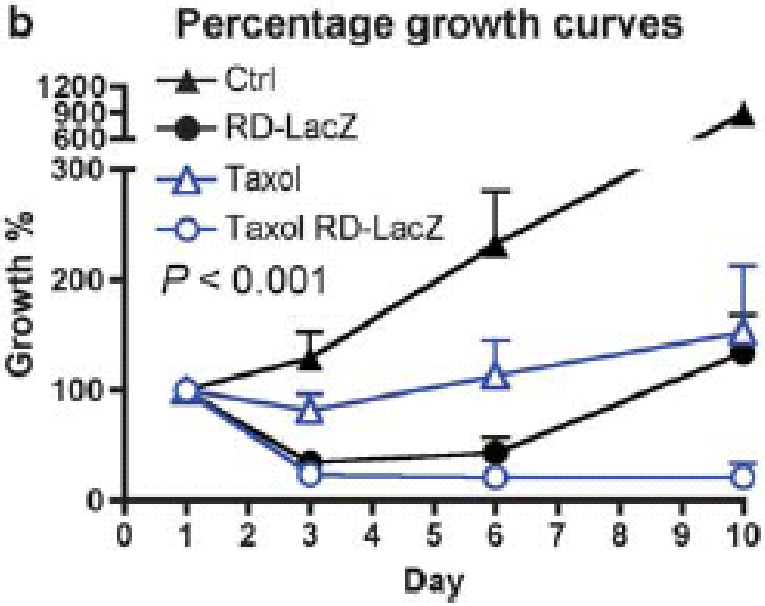

Latvijas Biomedicīnas pētījumu un studiju centrs
biomedicīnas pētījumi un izglītība no gēniem līdz cilvēkam

Gēnu terapijas problēmas:

1.gēnu piegāde (*biodistribution*)
2.efektivitāte
3.biodrošība

Alfavīrusu vektori

gēnu piegāde
- nespecifiskā piegāde
- pret vektora imunitāte atkārtotas infekcijas gadījumā
+ inficē lielu šūnas spektru
+ inficē dendrītu šūnas
+ nav preimunitātes

biodrošība
- relatīvi bīstami
+ neintegrējās genomā
+ replikācijas gadījumā neizraisa smagu saslimšanu

efektivitāte
- ierobežots ekspresijas laiks
+ augstais produkcijas līmenis
+ inducē apoptozi
+ ātra terapeitiskā gēna ekspresija

In vivo pētījuma uzdevums:

Latvijas Biomedicīnas pētījumu un studiju centrs
biomedicīnas pētījumi un izglītība no gēniem līdz cilvēkam

Pārbaudīt un optimizēt SFV vadītas Luciferāzes gēna ekspresiju *in vivo*

Stratēģija

1.Izpētīt transgēna biosadale in vivo izmantojot:
✓Vīrusu daļiņas
✓Brīvas RNS ievadīšanu
✓RNS/liposomu kompleksu ievadīšanu

2. Modelēt transgēna ekspresiju *in vivo* izmantojot dažādas transgēna ievadīšanas kombinācijas:
✓Infekcija+brīvā RNS
✓Infekcija + RNS/liposomu komplekss + brīvā RNS....

3. Alfavīrusu gēnu piegādes un onkolitiskās īpašības raksturošana peļu audzēju modeļos

Alfavīrusa spēja mērķtiecīgi piegādāt transgēnu vēža šūnām

Maģistra darbs:
"Alfavīrusu vektori kā gēnu piegādes līdzekļi pretvēža terapijai", autore Jeļena Vasiļevska, darba vadījājs Anna Zajakina, recenzente Jekaterina Ērenpreisa. Latvijas Universitāte, Mol. Biol. katedra. Jūnijs, 2011.

Konferenču tēzes:
A. Zajakina, Y. Vasilevska, D. Skrastina, and T. Kozlovskairal, "Semliki Forest Virus Vector distribution in mice" "Vectors in Gene Therapy", Finland, Kuopio, 2010.

A. Zajakina, J. Vasiļevska, D. Skrastiņa, T. Kozlovska „Rekombinanto alfavīrusu vektoru pretvēža aktivitāte *in vivo*", stenda ziņojums. Apvienotais Pasaules latviešu zinātnieku III kongress un Letonikas IV kongress „Zinātne, sabiedrība un nacionālā identitāte". Latvijā, Rīgā, 2011. gada 24.-27. oktobrī.

Publikācija

Alphavirus biodistribution in healthy and in 4T1 mammary tumour bearing mice: efficient tumour targeting by systemic virus injection. J.Vasilevska, D.Skrastina, T.Kozlovska, A.Zajakina. *Cancer Gene Therapy*, in preparation. 2011