



Recombinant Viruses

- Viruses produced by recombinant DNA technology
- Naturally occurring recombinant virus (= when more than one virus strain infects the same cell and genetic information is exchanged)



Common recombinant viruses used in biomedical research

- Baculovirus
- Adenovirus
- Adeno-associated virus
- Lentivirus/Retrovirus
- Many more: Vaccinia virus, Semliki Forest virus



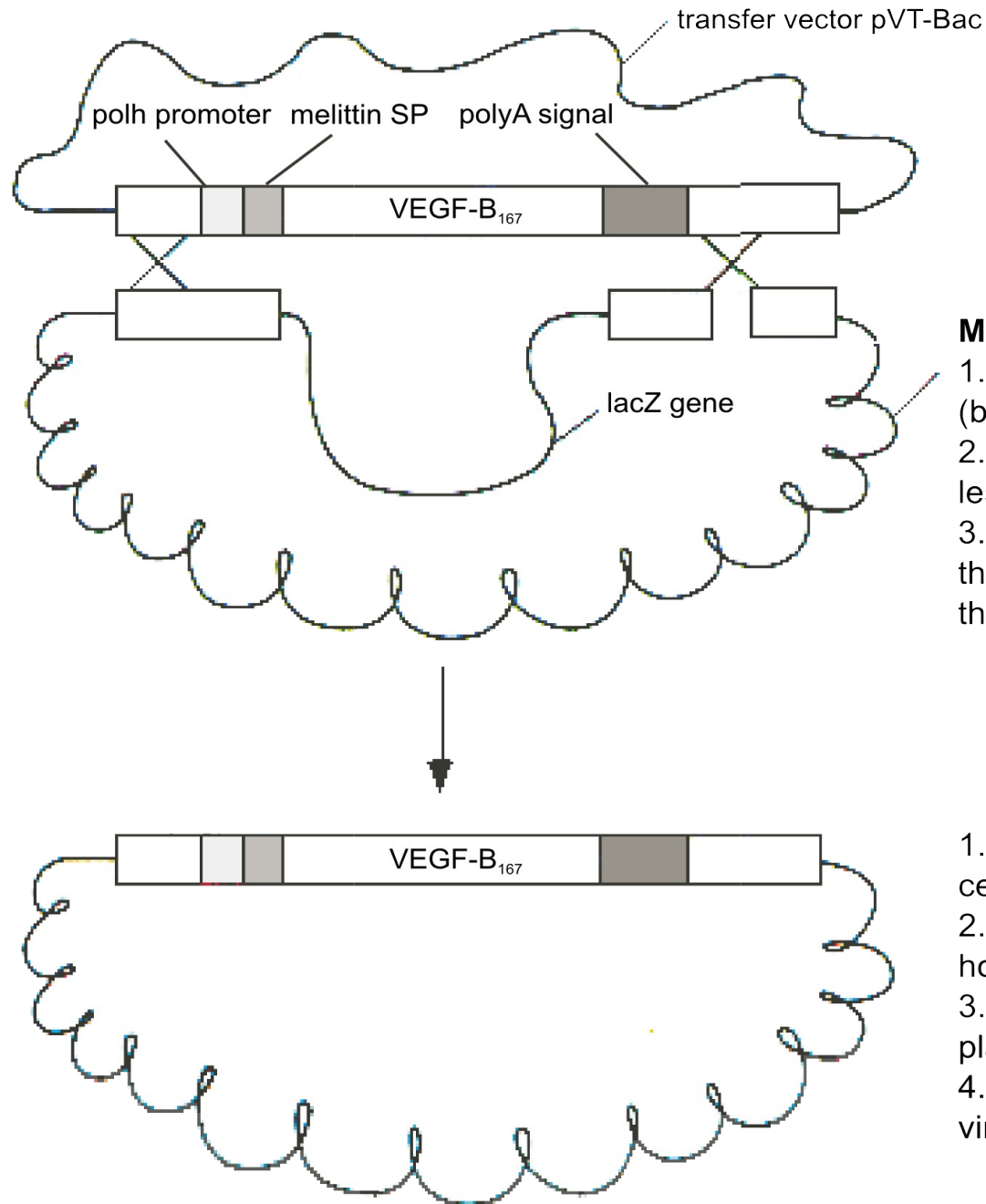
Common recombinant viruses used in biomedical research

Viral vectors

- Gene delivery for difficult-to-transfect cells; to make stable cell lines or transgenic animals (Lentivirus, retrovirus): over-expression & knock-down
- for recombinant protein expression (e.g. papillomavirus vaccine)
- as vaccines (since Jenner & others in the second half of the 18th century: mild cowpox infection protects against serious small pox infection); increased safety due to more control over virulence
- as therapeutics (e.g. oncolytic viruses - evidence for the possibility, but mechanistically not yet well enough understood to turn into an efficient drug)
- to express proteins in-situ in animal models



Recombinant baculoviruses



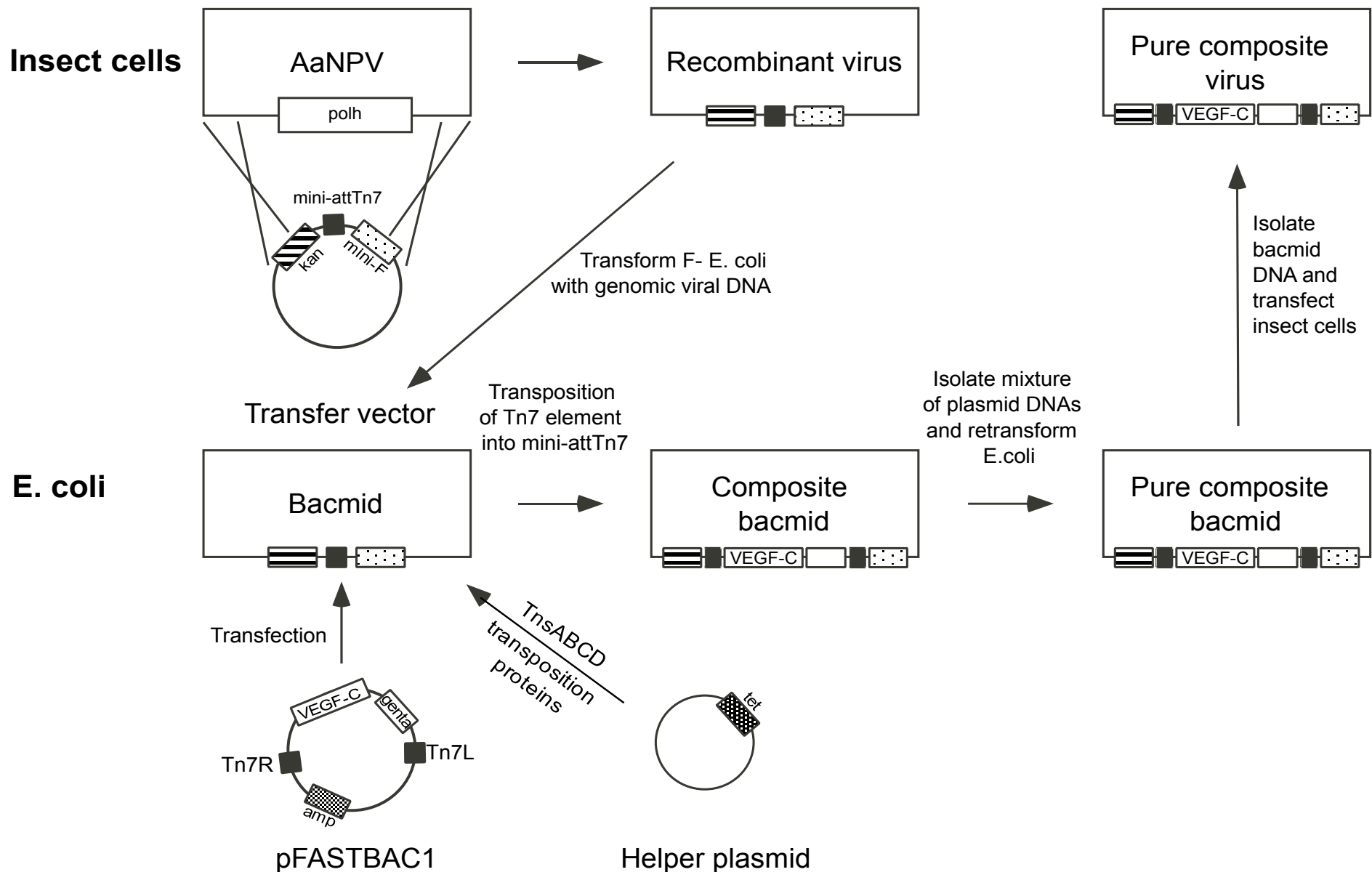
Modified AcMNPV

1. lacZ replaced by VEGF-B (blue/white screening)
2. linearized (linear DNA is much less infective than circular DNA)
3. containing a lethal deletion that must be complemented by the transfer vector

1. Cotransfection of insect cells (Sf9 or HighFive)
2. Allelic replacement by double homologous recombination
3. Screening for the correct plaque phenotype (white)
4. Plaque purification and virus amplification

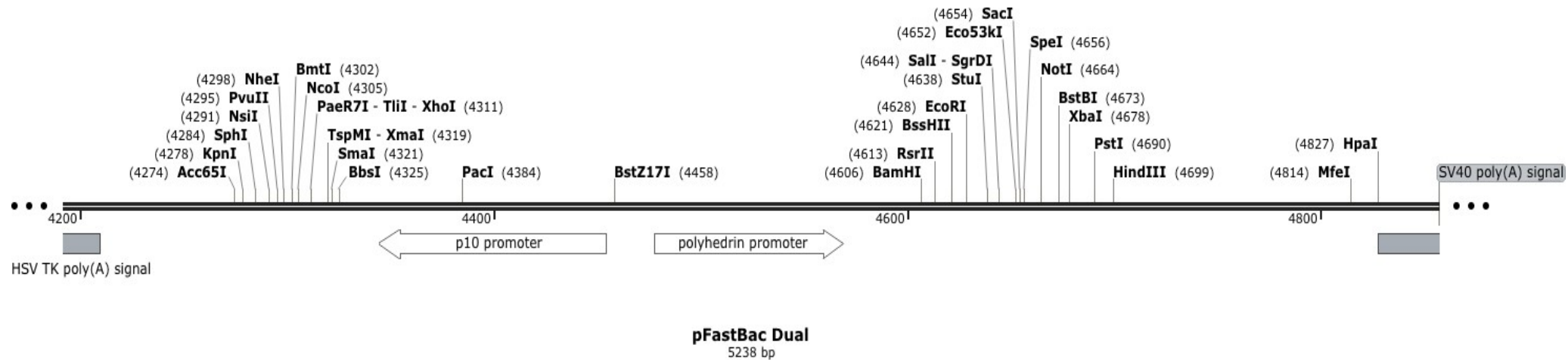


Bac-To-Bac[®] (LifeTechnologies, developed by Monsanto)





Baculovirus system enhancements

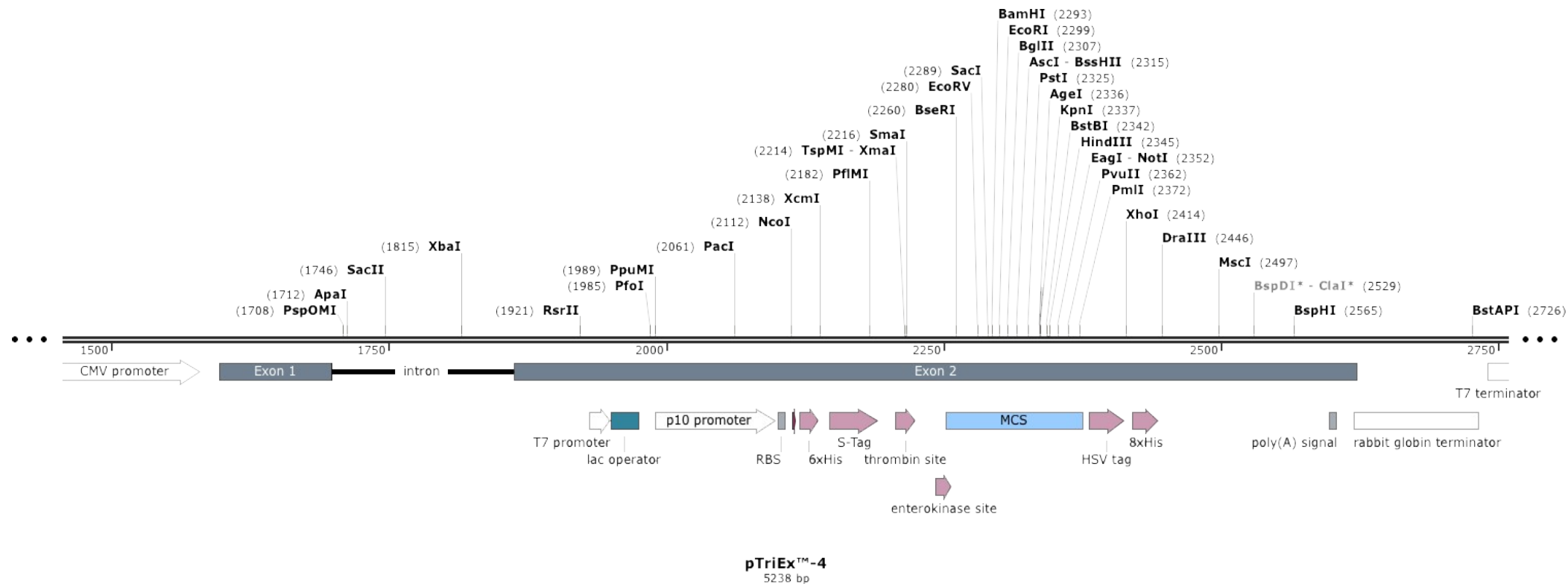


More than one GOI

- 2 GOIs: pFastBac Dual (**Life Technologies**)
- Many GOIs: MultiBac (**Geneva Biotech**)



Baculovirus system enhancements



The same plasmid for bacterial, baculoviral and mammalian expression

- pTriEx (Novagen→Merck/Millipore)
- pQE-TriSystem (Qiagen)



Baculovirus system enhancements

Mammalian-type glycosylation

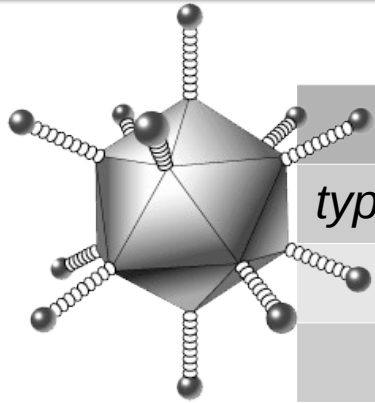
- SweetBac™ ([Geneva Biotech](#))
- Sf9 Mimic™ ([LifeTechnologies](#))

Protein quality/folding enhancements

- Adding enzymes (foldases/chaperones/protein disulfide isomerases): ProFold™ ([AB Vector](#))
- Deleting enzymes (viral proteases):
flashBAC ([Oxford Expression Technologies](#))



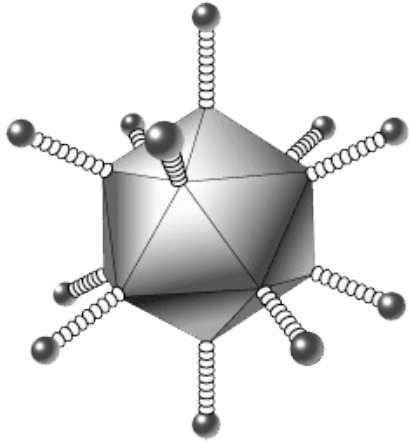
Adenovirus & Adeno-associated virus



	AV	AAV
<i>type</i>	pathogenic	non-pathogenic
	non-enveloped	non-enveloped
	replicating	replication-defective
<i>genome</i>	26-48 kb dsDNA	4.8 kb ssDNA
<i>immune response</i>	strong	mild
<i>expression</i>	short-lived	long-lived (possible integration into host genome)
<i>diameter (nm)</i>	~90	~20
<i>receptor</i>	coxsackievirus adenovirus receptor (CAR)/ $\alpha 5$ integrin	different (dependent on serotype)
<i>cargo (kb)</i>	up to 10	~4.8
<i>biosafety level (BSL)</i>	2	1 (in absence of helper virus)



Adenovirus



Crystal et al. Adenovirus: **The First Effective In Vivo Gene Delivery Vector.**
Human Gene Therapy. 25, 2014: 3-11.

Sandmair et al. **Adenoviruses as Gene Delivery Vectors.**
Advances in Experimental Medicine and Biology. 465, 2002: 423-429.

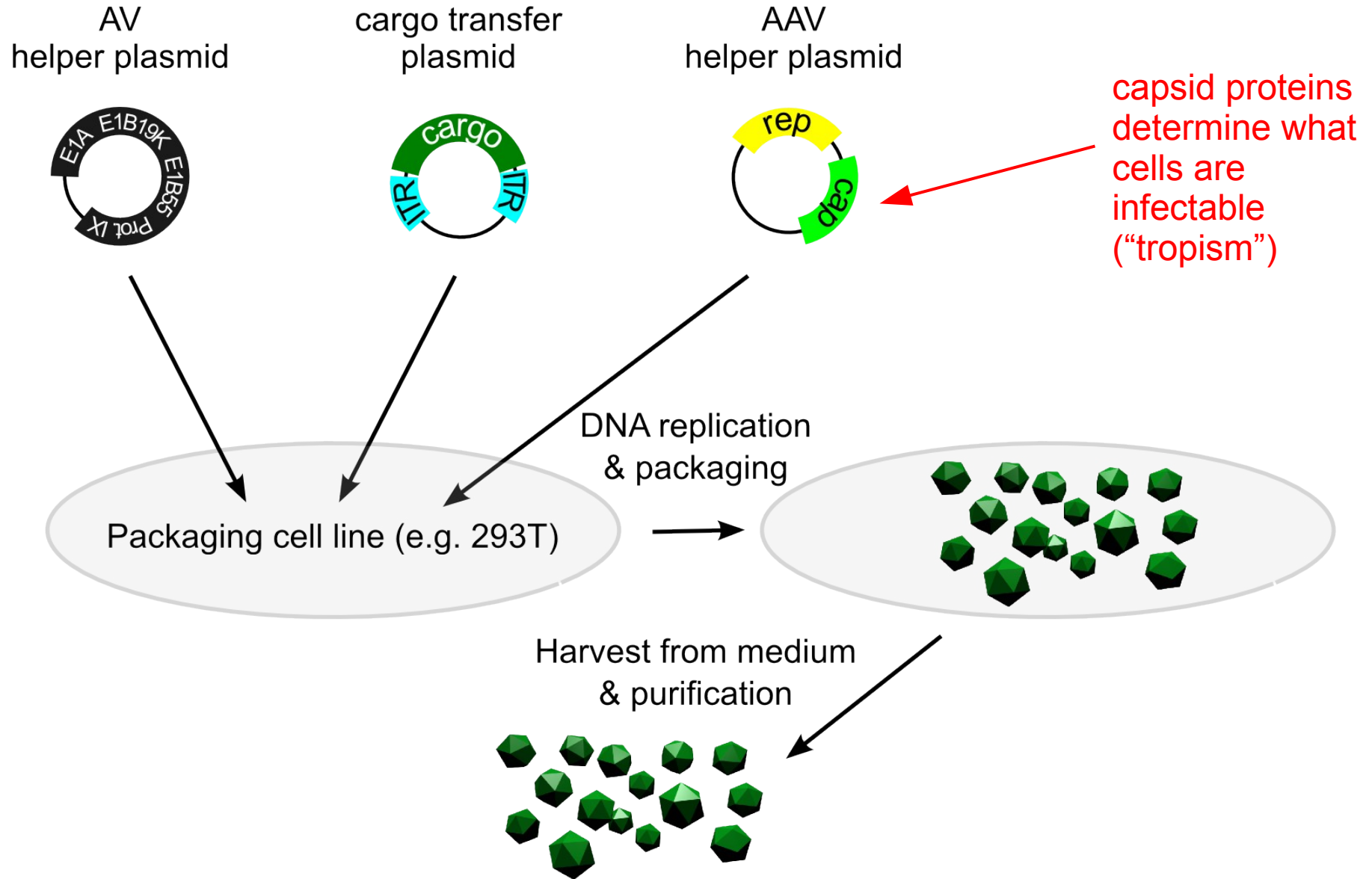
AdEasy System (developed in Bert Vogelstein's Lab)

He et al. **A simplified system for generating recombinant adenoviruses.**
PNAS. 95, 1998: 2509–14.

- Works similar to the Bac-to-Bac system
- Modified adenoviral DNA maintained as a plasmid in E.coli (AdEasier-1 or BJ5183 cells)
- Recombination with a shuttle vector to generate recombinant virus
- DNA prep to isolate viral DNA
- Transfection of 293 cells (packaging cells) to generate virus
- Non-proprietary (**Addgene**), Kit from **Agilent**



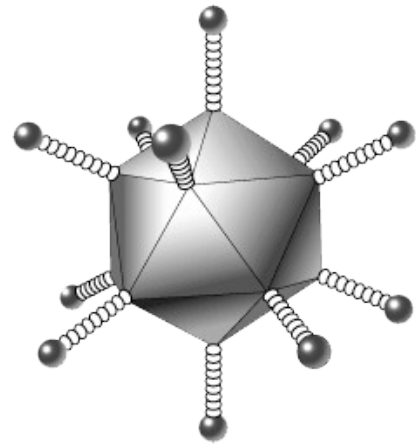
Adeno-associated virus



Büning et al. Recent developments in adeno-associated virus vector technology. Journal of Gene Medicine. 10, 2008: 717–33.



Adeno-associated virus



*Liver: AAV4, AAV7,
AAV8, AAV9*

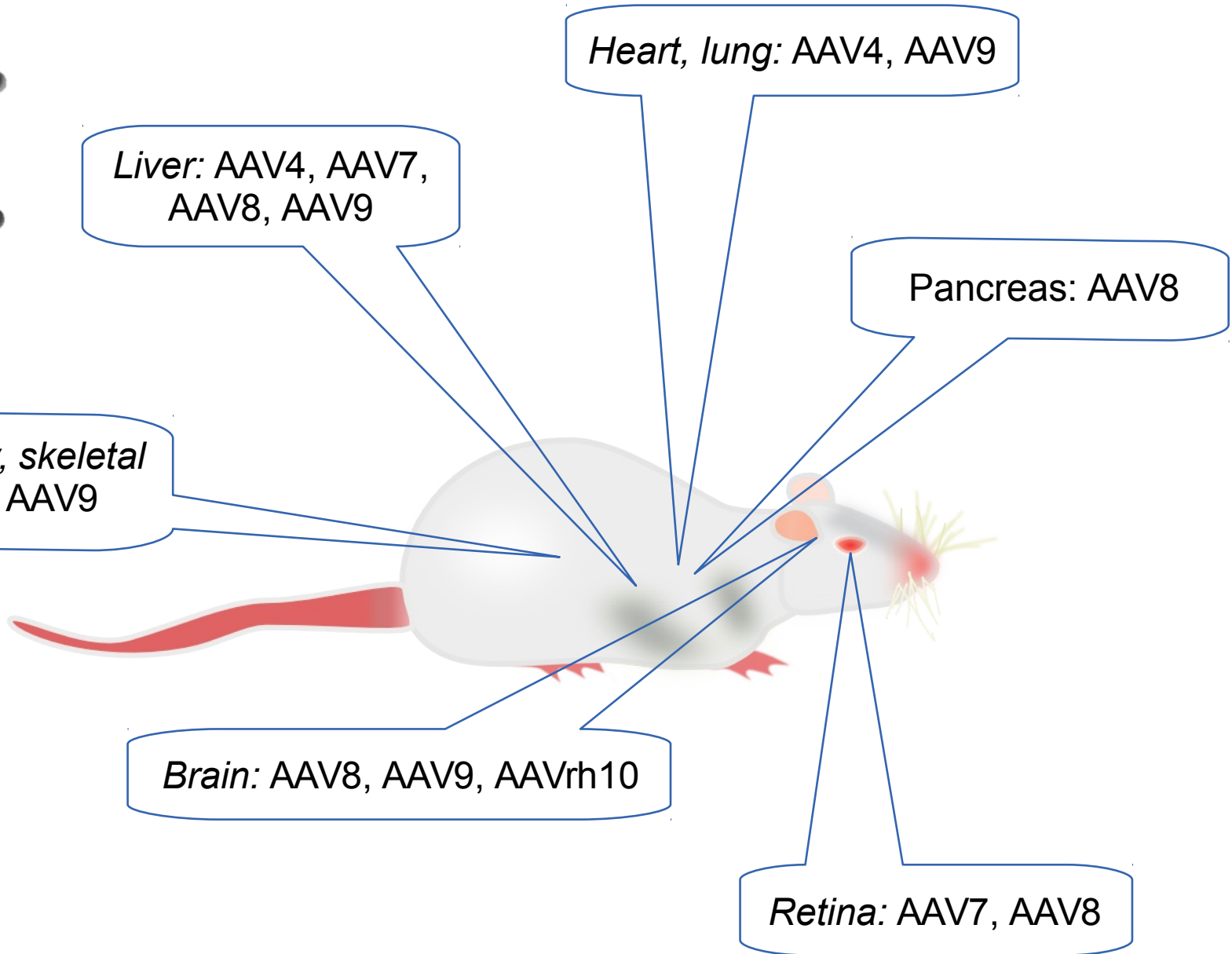
Heart, lung: AAV4, AAV9

Pancreas: AAV8

*Systemic delivery, skeletal
muscle: AAV8, AAV9*

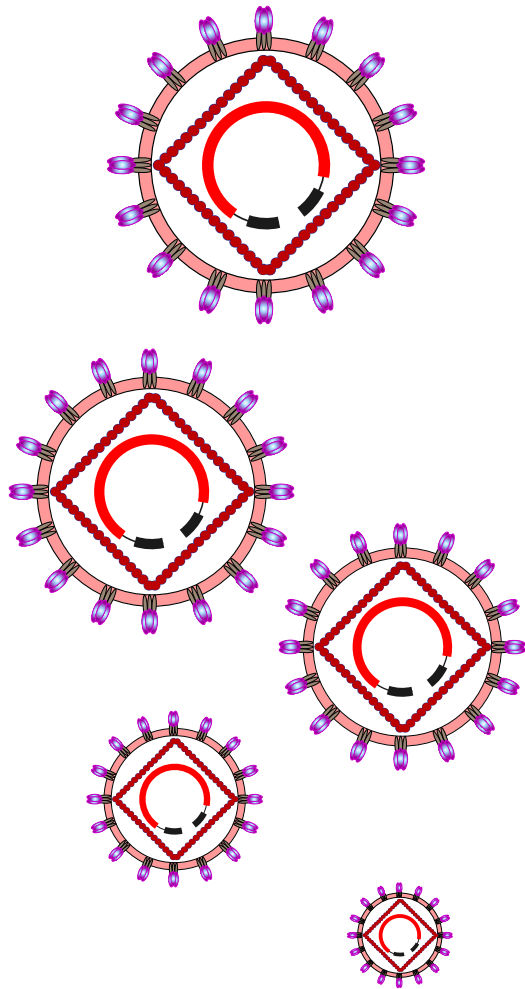
Brain: AAV8, AAV9, AAVrh10

Retina: AAV7, AAV8





Recombinant Retroviruses

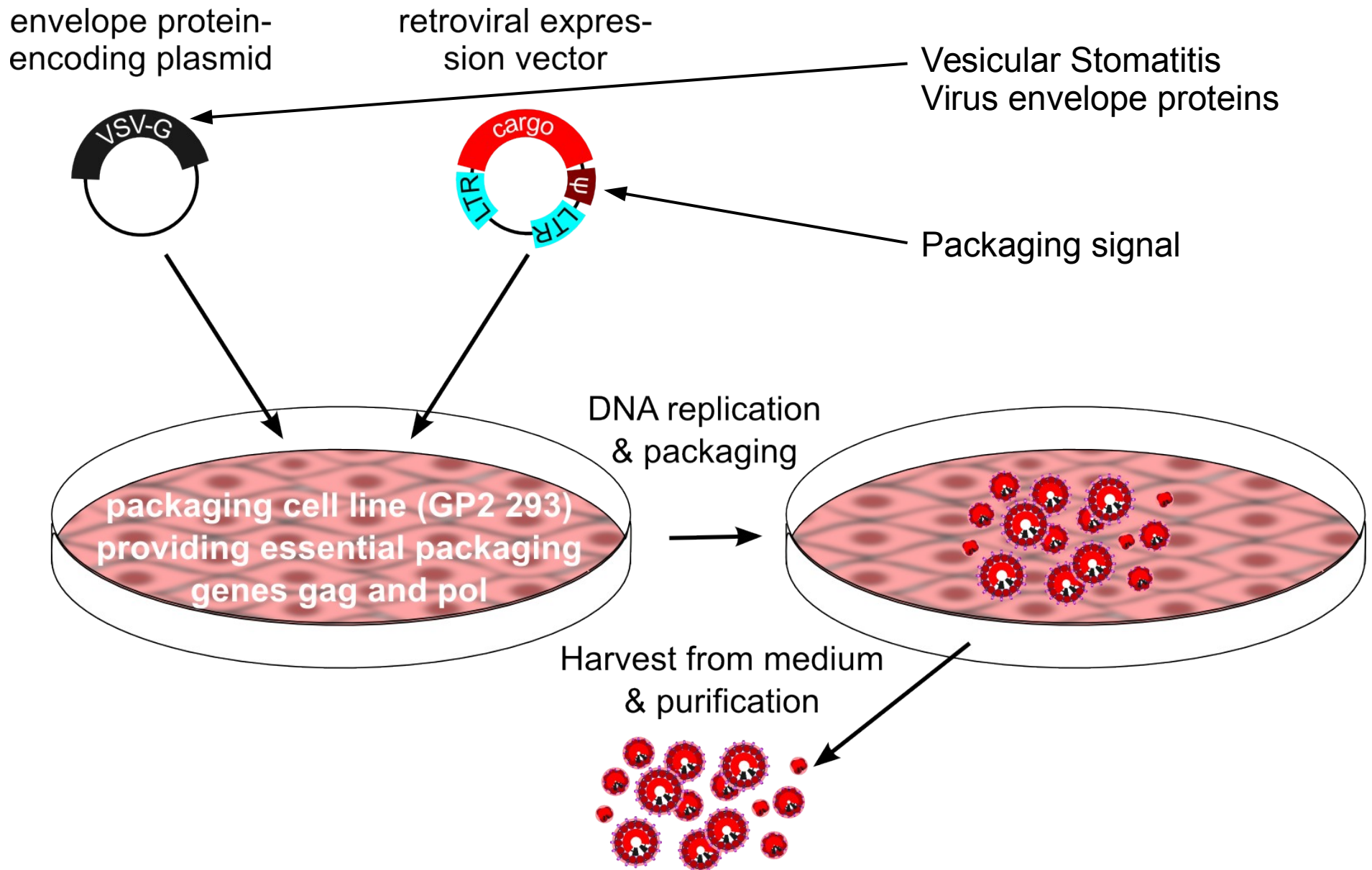


Recombinant Retroviruses

- Enveloped, ssRNA (= mRNA) genome
- Most commonly used recombinant retroviruses:
 - Moloney murine leukemia virus (MMLV)
 - Murine stem cell virus (MSCV)
- Narrow host range (ecotropic) vs wide host range (amphotropic)
- Host range is determined by the packaging cell line
- Infects efficiently only dividing cells
- BSL-2 (some experiments require BSL-3)
- Cargo up to ~8 kb
- Gene therapy: X-SCID, insertional activation of proto-oncogenes resulted in acute T-cell leukemia in a substantial proportion of patients
- Services available from **FUGU**

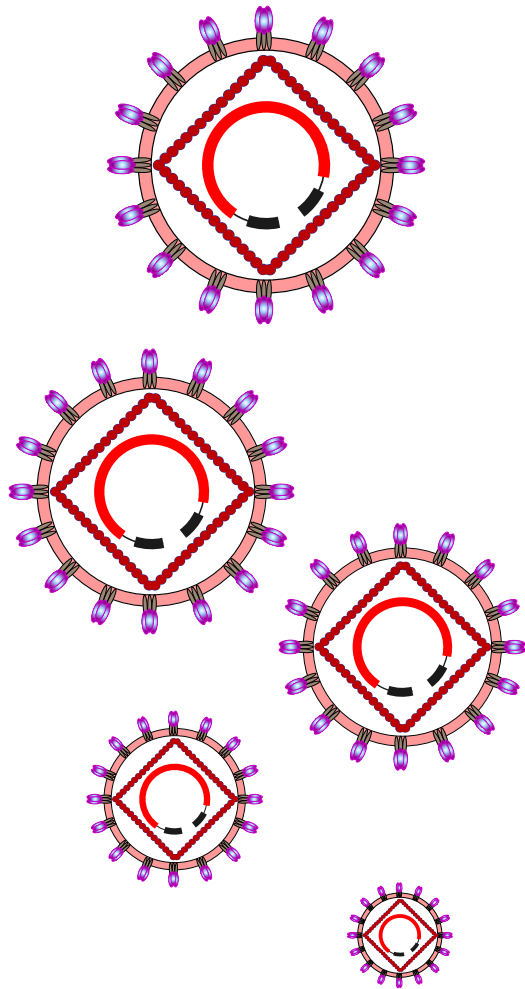


Recombinant Retroviruses





Recombinant Lentivirus



Recombinant Lentiviruses

- Enveloped, ssRNA (= mRNA) genome
- Most commonly used recombinant lentivirus:
 - HIV-1-based
- Transduces dividing and non-dividing cells
- BSL-2 (some experiments require BSL-3)
- Cargo up to ~8 kb
- Services available from **FUGU**



Missed a lecture to much?

Self-study the lecture you missed and take an exam!

- a) Inform the **course organizer** which lecture you would like to self-study.
- b) Agree with the **organizer** when to take the exam (Dec. 1st and 12th).

Practical course

- a) Starts on Monday, December 1st am 9:00 am,
Biomedicum Helsinki A509a2
- b) Schedule sent by e-mail