Construct Design for Mouse Models: Lentiviruses and Adenoviruses

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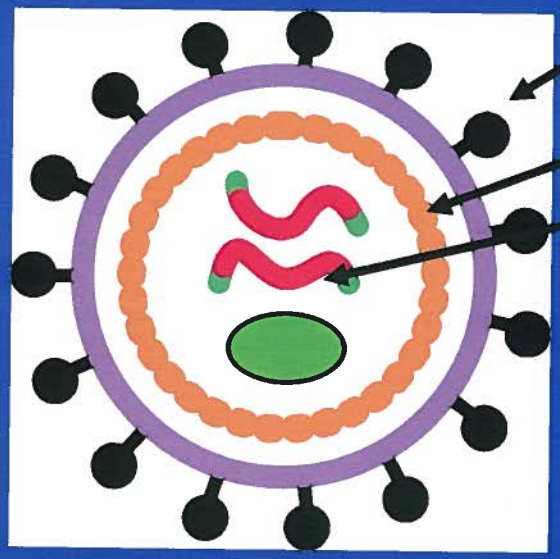
Lentiviral and Adenoviral Vectors

Retro

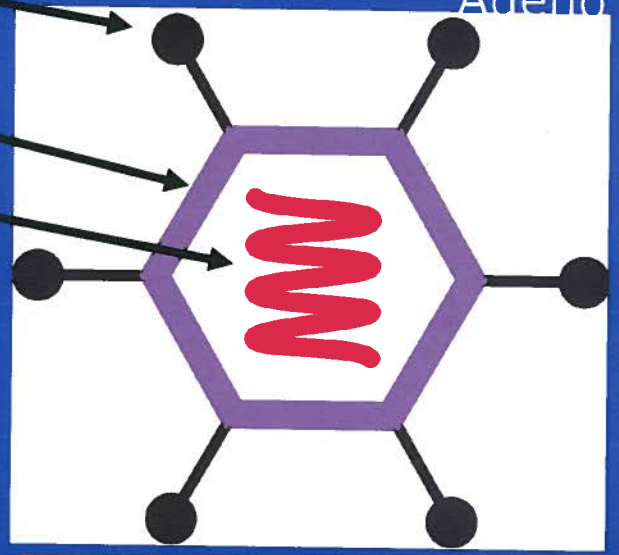


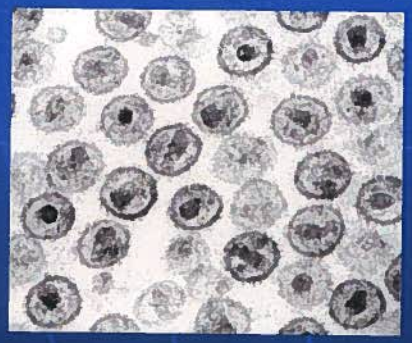
Surface Protein

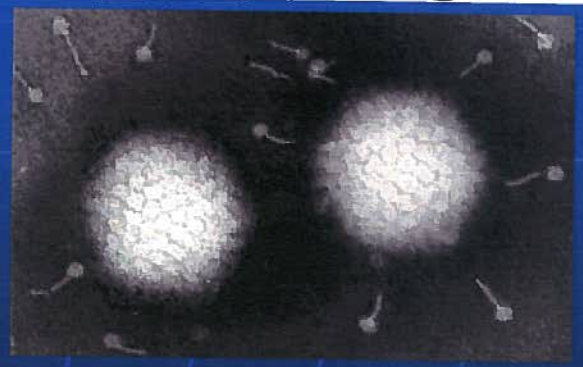
Capsid

Genome

Adeno



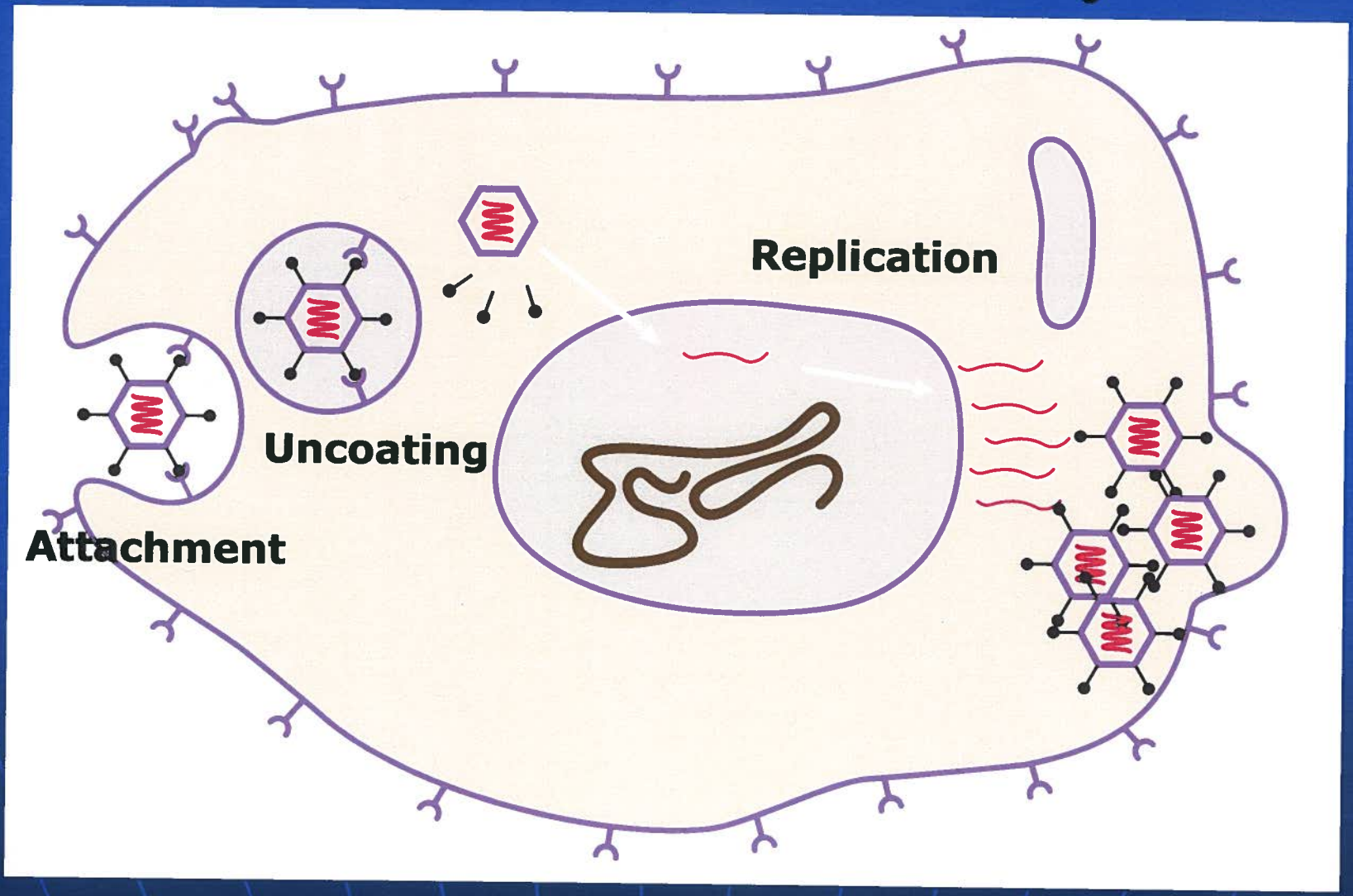




- Moderate Titer
- Broad tropism
- "Permanent expression"

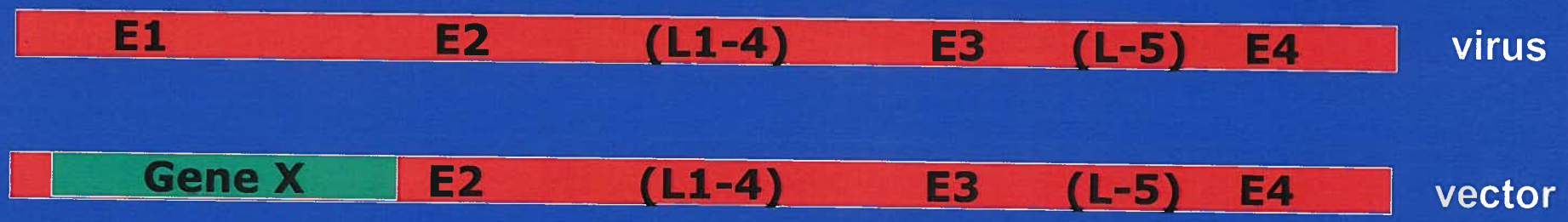
- High Titer
- Broad tropism
- Transient expression

Adenovirus Replication Cycle



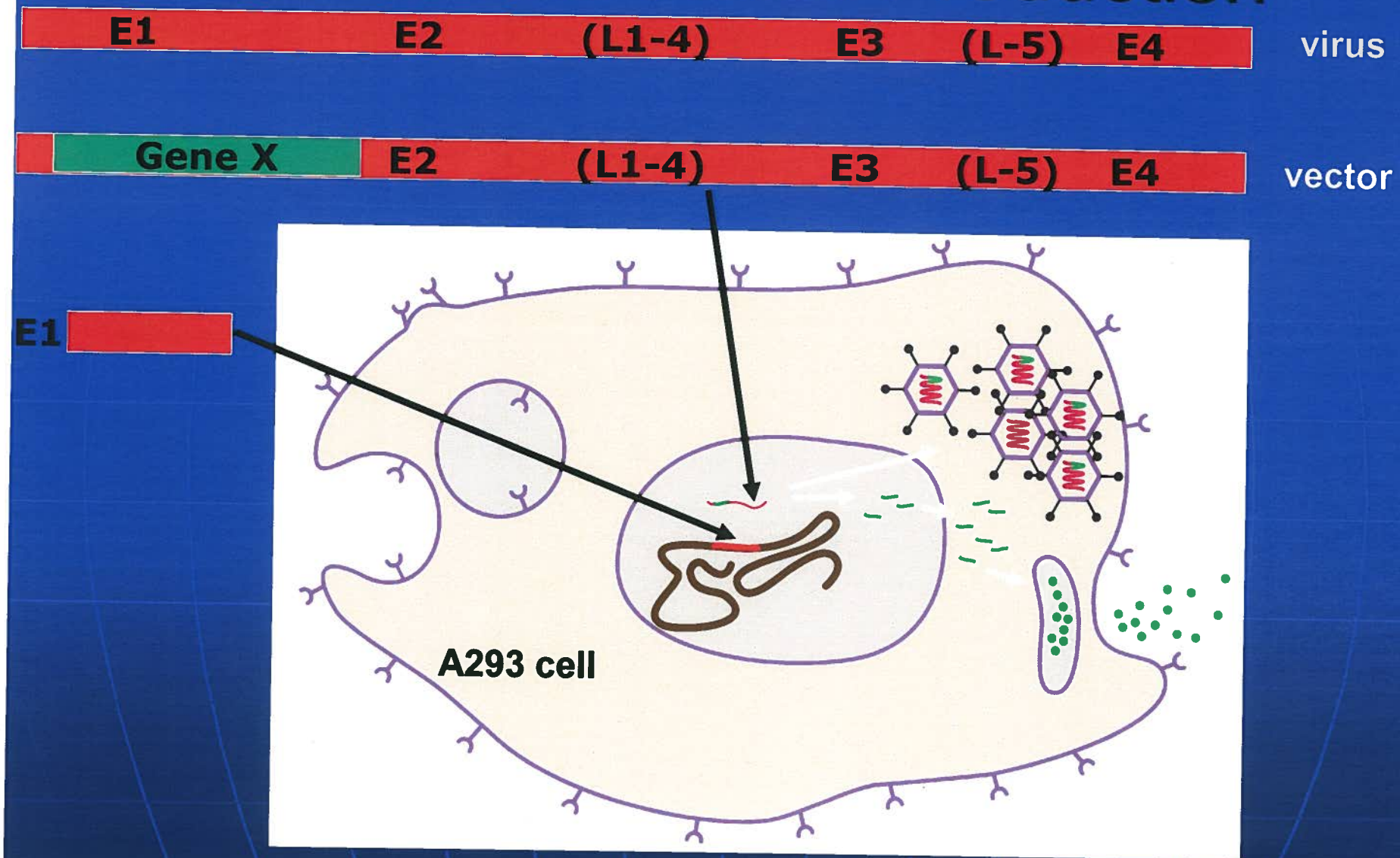
Unmodified retroviruses are "replication competent (production of progeny viruses that are infectious)

Making an Adenoviral Vector



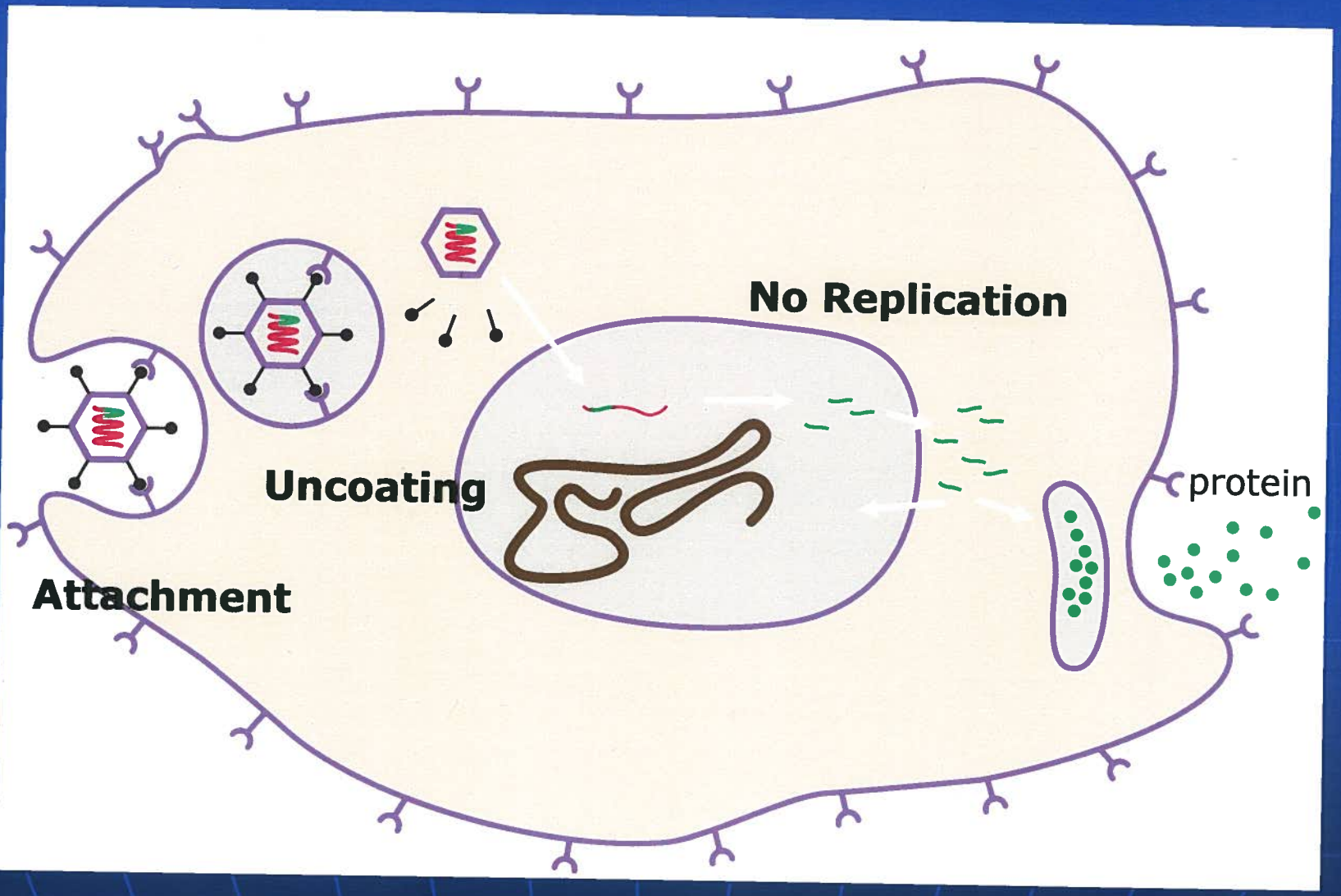
- Deletion of an essential gene
  - Make vector replication defective
- Vector capacity: 6Kb
- Transient expression
- High titer stocks (>10^{10}/ml)
- Uses:
  - Expression of CRE recombinase + GFP
  - Other

Adenoviral Vector Production



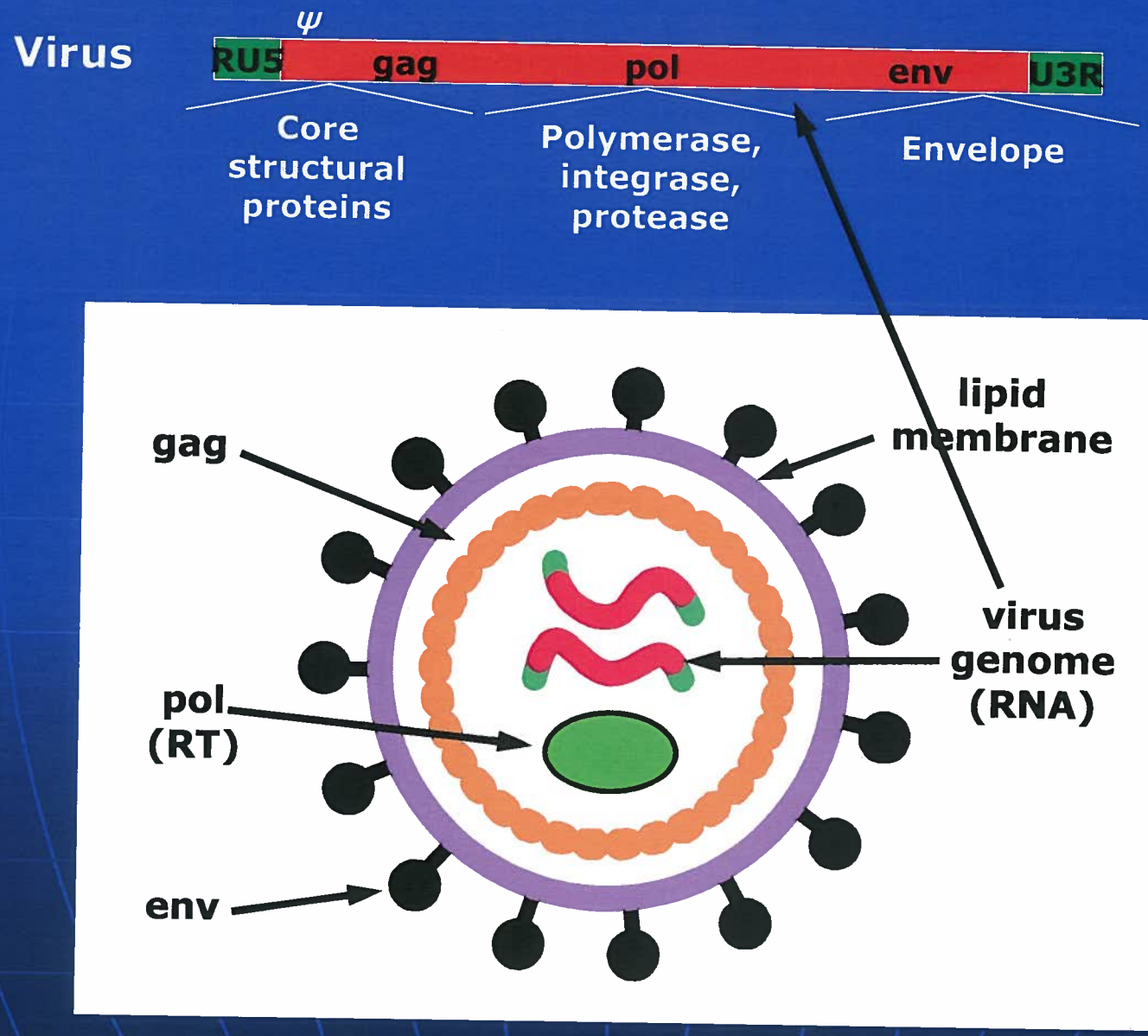
Adenoviral vector production takes place in a helper cell (A293 cells) that complements the essential viral gene missing from the vector.

Adenovirus Vector Infection

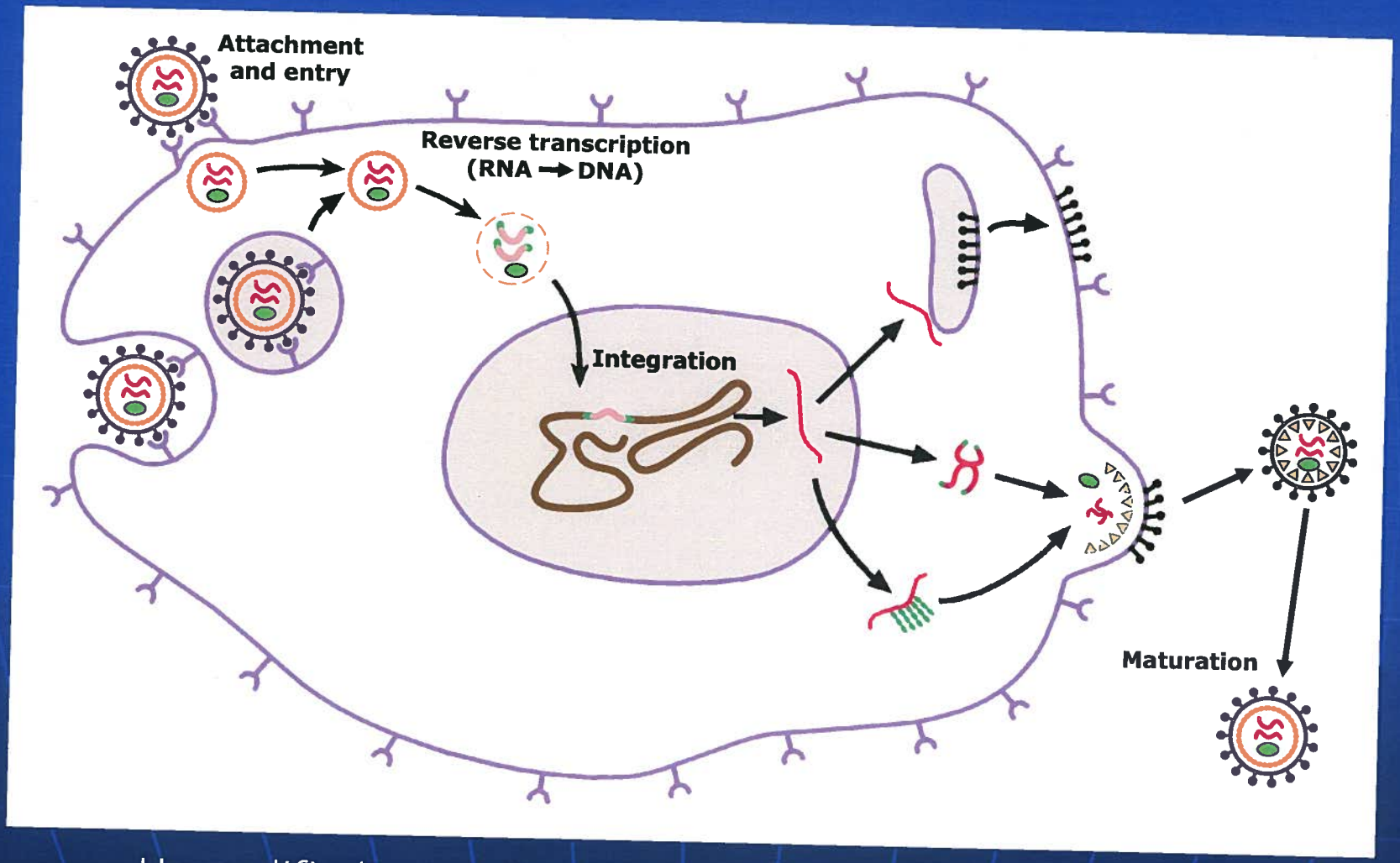


If used correctly, no progeny virus are produced--the adenovirus vector is considered “infectious”, but “replication defective”.

Lentivirus Structure



Lentivirus Replication Cycle

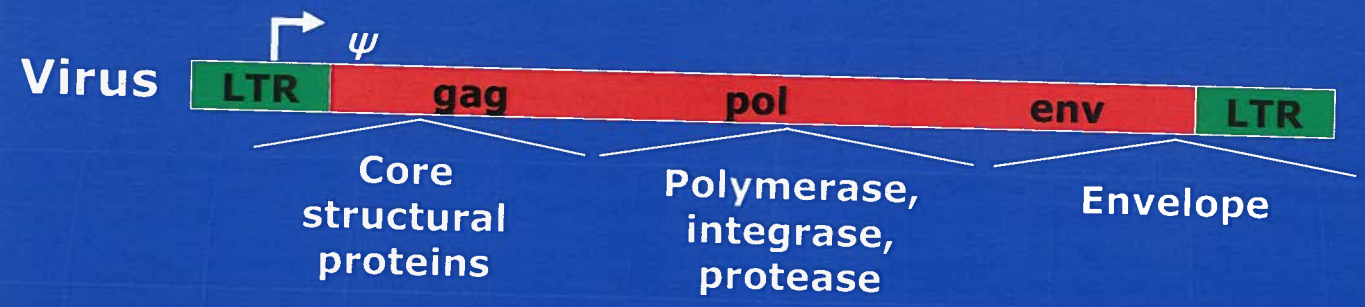


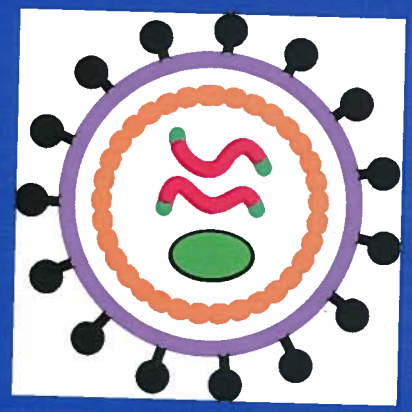
Unmodified retroviruses are replication competent (production of progeny viruses that are infectious)

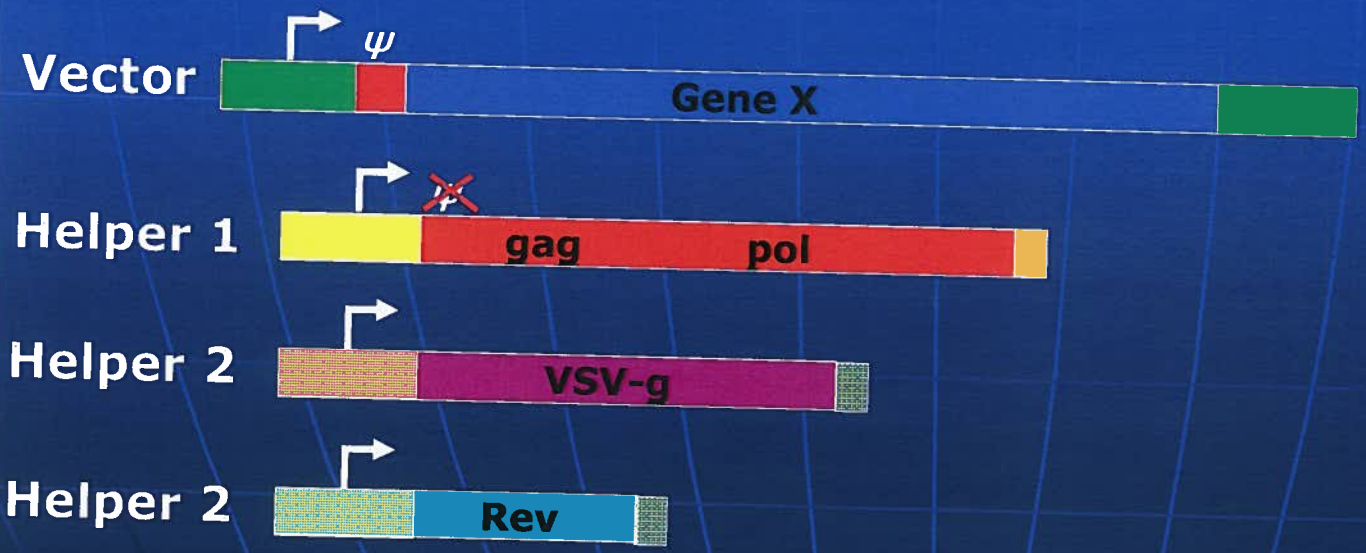
Lentiviral Vector

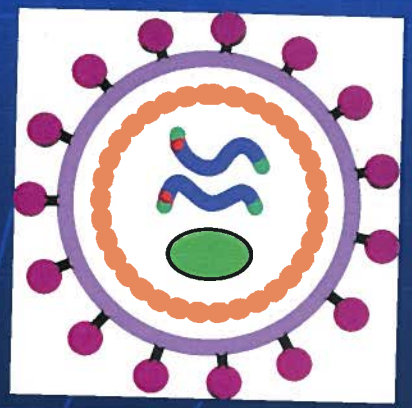
- Deletion of an essential genes and replication elements
  - Make vector replication defective
- Vector capacity: 7Kb
- Stable expression
- Moderate titer stocks (>10^7/ml)
- Broad tropism

Lentiviral Vector Production (Improved)

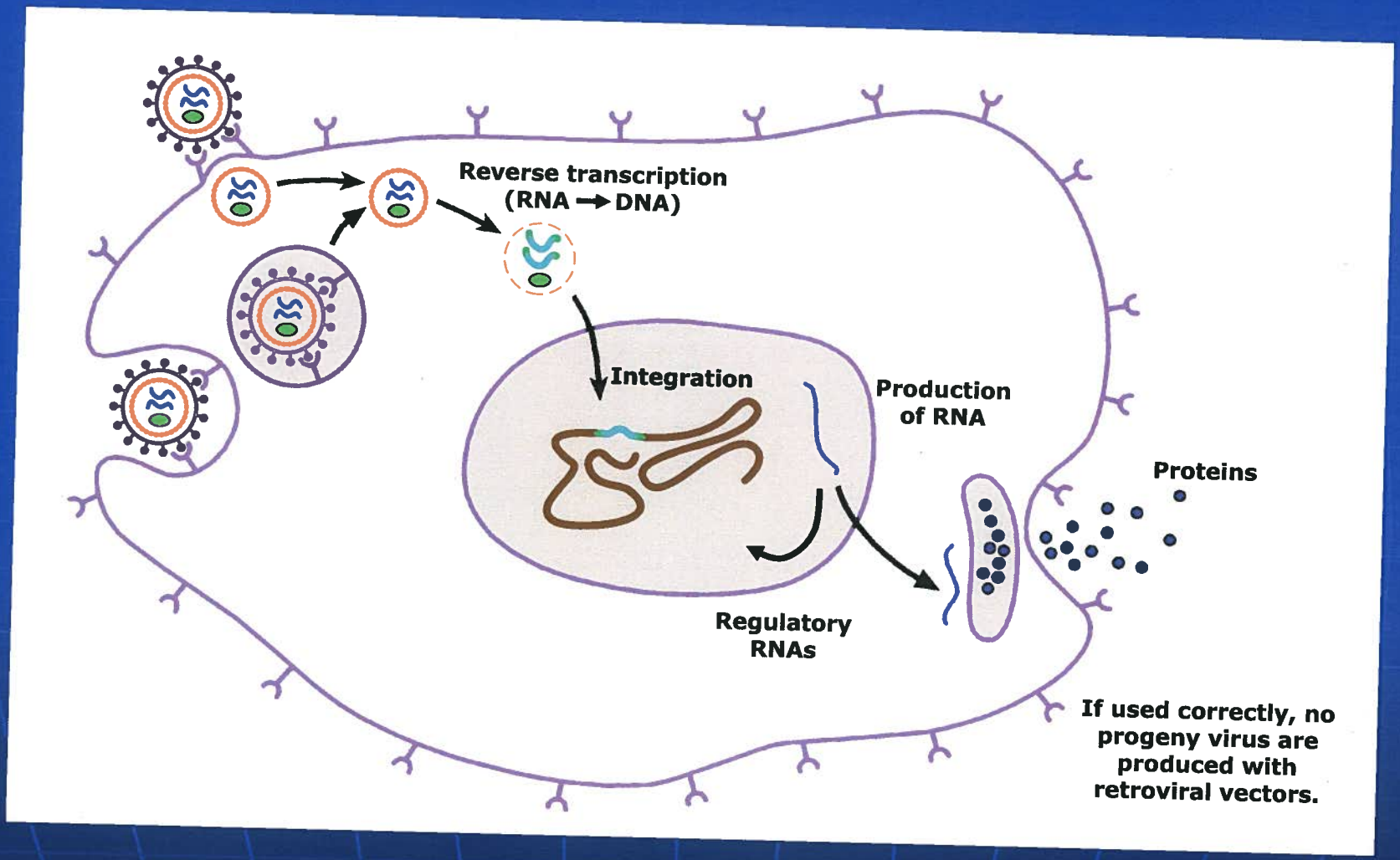






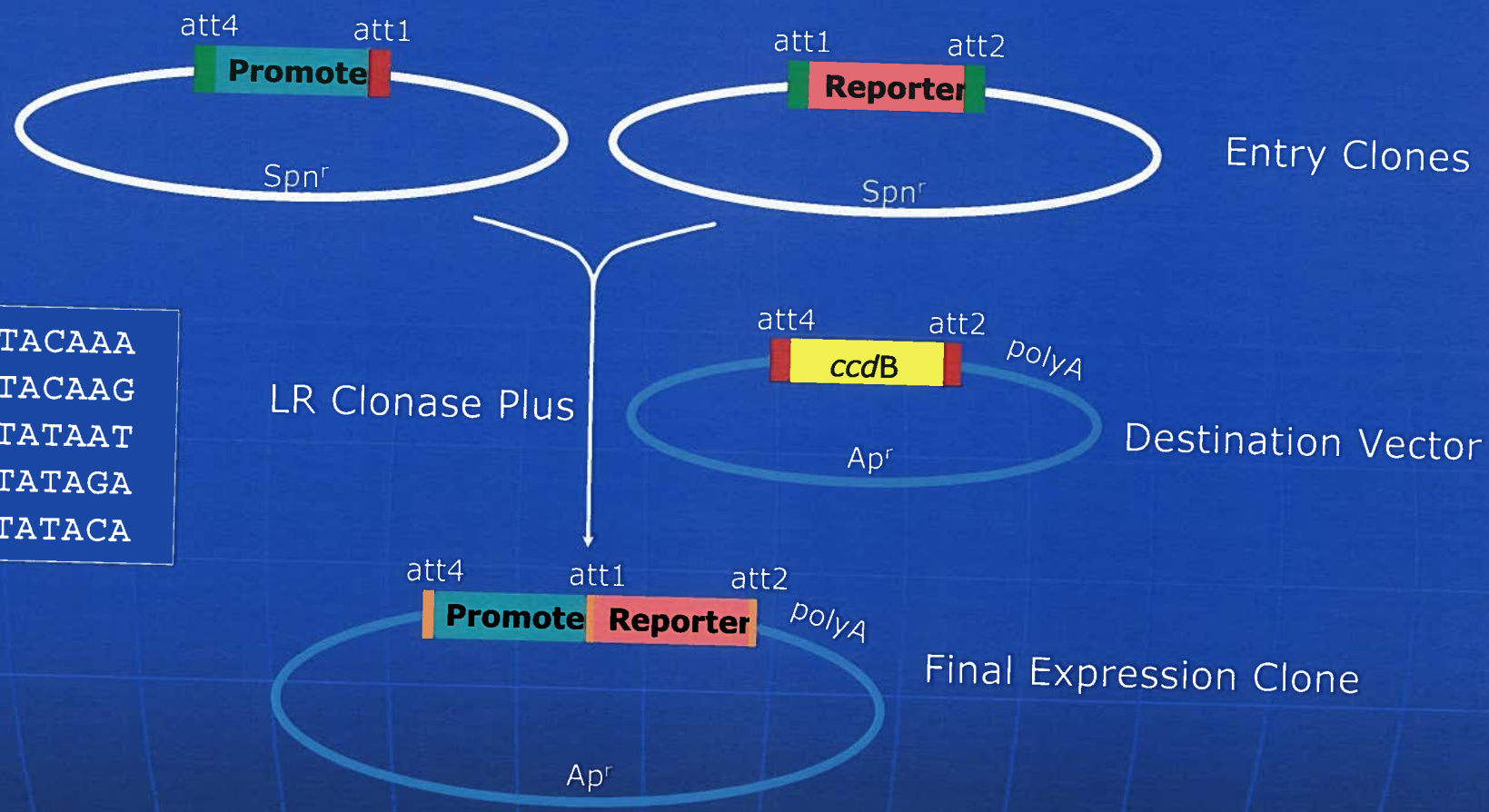


Lentiviral Vector Infection



Retroviral vectors are designed to be "replication incompetent" (doesn't make more infectious progeny)…but only if manufactured correctly.

Gateway Multisite Cloning

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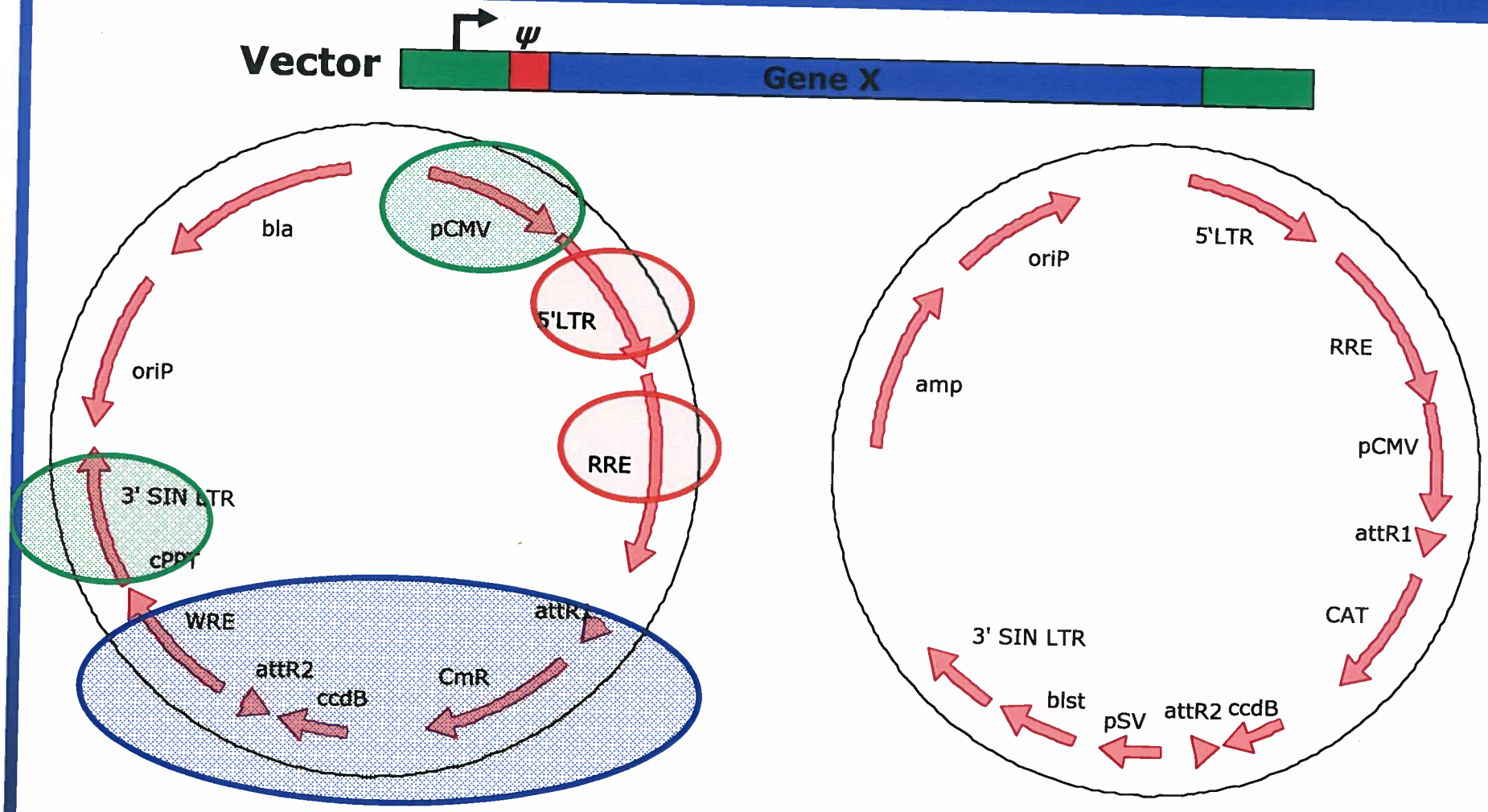
att1: GTACAAA
att2: GTACAAG
att3: GTATAAT
att4: GTATAGA
att5: GTATACA
```

Sequence verified Entry clones of promoters, reporters, or markers can be used to make large numbers of final expression constructs in parallel, and with completely identical vector backbones!

Lentiviral Expression

- Lentivector Backbone (all SIN)
  - pLenti4/6 (IVGN)
  - pFUGW (Baltimore lab, WPRE, cPPT)
  - pLKO.1 (siRNA)
- Vector Options
  - selectable markers (neo, blast, zeo, puro, hygro)
  - coexpression markers (IRES-FPs, FPs with promoters)
  - promoters (library of 15 available, others can be made)
- Cloning Issues
  - stabilization of inverted LTR repeats
  - location of elements
  - maximum size of inserts

Comparison of Lentivector Backbones



Both 2nd generation lentivectors, SIN, Tat-independent, require Gag/Pol and Env helper plasmids

Standard Gateway Lentivectors

- pLenti6 (blasticidin resistance)
  - pDest-690 (CMV promoter, C-term V5 tag)
  - pDest-691 (UbC promoter, C-term V5 tag)
  - pDest-694 (no promoter, for pU6-shRNA clones)
  - pDest-688 (CMV promoter, C-term V5 tag, IRES2-eGFP)
- pLenti4 (zeocin resistance)
  - pDest-689 (CMV promoter, C-term V5 tag)
  - pDest-692 (no promoter, for shRNA clones)
  - pDest-697 (no promoter, pCAG-ffLuc-mRFP1)
  - pDest-693 (no promoter, mRFP1 replaces zeocin)
- pFUGW
  - pDest-673 (CMV promoter, neo resistance)
  - pDest-677 (no promoter, pUbC-eGFP)
  - pDest-674 (CMV-Tet, neo resistance, rtTA3)

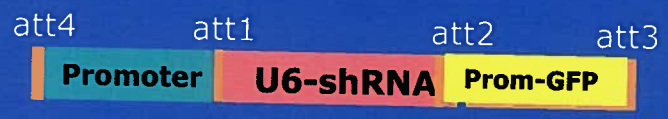
Multisite Gateway Lentivectors

no promoter—designed for use with att4-att1 promoter libraries

- pLenti6 (blasticidin resistance)
  - pDest-682
- pFUGW (no resistance marker)
  - pDest-671 (IRES2-ffLuc2-eGFP)
  - pDest-683 (pTyr290-mRFP1)
  - pDest-685
- pFUGW (neomycin resistance)
  - pDest-674 (IRES2-eGFP)
  - pDest-675 (IRES2-DsRedExpress2)
  - pDest-686
  - pDest-684 (WRE moved before gene of interest)

Multisite Gateway Lentivectors

new att4-att3 design for additional element inclusion



For easier vector construction, element can be:

- IRES-FP
- promoter-FP
- selectable marker

Vectors for shRNA expression:

- U6 promoter
- H1 promoter
- Tet-regulated U6 promoter

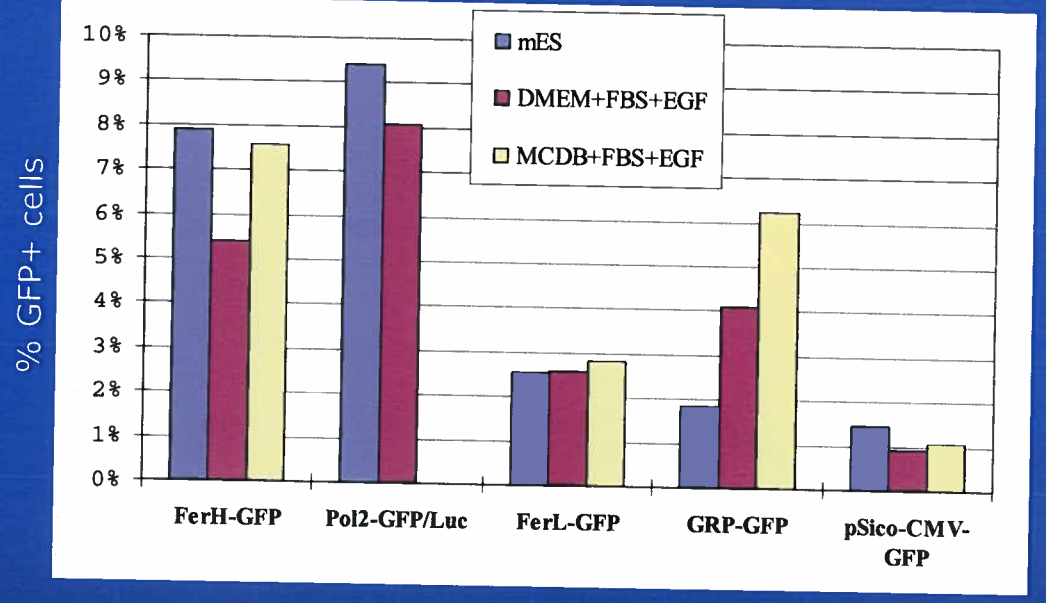
- Available in pFUGW backbone with various resistance
  - pDest-653 (no marker)
  - pDest-667 (puromycin)
  - pDest-668 (zeocin)
  - pDest-669 (hygromycin)
  - pDest-676 (neomycin)

Lentiviral Results

HGF-mouse Melanoma Cell Line AN5799 in Serum-free DMEM

	Ratio of GFP+ cells	Median GFP intensity
No infection	0.31%	23.29
pSico-CMV-GFP	89.26%	423.60
Pol2-Luc/GFP	96.34%	2121.11
FerH-GFP	97.66%	4756.30
FerL-GFP	94.51%	2600.00

Rhabdo #5951 (Rhabdomyosarcoma Cell Suspension) in Various Media



In mice, only FerH and Pol2 promoters gave good signals in tumors, and only Pol2 was highly persistent after 4 weeks.

Other promoters were silenced very quickly both in primary cells in culture and in the live animals

Day and Merlino (NCI)

Reporter Constructs

- Fluorescent proteins
  - eGFP, eYFP, eCFP, BFP, CyPet, Venus
  - mOrange, DsRed2, DsRedExpress2, mRFP1, mCherry, mKate
  - paeGFP, pamCherry, mEos2, tdEos2
- Luminescence reporters
  - firefly luciferase (ffLuc2)
  - Renilla luciferase (rLuc)
- Multifunctional reporter fusions
  - ffLuc2-eGFP
  - ffLuc2-mRFP
  - rLuc-mRFP
  - ffLuc2-mCherry
  - ffLuc2-tdRFP2(12)

and we're making more every day...

Contact Information

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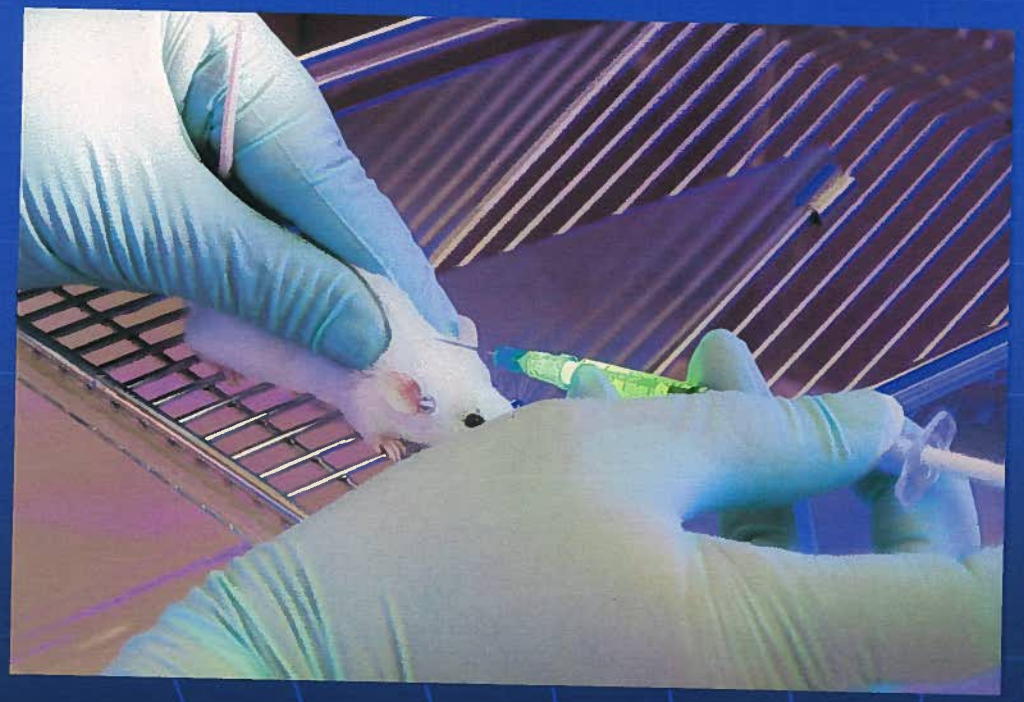
Injection Technique Can Greatly Increase Risk

- Which technique is most likely to cause an accident?