

# Non-viral delivery Polyplus® solutions

## Guidelines for Nucleic Acid Delivery in Mice



With a proven track record (over 600 publications), our *in vivo* reagents have been used to target a wide range of organs using various administration routes.

General considerations	<i>in vivo-jetPEI</i> ®	<i>in vivo-jetRNA</i> ®+ - NEW
Complexation Buffer	5% glucose	mRNA buffer
Reagent volume	0.12 to 0.16 µL/µg of nucleic acid (N/P= 6 to 8)	1:2 ratio (µgmRNA: µLreagent)
Nucleic acid maximal concentration in final injection volume	0.5 µg/µL	0.3 µg/µL

Animal experiments must be approved by the local ethics committee.

At Polyplus®, mice were anaesthetized by inhalation using anaesthetic metoxyflurane or by intraperitoneal injection of pentobarbital or ketamine/xylazine.

Standard conditions are given for a 20-25g mouse.

Our reagents:

- in vivo-jetPEI*®: Ready-to-use cationic polymer reagent recommended for *in vivo* delivery of various types of nucleic acid (DNA, siRNA, miRNA, shRNA and other oligonucleotides)
- in vivo-jetRNA*®+: Ready-to-use lipid-based transfection reagent composed of preformed liposomes specifically developed for *in vivo* mRNA delivery

	Reagent size	Buffer size	Part number
<i>in vivo-jetPEI</i> ®	0.1 mL	10 mL	101000040
	0.5 mL	2 x 10 mL	101000030
<i>in vivo-jetRNA</i> ®+	1 mL	60 mL	101000122

To get the complete protocols

Contact our Scientific Support

## Recommended starting conditions

### Nasal Instillation

mRNA: 5 µg  
*in vivo-jetRNA*®+: 10 µL  
Injection volume: 50 µL, mRNA buffer

Other Nucleic acid: 20 µg  
*in vivo-jetPEI*®: 2.4-3.2 µL  
N/P ratio: 6-8  
Injection volume: 50-100 µL, 5% glucose

**Method:** The mouse is held supine at an angle of 45° with pressure applied to the lower mandible to immobilize the tongue and prevent swallowing. Complexes in solution are then introduced to the nasal planum using a micropipet.

#### References:

- in vivo-jetPEI*®:
- Manček-Keber M. et al. (2021) *FASEB J* 35 (DNA, airway tract)
  - Dileepan M. et al. (2020) *Exp. Lung. Res.* 46 243-257 (DNA, lungs)
  - Siddiqui MR. et al. (2019) *Am J Respir Cell Mol Biol.* 61(2): 257-265 (miRNA, lungs)

### Topical application

Please contact our Scientific Support for starting conditions.

#### References:

- in vivo-jetPEI*®:
- Yan, M. et al. (2017) *Wound Repair Regen* 25 933-943 (siRNA, skin)
  - Cabrera JR et al., (2015) *PLoS Pathog* (DNA, skin)

### Subcutaneous application

mRNA: 5 µg  
*in vivo-jetRNA*®+: 10 µL  
Injection volume: 100 µL, mRNA buffer

Other Nucleic acid: 20 µg  
*in vivo-jetPEI*®: 2.4-3.2 µL  
N/P ratio: 6-8  
Injection volume: 100-200 µL, 5% glucose

#### References:

- in vivo-jetPEI*®:
- Zheng M. et al. (2020) *Sci Rep* 10 17622 (siRNA, skin)
  - Heidegger, S. et al. (2019) *EBioMedicine* 41, 146-155 (pRNA, immune cells)

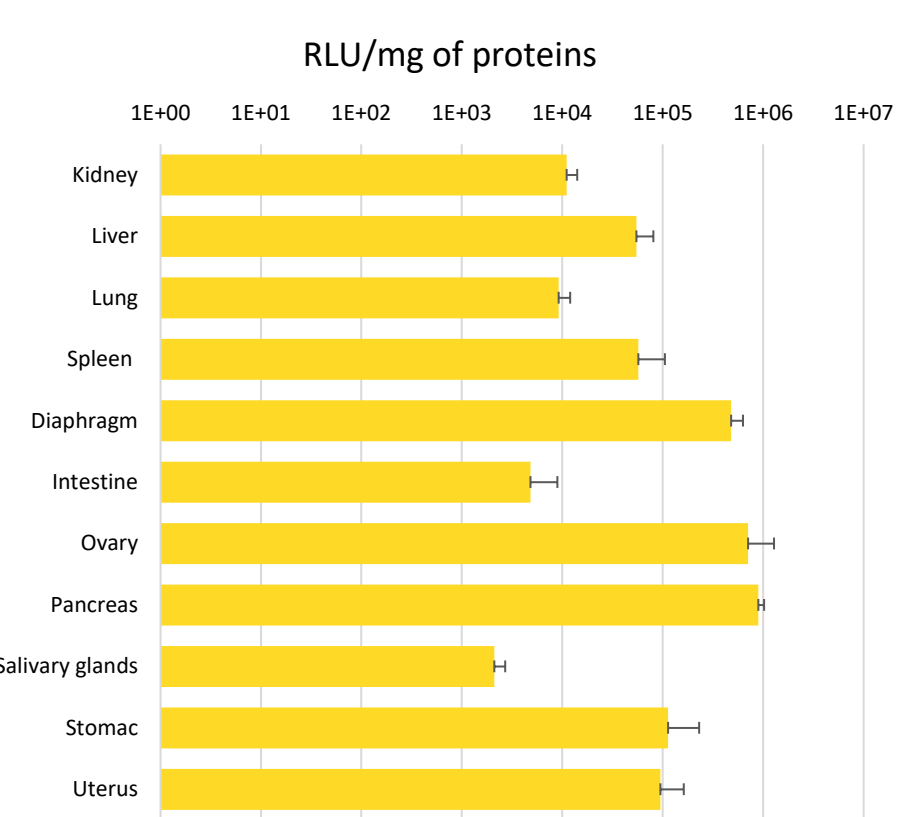
### Intraperitoneal injection

mRNA: 10-20 µg  
*in vivo-jetRNA*®+: 20-40 µL  
Injection volume: 500 µL, mRNA buffer

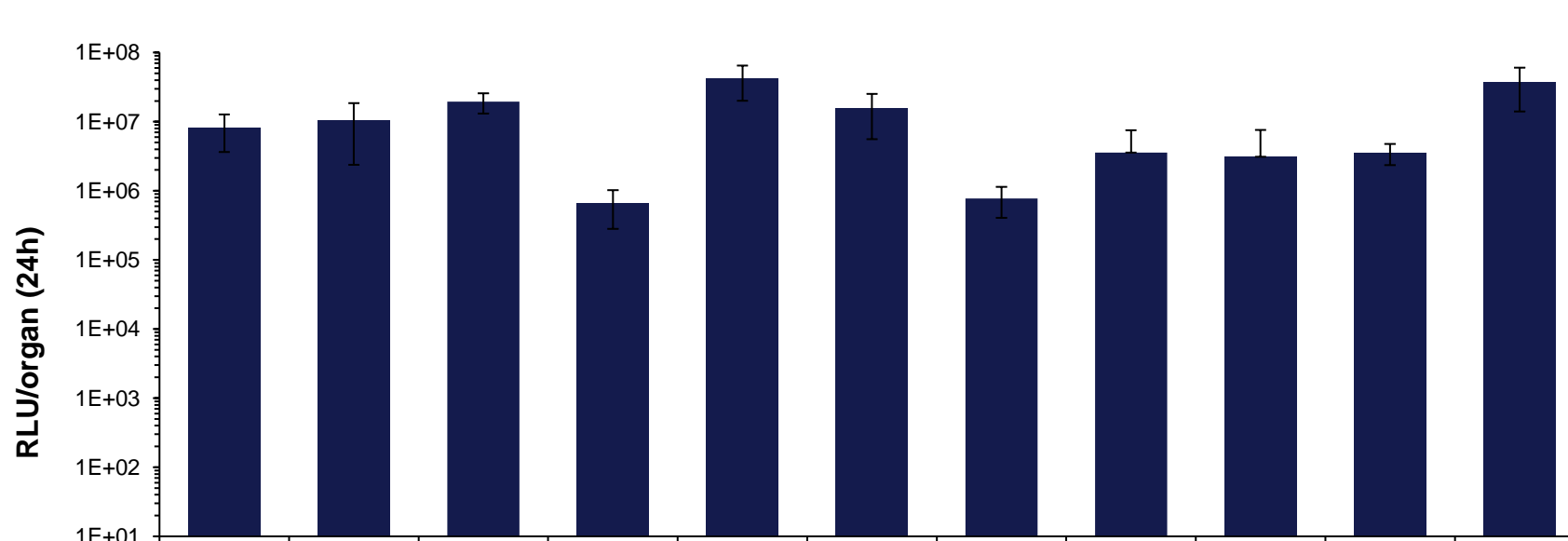
Other Nucleic acid: 100 µg  
*in vivo-jetPEI*®: 12-16 µL  
N/P ratio: 6-8  
Injection volume: 0.4-1 mL, 5% glucose

**Method:** Complexes in solution are injected into the peritoneal cavity over 10 sec, using a ½ inch 26G needle and a 1 mL syringe.

**Figure:** pCMVLuc (100 µg) was complexed with *in vivo-jetPEI*® at an N/P ratio of 8, in 1 mL of 5% glucose solution and injected intraperitoneally.



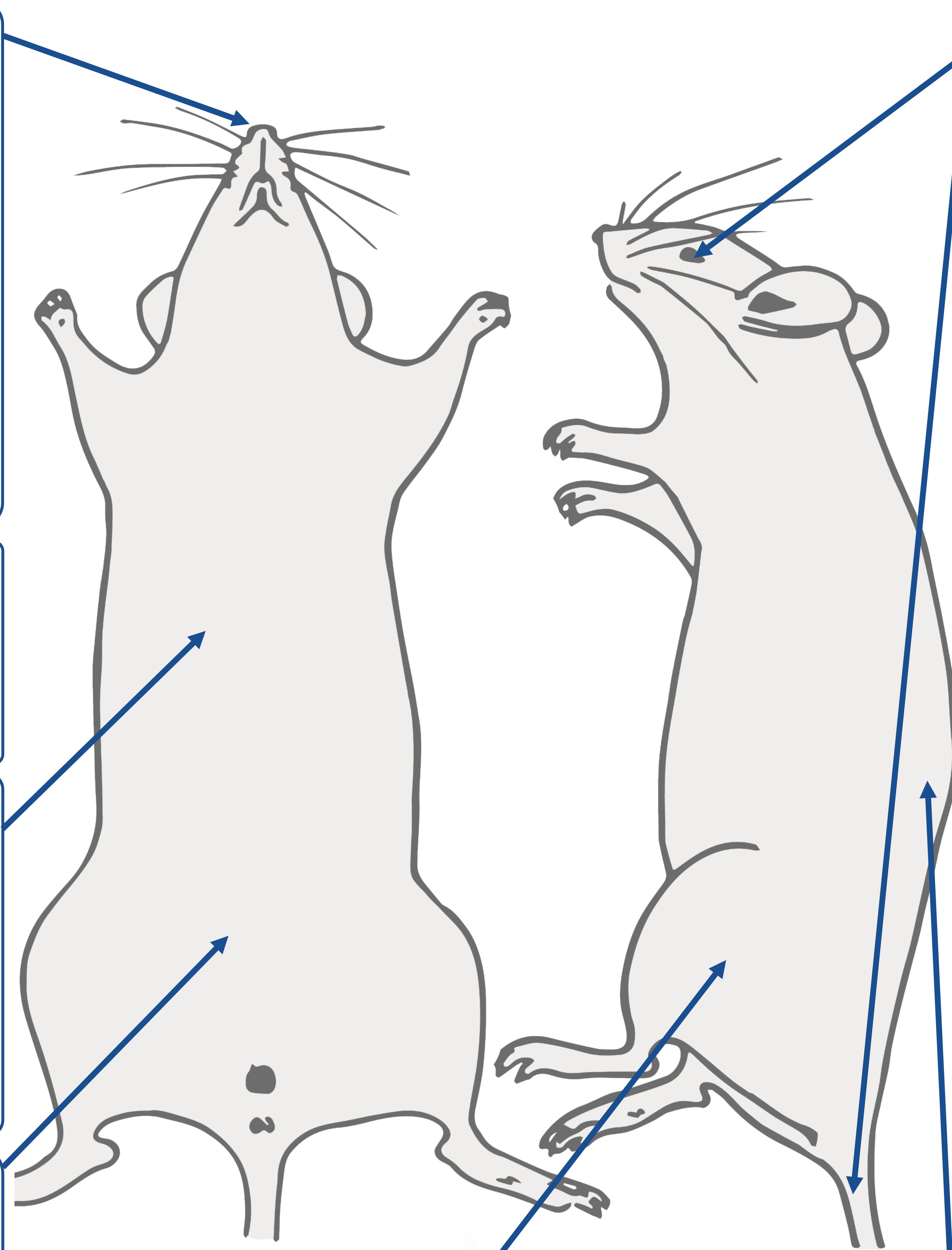
24 h after injection, organs were extracted, and luciferase expression was measured and expressed relative to the amount of total proteins.



**Figure:** Complexes were formed with a Luciferase encoding mRNA/*in vivo-jetRNA*®+ ratio of 1:2 (µgmRNA:µLreagent) in mRNA Buffer using 20 µg mRNA for intraperitoneal (IP) injection. 24 hours after injection, different organs were extracted, and luciferase expression was measured.

#### References:

- in vivo-jetPEI*®:
- Lupse B. et al. (2021) *Cell Rep* 36 109490 (DNA, pancreas)
  - Chin WX. et al. (2021) *Vaccines* 6 20 (DNA, immune cells)
  - Takao T. et al. (2020) *Proc Natl Acad Sci U S A* 117 28579-28581 (DNA, sgRNA, CRISPR, uterus)



### Intravenous injection

(retro-orbital or tail vein injection)

mRNA: 10-20 µg  
*in vivo-jetRNA*®+: 20-40 µL  
Injection volume: 200µL, mRNA buffer

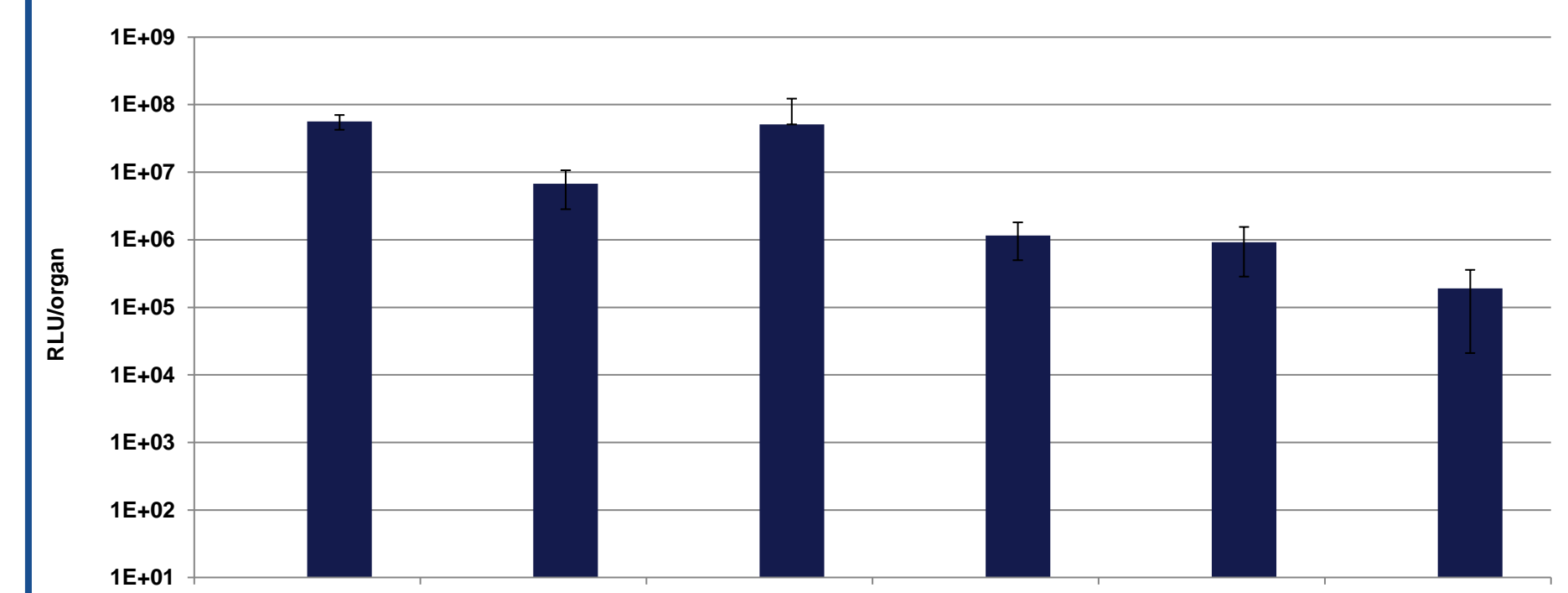
Other Nucleic acid: 40 µg  
*in vivo-jetPEI*®: 4.8-6.4 µL  
N/P ratio: 6-8  
Injection volume: 200-400 µL, 5% glucose

**Method Retro-orbital injection:** A 27G hypodermic needle is introduced carefully in front of the eye. The edge of the orbit is followed down until the needle tip reaches the base beneath the eye. Inject complexes in solution over 2 sec. If performed carefully, there will be little or no bleeding. The capillary nexus will take up the injected solution rapidly.

**Method Tail vein injection:** The mouse is placed in a restrainer and 70% ethanol is applied on the tail to slightly swell the vein. Complexes in solution are injected into the tail vein over 10 sec, using a ½ inch 26G needle and a 1 mL syringe.



**Figure:** pCMVLuc (40 µg) was complexed with *in vivo-jetPEI*® at an N/P ratio of 8, in 200 µL of 5% glucose solution and injected through retro-orbital sinus. 24 h after injection, organs were extracted and luciferase expression was measured and expressed relative to the amount of total proteins.



**Figure:** mRNA encoding for Luciferase was injected into mice using *in vivo-jetRNA*®+. Complexes were formed with a mRNA/*in vivo-jetRNA*®+ ratio of 1:2 (µgmRNA:µLreagent) in mRNA Buffer using 10 µg mRNA for intravenous (retro-orbital) injection. Luciferase expression was assessed 24h post-injection.

#### References:

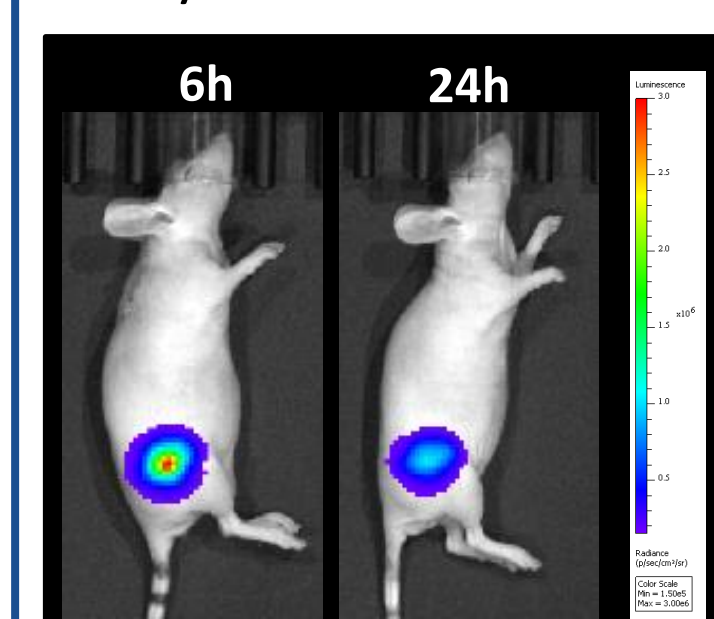
- in vivo-jetPEI*®:
- Li S. et al. (2021) *Cell Rep* 34 108631 (5'-PPP-dsRNA, immune cells)
  - Garg M. et al. (2021) *Cell Rep* 34 108736 (siRNA, lungs)
  - Hsu YL. et al. (2020) *Oncogene* 39 739-753 (miRNA, tumors, RO)

### Intramuscular injection

mRNA: 5-10 µg  
*in vivo-jetRNA*®+: 10-20 µL  
Injection volume: 100 µL, mRNA buffer

Other Nucleic acid: 10 µg  
*in vivo-jetPEI*®: 1.2-1.6 µL  
N/P ratio: 6-8  
Injection volume: 50-100 µL, 5% glucose

**Method:** The mouse should be properly restrained or anesthetized prior injection. Administration is performed into the caudal thigh muscle to avoid the sciatic nerve and femur. Upon inserting the needle, bevel up and aspirate to ensure that blood vessel is not damaged. Inject the complexes slowly.



**Figure:** mRNA encoding for Luciferase was injected into mice using *in vivo-jetRNA*®+. Complexes were formed with a mRNA/*in vivo-jetRNA*®+ ratio of 1:2 (µgmRNA:µLreagent) in mRNA Buffer using 5 µg mRNA for intramuscular (IM) injection. Luciferase expression was assessed 6h and 24h post-injection.

#### References:

- in vivo-jetPEI*®:
- Robinson ER. et al. (2021) *J Control Release* 335 281-289 (minicircle plasmid, tumor)
  - Pan T. et al. (2019) *Theranostics* 9 405-423 (siRNA, muscle)

### Intratumoral injection

mRNA: 2 µg  
*in vivo-jetRNA*®+: 4 µL  
Injection volume: 20-50 µL, mRNA buffer

Other Nucleic acid: 10 µg  
*in vivo-jetPEI*®: 1.2-1.6 µL  
N/P ratio: 6-8  
Injection volume: 20-100 µL, 5% glucose

#### References:

- in vivo-jetPEI*®:
- Brown MC. et al. (2021) *Nat Commun* 12 1858 (Poly(I:C))
  - Dasgupta S. et al. (2021) *Cell Death Differ* (siRNA)
  - Cho J. et al. (2021) *J Clin Invest* 131 e136779 (siRNA)

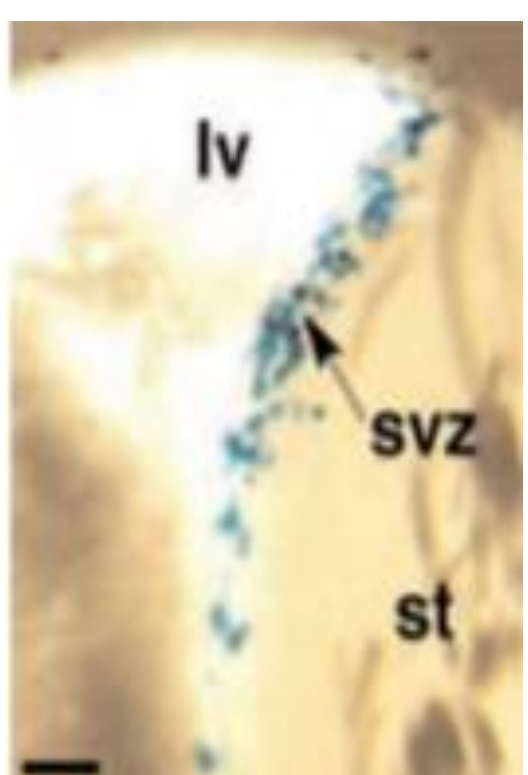
### Intracerebral injection

mRNA: 0.5 µg  
*in vivo-jetRNA*®+: 1 µL  
Injection volume: 5µL, mRNA buffer

Other Nucleic acid: 1 µg  
*in vivo-jetPEI*®: 0.12-0.16 µL  
N/P ratio: 6-8  
Injection volume: 4-5 µL, 5% glucose

**Method:** Perform single injection into either lateral ventricle (0.2 mm posterior to the bregma line, 1.1 mm lateral, and 2.2 mm deep from the pial surface) or stereotaxical injection.

**Figure:** Example of transfected cells expressing the β-galactosidase found in the anterior subventricular zone (1 week after intraventricular injection of pCMV-LacZ). lv: lateral ventricle, svz: subventricular zone, str: striatum. Courtesy B. Demeneix.



#### References:

- in vivo-jetPEI*®:
- Saha P. et al. (2019) *J Neurotrauma* (DNA, brain)
  - Bacq A. et al. (2018) *Mol Psychiatry* (shRNA plasmid, brain)