Non-viral transfection in mice

Guidelines for Nucleic Acid Delivery Experiments in Mice

With a proven track record (over 500 publications), our *in vivo* reagents have been used to target a wide range of organs using various administration routes.

General considerations	*in vivo*-jetPEI®	*in vivo*-jetRNA®
Complexation Buffer	5% glucose	mRNA buffer
Reagent volume	0.12 to 0.16 µL/µg of nucleic acid (N/P= 6 to 8)	1:1 ratio (µgmRNA: µLreagent)
Nucleic acid maximal concentration in final injection volume	0.5 µg/µL	0.1 µg/µL

Animal experiments must be approved by the local ethics committee.

At Polyplus-transfection®, 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®: delivery of multiple type of nucleic acid (DNA, siRNA, miRNA, oligonucleotide) in various tissues.
- *in vivo*-jetRNA®: is the reagent of choice to deliver mRNA to various organs using different administration routes.

	Reagent size	Buffer size	Part number
***in vivo*-jetPEI®**	0.1 mL	10 mL	101000040
	0.5 mL	2 x 10 mL	101000030
***in vivo*-jetRNA®**	0.3 mL	2 x 10 mL	101000013
	1 mL	60 mL	101000021

To get the complete protocol

Contact our scientific support

Recommended starting conditions

Nasal Instillation

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

Other Nucleic acid: 20 µg
***in vivo*-jetPEI®:** 24-32 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 mandibule to immobilize the tongue and prevent swallowing. Complexes in solution are then introduced to the nasal planum using a micropipet.

References:

***in vivo*-jetRNA®:** *Hassert M. et al. (2020) PLoS Pathog. Dec 16;16(12):e1009163*

***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 adapted to your application.

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

Please contact our scientific support for starting conditions adapted to your application.

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)*

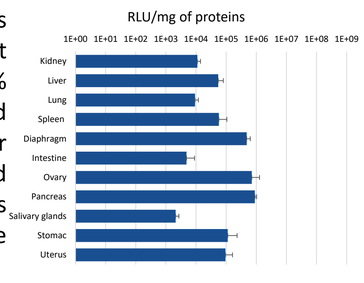
Intraperitoneal injection

mRNA: 10-20 µg
***in vivo*-jetRNA®:** 10-20 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 1: 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.



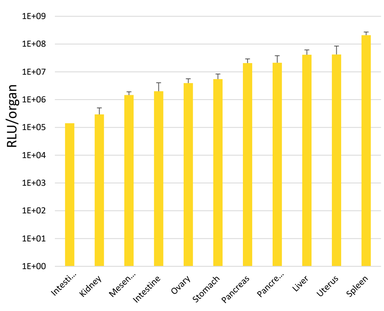
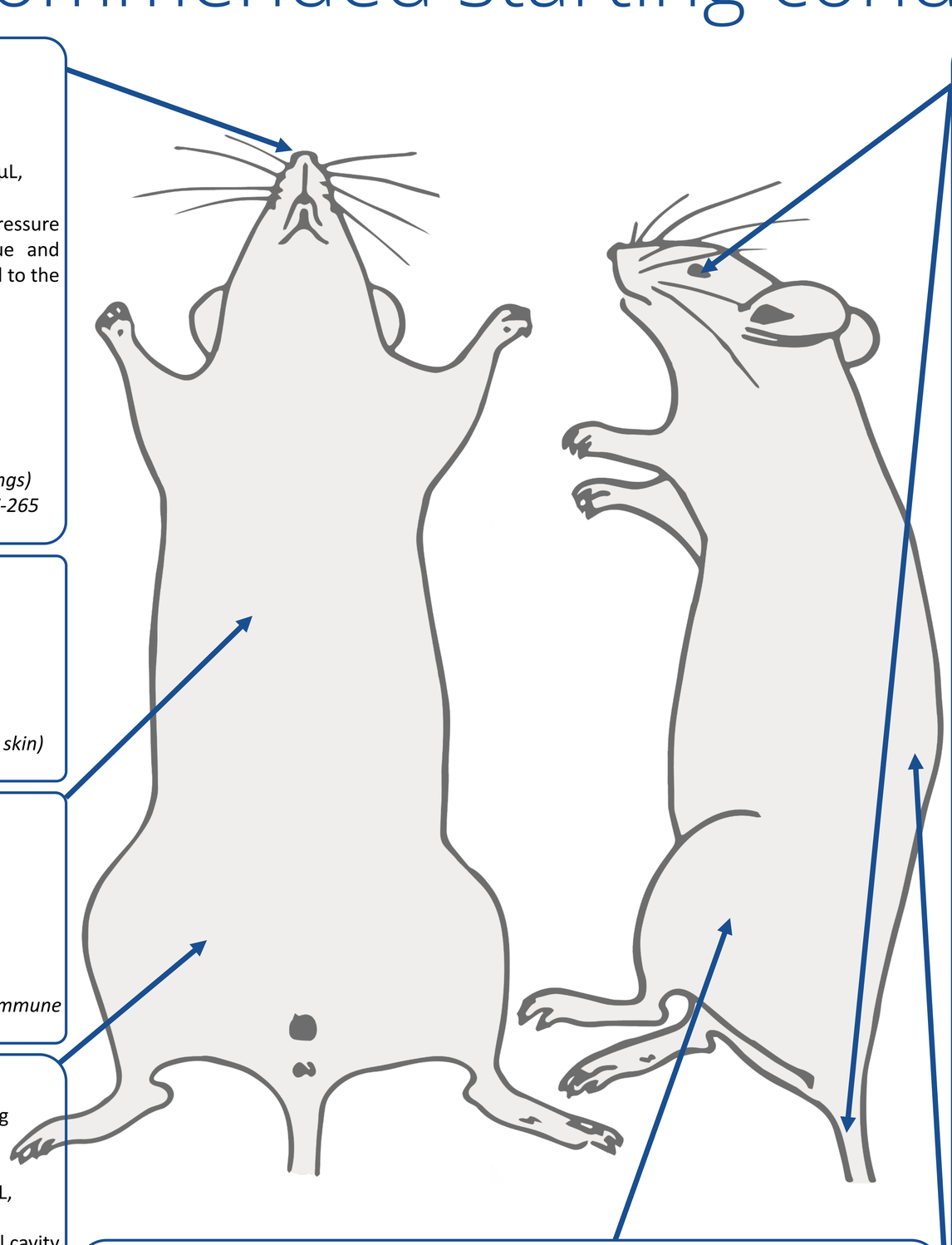


Figure 2: Complexes were formed using the recommended conditions with luciferase mRNA/*in vivo*-jetRNA® and were injected via intraperitoneal (IP) administration routes in mice. 24 hours after injection, different organs were extracted and luciferase expression was measured.

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®:** 10-20 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: 100-200 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.

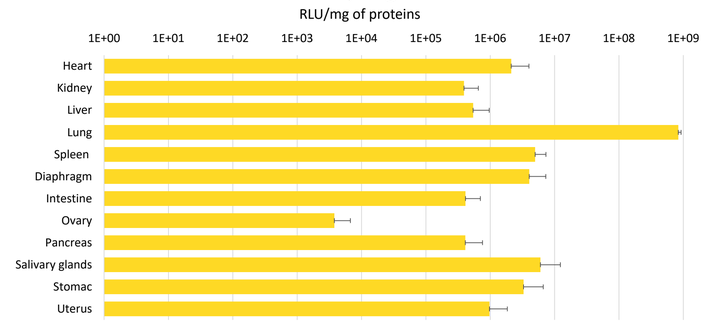


Figure 1: pCMVLuc (40 µg) was complexed with *in vivo*-jetPEI® at an N/P ratio of 8, in 200 µL of 5% glucose solution and injected through retro-orbital sinus. 24 h after injection, organs were extracted and luciferase expression was measured and expressed relative to the amount of total proteins.

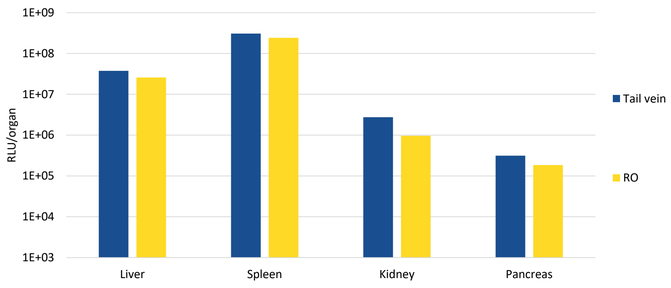


Figure 2: Complexes were formed using the recommended conditions with luciferase mRNA/*in vivo*-jetRNA® and were injected via intravenous (retro-orbital - RO or tail vein) administration routes in mice. 24 hours after injection, different organs were extracted and luciferase expression was measured.

References:

***in vivo*-jetRNA®:** *Hassert M. et al. (2020) PLoS Pathog. Dec 16;16(12):e1009163*

***in vivo*-jetPEI®**:

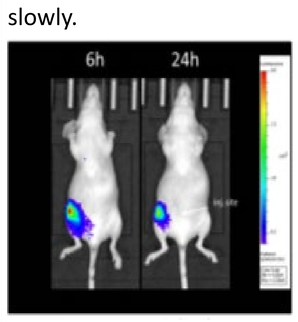
- *Li S. et al. (2021) Cell Rep 34 108631 (5'-PPP-dsRNA, immune cells)*
- *Garg M. et al. (2021) Cell Rep 34 108736 (siRNA, lungs)*
- *Hsu YL. et al. (2020) Oncogene 39 739-753 (miRNA, tumors, RO)*

Intramuscular injection

mRNA: 5-10 µg
***in vivo*-jetRNA®:** 5-10 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 anesthesized 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.



Cy7 labeled mRNA encoding for Luciferase was injected into mice using *in vivo*-jetRNA®. Complexes were formed with a mRNA/*in vivo*-jetRNA® ratio of 1:1 (µgmRNA:µLreagent) in mRNA Buffer using either 2.5 µg, 10 µg or 20 µg of mRNA for respectively intramuscular. Fluorescence signals were observed with Bioluminescence imaging using IVIS system and Luciferase (on the right)

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®:** 2 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)*

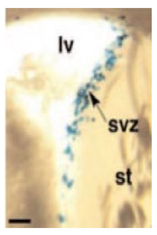
Intracerebral injection

mRNA: 0.5 µg
***in vivo*-jetRNA®:** 0.5 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 ß-galactosidase found in the anterior subventricular zone (1 week after intraventricular injection of pCMV-LacZ). lv: lateral ventricle, svz: subventricular zone, str: stratium. 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)*