

Technical Note

Guidelines to set up Nucleic Acid Delivery Experiments in Mice

+ General considerations

- High-quality preparations of nucleic acid are required.
- Resuspend nucleic acid in water or low salt buffer at high concentration (3-7 µg/µl for DNA and 5-10 µg/µl for siRNA).
- Form *in vivo-jetPEI*TM/nucleic acid complexes in 5% glucose (final concentration).
- The volumes of the *in vivo-jetPEI*TM solution and of the nucleic acid solution should be similar to ensure homogenous complex formation upon mixing.
- The concentration of nucleic acid in the final injection volume should not exceed 0.5 µg/µl.
- The volume of *in vivo-jetPEI*TM is determined by the N/P ratio which is a measure of the ionic balance of the complexes, referring to the number of nitrogen residues of *in vivo-jetPEI*TM per phosphate in the nucleic acid.
- Animal experiments must be approved by the local ethics committee and carried out humanly. At Polyplus, mice are anaesthetized by inhalation using anaesthetic metoxyflurane or by intraperitoneal injection of pentobarbital or Ketamine/xylasine.

| Reagent | Cat. N° | Size | 10% Glucose solution |
|-------------------------------------|---------|--------|----------------------|
| <i>in vivo-jetPEI</i> TM | 201-10 | 0.1 ml | - |
| | 201-10G | 0.1 ml | 10 ml |
| | 201-20 | 0.2 ml | - |
| | 201-20G | 0.2 ml | 10 ml |
| | 201-50 | 0.5 ml | - |
| | 201-50G | 0.5 ml | 2 x 10 ml |

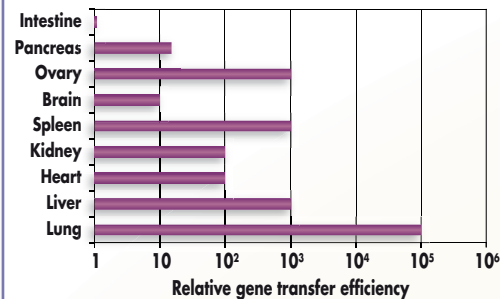
Bulk quantities are available upon request.

The use of polyethylenimine (PEI) or polypropylenimine (PPI) or cationic polymers similar in structure thereto for transfecting cells, as well as compositions comprising these cationic polymers and at least one nucleic acid, are the subject matter of U.S. Patent No. 6,013,240, EP Patent No. 0770140 and foreign equivalents, for which Polyplus-transfectionTM is the worldwide exclusive licensee.

Tail vein injection

Nucleic acid: 50 µg
***in vivo-jetPEI*TM:** 5-8 µl
N/P ratio: 5-8
Injection volume: 200-400 µl, 5% glucose

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



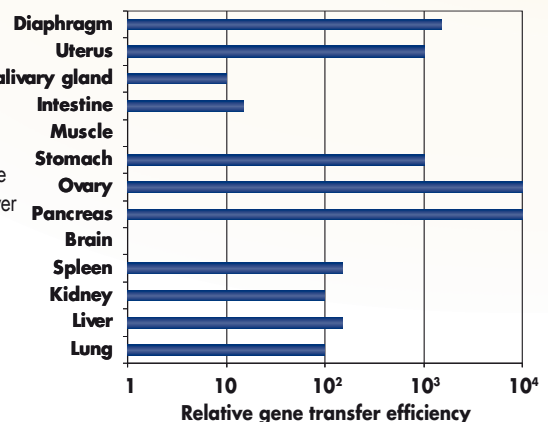
Tissue distribution of luciferase transgene expression 24 h following tail vein injection.

Intraperitoneal injection

Nucleic acid: 100 µg
***in vivo-jetPEI*TM:** 12-16 µl
N/P ratio: 6-8

Injection volume: 400 µl to 1 ml, 5% glucose

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

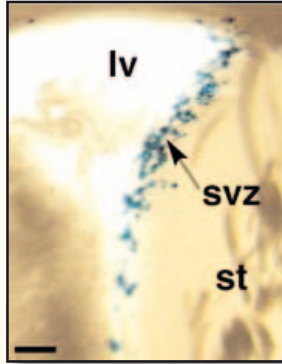


Tissue distribution of luciferase transgene expression 24 h following i.p. injection.

Intracerebral injection (stereotaxic injection)

siRNA: 0.1 µg/µl using **jetSI™ 10 mM** (see page 51)
Injection volume: 1-4 µl
Method: Single injection into either lateral ventricle or stereotaxical injection.

DNA: 1 µg (for 8-12 week-old mice)
in vivo-jetPEI™: 0.12 µl
N/P ratio: 6
Injection volume: 5 µl, 5% glucose
Method: Single injection (5 µl) 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) to pentobarbital anesthetized mice (65 mg/kg).

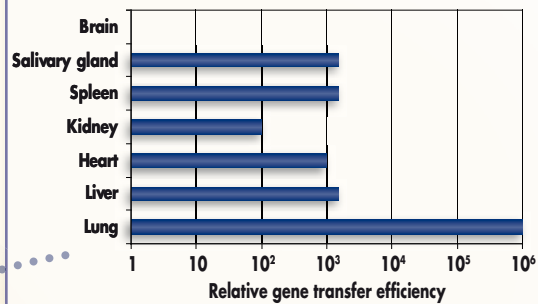


Example of transfected cells expressing the β-galactosidase and found in the anterior subventricular zone (1 week after intraventricular injection of pCMVlacZ).
 Courtesy B. Demeneix

Retro-orbital injection

Nucleic acid: 40 µg
in vivo-jetPEI™: 6.4 µl
N/P ratio: 8
Injection volume: 200-400 µl, 5% glucose

Method: The tip of a 27 g hypodermic needle is introduced carefully in front of the eye. Follow the edge of the orbit down until feeling the needle tip at the base beneath the eye. Inject complexes in solution within 2 sec. If performed carefully, there will be little or no bleeding. The capillary nexus will take up the injected solution rapidly.



Tissue distribution of luciferase transgene expression 24 h following retro-orbital injection.

Nasal instillation for trachea and lung delivery

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

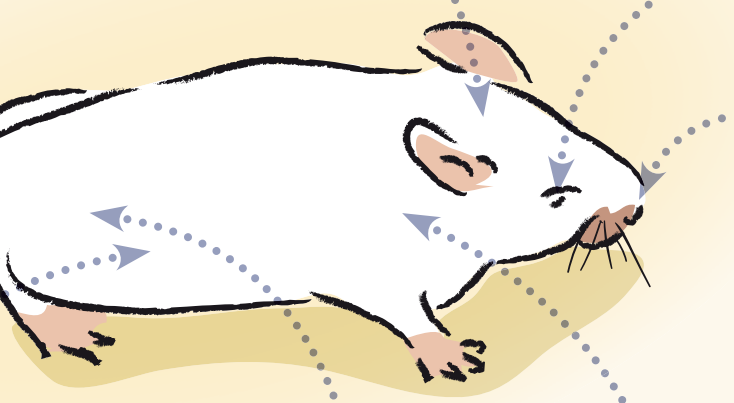
Method: Mice are held supine at an angle of 45° with pressure applied to the lower mandible to immobilize the tongue and prevent swallowing. Complexes in solution are then introduced to the nasal planum using a micropipet.

Intratumoral injection

Nucleic acid: 10-20 µg
in vivo-jetPEI™: 1.2-3.2 µl
N/P ratio: 6-8
Injection volume: 50-100 µl, 5% glucose
Method: For implanted subcutaneous tumors (size > 5 mm³), perform multiple injections of 10-20 µl complexes at different sites of the tumor to avoid reflux.

Subcutaneous injection

Nucleic acid: 3-5 µg
in vivo-jetPEI™: 0.3-0.7 µl
N/P ratio: 5-7
Injection volume: 10 µl, 5% glucose
Method: Mice are restrained and complexes are injected subcutaneously in the region of interest.



For other delivery routes or animal models, please contact our Delivery Experts through the contact form on the Polyplus website. They will be pleased to assist you.