In vivo Magnetofection™

Short protocols

Target tissue	Route of injection	Site of injection	Acid nucleic amount / injected volume	Kind of magnet	Magnet position
Tumor	Intravenous, Intratumoral	Tail vein Tumor	40 µg in 200 µL 10 to 50 µg in 0.5 µL/mm^3	All kind	External (subcutaneous tumor, brain tumor, well localized tumor) Internal (interne organ tumor)
Endothelial cells	Intravenous Intra-arterial	Vessel of interest Ear artery (rabbit) Femoral artery (mouse)	400 µg in 1.5 mL (rabbit) 5 µg in 200 µL (mouse)	All kind	Internal (deep vessels) External (ear artery)
Heart	Intravenous, Intra-arterial	Tail vein Carotid artery	50 µg in 200 µL	Cylinder	Internal (in the chest) External (on the chest)
Liver	Intravenous, Intra-arterial	Tail vein Carotid artery	40 µg in 200 µL	Cylinder, Square	External (on the right flank) Internal (for focalized gene transfer)
Lung	Intravenous	Tail vein	40 µg in 200 µL	Square	External
Intestine	Ileum lumen	Intestine (rat)	200 µg in 1 mL	Cylinder, Square	Internal
Brain	Intraventricular	Brain ventricle	0.5 µg in 2 µL	Small Cylinder	External

Quantities are given for mouse (20 g) excepted indications

NB: Magnet can be positioned:

- externally for large organs or isolated organs (liver, brain, muscle, subcutaneous tumor)
- internally for deep organs or focalized gene transfer

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Short protocols for Rat:

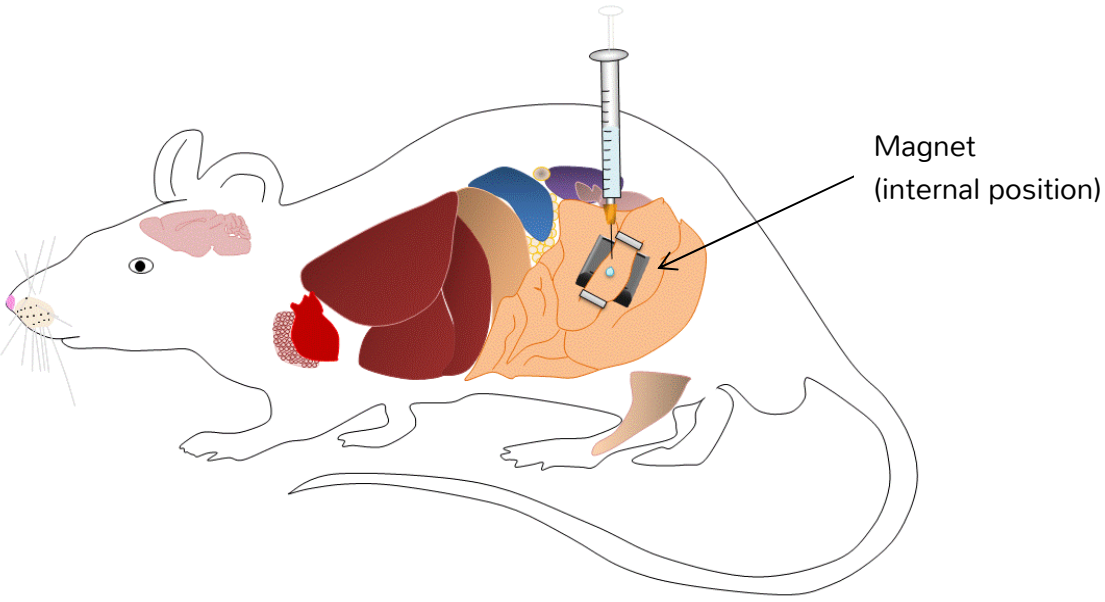
icv injection in rat embryo

- Anesthetize your timed pregnant rat [embryonic day 15]
- Mix 10 l of adenoviral particles (10^7 infectious particles), 15 l of ***In vivo*** **ViroMag** and 2 l of dye
- Incubate 15-30 min at room temperature
- Expose the uterine horns
- Inject 2 to 3 l of the complexes to each embryo brain via pulled glass capillaries and a microinjector
- Apply the small cylinder magnet for 30 s on one side of the embryo cranium
- After surgery, replace the uterine horns
- Proceed to analyses 2 days after surgery

Short protocols for Rat:

Transfection of rat intestine

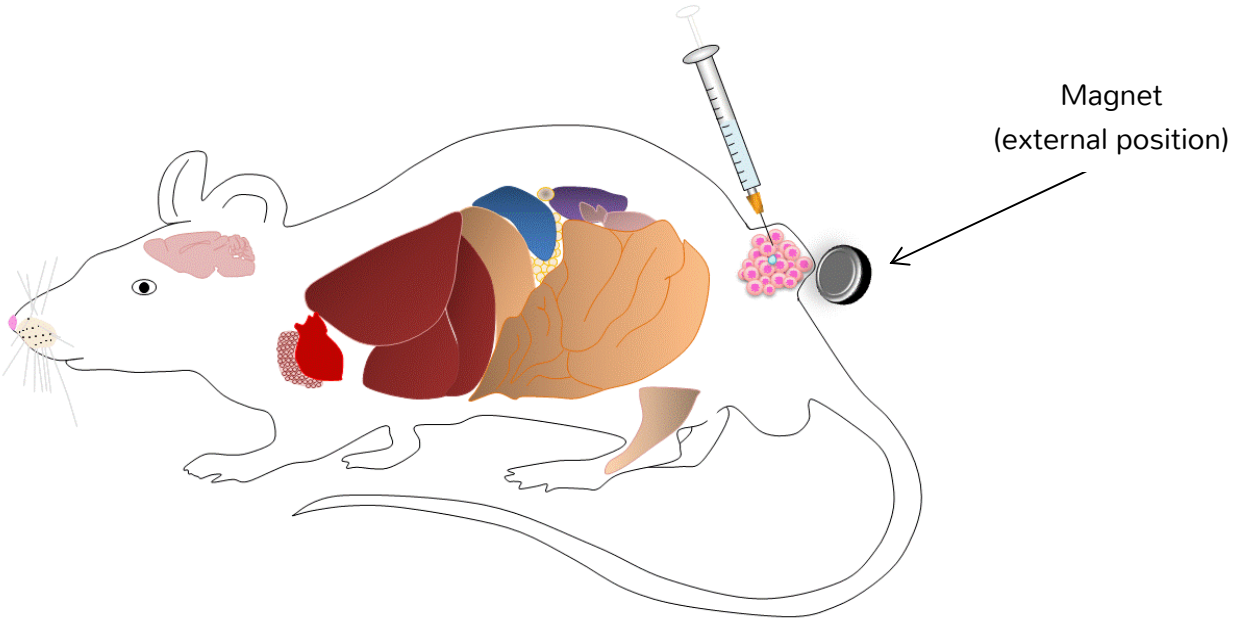
- Anesthetize your rat
- After laparotomy in the linea alba region, expose ileum and caecum
- Clamp off the guts 8 cm in the oral direction of the ileo-caecum junction
- Carefully rinse ingested material towards the caecum by application of 1 mL of isotonic saline
- Place a second clamp 3 cm aborally from the first one.
- Mix 200 µg of DNA and 200 µL of ***In vivo*** **PolyMag**
- Incubate for 20 min at room temperature
- Inject the complexes with a 20-G needle adjacent to the first clamp
- Place the square magnet under the clamp off section
- Remove the clamps 5 min after injection
- Let stand the magnet for 15 more min
- Return the guts into abdominal cavity
- Proceed to analyses after 48h



Short protocols for mouse:

Intra-tumoral injection for gene delivery to subcutaneous tumor

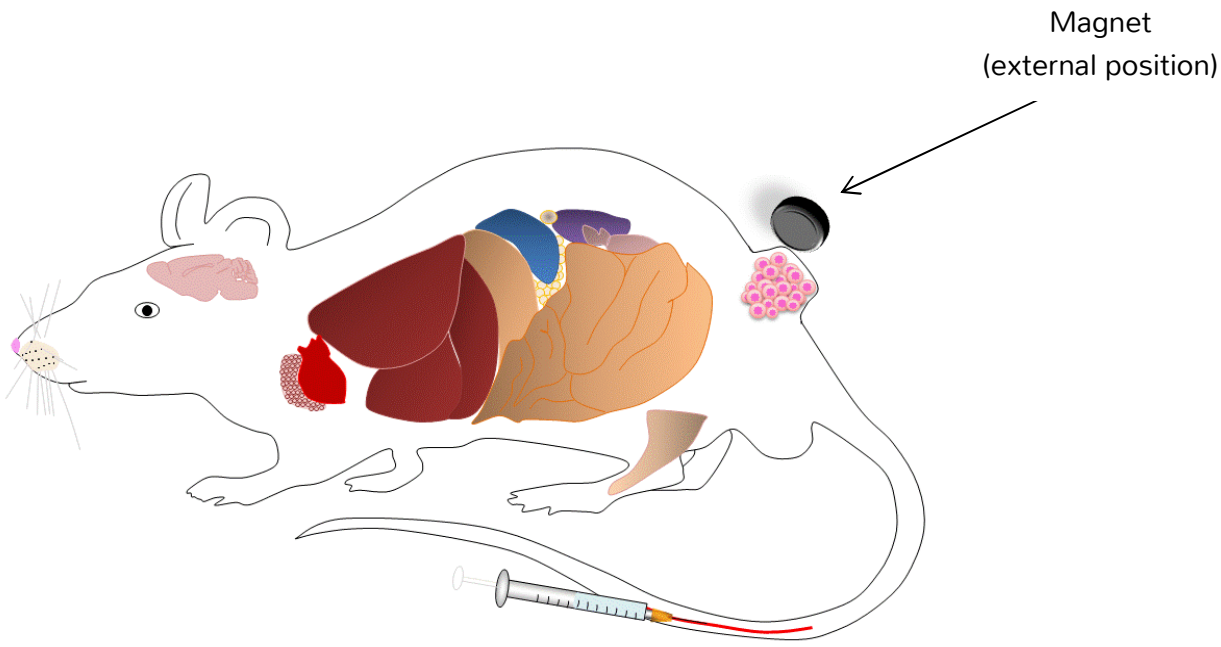
- Anesthetize your mouse
- Mix 20 g of DNA with 20 l of **Dogtor**
- Incubate 5 min at room temperature
- Combine the mixture to 20 µL of ***In vivo*** **CombiMag**
- Incubate 20 min at room temperature
- Inject 100 l of the mixture intratumorally using a 28-G needle
- Hold the cylinder (or the square) magnet onto the tumor surface for 30 min following the injection
- Proceed to analyses after 24h



Short protocols for mouse:

iv injection for gene delivery to subcutaneous tumor

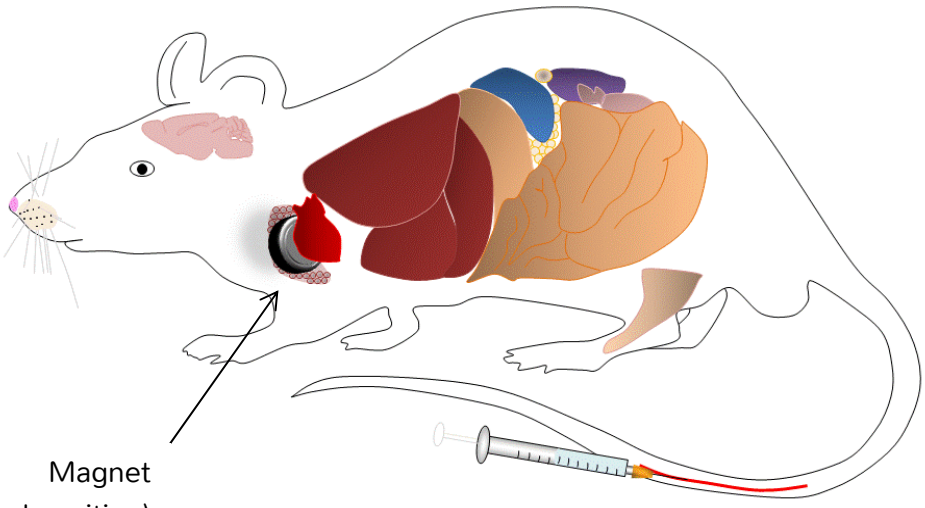
- Anesthetize your mouse
- Mix 50 g of DNA with 50 l of **Dogtor**
- Incubate 5 min at room temperature
- Combine the mixture to 50 µL of ***In vivo*** **CombiMag**
- Incubate 20 min at room temperature
- Inject slowly 200 l of the mixture via the tail vein
- Hold the cylinder magnet onto the tumor surface for 1 min throughout the infusion and for 15 min following the injection
- Proceed to analyses after 24h



Short protocols for mouse:

iv injection for gene delivery to the heart

- Anesthetize, intubate and ventilate your mouse
- Expose the heart via left thoracotomy
- Put the cylinder magnet in the chest of the mouse between the heart and lung
- Mix 50 µg of DNA and 50 µL of ***In vivo*** **PolyMag**
- Incubate for 20 min at room temperature
- Inject 200 µL of the mixture via the tail vein
- Remove the magnet after 20 min
- Proceed to analyses after 72h

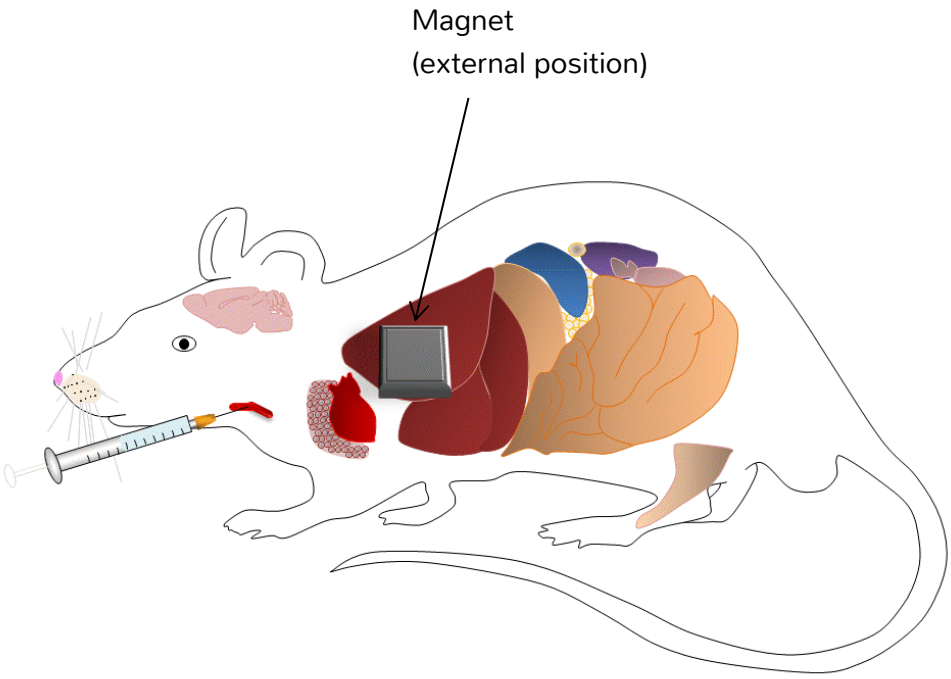


Magnet
(internal position)

Short protocols for mouse:

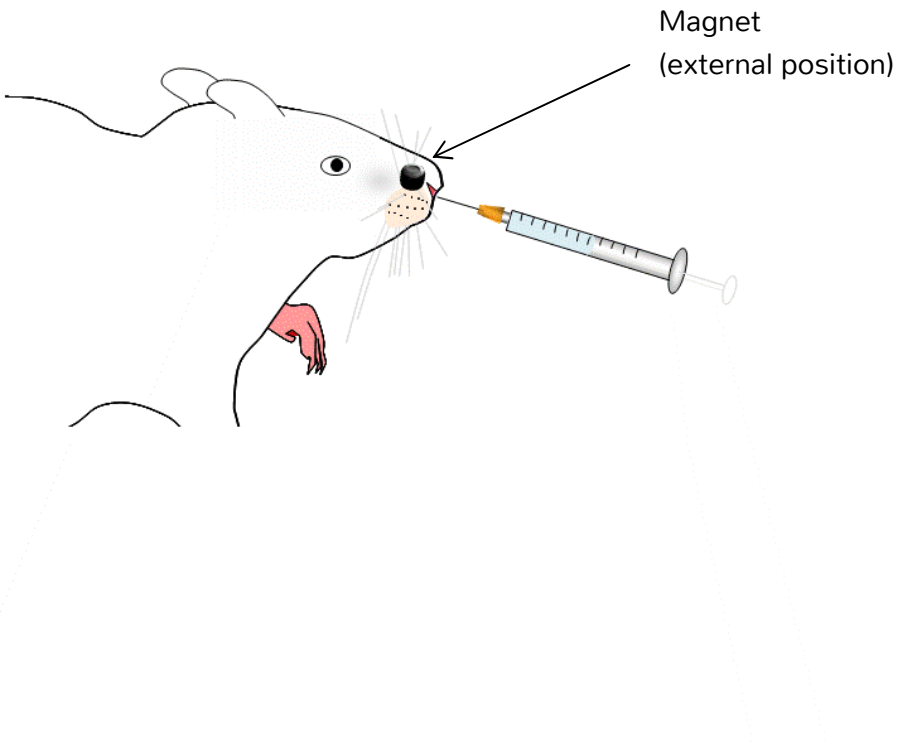
Intra-arterial injection for gene delivery to the liver

- Anesthetize your mouse
- Mix 2 x10^8 lentiviral particles with 20 l of ***In vivo*** **ViroMag**
- Incubate 20 min at room temperature
- Place a square magnet externally at the right abdominal wall close to the liver
- Inject the complexes via a catheter into the carotid artery
- Let stand the magnet for 30 min
- Observe the tissue after 48h



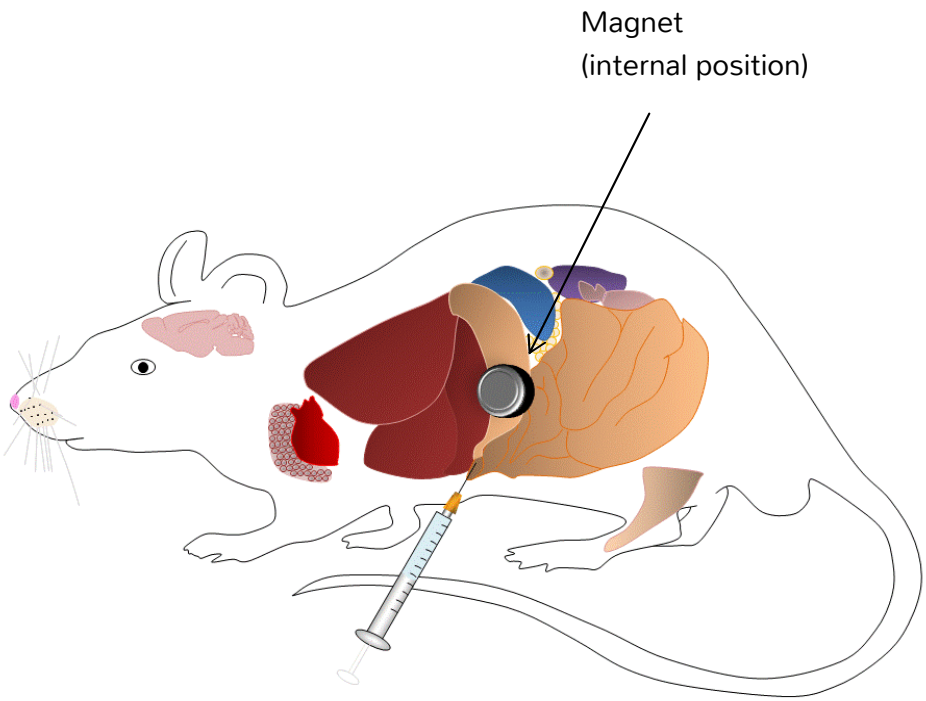
Transfection of mouse nasal epithelium

- Anesthetize your mouse
- Mix 50 g of DNA with 50 l of **Dogtor**
- Incubate 5 min at room temperature
- Combine the mixture to 50 µL of ***In vivo*** **CombiMag**
- Incubate 20 min at room temperature
- Place the magnet externally in direct contact onto the nostril (to attract the vector onto the septum during perfusion)
- Insert 2.5 mm of a fine tip catheter (˜0.5 mm in diameter) into the nasal cavity
- Slowly inject the complexes
- Let stand the magnet during injection and 15 min later
- Observe the tissue 48h after transfection



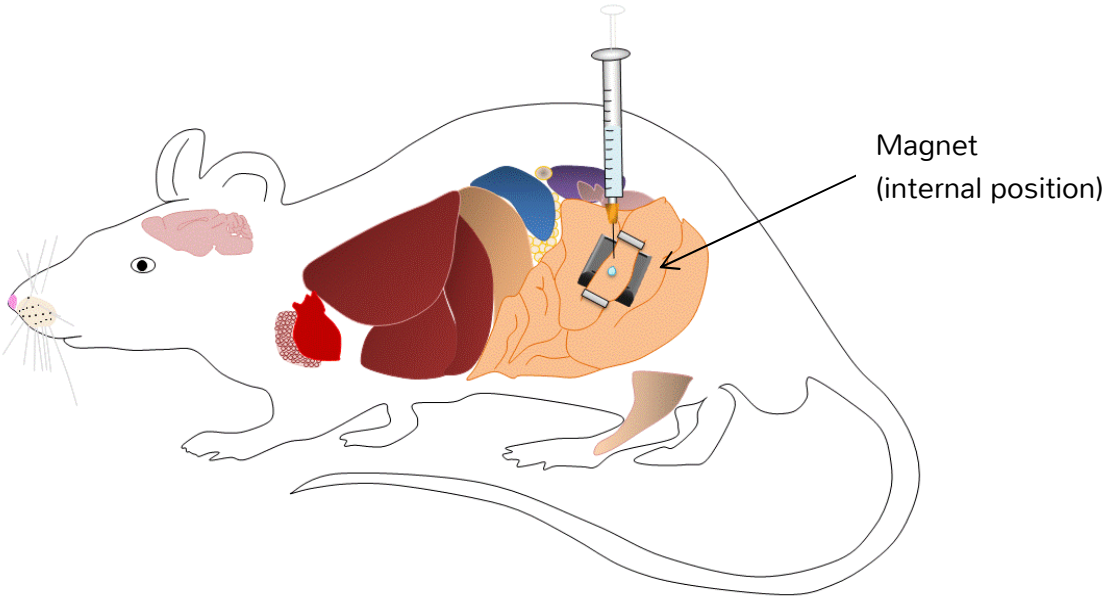
Transduction of mouse stomach cells

- Anesthetize your mouse
- Expose the stomach and duodenum after laparotomy at the left costal margin
- Mix 10^8 p.f.u of adenovirus with 20 l of ***In vivo*** **ViroMag**
- Incubate 15-30 min at room temperature
- Dilute the complexes in 1mL PBS
- Inject the complexes with a 27-G needle via the duodemun into the curvature of the stomach
- Position a cylinder magnet under the area of gastric fundus for 20 min
- Return the stomach into abdominal cavity
- Proceed to analyses after 48h



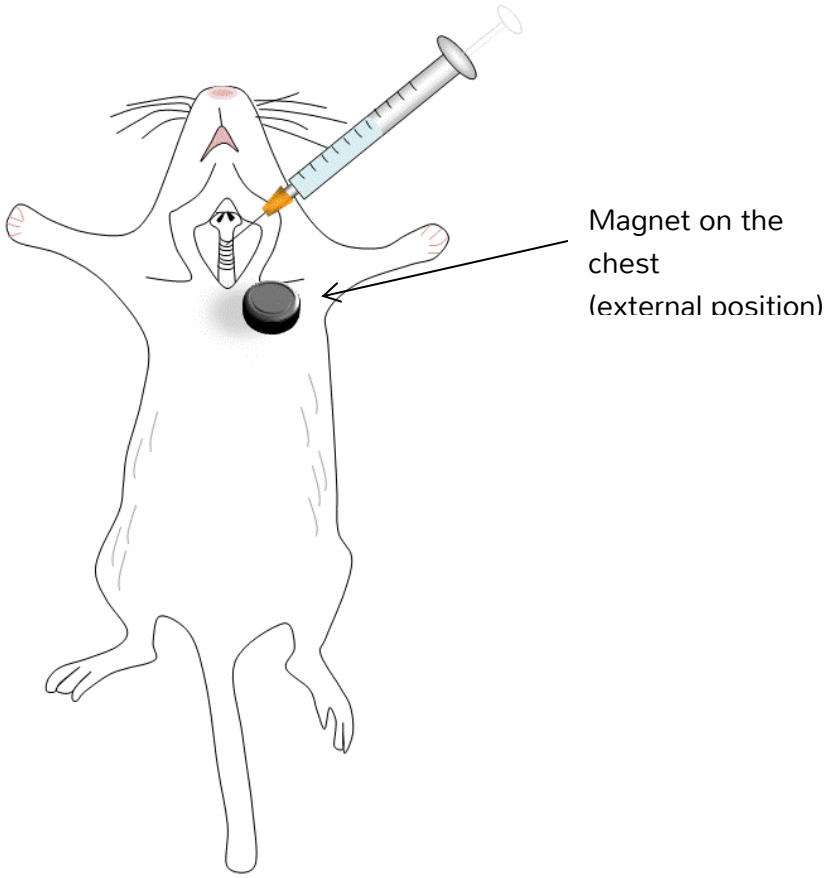
Transduction of mouse intestinal cells

- Anesthetize your animal
- After laparotomy in the linea alba region, expose duodenum and jejunum
- Clamp off the guts 5 cm in the oral direction of the stomach
- Carefully rinse ingested material towards the caecum by application of 1 mL of PBS
- Place a second clamp 3 cm above the first one.
- Mix 10^8 p.f.u of adenovirus with 20 l of ***In vivo*** **ViroMag**
- Incubate 15-30 min at room temperature
- Inject the complexes with a 20-G needle adjacent to the first clamp
- Place the square magnet under the clamp off section
- Remove the clamps 5 min after injection
- Let stand the magnet for 15 more min
- Return the guts into abdominal cavity
- Proceed to analyses after 48h



Transduction of mouse lung

- Anesthetize your animal
- Expose the trachea
- Mix 10^8 p.f.u of adenovirus with 20 l of ***In vivo*** **ViroMag**
- Incubate 15-30 min at room temperature
- Carefully inject 1 mL of PBS through a 20-G bent needle in order to wash the whole lung
- Immediately after washing, dilute the complexes in 300µL of PBS and inject with a 20-G bent needle
- Place the square magnet on the chest for 20 min
- Proceed to analyses after 48h



icv injection in adult mouse brain

For retro/lentiviral particles:

- Anesthetize your animal
- Mix 10 µL of ***in vivo* ViroMag** with 10 µL of lentivirus (10^9 Viral particles / mL)
- Incubate 20 min at room temperature
- Inject 2 µL / mouse
- Apply the square magnet up to 5 minutes

For adenoviral particles:

- Anesthetize your animal
- Mix 15 µL of ***in vivo* ViroMag** with 10 µL of adenoviral particles (10^7 infectious particles)
- Incubate 20 min at room temperature
- Inject 2 µL / mouse
- Apply the square magnet from 30s to 5 min.