**Shuttle/Cas9 RNP mix preparation**

**Delivery conditions**

* Feldan Shuttle 40 µM
* Cas9-NLS 2.5 µM / crRNA-tracrRNA 2µM

**Material**

* D10 (S10 or FS66d2) Feldan Shuttle 250 µM stock
* D237 (FSD237) Feldan Shuttle 250 µM stock
* Cas9-NLS protein 61 µM stock
* Alt-R® CRISPR-Cas9 crRNA XT (SpyCas9 g-loxP2\_C9):

Target sequence 5’– CATTATACGAAGTTATATTA – 3’ (stock 100µM)

* Alt-R® CRISPR-Cas9 trcrRNA (stock 100 µM)
* Duplex buffer
* PBS 1X: regular PBS (NaCl/Na2HPO4/KH2PO4/KCl), **needs to be Ca2+-free** (see PBS options at the end of the protocol if needed)
* **Thaw and keep material stock on ice**
* **Scale up the volumes of reagents to accommodate the number of mice to be instilled.**

**To prepare RNA duplex, combine:**  
crRNA, 1 ul (100 uM stock)  
trcrRNA, 1 ul (100 uM stock)

Duplex buffer, 8 ul  
Mix gently by pipetting.

Total volume of 10 ul, and final concentration of RNAs 10 uM.

Heat at 95 °C for 10 min, cool down to RT.

**To prepare RNP, 20 ul, combine:**

RNA duplex, 10 ul (10 uM)

Cas9, 2.1 ul (61 uM stock)

PBS, 7.9 ul

Mix gently by pipetting.

Total volume of 20 ul, and final concentrations: 5 uM RNAs, 6.25 uM Cas9.

Incubate at least 15 min at RT. (Can be kept at RT up to 2h).

**Prepare the Shuttle mix. Combine in order**

PBS 1X, 6 ul

Water, 16 ul

Peptide, 8 ul (250 uM stock)

Mix gently by pipetting.

Add the 20µL of RNP immediately before instillation.

Mix gently by pipetting.

Total volume of 50 ul, and final concentrations: 40uM Peptide, 2 uM RNAs, 2.5 uM Cas9.

Instill 50 ul on the nares of an anesthetized mouse.

**PBS options:**

You can use:

1x DPBS by Gibco (calcium-free, magnesium-free) - <https://www.thermofisher.com/order/catalog/product/14190144#/14190144>

or PBS preparation

For 1 liter of PBS 10X:

* NaCl 80 g
* Na2HPO4 14.2 g
* KH2PO4 2.4 g
* KCl 2 g

1. Sterilize the PBS 10X (autoclave)
2. Dilute in MilliQ water to obtain PBS 1X
3. Sterilize the PBS 1X (autoclave)

Gives a PBS 1X solution including:

* NaCl 137 mM
* Na2HPO4 10 mM
* KH2PO4 1.8 mM
* KCl 2.7 mM