Purification of Lenti-Cas9 and Lenti-Cpf1, and secreted Cas9 and Cpf1

- **pCAGGs-sigseq-Cas9-histag:**
  - Whole cell lysate
  - Purification from the medium
- **pCAGGs-sigseq-Cpf1-histag:**
  - Whole cell lysate
  - Purification from the medium
- **Lenti-Cas9:**
  - Whole cell lysate
- **Lenti-Cpf1:**
  - Whole cell lysate

Ni-Nta bead protein extraction of medium of secreted cas9/cpf1

- 30 ul Ni beads in 1,5 mL epp
- Add 1 mL media.
- plate rocker for 3 hours
  - *Preheat hotplate*
- @ 800 g 1 min
- remove supernatant
- wash with 1 mL P1 buffer
- @ 800 g 1 min
- remove supernatant
- add 50 ul sample buffer (laemmli sample buffer with 2-ME, 1:9)
- boil sample: 98ºC hot plate 3 min, vortex and 98ºC hot plate 3 min. @ max speed 3 min and transfer supernatant to a new tube
  - No nanodrop!
- store at -20ºC for protein gel analyses

Whole cell lysate

- *Preheat heatblock to 98 degrees Celsius*
- Wash cells with PBS
- Add 250 uL 1x laemmli loading dye (probably needs to be diluted to 1x beforehand! And you need to add 2 beta mercaptoethanol (1:10) To each well, this will lyse them and it might be very viscous
- Transfer to 1,5 mL tube, 98ºC hot plate 3 min, vortex and 98ºC hot plate 3 min.
- Centrifuge max speed 3 min and transfer supernatant to a new tube
  - After this point you could store the 1,5 mL tubes in the fridge, to use them the next day (or store in freezer for longer storage)