iGEM 2017 Collaboration –
Utrecht University and Wageningen University

This collaboration consists of two parts and was meant to verify the secretion of sCpf1 and sCas9. Part 1 will be done at Utrecht University. Part 2 will be done at Wageningen University.

Part 1:
Cells transfected with

1. **GFP (control)**
2. **GFP + Cpf1**
3. **GFP + dCpf1**
4. **GFP + sCpf1**
5. **GFP + Cas9**
6. **GFP + sCas9**

Transfection success will be confirmed by visualizing GFP.
The medium of the cells will be taken and centrifuged (full speed 3min). The supernatant of this will be mixed with 1/5 volume 5x Laemmli buffer.
The cells will be washed with PBS (2x), before resuspending in PBS and then centrifuging (800 RPM 3min). The pellet is resuspended in 500 uL PBS. Dilute 4x in PBS. Add 1/5 volume 5x Laemmli buffer.

Boil the samples (5 min 95C). These samples can then be transported safely and are ready to load onto a polyacrylamide gel. **Note:** when transporting on ice, reboil the samples before use (5 min 95C)

Part 2:
Materials:

- Samples (supplied by iGEM Utrecht)
- Primary antibody (anti Cas9) (supplied by iGEM Utrecht)
- Primary antibody (anti Cpf1) (supplied by iGEM Utrecht)
- Secondary antibodies (supplied by iGEM Utrecht)

- Transfer Buffer
- Blotting Buffer
- TBST
- Milk powder
- Polyacrylamide gel
- Protein ladder
- Westerblot membrane
- SDS-PAGE and Westernblot equipment
- Chemiluminecence imager
- Ponceau S solution (optional)
- Chemiluminecence substrate (HRP)

Protocol:

1. Prepare a polyacrylamide gel 4/12% (15 slots, 1,5 mm)
2. Load protein ladder (we used 8uL SeeBlue Plus2 Pre-stained Protein ladder) and samples (20 uL each) (see next page for order)
3. Run the gel at 100V until the dye front runs out of the gel

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4. Transfer the protein from the gel to a nitrocellulose membrane (60 min at 100V)
5. Rinse blot in demi-water and stain with Ponceau S solution
6. Rinse off the stain by three washes TBST
7. Block in 5% (w/v) milk powder (1hour at RT)

8. **Cut the membrane into two parts; now you have a membrane for anti-Cas9 and a membrane for anti-Cpf1**
9. Incubate primary antibody overnight at 4C (1:2000 dilution)
    a. Anti-Cas9 for the membrane with the Cas9 proteins
    b. Anti-Cpf1 for the membrane with the Cpf1 proteins
10. Rinse 3- 5 times for 5min with TBST
11. Incubate with secondary antibody for 1 hour at RT (1:10 000 dilution)
    a. Secondary antibody works for both primary antibodies
12. Rinse 3- 5 times for 5min with TBST

13. Apply chemiluminescence substrate (according to kit protocol)
14. Image chemiluminescence to verify secretion of sCas9 and sCpf1 (and no secretion of Cas9 and Cpf1)

Gel loading order:

1. Ladder
2. Cpf1 (medium)
3. dCpf1 (medium)
4. sCpf1 (medium
5. Cpf1 (cells)
6. dCpf1 (cells)
7. sCpf1 (cells
8. GFP control (cells)

_____ cut membrane along this line

9. Ladder
10. Cas9 (medium)
11. sCas9 (medium)
12. Cas9 (cells)
13. sCas9 (cells)
14. GFP control (medium)
15. GFP control (cells)