**Northern Blot protocol for Cas9 guideRNA**

**Probe radiolabeling :**

- Order a DNA probe of 70nt in size that is antisense to the target RNA (in our case, we used ER#415 which is complementary to the conserved part of the gRNA).

- Dilute the probe to 10µM final.

- Prepare a PNK reaction as follows : 3µl of the 10µM probe, 2µl of the PNK 10X buffer, 5µl of 32P-γATP, 1µl of T4 PNK and 9µl of water.

- Incubate 1 hour at 37ºC.

- Add 40µl of AMPure XP beads and mix by pipetting. Incubate 5 minutes at RT.

- Place sample on the magnet and wash beads twice with 200µl of 70 % ethanol.

- Resuspend in 100µl of water, incubate at RT for 5 minutes and recover the aqueous phase.

**Acrylamide denaturing gel:**

- Prepare a 10% acrylamide (19:1), 8M UREA, 0.5X TBE gel.

- Warm-up the gel at 35 watts (~700 volts) for 45 minutes.

- Load 5µg (up to 50µg) of RNA in each well.

- Run the gel for 1 hour at 35 watts.

**Semi-dry RNA transfer to a nitrocellulose membrane:**

- Prepare a sandwich as follows: 2 layers of 3M whatman paper, one layer of Nitrocellulose membrane, gel, 2 layers of 3M whatman paper. Everything has to be pre-wet in 0.5X TBE.

- Transfer at 300mA (maximum 30volts) for 1 hour at RT.

- Recover the nitrocellulose membrane and irradiate it in the stratalinker for 1 minute.

- Bake the membrane at 80ºC for 30 minutes.

- Store the membrane between two 3M whatman sheets.

**Probe Hybridization:**

- Incubate the membrane in 50ml of Church Buffer for 30 minutes at 37ºC.

- Wash the membrane twice in Church buffer and resuspend it in 5ml of Church buffer.

- Add 100µl of the radiolabeled probe (heat denature the probe at 95ºC for 5 minutes before using it).

- Incubate the membrane with the probe O/N at 37ºC.

- Wash the membrane twice in 50ml wash buffer (1X SSC + 0.1% SDS).

- Take the membrane out of the hybridization bottle and wash it in 100ml of wash buffer twice.

- Wrap the membrane in Saran plastic wrap and expose it.

**Buffer composition:**

Church buffer (for 500ml):

16.75g of Na2HPO4\*7H@)

500µl of 85% Phosphoric Acid

1ml of 0.5M EDTA pH 8.0

Heat to dissolve

35g of SDS

5g of BSA