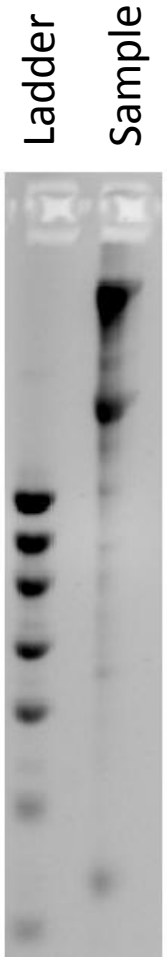
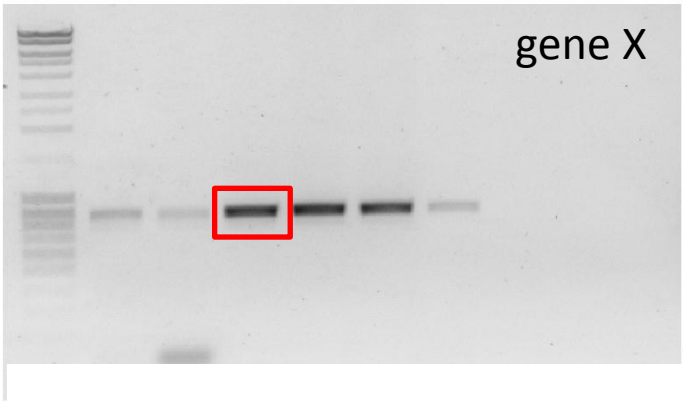
Total RNA extraction on neuronal tissue (Rneasy kit)

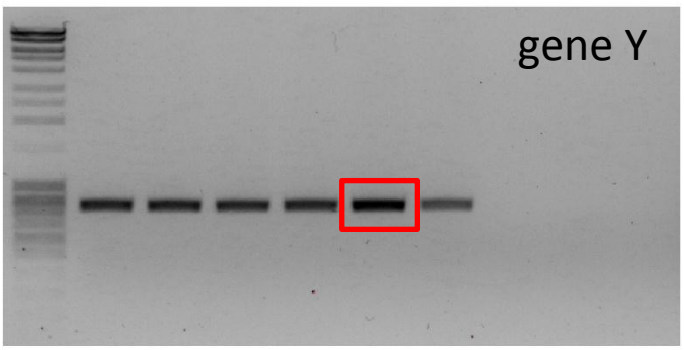


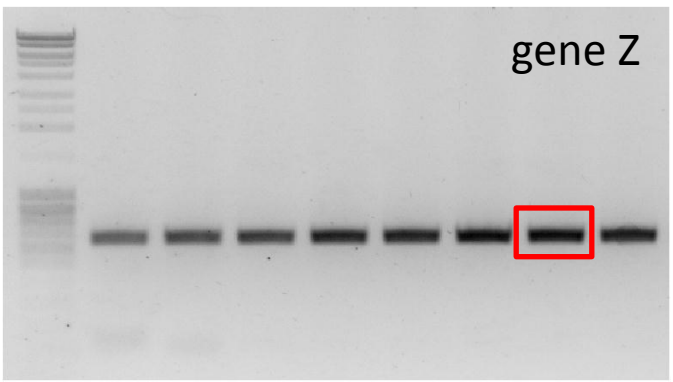
Extraction OK

→ Reverse transcription of the sample (Superscript II kit)

Gradient PCR 60°C+/-10°C on the previously synthetized cDNA
Test with T3-Forward and T7-Reverse primers







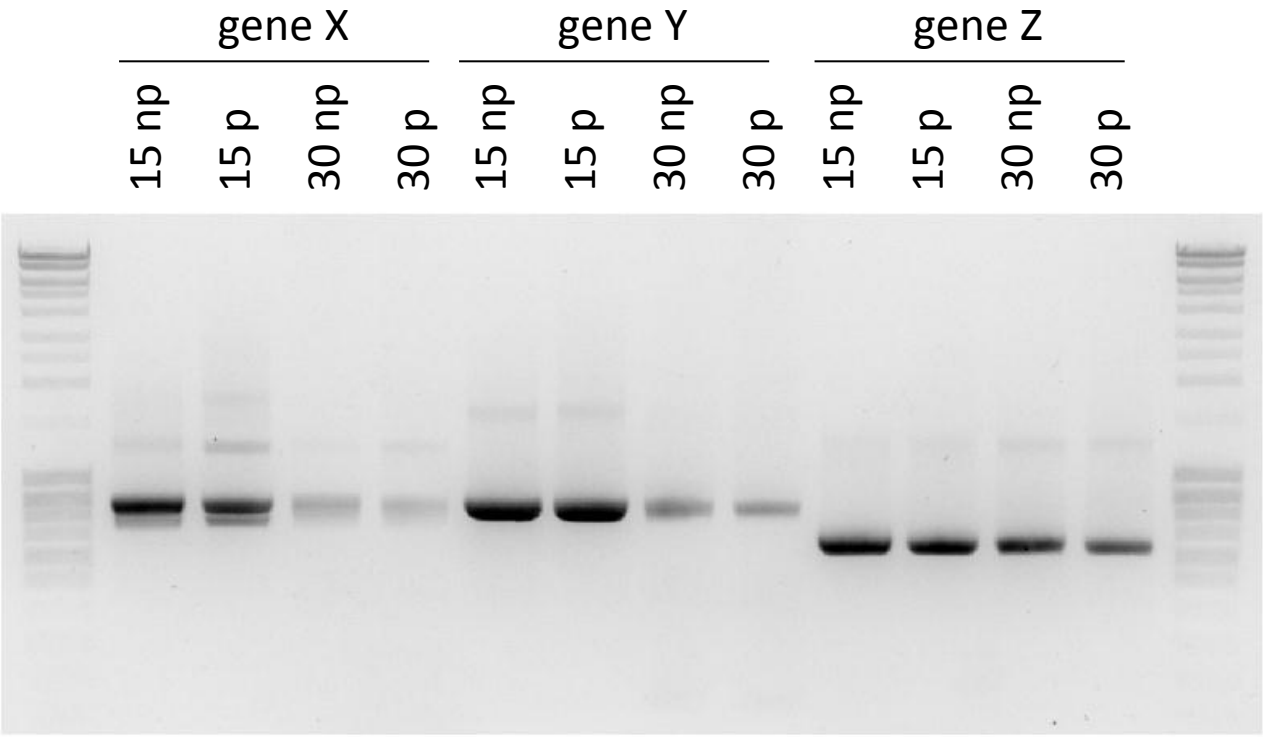
50°C → 70°C

Chosen annealing temperature

Size of the bands is OK.

→ DNA extraction on gel for PCR #2

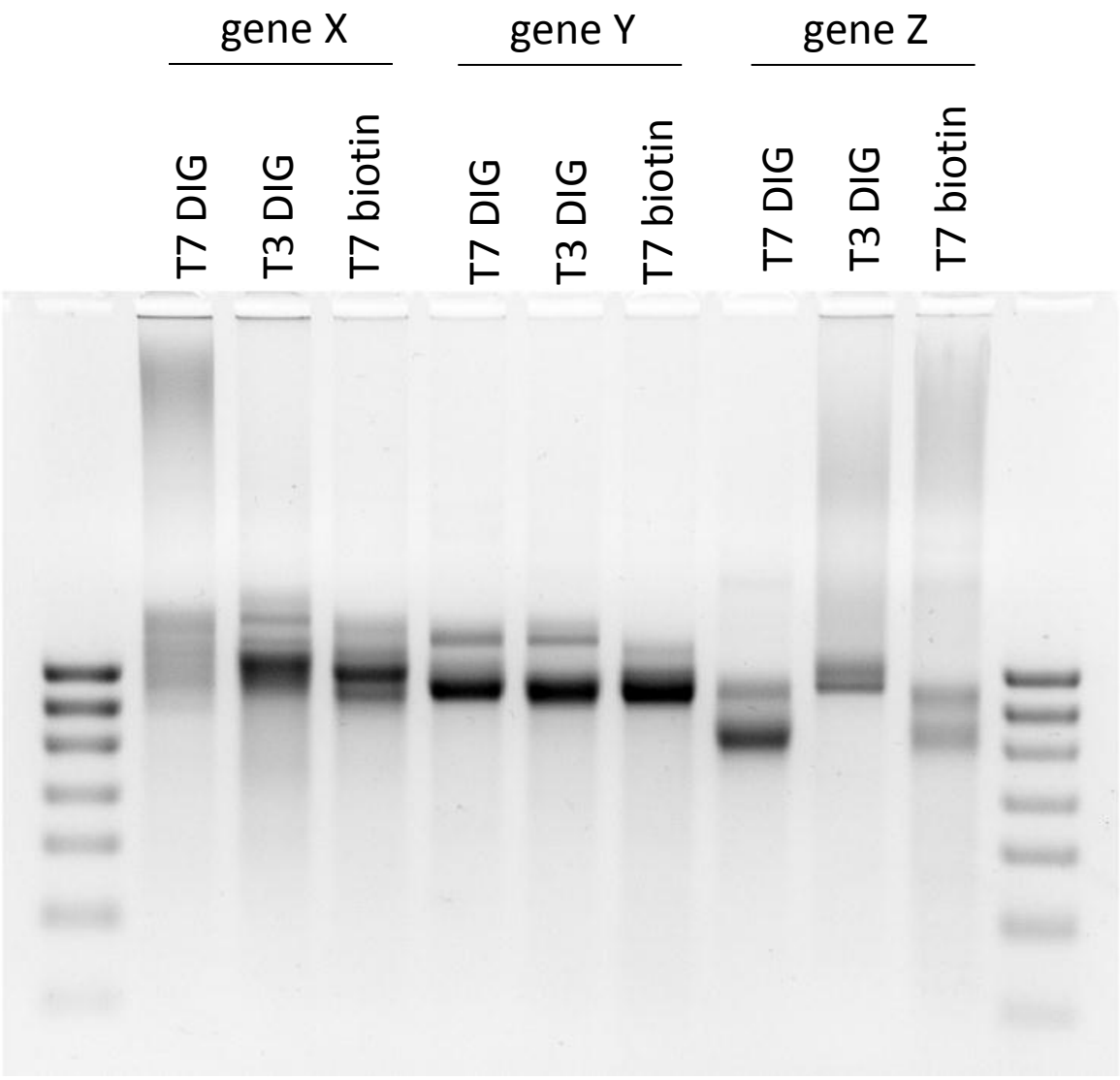
PCR #2 on gel extracted DNA from PCR #1 (same primers)



15 = PCR #2 with 15µl DNA extracted from gel after PCR #1
30 = PCR #2 with 30µl DNA extracted from gel after PCR #1
np = non purified PCR #2 product
p = purified PCR #2 product (in the same volume)

Faint non specific products ???

Purified PCR products ("15µl" samples) used for in vitro transcription



Smearing of the RNA samples

Products are highers than expected

Multiple bands

1,5% agarose gel, RNA staining with GelRed
1000-100b RNA ladder

IN VITRO TRANSCRIPTION PROTOCOLE :

Transcription mix (prepare on ice) :

- 1µg purified DNA template
- H_2O RNase-free (up to 13,5µl, for a 20µl final volume)
- 2µl 5X transcription buffer (1X final)
- 2µl DIG *or biotin* RNA Labeling Mix (1mM final, 0,65 and 0,35mM for UTP and DIG-UTP respectively)
- 2µl T7 *or T3* RNA polymerase (2U/µl final)
- 0,5µl RiboLock RNase Inhibitor (1U/µl final)

Incubate 2h at 37°C
Add 2µl DNase I (2U) and incubate 15min at 37°C
Stop the reaction with 1µl 0,5M EDTA pH8

Work in ice from this time

Probes purification :

Add 2,5µl 4M LiCl
Add 75µl cold 100% EtOH
Incubate 1h at -80°C
Spin 30min at 16000 x g, 4°C
Pipette out the supernatant
Wash with 100µl cold 100% EtOH
Spin 5min at 16000 x g, 4°C
Pipette out the supernatant and let the tubes dry under the hood, at room temperature
Resuspend pellet in 50µl RNAse-free H_2O