LAB NOTEBOOK

PATRAS MED

Patras Medicine iGEM 2026

ICHNOS LAB NOTEBOOK

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WET LAB RECORD - Patras Medicine iGEM 2026

LAB NOTEBOOK

ICHNOS | *Patras Medicine iGEM 2026* | Wet Lab Team

STRAIN, MEDIA AND STANDARD REACTIONS

STRAIN

- Saccharomyces cerevisiae CEN.PK2-1C (EUROSCARF), his3Δ1. Unmodified cells of the same strain are referred to as WT.
- Three strains were built, one per sensing promoter: pSynOS4(alt1) tested with H_2O_2, pKAR2 with DTT and pCUP1 with $CuSO_4$. The cassette is integrated at the HO locus.

MEDIA

- YPD for routine growth and for competent cells.
- SD−His: yeast nitrogen base without amino acids and without ammonium sulfate, ammonium sulfate, CSM−His, and 2% glucose or galactose. Plates for selection, liquid for the cultures.
- The sugar is dissolved in a small part of the water and autoclaved in its own bottle. The two bottles are combined after cooling.

COMPONENT	WITH GLUCOSE, 200 mL	WITH GALACTOSE, 60 mL
Yeast nitrogen base w/o amino acids and ammonium sulfate	0.34 g	0.102 g
Ammonium sulfate	1.00 g	0.300 g
CSM−His	0.154 g	0.046 g
Water, first bottle	about 180 mL	about 54 mL
Sugar, separate bottle	4.0 g glucose in about 20 mL	1.2 g D-galactose in about 6 mL

STANDARD PCR REACTION (20 µL)

- KAPA Taq DNA Polymerase (5 U/µL) with 10× Buffer A, which contains 1.5 mM $MgCl_2$ at 1×; no $MgCl_2$ added separately.
- 18 µL of master mix and 2 µL of DNA. Master mix: 0.1 µL enzyme, 2 µL buffer, 0.4 µL dNTPs (10 mM each), 0.8 µL of each primer (10 µM) and 13.9 µL water.
- Annealing at 55 °C unless stated otherwise; remaining cycling parameters per the KAPA Taq protocol.

GROWTH, QUANTIFICATION AND EQUIPMENT

- Cells grown at 30 °C, shaken in liquid, plates incubated upside down.

- OD600 read against a blank of the same medium; samples above 1.0 diluted 1:10 in the cuvette.
- DNA and RNA quantified on a NanoDrop, 1 µL per reading, several readings per sample.
- NucleoSpin Gel and PCR Clean-up kit and NucleoSpin RNA kit (Macherey-Nagel). Primers from Microsynth, ordered through Bioline.
- Thermal cycler, table-top centrifuge, water bath at 42 °C, heating block at 95 °C, shaking incubator at 30 °C, Olympus CKX41 inverted fluorescence microscope.

Day 1 – 23.09.2026 STRAIN REVIVAL AND STOCK CULTURES

REVIVAL

- The strain arrived from EUROSCARF as a streak on a YPD agar slope in a screw-capped vial, containing approximately 0.01 mg of cells.
- Cells were revived on YPD and grown at 30 °C.
- Solid and liquid YPD stocks were prepared and kept for the work that followed.

Day 2 – 24.09.2026 FIRST PCR OF FRAGMENTS 1–8, CLEANUP AND QUANTIFICATION

TEMPLATE PREPARATION

- The synthetic DNA was dissolved following IDT's instructions.
- Seven fragments were supplied by IDT as gBlocks HiFi. Fragment 5 was supplied as a custom gene inside a carrier plasmid and is recovered from it by PCR.

FRAGMENT	CONTENT	LENGTH (bp)	USED IN
1	HO left flank (TS1) and HIS3 marker	1473	All strains
2	pCUP1 sensing module	1062	pCUP1
3	pKAR2 sensing module	1598	pKAR2
4	pSynOS4(alt1) sensing module, first part	144	pSynOS4(alt1)
5	pSynOS4(alt1) sensing module, second part	650	pSynOS4(alt1)
6	TetR cassette (pGAL1-D12, TetR, CYC1 terminator)	1437	All strains
7	Reporter cassette, first half	1281	All strains
8	Reporter cassette, second half, ending in HO right flank (TS2)	1735	All strains

AMPLIFICATION

- Each fragment was amplified with its own primer pair (Frag1_FWD/REV to Frag8_FWD/REV), designed with Tm of about 60–62 °C and a G or C at the 3′ end.
- Standard 20 µL reaction, 2 µL of dissolved synthetic DNA as template, annealing at 55 °C. For Fragment 5 the template was the carrier plasmid.

PCR CLEANUP

- Every reaction was cleaned with the NucleoSpin Gel and PCR Clean-up kit, following the protocol for PCR products:

- Sample mixed with binding buffer and loaded on the silica column.
- Washed twice with the ethanol-containing buffer.
- Membrane dried by centrifugation.
- Eluted in a small volume of elution buffer.

QUANTIFICATION

- Each crude reaction was read on the NanoDrop in the afternoon and each cleaned eluate in the evening.
- Several readings were taken per sample, recording the concentration and both purity ratios, A260/280 and A260/230.

NOTE: The products were not run on a gel at this stage.

Day 3 – 25.09.2026 NanoDrop QUANTIFICATION REPEATED

RE-MEASUREMENT

- Fragments that had given doubtful readings the evening before were re-measured, 1 µL per reading.
- Fragments 2, 3 and 5 were not re-measured.
- The second set of readings was adopted as the working set.

PRACTICE ADOPTED: From this day on, every sample was read more than once and no single NanoDrop reading of a PCR product was used on its own.

Day 4 – 26.09.2026 FRAGMENT 4 RE-AMPLIFICATION

AMPLIFICATION AND CLEANUP

- Fragment 4 was amplified on its own in a separate PCR, annealing at 52.7 °C.
- The product was cleaned with the same kit and eluted in 30 µL.
- The eluate was quantified on the NanoDrop and kept as the Fragment 4 stock for both transformations.

Day 5 – 28.09.2026 COMPETENT CELLS AND FIRST TRANSFORMATION

DNA PER REACTION

- 150 fmol of each fragment was used, inside the usual 100–200 fmol range for in vivo assembly.
- Mass in ng = 150 fmol × length in bp × 650 g/mol per bp ÷ 1,000,000. The volume pipetted is this mass divided by the concentration measured on the day.

FRAGMENT	1	2	3	4	5	6	7	8
Length (bp)	1473	1062	1598	144	650	1437	1281	1735
Mass for 150 fmol (ng)	143.6	103.5	155.8	14.0	63.4	140.1	124.9	169.2

- Total DNA per reaction: 681 ng for pCUP1, 734 ng for pKAR2, 655 ng for pSynOS4(alt1).

COMPETENT CELLS

- Lithium acetate / single-stranded carrier DNA / PEG method, in the version adapted for our laboratory.
- A single of the unmodified strain was grown overnight in 5 mL YPD at 30 °C, 200 rpm.
- The overnight culture was diluted 1:100 into fresh YPD and grown 4–6 h to OD600 0.6–0.8.
- Cells were collected at 3000 × g for 5 min, washed with sterile water and resuspended in 100 mM lithium acetate.
- All strains were set up from the same pool of cells.

TRANSFORMATION

- Four reactions were run: the three sensing strains and a WT reaction receiving no DNA. Each used 500 µL of competent cells.
- Carrier DNA was boiled 5 min at 95 °C and held on ice at least 2 min.
- 890 µL of master mix per reaction: 600 µL 50% PEG 3350, 90 µL 1 M lithium acetate, 75 µL boiled carrier DNA, 125 µL sterile water.
- Master mix and the fragments of the strain were added to the cells and mixed by vortexing.
- Incubated 30 min at 30 °C, then heat-shocked 15 min at 42 °C in the water bath.
- Pelleted at 3000 × g for 2 min, PEG and lithium acetate removed, cells resuspended gently in sterile water.

PLATING AND STORAGE

- Suspensions were spread on SD−His. The WT reaction was also plated on YPD.
- Plates were incubated at 30 °C, upside down, for two to four days.
- Unused competent cells were mixed with glycerol and frozen at −20 °C in 100 mM lithium acetate, in two tubes of about 900 µL.

DEVIATION: Fragment 4 was to be added from a 1:10 dilution of its stock. The dilution was skipped and 1.8 µL of the concentrated stock was added instead, about ten times the intended mass. The same 1.8 µL of concentrated stock was used deliberately in the second transformation.

Day 6 – 01.10.2026 RESTREAKING OF TRANSFORMANT COLONIES

RESTREAKING

- Colonies were picked from areas of the plates that were free of contaminating growth.
- They were restreaked on fresh SD−His, three plates per strain, to obtain single, well-separated colonies.
- Plates were labelled by strain, carrying over the label from the day of the transformation.
- Plates were incubated at 30 °C for two to three days.

NOTE: The colony PCR primers sit in sequence shared by all three cassettes and cannot distinguish the strains, so strain identity rests on these plate labels.

Day 7 – 02.10.2026 SECOND ROUND OF FRAGMENT PCR

AMPLIFICATION, CLEANUP AND QUANTIFICATION

- Fragments 1, 2, 3, 5, 6, 7 and 8 were amplified again with the same primers and the standard reaction.
- Fragment 4 was not repeated; the stock from Day 4 was used.
- Products were cleaned with the same kit and each eluted in 25 µL.
- Three to five NanoDrop readings were taken per fragment, 1 µL each. For each fragment the largest group of readings agreeing within a coefficient of variation of 15% was kept, and the mean of that group was used as the working concentration.

Day 8 – 03.10.2026 SECOND TRANSFORMATION, FROM FROZEN COMPETENT CELLS

FRAGMENT 8 RE-QUANTIFICATION

- Fragment 8 was read three more times in the morning before the working concentration was fixed.

CELLS AND MASTER MIX

- The frozen competent cells were thawed at room temperature, pooled and mixed gently, giving about 1.8 mL.
- The pool was divided equally between five reactions: one per strain and two WT controls receiving no DNA, 360 µL of cells each.
- The master mix was scaled to the cell volume at the proportions of Day 5 (1.78 mL of mix per mL of cells): 432 µL PEG 3350, 64.8 µL 1 M lithium acetate, 54 µL boiled carrier DNA and 90 µL sterile water per reaction.

FRAGMENT VOLUMES PER REACTION

FRAGMENT	VOLUME PER REACTION (µL)	USED IN
1	1.74	All strains
2	1.91	pCUP1
3	2.85	pKAR2
4	1.8, from the undiluted stock	pSynOS4(alt1)
5	0.95	pSynOS4(alt1)
6	1.97	All strains
7	2.13	All strains
8	4.49	All strains

- A fresh pipette tip was used for every fragment.
- Incubation, heat shock, centrifugation and resuspension followed Day 5.
- Strains were plated on SD−His, WT controls on SD−His and YPD, and all plates were kept at 30 °C for at least three to four days.

Day 9 – 05.10.2026 GALACTOSE SWITCH, CULTURE ROUTINE AND FIRST H_2O_2 DOSE RESPONSE

CULTURE ROUTINE

- Two independent colonies of the strain were each inoculated into 5 mL SD−His with glucose in a 15 mL tube and grown with shaking at 30 °C overnight or longer. Only the healthier culture was carried forward.
- On the day of the experiment the OD600 of both cultures was read.
- The culture was diluted to OD600 about 0.25 in 5 mL fresh SD−His with glucose, using (0.25 × 5) ÷ OD600 of the old culture.
- It was grown at 30 °C for two to four hours, with a reading every 45–60 min, to OD600 0.5–0.8. Values of 0.4–1.0 were accepted when time was short and were recorded.
- Cells were centrifuged at 3000 rpm for 3 min, and 2 min more if the supernatant was still cloudy. The supernatant was removed completely.
- The pellet was resuspended in the same volume of SD−His with galactose and incubated at 30
  °C for 3 hours before any stressor was added.

NOTE: pGAL1-D12 drives both TetR and the reporter and is repressed by glucose, so the galactose step precedes every dose response. Strict sterile technique was kept up to this point.

SPIKE PREPARATION

- Every stressor was added as a 100× spike, so the spike is 1% of the culture volume.
- Each spike was made from the concentrated stock or intermediate, never from another spike, water first and reagent last, and used within about half an hour. Leftovers were discarded.
- H_2O_2 intermediate: 1980 µL of water in a 5 mL tube, 20 µL of the 30% (w/w) solution added into the water and mixed, giving about 98 mM.

FINAL DOSE	SPIKE (100×)	98 mM INTERMEDIATE	WATER
0 µM	water only	none	1000 µL
20 µM	2 mM	20 µL	980 µL
75 µM	7.5 mM	75 µL	925 µL
300 µM	30 mM	300 µL	700 µL
600 µM	60 mM	600 µL	400 µL

DOSING AND SAMPLING

- Five tubes, one per dose, each with 198 µL of galactose-adapted culture.
- 2 µL of spike was added to every tube one after another within about a minute; the time of the first addition was recorded as T. The zero-dose tube received 2 µL of water.
- Tubes were loosely capped and returned to the shaker at 30 °C.

- At nominal 0 h (in practice about 30 min after T), 30 min, 1 h, 2 h and 3 h after T, a 2–5 µL drop was taken from each tube onto a clean slide under a coverslip and the tube returned to the shaker.
- All slides for one time point were prepared before going to the microscope.

IMAGING

- Olympus CKX41 inverted microscope, 130 W mercury lamp with fiber light guide, cooled QImaging MicroPublisher 3.3 RTV camera, Image Pro Plus 7. Blue cube for sfGFP, green cube for mCherry.
- Every slide was imaged in brightfield, sfGFP and mCherry, in one or two fields of view. Imaging ran from 51 to 221 min after T, because the slides of one time point were prepared first and imaged in one visit afterwards.
- Slides were prepared and labelled by the team; imaging was performed by a graduate student from a neighbouring laboratory.

ACQUISITION SETTINGS REQUESTED BY THE DRY LAB

- Black level (offset) between 10 and 50.
- Saturation warning switched on; if it fires, the series is retaken at a shorter exposure.
- Any brightness-rescaling macro on the fluorescence setting switched off.
- 40× objective for fluorescence, correction collar set to the slide thickness.
- Daily record of lamp-on time, lamp setting, objective, collar position, container, time T and offset; per-image record of dose, sampling time, file name, exposure and gain.
- Original TIFFs kept and named day / time in minutes / dose / field / channel, e.g. d1_t30_dose300_f2_GFP.tif.

Day 10 – 06.10.2026 H_2O_2 WITH LATE READINGS AND FIRST DTT DOSE RESPONSE

pSynOS4(alt1) WITH H_2O_2, LATE READINGS

- Same five doses and the same spikes as Day 9. The cells were already in galactose medium.
- 1 mL of culture per dose was placed in a 15 mL tube and 10 µL of spike added, instead of 198 µL and 2 µL, so that the tubes hold enough air over a long incubation.
- Readings were taken at 6 and 7 hours after dosing and imaged from 357 to 430 min after T.

DTT STOCK

- DTT was supplied as a solid in a 1 g vial (Biotium 91050, M = 154.2).
- The vial was brought to room temperature unopened before weighing and returned to the freezer with its desiccant immediately afterwards.
- 154 mg was weighed and about 880 µL of water added, giving about 1 mL of 1 M stock. The stock was kept on ice while the spikes were made, then stored at −20 °C.

FINAL DOSE	SPIKE (100×)	1 M STOCK	WATER
0 µM	water only	none	1000 µL
100 µM	10 mM	10 µL	990 µL
250 µM	25 mM	25 µL	975 µL
500 µM	50 mM	50 µL	950 µL
1000 µM	100 mM	100 µL	900 µL
2000 µM	200 mM	200 µL	800 µL

pKAR2 DOSING AND SAMPLING

- Six tubes, a zero dose and five doses, each with 198 µL of culture and 2 µL of spike, as on Day 9.
- Cells were moved from glucose to galactose medium and dosed 3 hours later.
- Slides were made at six time points and imaged from 17 to 395 min after T.
- Both runs were imaged in brightfield, sfGFP and mCherry, in one or two fields of view.

Day 11 – 07.10.2026 H_2O_2 AND DTT DOSE RESPONSES REPEATED

RUNS

- pSynOS4(alt1) was run again against H_2O_2 at the same five doses, with slides made in four sets and imaged from 55 to 457 min after T.
- pKAR2 was run again against DTT at the same six doses, with slides made in three sets and imaged from 56 to 319 min after T.
- All slides were imaged in brightfield, sfGFP and mCherry, in three neighboring fields of view.

Day 12 – 08.10.2026 UNMODIFIED STRAIN UNDER H_2O_2 AND DTT

WT MEDIUM

- WT cells had been growing in 10 mL YPD, which fluoresces, so they were moved to synthetic medium with galactose.
- The WT strain requires histidine. A 20 mL base identical to the sensing-strain medium was prepared and split into two 10 mL portions after autoclaving.
  - Bottle 1: 0.034 g yeast nitrogen base w/o amino acids and ammonium sulfate, 0.100 g ammonium sulfate and 0.015 g CSM−His in 16 mL water.
  - Bottle 2: 0.400 g D-galactose in 4 mL water.
  - Bottle 3: 0.020 g L-histidine in 10 mL water (2 mg/mL).
- All three bottles were autoclaved at 121 °C for 15 min with caps loosened, cooled and combined under sterile conditions.
- One 10 mL portion received 100 µL of the histidine stock (20 mg/L final); the second received 100 µL of sterile water, so the dilution is the same in both.

EXPOSURE AND IMAGING

- WT cells were centrifuged at 3000 rpm for 3 min to remove the YPD and resuspended in the WT medium.

- They were brought to OD600 0.5–0.8 and kept in galactose for 3 hours before dosing.
- Doses were those of Day 9 for H_2O_2 and Day 10 for DTT, imaged from 13 to 153 min after T for H_2O_2 and from 26 to 303 min after T for DTT.
- Slides were prepared and imaged in the same way. A drop of sterile medium without cells was also imaged on 5 and 8 October, as a blank for the medium itself.

Day 13 – 10.10.2026 pCUP1 WITH COPPER SULFATE

COPPER STOCK

- Copper sulfate pentahydrate (249.68 g/mol), 0.250 g in 10 mL of water, giving a 100 mM intermediate.

FINAL DOSE	SPIKE (100×)	100 mM INTERMEDIATE	WATER
0 µM	water only	none	1000 µL
10 µM	1 mM	10 µL	990 µL
25 µM	2.5 mM	25 µL	975 µL
50 µM	5 mM	50 µL	950 µL
100 µM	10 mM	100 µL	900 µL

NOTE: The dose range was taken from published characterizations of the CUP1 promoter, inside the linear range and a little above it, well below the concentrations at which copper is toxic.

DOSING AND SAMPLING

- Five tubes, a zero dose and four doses, each with 198 µL of galactose-adapted culture and 2 µL of spike, as on Day 9.
- Slides were prepared at the time of dosing and at 1, 2, 3 and 4 hours after T, and imaged from 10 to 258 min after T.
- All slides were imaged in brightfield, sfGFP and mCherry, in one or two fields of view.

Day 14 – 12.10.2026 COLONY PCR ACROSS THE LEFT JUNCTION

PRIMERS

- Colony_OUT_FWD (GTAGACGTGTGTGTCTCATGG) anneals in the native genome 180 bp upstream of the insertion site, outside any synthetic fragment.
- Colony_IN_REV (TTTAAGAGCTTGGTGAGCGC) anneals at the 3′ end of Fragment 1.
- A correct integration gives a single product of 1.65 kb.

LYSIS AND PCR

- 20 µL of freshly made 20 mM NaOH was placed in a PCR tube.
- A small amount of colony was picked with a sterile tip and resuspended in it.
- Tubes were heated at 95 °C for 10 min in the thermal cycler.
- They were centrifuged at 10,000 × g for 1 min and 2 µL of the clear supernatant was used as template, without disturbing the pellet.

- The standard 20 µL reaction was run with the two primers, annealing at 55 °C.
- Products were run on an agarose gel, with an unmodified WT colony as negative control.

OUTCOME: No bands were obtained.

Day 15 – 14.10.2026 TRANSCRIPT EXPERIMENT SET-UP AND CULTURES

DESIGN

- Transcripts are measured by RT qPCR 60 minutes after the stress, comparing stressed and unstressed samples of the same strain.
- Genes: UBC6 as reference, sfGFP for the reporter cassette, GB1 as a proxy for the TIP transcript, and TetR in a second run. Amplicons are 100–120 bp.
- Five bead tubes were available, so five samples were planned, each at the highest dose of its stressor.

CODE	STRAIN	CONDITION	FINAL DOSE
S1	pSynOS4(alt1)	Water, no stress	0
S2	pSynOS4(alt1)	H_2O_2	600 µM
S3	pKAR2	Water, no stress	0
S4	pKAR2	DTT	2000 µM
S5	pCUP1	$CuSO_4$	100 µM

- Each stressed and unstressed pair comes from the same culture tube. The copper sample has no unstressed partner in this round.

CULTURES

- 5 mL cultures of the three strains were started in SD−His with galactose, to be used later in the week as they were.

Day 16 – 16.10.2026 RNA EXTRACTION, S1 AND S2, WT WITH $CuSO_4$

DOSING

- Both samples came from the same pSynOS4(alt1) culture started on Day 15, used undiluted.
- The culture was mixed with the pipette and 1 mL placed into each of two 15 mL tubes, S1 and S2.
- At T, S1 received 10 µL of sterile water; one minute later S2 received 10 µL of a 60 mM H_2O_2 spike, giving 600 µM in the culture.
- The spike was made as 10 µL of 30% H_2O_2 into 990 µL of water, then 120 µL of that into 80 µL of water.
- Both tubes were returned to the shaker at 30 °C with loose caps.

HARVEST

- Each sample was collected 60 minutes after its own dose.
- The culture was mixed with the pipette, 0.5 mL moved to a 1.5 mL tube and centrifuged 1 min at 11,000 × g.

- All supernatant was removed and the cells lysed immediately in 350 µL of buffer RA1.

RNA EXTRACTION — NucleoSpin RNA WITH BEAD TUBES TYPE B

- All centrifugation steps at 11,000 × g.
  - The pellet in 350 µL RA1 was mixed to a smooth suspension and transferred completely to a bead tube Type B, closed tightly.
  - Vortexed in 8 rounds of 1 min, with 1 min on ice between rounds.
  - Centrifuged 1 min; the clear lysate was moved to a clean 1.5 mL tube, leaving the beads behind.
  - 350 µL of 70% ethanol added and mixed with the pipette.
  - Loaded on a NucleoSpin RNA column (light blue ring), 30 s; flow-through discarded.
  - 350 µL MDB added, 1 min, to dry the membrane.
  - rDNase mix (10 µL rDNase + 90 µL reaction buffer) prepared shortly before use; 95 µL applied to the centre of the membrane and left 15 min at room temperature.
  - 200 µL RAW2 added, 30 s.
  - 600 µL RA3 added, 30 s; then 250 µL RA3, 2 min to dry the membrane.
  - Column transferred to an RNase-free 1.5 mL tube and eluted with 40 µL RNase-free water, 1 min. The eluate was kept on ice.
- Each sample was read three times on the NanoDrop, 1 µL per reading.

RNASE PRECAUTIONS: Gloves and filter tips were used throughout, tubes kept closed as much as possible, and the bench and pipettes wiped with 70% ethanol before starting.

UNMODIFIED STRAIN WITH COPPER SULFATE

- WT cells were prepared as on Day 12 and exposed to copper sulfate at the doses of Day 13.
- Dosing and the first slides were at 10:41, then slides were made every hour up to 4 hours after T.

Day 17 – 17.10.2026 RNA EXTRACTION S3–S5, RT qPCR AND GEL ELECTROPHORESIS

SPIKES

- DTT, 2000 µM final, 200 mM spike: 160 µL sterile water and 40 µL of the 1 M DTT stock.
- Copper sulfate, 100 µM final, 10 mM spike: 180 µL water and 20 µL of the 100 mM stock.
- Both were made shortly before dosing and kept on ice.

DOSING AND EXTRACTION, S3 TO S5

- S3 and S4 came from the same pKAR2 culture tube, S5 from the pCUP1 culture, both used as they were.
- 1 mL was placed into each 15 mL tube. S3 received 10 µL of water at T, S4 received 10 µL of the DTT spike one minute later, and S5 received 10 µL of the copper spike at T + 15 min.

- Each sample was collected 60 minutes after its own dose, with 0.5 mL of culture per sample.
- Extraction was carried out exactly as on Day 16, with the same buffers, volumes and times, eluting in 40 µL.
- The three eluates were read on the NanoDrop, 1 µL per reading.

PRIMER RECONSTITUTION

- The RT qPCR primers arrived as a dry powder and were dissolved in the volume of PCR-grade water stated on the datasheet, giving 100 µM stocks.
- 10 µM working solutions were made from each stock, 5 µL of stock and 45 µL of water.
- All primer stocks are stored at −20 °C.

RT qPCR

- One-step RT qPCR with the Power SYBR Green RNA-to-CT 1-Step Kit, 20 µL per reaction, on a Rotor-Gene Q with the 36-well rotor.
- The master mix was prepared separately for each gene, with one extra volume: N+1 = 16 for each target gene and N+1 = 19 for UBC6, which also takes the WT sample.

COMPONENT	PER REACTION	N+1 = 16	N+1 = 19
2× RT-PCR Mix	10 µL	160 µL	190 µL
RT Enzyme Mix, 125×	0.16 µL	2.56 µL	3.04 µL
Forward primer	0.4 µL	6.4 µL	7.6 µL
Reverse primer	0.4 µL	6.4 µL	7.6 µL
Master mix total	10.96 µL	—	—

- Each reaction received 20 ng of RNA, made up to 9.04 µL with nuclease-free water. Primers are at 200 nM each in the reaction.

SAMPLE	S1	S2	S3	S4	S5	WT
RNA (µL)	1.20	1.59	1.23	2.13	2.31	3.19
Water (µL)	7.84	7.45	7.81	6.91	6.73	5.85

- Controls: a no-template control for every gene (4 µL of water in place of RNA); one reaction without RT enzyme (GB1, S1); and WT RNA for sfGFP and GB1 as a specificity control.

OUTCOME: No reaction gave a true exponential amplification curve, and the melt curves lacked the specific high-temperature peaks expected for the products. UBC6 amplified late in the WT RNA and did not cross the threshold in the experimental samples. sfGFP and GB1 gave late signals with scattered technical replicates and melt peaks below the expected amplicon melting points, and TetR gave no valid peak. The 20 ng of total RNA per reaction was most likely below the effective sensitivity range of the assay.

GEL ELECTROPHORESIS OF THE RNA PREPARATIONS

- A 2% agarose gel was cast with 1µL EtBr2 and 120 TBE
- 20µL of every PCR product from S1 to S5 were added to 4µL loading buffer.
- For every sample 6 µL were added in the gel, at 100 voltage for 40 minutes

OUTCOME: The ladder resolved normally over its whole range. No bands were obtained in any of the five sample lanes.