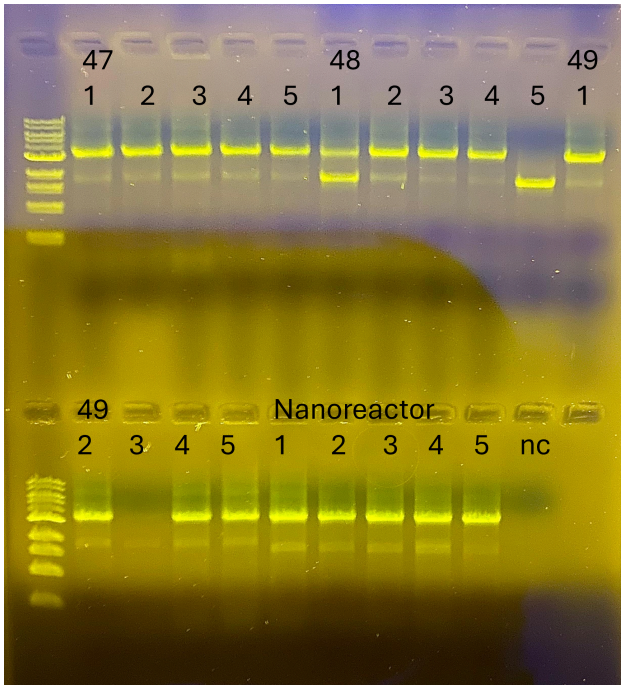
cPCR_Level2.png




Full plasmid seq of Level 2 Destination tspurple correct!

**MONTAG, 3.8.2026**

- [x] overnight of the level2 plasmids 47 c5, 48 c4, 49 c1
- [x] overnight of yeast preculture with chloramp

Seq of 47, 48, 49 correct!

**DIENSTAG, 4.8.2026**

- [x] MiniPrep 47, 48, 49
- [x] Yeast Trafo 47, 48, 49 S. cerevisiae Transformation

  47/ 48 -> 55ng/ul -> 9ul

  49 -> 70ng/ul -> 7ul

**MITTWOCH, 5.8.2026**

- [x] prepare glycerols for 47,48,49

**FREITAG, 7.8.2026**

- [x] cPCR 47, 48, 49

LP022, LP034

3600bp

10min denaturation

--> no bands visible, propably the cell could not open

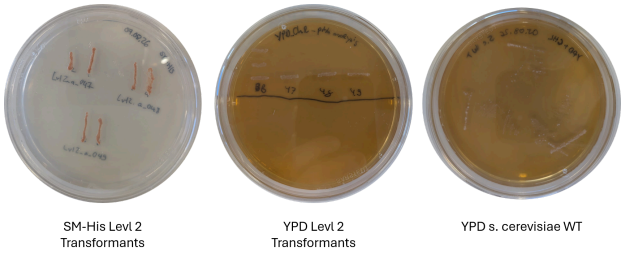
- [x] full plasmid seq for Lvl2_a_047 number: 30372994

- [x] transfer one pink yeast colonies from all three trafos and the backbone trafo onto ypd to see if it stayes pink (ts purple ) if not it is probably selection stress that causes the color

## MONTAG, 10.8.2026

- [x] overnight 47,48,49 for miniprep

**Transfer of pink colonies**




after two days of incubation at 30 °C the color of the colonies on YPD (transfered from Selection Media) is very similar to the color of the S. cerevisiae wild type. Therefore the pink color is likely caused by selection stress and not by ts purple being present.
possible explanation: De novo adenine biosynthesis, and an intermediate (AIR) accumulates and oxidizes into a red/pink pigment [1][2]. On adenine-rich medium this stays suppressed by feedback inhibition, but SD dropout media typically contain only a small amount of adenine (~20 mg/L), which quickly becomes limiting in dense colonies, so colonies turn increasingly pink after a few days [2]. BY4741/BY4742 are normally ADE2+ (i.e., white), but some laboratory backgrounds (e.g. W303 derivatives) carry ade2-1.
Sources:
ADE2 | SGD
Frequently Asked Questions--Yeast Strains

## DIENSTAG, 11.8.2026

- [x] Mini Prep 47,48,49

**Miniprep results 11.08**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | number | name | concentration / ng/µL | A260/A280 | A260/A230 |
| 2 | 1 | 47 | 381.5 | 1.89 | 2.4 |
| 3 | 2 | 48 | 390 | 1.89 | 2.42 |
| 4 | 3 | 49 | 423 | 1.89 | 2.3 |

- [x] yeast cPCR from new plate different methods for cell lysis Taq colony PCR

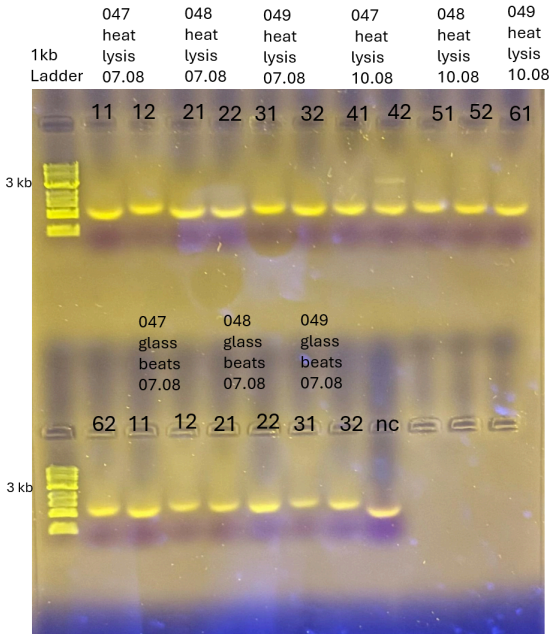
--> there were no colonies on the n.c plate and nicely distributed ones on the Trafo plate.
test two new colonies from the new Trafo plate with heating up
cycler 1: before PCR heat colony diluted in Mili Q for 10 min at 98 °C (20 µl in PCR tube) --> 12 reactions

cycler 2: normal cPCR protocol but with 2 µl supernatant as template after cell lysis with glass beads --> 6 reactions

Workflow:
reuse the colonies from last week to compare the cell lysis mechanism (visible cell mass needed)
and PCR tube 20 µl MilliQ --> place in thermocycler for 10 min at 98°C after mixing
transfer to Eppi with 30 µl steril Milli Q
to the rest add a small amount of glass beads
vortex 3 min --> if the tube gets hot vortex for 1 min place on ice for 1 min repeat 3 times
centrifuge for 1 min at max speed
use 1 µl of the supernatant as template for the PCR
Primer: LP 22 and LP 34 10 µM
elongation: 4 min

1% agarose gel with 1kb ladder

Yeast cPCR




no fragments visible on the gel except for the Taq smear and one possible positive colony (number 8 --> contruct 47 colony 2 from 10.08)

**MITTWOCH, 12.8.2026**

- [x] redo cPCR

reuse the cells from the heat lysis --> spin down again and use 1 µl of supernatant and dilute 1 µl in 9 µl MiliQ --> use 1 µl of this dilution as well
only one colony for each construct --> total of 3 (tube 4.2 (possibly correct acording to previous gel), 5.1 and 6.1) plus one colony with pESC domesticated
eight total reactions use Q5 Master Mix
add 3% DMSO to reaction
Primer LP 22 and LP 34
Tm:64 °C
elongation: 1 min 50 sec
sadly there were no fragments visible on the gel

- [x] prepare drop out media for overnight and plates

**DONNERSTAG, 13.8.2026**

- [x] new method for yeast cPCR:

use new Primer dilution 10µM
pick from masterplate

1. Prepare a 1.5 ml centrifuge tube with 10 ul sterile deionized water.
2. Use a 10 ul petite tip to pick a quinoa sized yeast colony. My plate is about 2 weeks old.
3. Smear the colony inside the bottom of the 1.5 ml centrifuge tube and stir the water. You should see the water become murky.
4. Put the tube in -20 Celsius freezer for 2 mins.
5. Take the tube out of the freezer, vortex for 4 sec. Then put it in a 95-celsius water bath, boil 2 mins.
6. Take the tube out, vortex, then put in -20 Celsius freezer for 2 mins.
7. Take out, then put it in a 95 Celsius water bath, boil 2 mins.
8. Spin down the water inside from the top of the lid. Use a petite to make the liquid murky again.
9. Use 2 ul of the murky liquid in your 25 ul volume PCR reaction as the DNA template.

annealing: 50 °C
elongation: 3:40 min
LP22 and LP34 new dilution

**Table3**

| | A | B |
|---|---|---|
| 1 | 1.1 | 47 c1 (07.08) |
| 2 | 1.2 | 47 c2 (10.08) |
| 3 | 2.1 | 48 c3 (07.08) |
| 4 | 2.2 | 48 c2 (10.08) |
| 5 | 3.1 | 49 c5 (07.08) |
| 6 | 3.2 | 49 c2 (10.08) |
| 7 | nc | tspurple bb |

--> order new primer that yield a shorter fragment