Appendix 1 – Kit supplies

Materials Provided in the kit and preparation instructions

Below is a list of the materials provided in each student kit, as well as notes on modifications that could be done as needed. See Supplemental Materials Appendix 2 for details on preparation. Note that most supplies are in duplicate to provide enough for students to repeat the experiment if needed (e.g., because of contamination or mistakes).

- 1x Yeast stab culture (2mL tube with 1ml of YPD, yeast colony stabbed into media)
- 4 YPD plates. One is needed for streaking out yeast from the stab, and the other plates are extra in case of contamination issues during the initial streak.
- 8 YC-ura plates. These are uracil-minus media plates for selection of yeast that take-up the plasmid (plasmid contains the URA3 gene). Four of the plates are for transformations, the others are extra, these are uracil-minus media plates.
- 1 pack of Inoculating loop/Inoculation needles (e.g., Fisher Scientific 22-363-602)
- 10 sterile plastic cell spreaders (e.g., Fisher Scientific 14-665-230)
- Three pairs of large and three pairs of medium nitrile gloves (one for streaking out yeast, second for transformation day, additional pairs as extras)
- 1 mini pipette (fixed volume - 50 ul or 100 ul), e.g., Fisher Scientific S90244G
- 1 box of sterile pipette tips

Notes about the pipette and tips:

  o If preparing a large number of kits, advance ordering of specialized supplies such as the fixed-volume micropipettes is highly recommended.
  o Instead of using a fixed volume pipette, to reduce costs the kit could be supplied with small volume disposable pipettes (e.g., small volume disposable pipettes), although in this case we recommend increasing the volume of DNA aliquots students will pipette or pre- aliquoting the amounts of transformation mixture they will pipette into the DNA tubes.

- 1 foam tube rack (holding tubes with DNA mixtures, transformation mixture, & yeast stab)
- 1 felt tip marker for labelling plates and tubes
- 2 x 1.5 mL tubes with pML104+gRNA+HDR (each tube with about 8uL: 1ug gRNA & 250ng HDR)
- 2 x 1.5 mL tubes with pML104+gRNA (each tube with about 4uL, 1 ug of DNA)
- 2 x 1.5 mL tubes with empty pML104 (pML104 plasmid with no gRNA, each tube with about 4uL, 1 ug)
● 2 x 1.5 mL tubes with 500 μL of transformation mixture (40% PEG, 100μM Lithium acetate, 10mM Tris, 1mM EDTA, 100ng/μL salmon sperm DNA)

● 3-50mL conical tube with bleach crystals

● 3 sterilizing wipes (70% isopropyl alcohol), Dukal Corporation #852

● 8 pieces of parafilm for sealing plate to mail back to campus

● 1 piece of water-activated sealing tape for mailing back supplies in the original kit box

● 1 pre-paid return label to mail plate back to campus for colony isolation and sequencing

Below are some pictures of the materials stacked up prior to packing in the kit box. Note that in this picture the plates were not yet vacuum sealed for shipping. The picture on the right shows a single subset of DNA tubes (in the black foam rack).
Appendix 2 - Preparing Kit Samples

Table S1. Overview of kit preparation. The suggested preparation time is greater than the time it takes to prepare the supplies (compare third to second column); the reason for this is to allow time to test the kit materials one final time prior to shipping (e.g., set-up a transformation to observe the expected yeast colonies). *Preparation for these items can be done at the same time as media, 21 days prior to shipping. In our experience it is not recommended to have less than 21 days to prepare the kits.

| Materials to Prepare          | Approximate time to prepare for 250 kits | Suggested preparation time (days before shipping) |
|------------------------------|------------------------------------------|-----------------------------------------------|
| Yeast Stab Culture           | 7                                        | 14                                            |
| YPD Plates                   | 4                                        | 14                                            |
| YC-Ura Plates                | 10                                       | 21                                            |
| pML104+gRNA+HDR Tubes        | 3                                        | 24*                                           |
| pML104+gRNA Tubes            | 3                                        | 27*                                           |
| empty pML104 Tubes           | 3                                        | 30*                                           |
| Transformation Mixture       | 2                                        | 32*                                           |

Yeast culture and media

We used a haploid strain of yeast from the BY background (https://wiki.yeastgenome.org/index.php/Commonly_used_strains#BY4743). Yeast can be acquired from various companies, such as ATCC (for example, haploid BY4741 https://www.atcc.org/products/201388).

If a new yeast sample is purchased, we recommend the following:

- Streak out the yeast on a fresh YPD plate.
- Pick 2 to 3 single colonies and grow each in liquid media. Make a glycerol stock by mixing 930 ul of liquid culture with 70 ul of sterile DMSO. These can be kept in the freezer for several years.

Stab cultures:

Recipe for 1L of YPD stab media:

- 10g Bacto Yeast (Thermo Fisher 212750)
- 20g Bacto Peptone (Thermo Fisher 211677)
- 20g Dextrose (Fisher Scientific DF0155-07-6)
- 6g Agar (VWR 90000-786)
- 944mL ddH2O
- Autoclave for 1 hour, cool to 50C for 1 hour
- Aliquot into tubes (e.g., 1 ml of YPD into a 2 ml tube, or 3 ml in a 10 ml tube). Let these harden overnight and then leave out at room temperature.

Create stab cultures
● Touch yeast colony with needle (Fisher Scientific 22363597) and stab halfway down into YPD stab media

● Grow at 30C for 24 to 48 hours and then place at 4C

**YPD media and streaking yeast**

Recipe of 1L of YPD Plates:

- 10g Bacto Yeast (Thermo Fisher 212750)
- 20g Bacto Peptone (Thermo Fisher 211677)
- 20g Dextrose (Fisher Scientific DF0155-07-6)
- 20g Agar (VWR 90000-786)
- 930mL ddH20
- Autoclave for 1 hour, cool to 50C for 1 hour
- Pour 25mL per plate (Fisher Scientific 08-757-100D)
- Let these harden overnight and then leave out at room temperature.

A note about preparing media plates for the kits

The YPD and uracil minus plates are susceptible to contamination, so it was very important to ensure the plates were made using extremely sterile techniques. After the plates were solidified and sufficiently cooled, they were vacuum sealed for each student kit. They were then left for multiple weeks at room temperature to monitor for contamination prior to packaging the kits. Media was also made in several batches. Students were given duplicates of the plates needed for a single experiment, and care was taken to ensure the two sets of plates came from different batches.

**Streaking yeast on plates**

- Streak yeast onto YPD plate. Grow at 30C for 24 to 48 hours, then leave at 4C. Can also be grown at room temperature for 48 hours to 7 days.

**Uracil deficient media (-ura media)**

This media is used to streak transformations.

Recipe for 1L of uracil deficient media:

- 0.77g CSM-ura (MP Biomedicals 114511222)
- 20g Agar (VWR 90000-786)
- 800mL ddH20
- Autoclave for 1 hour, then cool to 50C
- Add 100mL of 20% Dextrose (Fisher Scientific DF0155-07-6)
- Filter sterilize stocks and store at room temperature (Filter, Thermo Scientific 1270020)
- Add 100mL of YNB Stock (Milipore Sigma Y0626)
  - Need 6.7g YNB in 100mL H20 to make YNB stock
- Filter sterilize (Thermo Scientific 1270020) and store at 4C
- Pour 25mL per plate
- Let these harden overnight and then leave out at room temperature
- Please see the note above about vacuum sealing plates for the kits, and monitoring for contamination.
Plasmids

Students will be performing 4 transformations. Note that “empty pML104” refers to plasmid without gRNA.

- 1 ug empty pML104 + yeast + 100uL of transformation mix (positive transformation control)
- 1 ug pML104+gRNA + 1 yeast colony + 100uL of transformation mix
- 1 ug pML104+gRNA+500ng HDR + 1 yeast colony + 100uL of transformation mix
- 1 yeast colony + 100uL of transformation mix (negative transformation control)

pML104 plasmid (Addgene plasmid #67638, https://www.addgene.org/67638/)

We followed the protocol outlined in Ulbricht et al. 2019 to clone the desired gRNA sequence into the plasmid. Two oligos were ordered, one that contained the 5’ GATC overhang necessary to ligate the hybridized oligos into the BclI restriction site. The oligos also contain the 5’ sequence of the scaffold RNA: the pML104 is missing part of the full scaffold sequence.

Oligos to clone into BclI/SwaI digested pML104:

Oligo 1: 5' GATCATTGGGACGTATGATTGTTGGTTTTAGAGCTAG 3'

Oligo 2: 3' TAACCCTGCATACTAACAACCAAAATCTCGATC 5'

Oligos can be ordered from a variety of companies, such as IDT (https://www.idtdna.com/). We recommend ordering the oligos with a 5’ phosphate for easier cloning into digested pML104.

Preparing the plasmids for student kits:

For planning purposes:

- Each student needs 2ug of pML104+gRNA, 1ug of pML104 w/o gRNA (empty pML104), 500ng of HDR template, and 100uL transformation mix per transformation

To prepare the aliquots of plasmid:

- Grow large volumes* of transformed E. coli (e.g. 1 L for E. coli+pML104, 1 L for E. coli+pML104+gRNA) in LB Broth with a final concentration of 50 ug/ml ampicillin.
- Extract plasmids using standard Qiagen Protocol or other similar plasmid purification protocol

*Decide on the volume of culture needed based on the amount of total DNA needed

- Empty pML104 cultures last yielded 300ng/uL
  - 10mL cultures may yield 15ug
  - 30mL cultures may yield 45ug
- pML104+gRNA cultures last yielded 600ng/uL
  - 10mL cultures may yield 30ug
  - 30mL cultures may yield 90ug
- If possible, dilute each pML104 plasmid down to 250ng/μL with sterile water
  - This is in preparation of giving each student 4μL or 1μg per tube of each plasmid
  - Extracted pML104 plasmids can be stored at 4°C for at least 2 months

**HDR Template**

The HDR template can be synthesized as two separate oligos and the synthesized oligos used as template for PCR to bulk up the amount of template, and then aliquot cleaned PCRs for the student kits.

The two oligo strands to synthesize are as follows:

Oligo 1:

5' CGGACAAAAACAAATCAAGTATGGATTCTAGAACAGTTGTATATTGGGAGGAGGAGGATAATTGTGACGTATGATTGTAGTAGCTCAACAGGCTCAACATTAAGACGGTAATACTAGATGCTGAAAATTCTCC 3'

Oligo 2 (written 5' to 3', but when aligned 3' to 5' it is the complement to oligo 1)

5' GGAGAATTTTACGATCTCTAGATTACCCTCTTTATGTTGAGCCTGGTTAGCTTACTCAACATCATACATCACAATTTATCCCCCTCCCAATATACCAACTGGTTCTAGAATCTAGTTGATTGTTTTGTCCG 3'

Below is an alignment of the top oligo sequence and the wildtype ADE2 (YOR128C systematic name, available at the yeastgenome.org site: [https://www.yeastgenome.org/locus/S000005654](https://www.yeastgenome.org/locus/S000005654))

The substitutions are as follows (listing wildtype nucleotide → HDR nucleotide)

#1 A → G, silent mutation
#2 C, missense
#3 G → T, missense
#4,5 GC → TA, missense (if both occur)
#6 A → T, silent

PCR primers used to make copies of the HDR template (they anneal to the absolute ends of the HDR template)
Forward primer: 5' CGGACAAAAACAAATCAAGTATG 3'
Reverse primer: 5' GGAGAATTTTACGATCTC 3'
Perform PCR using the following conditions:

\[(1x \ (95C \ for \ 2min) \ [40x \ (95C \ for \ 30sec, \ 48C \ for \ 30sec, \ 72C \ for \ 15sec)\] \]

Clean up each PCR sample using PCR Clean-up Kit (e.g. the \textit{Lambda Biotech Catalog#: D509}).

Cleaned HDR samples can be stored at 4C for at least 2 months.

For a 50 ul PCR, eluted in 15 ul from cleaning, the average yield may be around 35ng/ul. Each student needs approximately 500 ng for a single yeast transformation.

\section*{Transformation Mixture}

Each yeast transformation requires 100uL of transformation mix. In our experience, using only 50uL of transformation mix per transformation results in ~6x fewer colonies so we recommend planning to provide 100 uL of transformation mix per transformation.

Transformation mix, final concentrations:

- 100mM Lithium Acetate (\textit{Sigma-Aldrich L6883})
- 40\% PEG (\textit{Spectrum Chemical PO125})
- 10mM Tris (\textit{Fisher Scientific BP152-10})
- 1mM EDTA (\textit{Fisher Scientific BP1201})
- 0.1mg/mL Salmon Sperm DNA (\textit{Thermo Fisher 15632011})

- Make sure lithium acetate, Tris, and EDTA stocks are filter sterilized before adding to the transformation mixture. We used rapid-flow sterile single use vacuum filter units (\textit{Fisher Scientific 09-740-3A}).

- We make a 50\% PEG stock solution. The 50\% PEG solution can be autoclaved for 1 hour in a glass bottle, instead of being filter sterilized, since the filtering process can be very slow with PEG.

- The final transformation mixture can be stored at 4C for at least 2 months.

\section*{Preparing the tubes of aliquots for kits}

Below is an example of how the aliquots were set up for a single student kit. All tubes are sterile 1.5 mL microfuge tubes (e.g., \textit{Fisher Scientific 05408129}).

We have found that the transformation works best if smaller volumes and more concentrated amounts of DNA are used. For example, 4 uL of concentrated plasmid resulted in about 8x more colonies than using more dilute, 25 uL of plasmid.

Stock ingredients:

- pML104 empty plasmid 250 ng/uL (“empty” refers to no gRNA in the plasmid)
- pML140-gRNA plasmid 250 ng/uL
- HDR template 35 ng/uL
| Tube # | Contents                                      | Use               |
|--------|-----------------------------------------------|-------------------|
| 1      | 4uL of empty pML104                           | Control transformation |
| 2      | 4uL of pML104+gRNA                            | NHEJ edits        |
| 3      | 4uL of pML104+gRNA + 14uL of HDR into tube    | HDR edits         |
| 4      | 500 uL transformation mix                     |                   |

A note about preparing DNA aliquots for kits:

When we prepared the DNA samples 1-2 months in advance, and stored at room temperature in prepared kits, some of the aliquots experienced significant evaporation, leaving very little liquid in the tubes. In this case, we encourage students to mix the tubes with DNA, transformation mixture, and yeast very well to ensure sufficient dissolving of any dehydrated DNA in the tube.

Processing returned samples of transformed yeast colonies for sequencing

Ultimately, a cleaned PCR product needs to be sequenced. PCR may be performed on crude extracted DNA from a colony, crude extracted DNA from an overnight culture, or with direct colony PCR. We have had the best success at growing the colonies overnight in liquid media, then extracting genomic DNA and using as a template for PCR. It would be wise to re-streak any colonies received onto a fresh YC-ura plate, in case PCR/DNA extraction needs to be repeated.

For growing colonies in liquid culture overnight prior to DNA extraction:

- May be grown in 2mL of YC-ura liquid media (YC-ura is the uracil minus media necessary for selection of cells carrying the pML104 plasmid). Recipe for 1L of YC-ura media:
  - 0.77g CSM-ura (Fisher Scientific MP114511222)
  - 820mL ddH20
  - Autoclave for 1 hour, then cool to 50C
  - Add 100mL of filter-sterilized 20% Dextrose (Fisher Scientific DF0155-07-6)
  - Add 100mL of filter-sterilized YNB Stock (Sigma Aldrich Y0626)
    - Need 6.7g YNB in 100mL H20 to make YNB stock. Filter sterilize and store at 4C
- Dispense 2mL of the YC-ura media in a sterile manner into test tubes or microcentrifuge tubes
- Add one yeast colony to 2mL of YC-ura liquid media and grow for 24 to 48 hours, shaking at 200rpm, at 30C. Store at 4C after incubation.

- Crude genomic DNA may be extracted from liquid culture or from large yeast colony.
Crude genomic DNA extraction protocol

For liquid culture DNA extraction:
- Transfer 200uL of re-suspended YC-ura yeast culture to a 1.5mL tube
- Centrifuge for 45sec at 10k RPM
- Pipette out the supernatant
- Proceed to “next steps” below

For colony DNA extraction:
- Add 1 large colony to the bottom of 1.5mL tube
- Proceed to “next steps” below

Next steps:
- Add 50uL of 10mM Sodium Hydroxide (NaOH, Fisher Scientific SS255-1) to pellet and pipette several times to resuspend pellet/colony
- Vortex tube for 10sec
- Place tube at 99C for 5min and 30sec
- Place tube on ice for 5min
- Freeze tube for later use or use as template for PCR

Amplify region of ADE2 from genomic DNA using PCR

We use a forward primer that anneals approximately 400 bp upstream of the ADE2 start codon (and approximately 500 bp upstream from the predicted Cas9 cut site for our gRNA. The reverse primer anneals approximately 430 bp downstream of the predicted Cas9 cut site. The resulting 890 bp product can then be cleaned and sent for sequencing.

PCR master mix for 1 sample with 50uL total volume in a 0.2mL PCR tube. We use GoTaq (Promega M7122)
- GoTaq Master Mix: 25uL
- 10uM forward primer: 2.5uL
  - 5’ CCTTTTGATGCAGGAATTGAC 3’ (can order from IDT)
- 10uM reverse primer: 2.5uL
  - 5’ CTTTACAACGAAGTTACCTCTTCCA 3’ (can order from IDT): Primer E
- 2.5uL of crude extracted DNA or 1 colony
- Sterile water: 17.5uL

Perform PCR using the following conditions:
- [1x (95C for 2min)] [20x (95C for 30sec, 50C for 30sec, 72C for 30sec)] [20x (95C for 30sec, 54C for 30sec, 72C for 30sec)]
- Primer dimers may show, but all of our cleaned PCR products have resulted in successful sequencing
  - Raising the annealing temperatures a few degrees might reduce non-specific amplification

Clean up each PCR sample using PCR Clean-up Kit (e.g. the Lamda Biotech Catalog#: D509).
- Elute in 15uL of Elution Buffer for most samples
- Yield is typically between 20-80ng/uL
● Sequence each sample using your preferred sequencing company or in-house methods. We use another primer for sequencing: 5’ TTGTTGCATGGCTACGAACC 3’
This primer anneals approximately bp upstream of the predicted Cas9 cut site. We have had the most success this way (PCR amplification with the sequencing primer as forward yields less successful PCR results).

● We can then use NCBI BLAST Global Alignment to compare the wildtype ADE2 gene sequence (https://www.yeastgenome.org/locus/S000005654) with the sequencing result. Examples of sequencing results can be found in Figure 1, main text.
Appendix 3 – Student Instructions

Below are the instructions we provided to students.

Experiment Overview

Your at-home CRISPR editing kit contains non-pathogenic yeast (Saccharomyces cerevisiae) as well as combinations of plasmid DNA (pML104) and a double-stranded DNA template (listed as HDR, homology directed repair, below) to do Cas9-directed editing of the Ade2 gene. The plasmid carries a promoter driving expression of the Cas9 gene. One sample contains the plasmid plus the gRNA molecule, which can also be expressed. The gRNA+Cas9 complex will target the Ade2 gene just downstream of the start codon. Ade2 is not essential for function, but when disrupted the yeast colonies turn red in color. The HDR DNA template is approximately 130 bp long. It is homologous to the Ade2 gene with six-point mutations, compared to the wildtype gene. HDR with this template should result in loss of function of Ade2. Yeast that successfully take up the plasmid will be able to grow on uracil minus (ura-) media. Below are the four transformations to be done:

| Transformation               | Purpose                                      |
|-----------------------------|----------------------------------------------|
| pML104 (no gRNA)            | Transformation control, no mutations introduced|
| pML104+gRNA + HDR           | Introduced targeted mutations into Ade2      |
| pML104+gRNA, no HDR         | Create NHEJ mutations in Ade2                |
| No DNA added (negative control) | Control for uracil-minus selection         |

There is also the opportunity to send transformed yeast back to our campus lab and have one colony selected for sequencing. In the campus lab, lab personnel will use PCR to amplify a portion of the Ade2 gene, and send the PCR product for sequencing. Sequencing results will be made available to the class for analysis to determine which mutations were introduced into the gene.

Brief Overview of Protocol*:

Day 1: Streak yeast (about 15 minutes) and let grow for 48 hours to 1 week at room temperature.
Day 3-8 (or more if initial growth was longer): Set-up transformation (about 30 minutes + 48-hour incubation)
Day 4-8: Plate transformations (about 30 minutes + 4 to 10 day incubation)
Day 8-17: Grow yeast, count colonies and enter results in class spreadsheet
Day 8-17: Isolate colony and mail back to UCSD

*Detailed protocol on subsequent pages. Also, please check your course schedule as your instructor may be planning to do certain experimental steps with you synchronously during lab time.
Materials Provided:

- Yeast stab culture (2mL tube with 1ml of YPD, yeast colony stabbed into media)
- 4 YPD plates (YPD is a rich yeast media). One is needed for streaking out yeast from the stab, and the other plates are extra in case of contamination issues during the initial streak.
- 8 to 10 YC-ura plates (four for transformations, the others are extra, these are uracil-minus media plates)
- Inoculating loop/Inoculation needle (1 pack)
- Cell spreader x 10
- Three pairs of large and three pairs of medium nitrile gloves (one for streaking out yeast, second for transformation day, additional pairs as extras)
- 1 mini pipette (fixed volume - 50 ul or 100 ul)
- Pipette tips
- 1 foam tube rack (holding tubes with DNA mixtures, transformation mixture, & yeast stab)
- 1 felt tip marker
- Sterilizing wipes (70% isopropyl alcohol)
- 2 x pML104+gRNA+HDR (each tube with 8uL, 1ug gRNA & 250ng HDR)
- 2 x pML104+gRNA (each tube with 1ug, 4uL)
- 2 x empty pML104 (pML104 plasmid with no gRNA, each tube with 1ug, 4uL)
- 2 tubes with 500 uL of transformation mixture (40% PEG, 100uM Lithium acetate, 10mM Tris, 1mM EDTA, 100ng/uL salmon sperm DNA)
- 3 x 50mL conical tube with bleach crystals
- 1 piece of water-activated sealing tape for mailing back supplies
- 1 piece of parafilm for sealing plate to send back to UCSD
- 1 return label

*Note: You have been provided with extra supplies in case you need to repeat the experiment. That is, extra media, extra DNA, extra spreaders, etc.

User supplied materials

- Lab coat
- Googles
Instructions:

- You may keep the kit at room temperature until needed.
- Some YPD and YC-ura plates are color coded by media type and batch number. Batch markings are on the other side of the plate and have various combinations of color and may have stickers on the bags identifying the media type. YPD plates may have a black+brown media type marking on the sides of the plate. YC-ura plates may have brown+brown+brown media type marking. Note the batch markings on your plates. Use plates from a single batch. If you experience contamination issues, you can restart the experiment with the second, different batch.
- Read the detailed protocol below very carefully well before you plan to do the work, so you can get an idea of what is involved and plan time accordingly. Check your lab schedule to determine which days are planned to start and work on the experiment during lab time.
- Do not throw out the box as you can reuse it to send yeast plates back to campus for disposal and sequencing.

Detailed Protocol:

Day 1: Create a streak of yeast

The 2 ml tube contains some solidified media (YPD media) into which a small amount of living yeast culture was stabbed (hence the name, stab culture). Stab cultures can be stored for longer periods of time in the fridge, providing a source of culture when needed. The goal of these steps in the procedure is to take some of the yeast from the stab culture and streak it onto a plate to create a fresh culture which you can then use for transformations (newly divided cells).

For each step using the yeast culture we will also be setting up a dilute bleach solution to decontaminate supplies prior to throwing them away (tips, spreaders).

1. Put on your lab coat, googles, and nitrile gloves.
2. Create a clean, and dry, work surface with the sterilizing wipe provided in the kit.
3. In a small cup, mix the bleach crystals from one of the bleach tubes with 50 ml of water until they have dissolved.
4. Place a YPD plate on your work surface (do not open the plate).
5. Use a sterile inoculating loop (the inoculating tool has a loop end and a needle end), pick some yeast from the stab culture.
6. Open the lid of the YPD plate just enough to put the inoculating loop inside, and streak the yeast all over the plate.
   *Opening the plate minimally helps reduce contamination.*
7. Place the used inoculating loop in the bleach solution. Incubate for 2 hours, then dispose of the loop in the trash and the bleach down the sink (do not mix with soap).
6. Incubate the plate at room temperature for 48 hours. You should see white colonies and smears of yeast growing on the plate after this time.

7. Sterilize the work area with a new sterilizing wipe. Wipe the exterior of your nitrile gloves with the wipe. Dispose of the gloves and wipe in the trash. Wash your hands thoroughly with soap and water. Store your lab coat and googles in a bag until the next session.

Day 3: Yeast transformation

8. Put on your lab coat, googles, and nitrile gloves.

9. Create a clean and dry work surface as above, and dissolve the bleach crystals from one of the bleach tubes in 50 ml of water.

10. Set out the labelled tubes containing the three different DNA samples for three of the transformations: pML104, pML104+gRNA, and pML104+gRNA+HDR (the fourth transformation, a negative control, will be set-up in step 12 below).

11. Pipette 100\(\mu L\) of transformation mix into each of the three separate microcentrifuge tubes from the previous step. Add the mixture in this way:

   Pipette the transformation mixture slowly along the inside walls of the tube so that any DNA that has spread to the sides of the tube becomes mixed into the solution. This is very important! During shipping and storage, the small volume of DNA often ends up on the walls of the tube and liquid can evaporate, leaving the DNA stuck to the walls of the tube.

   Because the pipette is 50 ul, you will need to pipette two volumes (2 x 50 ul) to achieve the desired 100 ul. Use a new tip for each aliquot to prevent contaminating the transformation mixture with any DNA.

   Any tip that was used to pipette Transformation Mixture can go into the trash. There is no need to incubate in bleach solution first.

12. Using a sterile inoculation needle, scrape enough yeast off the plate so that you have a clump of yeast that is a few millimeters thick and wide, and twirl into the tube containing 100\(\mu L\) transformation mix and DNA.

13. Place the used inoculation needle into the bleach solution.

14. Using a new pipette tip, quickly/vigorously pipette the mixture up and down for at least 1 minute. This step is very important to break up the yeast and improve transformation efficiency! Place the tip into the bleach solution.

15. Repeat steps 8, 9, and 10 for the remaining two tubes, using a new inoculating needle and new pipette tips for each sample.

16. To the Transformation Mixture tube, add a clump of yeast and pipette (steps 9 and 10). This tube is now your no-DNA negative control. Use the felt-tipped marker to label the tube.

17. Seal the tubes and put them at room temperature for about 48 hours.
18. After the inoculation loops and tips have incubated in the bleach solution for 2 hours, dispose of them in the trash and pour the bleach down the sink (do not mix with soap).

19. Clean up your work area as described in step 7 above.

48 hours later . . . .

20. Put on your lab coat, goggles, and nitrile gloves. Create a clean and dry work space as in step 2.

21. Dissolve the bleach crystals from one of the bleach tubes in 50 ml of water.

22. Label one YC-ura plate for each transformation (pML104, pML104+gRNA, pML104+gRNA+HDR, no DNA control).

23. Plate all of the liquid from a single transformation onto a uracil-minus media plate. You will need to pipette 2-3 times to get all the volume from the tube onto the plate:

  Only open the lid of the plate a very small amount, enough to pipette all the liquid from the transformation tube onto the media. Use a new pipette tip for each transformation.

19. Place the used pipette tips into the bleach solution.

20. Using a sterile spreader, spread the yeast all around the plate, trying to get an even distribution of liquid on the surface of the plate. Be sure to use a separate spreader for each transformation. Place the used spreader into the bleach solution.

21. To each emptied tube, add 500 ul of bleach. Seal the tube. Shake, and incubate for two hours. Then dump the contents down the sink and dispose of the tubes in the trash. Incubate the tips and spreaders in bleach for two hours. Throw the tips and tubes in the trash, and pour the bleach down the sink (do not mix with soap).

22. Grow the plate at room temperature for 4-10 days (at 4 days take note, if you see small colonies wait another 4-6 days to be sure about colony growth. At room temperature, after a transformation, it can take the yeast colonies several days to appear.

23. Clean up your work area as described in step 7 above.

Day 9-14: Checking colonies, sending cultures & pipettor back to campus

21. Once your yeast plates have visible colonies (about 1 mm wide minimum) count the colonies and enter results into your lab notebook and the shared class spreadsheet (find link on course site)

22. Take pictures of all your plates, put pictures in your lab notebook.
23. Identify one colony that you would like sequenced. Draw a circle around the colony on the back of the plate. Make note of which transformation it came from. Complete the “colony return spreadsheet” (find link on course site).

24. Write down the label # provided in the “colony return spreadsheet” on the plate and make note of this number in your lab notebook.

25. Seal all the plates using the parafilm by stretching the parafilm around the edge of the plate to seal the lid to the bottom of the plate.

**If you are mailing samples back:** place all of the sealed plates in the same box you received all of the supplies, and *if you do not want to keep the micropipette, please consider returning it as well so we can reuse them*. Add packing paper or newspaper to fill the box; the shipment may be rejected if the contents are not secure. Close the box with tape. Attach the prepaid label to the box, making sure to completely cover any old labels. Bring the package to your local FedEx location for shipment: [https://www.fedex.com/locate/index.html?locale=en_US](https://www.fedex.com/locate/index.html?locale=en_US)

**If you live on campus:** drop off the box containing the sealed plates instead of mailing: Bring the sealed and labelled plate *and pipette if you want to return it for reuse* to [REPLACE WITH BUILDING ROOM NUMBER]. There will be labelled bins outside of the room where you can place the items being dropped off.