Xu_UMBC lab Standard Operation Procedure (SOP) (molecular biology and cloning)

(updated 05/31/2018)

Be meticulous about everything you do, don't rush, be slow and ask around when you are not sure or just starting a new protocol. Try to improve/optimize your experiment every time you do, don't just follow the protocol. Think how you could do it better and faster. We are working with nanogram of DNA, a few microliters of enzymes and cells. Be patient, confident and demonstrate your fine-motor skills to pipette and transfer liquids. You will form a good habit and enjoy the benchwork. This is the basic training as a molecular biologist and microbiologist.

One of the important skill is parallel or multiplexed experimenting. Clear your mind and keep tracking/labelling every DNA and cell you have worked, plan ahead and use your notes/spreadsheets to keep tracking which plasmid goes to which cell, which cell goes to which plate/media. Rush and carelessness is the enemy of molecular experiment.

Symbol

● Steps should work under sterile conditions with gas burner or in biosafety hood

◘ Be careful of pipetting carry-over and cross-contamination

Ӝ suggestions (you may follow these suggestions to optimize your results)

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1. *E coli* Heat shock transformation

(1) Thaw one vial of chemically competent NEB5α cell on ice, divide 10 μL cell into each PCR tube. You only need 10 μL cell for each transformation. Keep the remaining PCR tube containing the competent cell back to -80 freezer.

(2) Mix 1 μL plasmid with the 10 μL cell, gently tap the PCR tube to mix DNA with cell, don't pipette up and down (◘)

(3) Incubate on smashed ice for 15 to 20 min

(4) Set up a 42C water bath (use thermometer to calibrate the water-bath, donot trust the digital display on the waterbath). Put the PCR tubes (containing cell and DNA) on a PCR racks and heat shock exactly at 42C for 30seconds (maximal 45 seconds)

(5) Immediately put the PCR tubes back to ice-bath, incubate for 2 min

(6) Add 150 μL SOC or other rice media, incubate at 37 C with shaking 200 rpm for 1 hour (●◘)

(7) If you work on a ligation reaction or Gibson assembly, plating all the transformation onto LB selective plate.

(8) If you transform a purified plasmid: Plating 80 µL transformation solution onto LB selective media (containing appropriate antibiotics) (●◘) and keep the remaining 80 µL transformation solution into 4C fridge. Caution, you may have too many colonies on the plate and you cannot get well-isolated colony the next day. For safe practice, try to dilution 10 times, for example, 10 µL transformation solution + 90 µL sterile H2O.

(8) Keep the agar plate upside down and grow at 37C (or 30C if temperature sensitive or expressing toxic protein) overnight.

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2. Make frozen stock (glycerol stock)

(1) Label each Cryotube clearly: host strain + plasmid + antibiotic abbreviation + date (For example, NEB5α with pYLXP'-yIIDP2, Amp, 12/18/2016)

(2) Transfer 750 µL of 30% sterile glycerol into each cryotube (●)

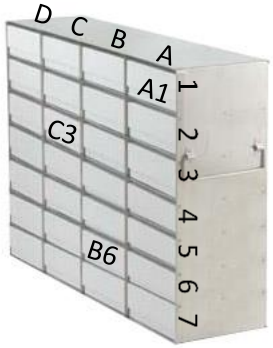
(3) Transfer 750 µL of cell culture and mix with the 750 µL 30% glycerol (● ◘)

(4) Tightly close the cap, and keep in your cryobox and store at -80 C freezer (need key access)

(5) Document the exact position of your cryotube stock in the dropbox spreadsheet the PI sent to you. (We have three levels, total of 18 racks in the freezer, each rack has 28 box positions, and each box has 81 tube positions, please label the box with your initials and the position as shown in the attached graph here,)

Rack 1	Rack 2	Rack 3	Rack 4	Rack 5	Rack 6
Rack 7	Rack 8	Rack 9	Rack 10	Rack 11	Rack 12
Rack 13	Rack 14	Rack 15	Rack 16	Rack 17	Rack 18

18 racks in the freezer



How you locate your box

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3. *E. coli* plasmid minipreparation (Zymo miniprep kits)

(1) Transfer 750 ul cell culture to a sterile mcirocentrifuge tube and keep at 4C fridge. This 750 ul cell will be used to make frozen stock if screening results are positive. Directly centrifuge the remaining *E. coli* cell culture at RT 4,000 rpm for 10 min with the Beckman centrifuge, be careful of centrifugation balance.

(2) Discard the supernatant in a beaker in the sink, bleach the cell culture

(3) Resuspend the cell pellet with 600 µL DI or MilliQ water and transfer the cell to an Eppendorf tube (◘)

(4) Add 100 uL of the 7X Lysis Buffer (Blue), invert or shake the tube vigorously and proceed with step (5) within 2 min (◘)

(5) Add 350 µl of cold Yellow Neutralization Buffer (4C fridge) and mix thoroughly (invert or shake the tube, do not vortex). (◘)

(6) Centrifuge at 14,000 x g for 4 minutes.

(7) Transfer the supernatant (~900 µl) into the provided Zymo-Spin™ IIN column. Avoid disturbing the cell debris pellet. (◘)

(8) Place the column into a **Waste Collection Tube** and centrifuge at 5000 rpm for 15 seconds. (◘).

(9) Discard the flow-through in a bottle and place the column back into the same **waste Collection Tube**. (◘) (Ж to increase the plasmid yield, you can re-apply the flow-through to the column and repeat step 8)

(10) Add 200 µl of Endo-Wash Buffer to the column. Centrifuge at 5000 rpm for 15 seconds. (◘)

(11) Add 400 µl of Zyppy™ Wash Buffer to the column. Centrifuge at 14,000 x g for 1 minute. (make sure that ethanol has been added into this Endo Wash buffer) (◘)

(12) Label 1.5 ml microcentrifuge tubes. Transfer the column into a clean 1.5 ml microcentrifuge tube, then add 40 µl of Zyppy™ Elution Buffer directly onto the center of the silica membrane, sitting at RT for 2min, and Centrifuge at 14,000 x g for 1 minute. (◘)

(13) Add 40 µl of Zyppy™ Elution Buffer directly onto the center of the silica membrane, sitting at RT for 2min, and Centrifuge at 14,000 x g for 1 minute. (◘)

(14) Labe each tube clearly with the plasmid name and keep the plasmid in your box at -20C. It is better to keep a spreadsheet of the location of all your DNA sample, so later you can easily retrieve them.

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4. DNA Gel Electrophoresis

(1) Dilute the 10XTAE buffer with MilliQ or DI water and keep the 1XTAE in a clean container

(2) The gel volume depends on the number of your DNA samples and your application (screening or DNA purification). For screening, each small gel (25 ml volume) can have 7 DNA samples and each large gel (45 ml volume) can have 15 DNA samples. For gel purification, each small gel (25 ml volume) can have 5 DNA samples and each large gel (45 ml volume) can have 10 DNA samples. Decide wisely how much 1XTAE buffer you will need.

(3) Transfer the appropriate amount of 1XTAE buffer into the gel bottle, add 1% (w/v) agarose (for example 0.5 g agarose add into 50 ml 1XTAE buffer), heat with microwave intermittently and gently shake until you get a clear hot solution (use special gloves and be cautious of the

heat). This step is crucial for a good separation. Must check visually there is no small undissolved particle in the solution**. To get a completely dissolved gel, make sure you see the gel solution is boiling for three times, each time lasting for at least 20 seconds.**

(4) Wait a few minutes and gently shake the solution until the gel is not too hot. Add 1/20000 gel volume of 10 mg/ml ethidium bromide, and mix thoroughly (use special gloves and be cautious of the heat). For example 2.25 µl for 45 mL gel; 5 µl for 100 mL gel.

(5) Clean the gel-casting tray (this is important if you try to recover your DNA) and install the tray properly into the casting support (the black strip should align with the comb position).

(6) Pour gel into the tray, get rid of air bubble with a 100 ul clean pipette tip and install the comb property (be cautious that comb has two direction which you can create small well for screening and large well for DNA recovery/purification)

(7) Wait at least 30 min until gel is completely solidified.

(8) Carefully remove the comb and transfer the gel with the tray to the electrophoresis box. Make sure the 1XTAE buffer completely immerge the gel. Load DNA samples into each well (make sure mix your DNA with loading dye to make your DNA sample settle down to the bottom of the well). Also load 1kb plus DNA ladder (0.05 ug/ul) in the middle lane as a reference, normally 10 ul for large well and 8 ul for small well. Later you can know the molecular weight of your DNA fragment. (Supercoiled DNA has a different migration rate than linear DNA!) (◘)

(9) Run the electrophoresis with 50V for 50 minutes or 100V for 30 minute (100 volts will generate lots of heat and the gel band may diffuse and become wider; 50 volts require long time also increase the chance of diffusion and wider band.). The thumb of rule is that the yellow dye moves to the bottom of the gel or the blue dye pass across the middle line of the gel. If you have multiple bands with few hundred-bps difference, try to run the gel at 50V for 15 min then switch to 100V for 20min to get a good separation between digested DNA bands. This is very important if you try to recover your DNA samples with identical size.

(10) For screening, visualize and image the gel with BioRad Geldoc and take picture. For DNA recovery, visualize the gel with the UV-transilluminator and use a scaple cut the expexted DNA band. Never cut your DNA bands on the BioRad Geldoc platform.

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5. DNA gel recovery with Zymoclean kits

(1) Excise the DNA fragment from the agarose gel using a razor blade, scalpel or other device and transfer it into a 1.5 ml microcentrifuge tube. Try to cut the DNA band as sharp as possible and no extra gel.

(2) Add 3 volumes of ADB buffer to each volume of agarose excised from the gel (e.g. for 100 µl (mg) of agarose gel slice add 300 µl of ADB). Estimate the volume of the gel by experience, normally the gel slice weights about 80 mg to 150 mg.

(3) Incubate at 50 °C for 5-10 minutes until the gel slice is completely dissolved. For DNA fragments > 5 kb, following the incubation step, add one additional volume (equal to that of the gel slice) of DI water to the mixture for better DNA recovery (e.g., 100 µl agarose, 300 µl

ADB, and 100 µl water). You can also add 1/20 gel volume of 2 M sodium acetate buffer to increase the DNA recovery ratio.

(4) Transfer the melted agarose solution to a Zymo-Spin™ Column in a Collection Tube.

(5) Centrifuge at 4000 rpm for 60 seconds. And transfer the flow-through liquid back to the Zymo-Spin™ Column again, centrifuge at 4000 rpm for 60 seconds and discard the flow-through. This two-step absorption process will increase the yield of DNA recovered from the gel.

(6) Add 200 µl of DNA Wash Buffer to the column and centrifuge at 4000 rpm for 60 seconds. Discard the flow-through.

(7) Repeat wash step (6) but centrifuge at 12,000 rpm for at least 60 seconds.

(8) Add 8 µl DNA Elution Buffer directly to the center of the column matrix. Place column into a 1.5 ml clean tube and incubate at 37C for 2 minutes. Centrifuge at 10,000 rpm for 1 min to elute DNA.

(9) Repeat step (8) with the same collection tube to receive the purified DNA. In the end, you should have 15 ul purified DNA fragment.

(10) Label your sample in this format: plasmid name-[RE1+RE2]; RE1 and RE2 is the restriction enzyme you have used to digest your plasmid. And Keep your sample at -20C in your box. It is better to keep a spreadsheet of the location of all your DNA sample, so later you can easily retrieve them.

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6. Yeast transformation protocol (lithium acetate and PEG4000)

(1) Prepare yeast transformation buffer master solution (this is for one single transformation, if you work with multiple transformation, scale-up by the number X)

Sterile water or 10xTris-HCl (pH 7)	10 µL
Sterile 50% PEG4000	80 µL
2 M Lithium acetate	5 µL
Boiled ssDNA (salmon sperm, denatured)	5 µl*

(*for good transformation, ssDNA should be boiled for three minutes and cool down before adding to the transformation buffer)

(2) Using a 200 ul sterile pipette tips, Scrape a large yeast colony (or the equivalent of a “yeast lawn”) from an overnight YPD plate. Transfer the yeast to the microcentrifuge tube containing the master transformation buffer, mix well by vortexing for 10 seconds

(3) Add your plasmid DNA or linear DNA, mix thoroughly by vortexing for 10 seconds each sample (for a good transformation, you should add at least 0.25~0.5 µg DNA for each plasmid)

(4a) For *S. cerevisiae* transformation, incubate the transformation mixtures at 37˚C for 30-45 min. Vortexing the transformation mixture for 10 seconds every 10 minute.

(4b) For Yarrowia transformation, incubate the transformation mixtures at 30˚C for 30-45 min. Vortexing the transformation mixture for 10 seconds every 10 minute, proceed with additional 10 min heat shock at 39 ˚C to increase transformation efficiency.

(5) Dilute the cell with 400 ul sterile water, plating the 100 ul diluted cell onto yeast selective media with a sterile glass rod. Grow the plate at 30C for one or two days. Keep the remaining 400 ul cell at 4C fridge.

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7. Plasmid digestion with restriction endonuclease

(a) for plasmid screening, check with VectorNTI and choose the enzyme pair or triplets that give you the unique DNA fragmentation pattern after digestion. It should give you a visible difference between your empty (control or recipient) plasmid and the one carrying recombinant DNA.

This is the recipe for one digestion, if you work with multiple digestions, scale-up by the number X and make a master solution.

Component	Volume (µl)
Plasmid DNA	1 to 2 ul
Enzyme 1	0.75
Enzyme 2	0.75
10XFD green buffer	1.5
MilliQ or DNA-free water	10.5
Total volume	~15 ul

Notes: Gently mix the reaction by tapping. Collect all liquid to the bottom of the tube by a quick spin if necessary.

Incubate the digestion reaction in a 37C water bath for 30 min. Then run gel to check the digestion pattern.

(b) for DNA recovery, check with VectorNTI to find **unique restriction digestion sites** that are compatible for both your recipient vector and your donor DNA.. Choose these enzyme pairs to digest your plasmid.

This is the recipe for one digestion, if you work with multiple digestions, find the common ingredients and scale-up by the number X and make a master solution, later you can dispense them into X tubes.

Component	Volume (µl)
Plasmid DNA	2 ul (for recipient) or 4 ul (for donor)
Enzyme 1	1.5
Enzyme 2	1.5
10XFD green buffer	3.0
MilliQ or DNA-free water	21.5
Total volume	~30 ul

Notes: Gently mix the reaction by tapping. Collect all liquid to the bottom of the tube by a quick spin if necessary. A golden standard is to use 150 ng recipient DNA and 250 ng donor DNA for each digestion.

Incubate the digestion reaction in a 37C water bath for at least 60 min. Then run gel and excise the desired DNA fragment (must have a clear mind what is the size of the fragment

you try to purify, you can have a sense by checking VectorNTI) to recover/purify the DNA fragment.

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8. T4 DNA ligation for RE based cloning or subcloning

(a) Set up the ligation as described below.

This is the recipe for one ligation, if you work with multiple ligations, scale-up by the number X and make a master solution.

Component	Volume (µl)
Donor fragment	3 ul
Recipient vector backbone	2 ul
T4 DNA ligasee	1 ul
10XT4 buffer	1 ul
MilliQ or DNA-free water	3.25
Total volume	~10 ul

(b) Keep reaction at Room temperature for 30 min or 1 hour.

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9. PCR amplification of your gene

(1) Site-directed mutagenesis PCR

Set up the PCR reaction on ice bath

Components	Volume (µl)
10 uM Forward primer	1.0
10 uM Reverse primer	1.0
DNA template (plasmid)	0.6 to 1.5
10xPfu buffer	3.0
25 mM dNTPs Mix	0.3
Pfu Ultra DNA polymerase	0.6
MilliQ or DNA-free water	23.5
Total volume	~30 ul

Notes: Gently mix the reaction by tapping. Collect all liquid to the bottom of the tube by a quick spin if necessary.

Set up the thermocycler program based on the polymerase protocol, choose the right annealing temperature based on your primer, and the right extension time based on the desired gene length and the speed of your polymerase.

(2) General PCR (Q5® High-Fidelity 2X Master Mix)

(a) Set up the PCR reaction on ice bath

Components	Volume (µl)
10 uM Forward primer	1.25 ul
10 uM Reverse primer	1.25 ul

DNA template	1.0 ul (plasmid DNA) or 2.5 ul (genomic DNA)
Q5 High-Fidelity 2X Master Mix	12.5 ul
DMSO (optional)	0.5 ul if your primer has dimer or high GC amplicon
MilliQ or DNA-free water	9 ul
Total volue	~25 ul

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary.

For plasmid template, use up to 20 ng of plasmid DNA; for genomic DNA template, use up to 200 ng of genomic DNA.

(b) Set up the thermocycler program based on the polymerase protocol, choose the right annealing temperature based on your primer, and the right extension time based on the desired gene length and the speed of your polymerase.

Transfer PCR tubes to a PCR machine and begin thermocycling.

Thermocycling Conditions for a Routine PCR using Q5 DNA polymerase:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 s for plasmid template 60 to 90 s for genomic DNA template
25–35 Cycles	98°C	10 s for plasmid template 15 to 30 s for genomic DNA template
	*50–68°C (Tm-5, should use the priming part Tm, not the Tm on IDT sheet)	25 s
	72°C	30 seconds/kb (calculate the extension time based on the exact size of your DNA, extend at least 15 s more to make sure your gene is fully amplified).
Final Extension	72°C	4 minutes
Hold	8–12°C	Avoid holding PCR reactions on thermocycler for > 2 hours. Freezer on thermocycler will lose efficiency.

(c) Run gel to check the size of your PCR products

Mix 3 ul of PCR products with 1 ul 10 x blue juice DNA loading dye (4C fridge) and 6 ul water.

Load all the 10 ul sample to a gel, (don't forget the ladder), run gel to check if your amplified gene has the correct size.

10. Accurate pipetting

(1) Make master mix of common ingredients except unique ingredients then aliquot

(2) If you transfer less than 10 ul solution, make sure you lift your tube and visually check the liquid is thoroughly withdrawed or released. This is very important if you set up digestion, ligation, transformation steps.

(3) Mix everything gently but thoroughly

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11. Gibson assembly protocol

(a) Prepare PCR reactions to amplify your gene with Q5 enzyme (25 µl reaction system)

(b) Run gel to check the size of your PCR products (load 3 µl PCR samples with 1 ul 10xblue juice loading dye and 6 ul sterile water, mix them well before loading to the gel)

(c) If your template DNA is a plasmid in the PCR reaction, do DpnI digestion to get rid of the template plasmid, set up DpnI digestion as below. This step is important to eliminate false negative clones if your destination vector carries same antibiotic marker with your PCR template.

Component	Volume (µl)
PCR reaction	~21 ul
DpnI	1.5
10XFD clear buffer	3.0
MilliQ or DNA-free water	5.0
Total volume	~30 ul

Keep reaction at 37C for at least 30 min, prefer 1 hour

Notes: if your template DNA comes from **genomic** DNA, do not need to do DpnI digestion, can directly proceed to DNA clean-up (step e)

(d) Double digestion of destination vector

Component	Volume (µl)
Destination vector	2~5 ul (about 250 ng)
Enzyme 1	1.5
Enzyme 2	1.5
10XFD green buffer	3.0
MilliQ or DNA-free water	21.5
Total volume	~30 ul

Notes: Gently mix the reaction by tapping. Collect all liquid to the bottom of the tube by a quick spin if necessary. A golden standard is to use 200 ng destination vector DNA for each digestion.

Incubate the digestion reaction in a 37C water bath for at least 60 min. Then run gel and excise the desired DNA fragment (must have a clear mind what is the size of the fragment you try to purify, you can have a sense by checking VectorNTI) to recover/purify the DNA fragment.

(e) DNA clean-up of PCR products from step (c), please follow the Zymo kits. Please check if the wash buffer has ethanol added.

In the last elution step, please elution twice each time with 8 ul elution buffer. In the end, you will yield about 15 ul cleaned PCR products. Check the DNA concentration with NanoDrop when necessary.

(f) Gel purification of digested plasmid backbone from step (d), follow the gel extraction protocol on this manual.

(g) Set up Gibson reaction as follows

Take one vial of 2x Gibson mix from -80, each vial contains 7.5 ul Gibson mix

Set up the reaction on ice or PCR cooler

Component	Volume (µl)
Inserts (Cleaned PCR products)	3.0 ul (at least 150 ng up to 300 ng)
Gel purified destination vector backbone (or PCR amplified vector backbone)	2.0 ul (at least 50 ng up to 150 ng)
2 X Gibson mix	7.5 ul
MilliQ or DNA-free water	2.5
Total volume	~15 ul

Notes: Gently mix the reaction by tapping. Collect all liquid to the bottom of the tube by a quick spin if necessary.

Keep reaction at 50C for 1 hour on Bio-Rad thermocycler, the cap temperature should be set at 60C, not 105C.

Immediately keep Gibson reaction on ice bath or PCR cooler when the reaction is done, and stored the reaction at -20C if you don't do the transformation at the same day.

(h) Heat shock transformation of 2.0 ul (up to 4.0 ul) Gibson reaction into 10 ul NEB5alpha **high efficiency competent cell**. Grow the plates overnight at 37C with appropriate antibiotic supplementation to select positive clones the next day.

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12. E. coli Colony PCR protocol with GoTaq G2 hot start green master Mix.

(1) Colony replication. Pick a single colony, resuspend with 10 ul sterile DI water in a sterile PCR tube, spot 2.0 ul cell suspension solution on a LB plate (with appropriate antibiotics). Grid and label the agar plates, so you know exactly the location of your labeled colony. Grow this plate at 37C overnight for strain recovery. (notes, you can spot about 20~40 colonies on a 9cm petri dish plate, label it correctly)

(2) Lyse the cell. Boil the remaining cell suspension at 95C for 5 min on a PCR block. Wait the solution cool down.

(3) Set up colony PCR reactions on ice bath with the remaining 8.0 ul cell suspension as template.

This is the recipe for one colony PCR, if you work with X colonies, find the common ingredients and scale-up by the number X and make a master solution, later you can dispense them into X tubes.

Components	Volume (µl)

10 uM Forward primer	0.75 ul
10 uM Reverse primer	0.75 ul
GoTaq G2 Green Master Mix (2X)	7.5 ul
Lysed cell suspension	8.0 ul
Total volume	~17 ul

Notes: Gently mix the reaction by tapping. Collect all liquid to the bottom of the tube by a quick spin if necessary.

(4) **Set up the thermocycler program** based on the GoTaq G2 polymerase protocol, choose the right annealing temperature based on your primer, and the right extension time based on the desired gene length and the speed of your polymerase.

Transfer PCR tubes to a PCR machine and begin thermocycling.

STEP	TEMP	TIME
Initial Denaturation	94°C	2 min
18–22 Cycles	94°C	25 s
	*50–68°C (Tm-5, should use the priming part Tm, not the Tm on IDT sheet)	25 s
	72°C	60 seconds/kb (calculate the extension time based on the exact size of your DNA, extend at least 15 s more to make sure your gene is fully amplified).
Final Extension	72°C	5 minutes

(5) Run gel to check the size of your PCR products. The reaction already contains green loading dye. You can directly load 6 ul of PCR products to DNA agarose gel and check the size your PCR products.

(6) If your colony PCR result is positive (means your amplified band contains your inserts with the right size), recover this colony from your replication plates and inoculate it into 3.5 ml LB liquid media (with appropriate antibiotics) and grow at 37C with shaking overnight.

(7) Miniprep the plasmid with Zymo kits, send the plasmid out for DNA sequencing with the correct sequencing primer.

(8) Analyze and align your sequencing results with VectorNIT, go back to your plasmid box and label the plasmid that contains correct DNA sequence. Throw away any plasmid that contains error or mutation. (Notes: silence mutation is acceptable and you can keep this one as positive clone). Make frozen stock of your positive clones that contain the correct DNA sequence and keep them into your frozen box. Don't forget to update your excel strain spreadsheet in your dropbox folder.

13. Yarrowia transformation (lithium acetate and PEG4000)

(1) Prepare a YPD plate, streak frozen stock yeast on YPD and grow at 30C for 16 hr to 22 hr.

(2) Prepare yeast transformation buffer master solution (this is for one single transformation, if you work with multiple transformation, scale-up by the number X)

Sterile water or 10xTris-HCl (pH 7)	10 µL
Sterile 50% PEG4000	80 µL
2 M Lithium acetate	5 µL
Boiled ssDNA (salmon sperm, denatured)	5 µl*

(*for good transformation, ssDNA should be boiled for three minutes and cool down before adding to the transformation buffer)

(3) Using a 200 ul sterile pipette tips, Scrape a "yeast lawn" from an overnight YPD plate. Transfer the yeast to the microcentrifuge tube containing the master transformation buffer, mix well by vortexing for at least 10 seconds. Aliquot 100 ul to each tube if you work with multiple transformation.

(4) Add your plasmid DNA or linear DNA, mix thoroughly by vortexing for 10 seconds each sample (for a good transformation, you should add at least 0.25~0.5 µg DNA for each plasmid)

(5) For Yarrowia transformation, incubate the transformation mixtures at 30˚C for 30-45 min. Vortexing the transformation mixture for 15 seconds every 10 minute, proceed with additional 10 min heat shock at 39 ˚C to increase transformation efficiency.

(6) Add 300 ul sterile DI H2O to dilute.

(7) Plating the 200 ul diluted cell onto yeast selective media CSM-Leu. Grow the plate at 30C for two days. Grow the remaining 400 ul cell at 30C with shaking overnight to enrich genetically mutant cell.