Cellulose Acetate

Project Journal

- 5/28/14
    - Created codon optimization program for acetobacter
    - Optimized wssFGHI DNA (acetylation) to be synthesized
    - Ordered gluconacetobacter hansenii (ATCC strain 53582)
    - Found a source for P fluorescens strain SBW25 from NZ/Houston
    - Found a plasmid (pGX2, CP004362.1) for use in acetobacter
    - Determined that before synthesizing or cloning wssFGHI genes, strong promoters would be categorized in acetobacter using constructs with GFP by cloning the 50bp upstream of multiple commonly transcribed genes
- 5/30/14
    - Modeling (Lydia):
        - Flux Balance Analysis (FBA) uses mathematical methods to examine how metabolites relate to each network and make predictions for the growth of the organism and product input outside of the cell. Allows user to see optimized network for cell growth.
        - Flux Variability Analysis (FVA): used to optimize the bioreactor for newly incorporated metabolic pathways. FVA combines the framework of metabolic pathways with enzymatic data to provide a computational platform for predicting what changes in metabolic levels resulting in an increased end product. (MATLAB-COBRA toolbox)
        - http://2012.igem.org/Team:Calgary/Project/OSCAR/FluxAnalysis
        - http://2012.igem.org/Team:WashU/Modeling
            - Flux Based analysis or Flux Variability Analysis can be used to determine the growth rate of e coli and predict

the optimum conditions and possible product output of cellulose grown from the e coli and acetobacter.
        - Also looking into combining MATLAB & GAMS in conjunction with each other
            - GAMS software (General Algebraic Modeling System) used for optimization
            - http://research.cs.wisc.edu/math-prog/matlab.html
            - http://2008.igem.org/Team:Wisconsin/Modeling
            - http://www.gams.com
        - See if we could contact U of Wisconsin about using their e coli database
- 6/2/14
    - Designed construct for testing promoters (in preparation for transforming into gluconacetobacter)
- 6/3/14
    - Finished designing constructs to test promoters (see geneious files attached). One of the files has 100bp upstream of 4 highly transcribed acetobacter genes (glucokinase, ribosomal protein, rna polymerase, acetyl-coa synthetase) separated out by restriction sites (for ligation into plasmids) and distinct primers for separate amplification. I also chose to add the lac promoter (shown to work in acetobacter) and a constitutive E.coli promoter. The other (two) construct(s) constitute a plasmid known to replicate in acetobacter (we engineered pGX2, commonly found in acetobacter and kept the origin of replication). They contain restriction sites for ligating together, a protein necessary for replication, the origin of replication, restriction sites to allow insertion of a promoter upstream of an acetobacter RBS and GFP (optimized for acetobacter) for use in fluorescence assays, and an ampicillin resistance gene.
    - Attempted to use a malachite green stain to assay for bacterial cellulose, but it seemed to be nonspecific (bound to glucose in the medium, maybe)
        - but I did get to use the solution as a fun ink! -Ross (womp)
    - Investigated use of calcofluor white for greater specificity
    - Contacted U of Wisconsin to use their database but really didn't get a positive answer so next step will be looking into doing a flux based analysis solely or looking at more ways to optimize cellulose production and acetobacter growth metabolically through modeling using matlab using COBRA toolbox. - Lydia
- 6/6/2014
    - I think we can possibly improve growth conditions by using mannitol instead of glucose:

http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2006.02055.x/full
    - Cellulose film has grown over big flask containing acetobacter, will continue to let it grow and test its properties next week
    - Smaller flasks have also been inoculated and we will monitor their growth (potentially for testing fusion proteins, etc.)
- 6/9/2014
    - Removed cellulose film from flask (after growing for one week)
        - approx. 2 mm in thickness when wet
        - transferred to aluminum foil, left to dry on roof
        - really dry/brittle/thin, should culture in a larger vat for longer period
    - Prepared enough dry mix for 5 L of liquid media
    - % acetylation assay: http://www.drugfuture.com/Pharmacopoeia/USP32/pub/data/v32270/usp32nf27s0_m14240.html
    - Cellulose Expression in Pseudomonas fluorescens SBW25 and Other Environmental Pseudomonads
    - http://cdn.intechopen.com/pdfs/45637/InTech-Cellulose_expression_in_pseudomonas_fluorescens_sbw25_and_other_environmental_pseudomonads.pdf
        - list of genes and connection that P. fluorescens has with E.coli
        - The Pseudomonas fluorescens SBW25 wrinkly spreader biofilm requires attachment factor, cellulose fibre and LPS interactions to maintain strength and integrity
        - http://mic.sgmjournals.org/content/151/9/2829.long

- 6/10/14
    - Tried to perform acetylation assay on dried cellulose, used old NaOH (not actually 1M) so assay failed. Made more 1M NaOH
    - Heard back about SBW25, cells are in the mail, should arrive tomorrow
    - Primers arrived for the amplification of specific promoters, in box in -30 in freezer room (iGEM Primer Box 1)
    - Considered whether or not to use anabaena as a fuel source for the acetobacter. One concern with producing cellulose acetate with acetobacter on a large scale is the cost of the glucose in the media (one reason why the current industrial method is preferred). However, if we co-cultured the acetobacter (which might have to express a sucrase enzyme, new project?) with the anabaena engineered to secrete sucrose, we could solve this problem with CO_2 and light energy. Will look into this more.
    - MATLAB modeling update:

        - Did demos to optimize growth conditions in aerobic and anaerobic environments
        - obtained e.coli core model from ucsd microbiology website and an e. coli model file from Dr. Jennie Reed at UW Madison
        - point of reference for modeling: http://2013.igem.org/Team:KU_Leuven/Project/Glucosemodel/MeS/Modelling-FBA

- 6/11/14
    - P. fluorescens arrived! Prepared LB media and have them incubating overnight at 30C
    - Designed primers for wssFGHI operon in P. fluorescens, we can now amplify these 4 genes together or separately. Primers are labeled wss Primers 1-10, and pairs correspond to different segments.
        - FGHI (1,2) (1,3), G (4,5), H (6,7), I (8,9), FGHI (1,2)
    - Started procedure to make competent acetobacter cells (http://2012.igem.org/Team:NYU_Gallatin/Project/Transforming), will be ready tomorrow, 6ml aliquot incubating overnight with shaking in 37°C
    - Acetobacter promoters to test also arrived, we have plasmids and will begin amplification tomorrow
    - Started experiment to optimize acetobacter growth medium (http://openwetware.org/wiki/Acetobacter_Xylinum_Culture)
        - In 100 mL each of liquid culture:
            - 1) control (standard broth)
            - 2) control + 0.5 g glucose
            - 3) control + 0.5 g sucrose
            - 4) control + 0.5 g mannitol
            - 5) control + 0.25 g sucrose + 0.25 g mannitol
            - 6) standard broth w/ sucrose, no glucose
            - 7) standard broth w/ mannitol, no glucose
        - Inoculated with 1000 µL of solution from BC-growing medium at 3:30 pm
    - Optimal conditions for cellulose production
        - Increased production of bacterial cellulose by Acetobacter sp. V6 in synthetic media under shaking culture conditions.
            - http://www.ncbi.nlm.nih.gov/pubmed/12688462
        - expression of sucrose synthase also increases the production of cellulose
            - Enhancement of cellulose production by expression of sucrose synthase in Acetobacter xylinum
                - http://www.pnas.org/content/96/1/14.full.pdf

        - http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2006.02055.x/full

- 6/12/14
    - Started PCR run to amplify promoters to test in acetobacter.
        - Used gBlock Ampli primers (F and R, diluted 1:24) to get the whole sequence. Used protocol from AmpliTaq instructions, melting temperature of 62C for the primers
            - 25ul AmpliTaq Gold Master Mix
            - 1ul DNA template (diluted to 5ng/ul)
            - 1ul Primer Mix (forward and reverse primers, diluted 1:24 from the tube)
            - 23ul MilliQ water
        - Then ran PCR to amplify out each of the different promoters from IDT DNA, same recipe as above (using annealing temperature of 57° this time)
        - These were very successful (GOOD GEL PICZ) and are ready for ligation into acetobacter plasmid (after cleaning up tomorrow)
    - Rest of DNA constructs arrived, are in -30C
    - Met with Dr. Dimandja (jdimandja@chemistry.gatech.edu) to talk about using tandem GCxGC for the analysis and characterization of our BCOAc polymer
        - will collect multiple samples throughout directed evolution process
        - send samples + final BCOAc + pure cellulose + pure COAc
    - Ordered primers for P. fluorescens wss operon

- 6/13/14
    - Finished cleaning up acetobacter putative promoters and put them in -30 in iGEM box
    - Ran PCR to amplify out acetobacter plasmid (both pGX2.1 and pGX2.2)
    - Gel was sad, will redo PCR on monday :(
- 6/16/14
    - Re-ran PCR to amplify acetobacter plasmid (both pGX2.1 and pGX2.2)
        - Used .5x primer solution at 3 degrees higher temperature
        - pGX2.2 PCR successful, pGX2.1 not (redone)
        - Performed gel extraction of 1.0kb (2.2) band
            - Nanodrop: 2.5 ng/µL
    - Still do not have high enough concentration of acetobacter cells to do transformation: re-inoculated media.
- 6/17/14

    - Made competent acetobacter cells, have about 25 40ul aliquots sitting in -80c (second shelf from the bottom)
    - Reran PCR for pGX2.1, confirmed need for gel extraction (which will be done tomorrow), sample in -30C
- 6/18/14
    - Gel Extraction for pGX2.1 successful! (14 ng/µL!)
- 6/19/14
    - Growth condition experiment flasks removed from media and allowed to dry
    - Ligated pGX2 plasmid
- 6/20/14
    - Growth condition experiment finished. Results:
        - 1) .094 g
        - 2) .264 g
        - 3) .029 g
        - 4) .359 g
        - 5) .357 g
        - 6) .111 g
        - 7) .043 g
    - Conclusion: Most effective to use 125% recommended sugar (using mannitol as additional 25%)
    - Nanodropped pGX2 (20.3 ng/µL!) and ran gel to confirm size
    - mixed and diluted primers for wssF,G,H,I,FGHI
- 6/23/14
    - Transformed competent acetobacter with pGX2 plasmid and plated on acetobacter media with and without ampicillin
    - Ran colony PCR with the wrinkly spreader P. fluorescens using primers for the wssFGHI operon (F, G, H, and I separately and FGHI together)
- 6/25/14
    - Acetobacter grew on no resistance, but not amp plates
        - replate with semilog concentration gradient of ampicillin concentration
        - Will see if there is more growth tomorrow
    - Tried to isolate total DNA from P. fluorescens cells but used 70% isopropanol instead of 100%, regrew overnight culture and will isolate DNA tomorrow
    - For future, might dye cellulose sheet w/ SYBR green and microscope to check for cells within the material
- 6/26/14
    - The old vat of cellulose was still growing! Took out the sheets and folded them 8x to see if we could alter material properties
    - Some info on mechanical and thermal properties of BC

    - Correctly isolated total DNA from P. fluorescens cells
- 6/27/14
    - Transformed and plated G. xylinum + pGX2 (and + control) on ampicilin .01X , .03X, .09X, .27X, .81X
    - PCRed wssF,G,H,I from P. fluorescens total DNA
- 6/30/14
    - G. xylinum grew on both plates up to .09X ampicillin, which shows that there is a concentration of amp between .09X and .27X that works but doesn't tell us anything about the success of our plasmid
    - Will repeat experiment with .10X, .14X, .18X, .22X, and .26X
    - On weighing cellulose, from here (http://www.google.com/patents/US5580783):
        - After washed with water to remove the medium components, the solids in each flask were treated in 1% NaOH solution at 110° C. for 20 minutes to remove the cells. The cellulose was washed with water until the washings approximated the neutrality, and then dried under vacuum at 80° C. to determine for the dry weight.
    - wssF and wssH were successfully isolated and cleaned; new primers needed for wssG and wssI
- 7/1/14
    - Sent off wssF, H for sequencing, ordered primers for adding prefix and suffix to them
    - Also ordered new wssG, I primers (at a different location from the genes)
- 7/2/14
    - wssG, I primers arrived, performed PCR and isolated them. Now have all wssF, G, H, I
    - Doesn't look like our plasmid is working. Found pUCD2 and pUCD4 shuttle vectors and ordered them from ATCC, should arrive soon.
    - Also ordered primers to get the acetobacter optimized GFP from our pGX2 plasmid to still test promoter constructs in acetobacter
- 7/3/14
    - Looks like there is growth on .22+ plate and not .22- plate!
    - Performed colony PCR on 3 colonies from the .22+ plate, 1 colony from the .18- plate (to see if there is a different), and pure plasmid DNA
    - No differences were found between the .22+ colonies and the .18- colonies, and neither had the band from the pure plasmid DNA, doesn't look like our transformation actually worked after all.
    - wssF and H biobrick primers arrived, as did the ones for getting GFP out from pGX2.1
- 7/7/14

    - Checked plates again and noticed growth on .26- and .81 plates! This is a bad result and suggests that something went wrong with our transformation or the construction of our plasmid. It could be that we neglected to include the toxin-antitoxin system in our plasmid (and so any acetobacter that take up our plasmid and eject their own [with the same replication origin] would die from the toxin) or something else, but we should look into other ways to get our genes into acetobacter.
    - Ordered wssG, I biobrick primers
- 7/8/14
    - Managed to amplify out GFP from pGX2.1 DNA, performed 6 digestion/ligation reactions (with HindIII) to get each promoter upstream of the GFP construct
- 7/9/14
    - wssG, I biobrick primers arrived
    - Amplified out wssF, G, H, and I with prefix and suffix, worked successfully
- 7/10/14
    - Shuttle vector arrived!
    - Grew up in liquid media and plates (LB and LB+amp)
    - Started two experiments for co-culture acetylation
        - 1) P. fluorescens w/ cleaned cellulose sheet
        - 2) co-culture of P. fluorescens with G. hansenii
- 7/11/14
    - PCRed more linear plasmid backbone, both from wax esterase plasmid DNA and from a random part from the distribution kit
        - Now have 5 tubes with 14ul of ~150ng/ul linearized backbone
    - Set up digestion reaction for wssF, G, H, I and the backbone to get these acetylation genes in pSB1C3 for BioBricking
        - Will digest ~1000ng of backbone with E, P, and DpnI (to get rid of any backbone that's not our linearized PCR product) and ~150ng of wss genes with E and P
        - Spilled digestion mixture, will try again monday
- 7/14/14
    - Retried digestion of wssFGHI and linear backbone with E+P
        - For backbone (20ul):
            - 5ul backbone
            - .5ul each enzyme (E, P, D)
            - 2ul CutSmart buffer
            - 11.5ul H2O
        - For wss genes (10ul):
            - (x)ul wss (5ul F, 1.5ul G, 1.5ul H, 2ul I)
            - .5ul each enzyme (E, P)

            - 1ul buffer
            - (8-x)ul H2O
    - After digestion performed ligation and transformation, plated onto LB+chlor plates, will check for growth (colony PCR, etc) tomorrow
    - Also transformed acetobacter with pUCD4 plasmid extracted through miniprep, will plate onto aceto media+amp plates and check for growth (successful transformation), will also try aceto+chlor if that doesn't work.
- 7/15/14
    - Performed colony PCR on four colonies from each plate (growth on all)
        - F1, G1 and G3, H1 and H2 and H3 and H4 all look good. Will grow up overnight culture and miniprep tomorrow, send off for sequencing.
        - Three of the four I bands showed up distinctly around 500bp, will send two of these off for sequencing along with wssI BioBricked DNA
        - F1, G1, G3, H2, H3, I2, I3 all grown up overnight
        - Will send for sequencing, wssI pure DNA with wssI F/R and wssI miniprepped plasmid with both wssI F/R and VF2/VR
        - Performed a colony PCR on wssI cells (and wssI BioBricked DNA) with wssI F and R primers (maybe something is off with the BioBrick VF2 and VR primers, we should see both of these around 1.1kb). This colony PCR showed a band in the correct place, so something is wrong with the VF2/VR primers and wssI, all BioBricks should be correct and we will send off for sequencing tomorrow.
    - Miniprepped more pUCD4 plasmid
    - Primers to get promoters into pUCD4 (with SacI site) arrived
- 7/16/14
    - Sent off samples for sequencing, anxiously awaiting results (F1, G1, G3, H2, H3, I2, I3 x2 (with VF2/VR primers) and I2, I3 (x1) with wssI F/R primers)
    - Made aceto media with tet, amp, spec, and kan to test pUD4
- 7/17/14
    - Transformed acetobacter again with pUCD4, plated both transformed and nontransformed bacteria on AM, Amp, Kan, Spec, and Tet plates to test resistances
    - Performed PCR on ligated promoter/GFP constructs (1-6) to add SacI restriction site
        - Constructs seem to be right length, although appear slightly dirty on the gel (a cleanup would probably help). This lets us know that our promoters worked. However, it might be

worthwhile to restart amplifying the promoter and GFP separately (adding restriction sites to each separately) before ligating together.
    - Sequencing data returned, will examine tomorrow along with order primers for ligation into pUCD4
- 7/18/14
    - Sequencing Data:
        - wssF is perfect
        - wssG contains a restriction site (EcoRI) in the middle, so when we digested with E and P and ligated into the plasmid, the first half of the gene flipped and ligated in backwards ('interesting' sequencing reads). Need to religate into backbone with X and P instead and perform mutagenesis PCR to make a viable biobrick
        - wssH has a few insertions (but the inserted bases are all duplicates…), could be PCR error, sequencing error, or actually just different in the organism from the sequence we found online (likely, because frame is preserved, amino acids inserted are of similar polarity, etc. etc.), will sequence straight from p. fluorescens DNA to see what is actually the asnwer
        - wssI has an insert into the backbone but it is only the tail end of wssI (starting after the restriction site and ending at the I reverse primer)
        - Turns out that there are more restriction sites we didn't catch. wssG has E, wssH has E and P, and wssI has E
- 7/21/14
    - Started order to sequence G from pure genomic DNA
    - Started overnight culture of F to miniprep out and BioBrick
    - A few colonies on tet and spec plates, will repeat transformation with a lower concentration of tet (15ug/ml rather than 50) and will test for presence of plasmid with primers in amp region of plasmid
    - Designed mutagenesis primers for G and I
- 7/22/14
    - Sent P. fluor DNA off to sequence H
    - Cleaned up SacI PCR on the promoter-GFP constructs and ran on gel, looked degraded, need to start and amplify promoters (with SacI and HindIII) and GFP (with HindIII and SpeI) separately and then ligate together, will start that tomorrow
    - Repeated digestion of G and I with X and P instead of E and P, ligated into backbone, and transformed. Plated onto Lb+chlor and will colony PCR/send off for sequencing tomorrow

    - pUCD4 verification primers arrived, ran colony PCR on one colony from tet (most likely at wrong tet concentration), two colonies from spec, pure pUCD4 DNA, and a colony from the non-transformed growth
    - Miniprepped wssF from overnight culture, wssF DNA tube (WN-) had great yield (~300ng/ul) and good 280/260 (1.8) and 280/230 (2.0) numbers, will start the process of submitting this to the registry
- 7/23/14
    - Performed colony PCR on wssG/I (but G looks very much like bacillus…)
    - Ran pUCD4 verification PCR on a gel and we have 4 equal bands at around 500bp for colonies 1-3 and the positive control (pure pUCD4 DNA) and no bands for the negative control (untransformed acetobacter)
- 7/24/14
    - Ran PCR with 6 promoters (with SacI), GFP (with SpeI), and the whole promoter chain (with gBlcok ampli, just to have more of it)
    - Also ordered primers for the entire wssFGHI operon and reran PCR with it
- 7/28/14
    - Made more competent acetobacter
    - Miniprepped a lot of pUCD4 plasmid
    - Retried transformation with wssG/I (last time contaminated)
    - Reamplified more of wssGHI with biobrick ends
    - Still need to call Elim about (lack of) sequencing results
- 7/29/14
    - Made more competent 5(alpha) E. coli
    - Redigested new wssG+I DNA with X+P, ligated into pSB1C3 and transformed
    - PCRed wssFGHI (failed) and GFP for ligation into pUCD4
- 7/30/14
    - Redigested wssG+I (other times we heat killed, PstI can't be heat killed!), cleaned up, ligated plasmid one more time and transformed
    - Made more linear backbone (pSB1C3)
    - Restriction enzymes arrived, so we can being ligating into pUCD4
    - Digested promoters 1-6, GFP for these promoters, GFP for Kam operon ligation, and wssFGHI for ligation into pUCD4. Tried digesting pUCD4, but yields were too low, will try tomorrow.
- 8/1/14
    - re-digested pUCD4, and extracted using phenol-chloroform extraction. Likely phenol residue remains; will use anyway

    - ligated all 6 promoter-gfp-pUCD4 constructs, as well as direct gfp-pUCD4 and wss-pUCD4 constructs, and transformed into e. coli; plated.

- 8/4/14
    - Growth was observed on wss-pUCD4 plate, as well as promoter-pUCD4 plates 1,2,3,6. Colony pcr for insert gave positive results for promoters 1,2,6 and wss. Overnight cultures from these colonies were started
    - Digested and ligated wssG/I
- 8/5/15
    - Overnight cultures failed to grow for wss and promoters 1 and 6. promoter 2 plasmid was minipreped; will pcr to confirm with others after another attempt at overnight culture.
    - This time, 6 colonies of wss-pUCD4 and 3 each of promoters 1 and 6 were taken for overnight culture. These were not first tested for insert; this will be done after miniprep (hence the redundancy)
    - Sent wssH off for sequencing (previous order failed)
    - Transformed E. coli with G and I and growing up over night
- 8/7/14
    - Replica plates checked: no growth on promoter 1; lots of growth on promoter 6; a few really tiny possible colonies on wss. 6 and wss colonies (4 each) were taken and grown up in lb-tet overnight cultures
    - wssG and I minipreped and sent for sequencing
    - Promoter 2 transformed into acetobacter
    - digestions for promoters 3, 4, and 5 (and corresponding gfp, pucd4) performed; will ligate and transform tomorrow
- 8/8/14
    - ligations and transformations into e. coli performed for promoters 3, 4, 5. Plates left at RT for weekend (on bench) [if these fail, digested products are in 4 C fridge]
    - No growth yet on promoter 2 acetobacter; check after weekend
    - sequence data for wssG and I look bad….
    - Growth in promoter 6 overnight cultures; these were miniprepped (need to do pcr to confirm insert; samples are in Cellulose Acetate box 2, labeled “promoter 6 pUCD4 ? Colony #” where # is 1-4)
    - No growth in wss overnight cultures :-(Will need to redigest monday, probably.
- 8/11/14
    - Promoters 3-5 are contaminated, no growth observed on Promoter 2 in acetobacter

    - wssG/I sequencing data reveals that ~700bp insert was actually wssF. We have two options now: we can either synthesize wssG and wssI (along with wssH) without restriction sites or we can make sure we have very pure backbone
        - UPDATE: Today we learned that when we make linear backbone, we lose the X+S sites, so this ligation has been futile. We are now going to a) digest miniprepped wssF plasmid with X+P (for immediate ligation) and S+X (for making more backbone) and b) order new primers for making it with both restriction sites
    - Digested wssF plasmid with X+P/X+S, left in -30C and will run on a gel and extract tomorrow. XP is for wssGI religations (which I will digest tomorrow morning) and XS is for making new linear backbone.
    - Ordered primers from Elim for adding entire prefix and suffix to linear backbone
        - Linear F: TACTAGTAGCGGCCGCTGCAGTCCGG
        - Linear R: CTCTAGAAGCGGCCGCGAATTCCAG
    - PCR amplified more wssGI with biobrick ends
- 8/12/14
    - Cleaned up backbone (XP), digested G/I, need to ligate and transform tomorrow
    - Cleaned up backbone (XS), PCRed with new primers (with anneal temp at 58/62), need to run on gel (made) and cleanup before use
    - PCRed more wss for pUCD4, need to cleanup, digest, and ligate into digested pUCD4
    - Still need to digest pUCD4 (with SacI and KpnI) and elute with hot water (80C) to allow the long plasmid to unbind the column, then transform into 5(alpha) cells
- 8/13/14
    - Ran wss and XS (at two different annealing temps) on a gel, looked good, cleaned up
    - Ligated wssGI into pSB1C3 and transformed, left to grow overnight
    - Digested wss, GFP, and pUCD4, cleaned up (using Kosuke's hot water elution), GFP looked good but the others didn't. Will try a ligation anyway tomorrow, will also cut more pUCD4 and do phenol/chloroform extraction.
- Tips on electroporation for G. xylinum:
    - Follow general electroporation protocol from: http://2013.igem.org/wiki/images/5/5c/The_iGEMer%E2%80%99s_Guide_to_the_Galaxy_%28Stanford-Brown%29.pdf
    - There are a very few aliquots of electrocompetent cells in box in the 2nd (maybe 3rd) to bottom shelf of -80C freezer. On the left hand side of this shelf, there are 4 15mL falcon tubes stuck directly into

the freezer. These are more electrocompetent cells! just aliquot them. (40 microL)

- Use 2500 V for electroporation. (from Acetobacter transformation paper)
- Use 5 microL plasmid mix (I have no justification for this, but it works)
- Fill cuvette to 100 microL. This usually means 40 cells + 5 plasmid + 55 water. To avoid heating, I normally add chilled water and plasmid to cell aliquot tube, then transfer everything to cuvette.
- Keep cuvette, and the black piece of the electroporator that holds the cuvette, in fridge right before use.
- Avoid moisture on cuvette surface! you might want to very gently and quickly (to avoid heating) go over cuvette with paper towel right before electroporation
- Incubate in SOC at RT, rather than 37 C. Do this for ~2 hrs, rather than 1 hr.