General Lab Summary

TUESDAY, 6/20/2017

Adil & Tinna grew the L. Shahii Cas13a E. Coli cells overnight.

WEDNESDAY, 6/21/2017

Adil & Tinna picked three colonies and did minpirep on the L. Shahii Cas13a bacterial cells.

THURSDAY, 6/22/2017

Adil & Tinna ran PCR on the L. Shahii Cas13a cells. Then we analyzed the 3 PCR products on the nanograph. Only one of the PCR products had a high enough concentration to do golden gate on.

FRIDAY, 6/23/2017

Adil & Tinna ran a gel on the three L. Shahii samples and looked at them under the blue light transluminator. The first two lanes gave weird results but the last four lanes (of the samples with the lowest concentrations) showed bright bands at the expanded length. They ran gel extraction on the last four lanes that gave good results.

SATURDAY, 6/24/2017

Adil & Tinna performed golden gate assembly to insert the L. Shahii Cas13a gene into a pDONR L1_LacZa_L2 backbone.

Tinna & Adil ran 2 PCR reactions on the Ms2 and Ms2-Ddx6.

SUNDAY, 6/25/2017

Adil & Tinna transformed the L. Shahii Cas13a plasmids. The transformation efficiency was good and they saw a mixture of blue and white cells on the plate. They picked three white colonies from the L. Shahii Golden Gate plate and grew them overnight.

Adil & Tinna ran a gel on the Ms2 & Ms2-Ddx6 samples. The gel gave them good results at the correct positions. Tinna & Zoe ran gel extractions on both samples and got weird nanographs, meaning these products can't be used for golden gate.

MONDAY, 6/26/2017

Adil, Nia, & Vienna performed miniprep on the 3 L. Shahii GG colonies they picked yesterday.

Tinna & Nia ran two PCR reactions on the L. Cas13a gene with linker and Ddx6 domain. They ran both PCR products on a gel and then placed both gels in the 4C fridge.

Tinna & Zoe ran another set of PCR reactions on Ms2 & Ms2-Ddx6. We ran both samples on a gel and put them in the fridge.

TUESDAY, 6/27/2017

Nia, Zoe, & Tinna looked at the two gels (L. Shahii Cas13 w/ linker & Ddx6, Ms2 & Ms2-Ddx6) under the transluminator. The strands were a little blurry but the bands were at the correct lengths. Adil did gel extraction on the two samples.

Vienna, Nia, & Tinna resuspended 72 primers and made sure to label them appropriately.

Adil & Ali performed golden gate assembly to insert the LS Cas13a with linker and Ddx6 domain into a pDONR L1_LacZa_L2 backbone.

WEDNESDAY, 6/28/2017

Adil & Ali got white and blue colonies on their golden gate plate.

THURSDAY, 6/29/2017

Nia & Vienna ran a restriction digest on the pENTR L1_LshCas13a_L2 plasmid we built using golden gate assembly. Even though the bands were faint, they still appeared at the expected lengths. Nia & Vienna decided to proceed with prepping the L. Shahii Cas13a plasmid to be sent off for sequencing.

FRIDAY, 6/30/2017

Adil & Ali did miniprep on the L. Shahii Cas13a-Ddx6 plasmid. Then, Nia & Ali analyzed the miniprepped samples on the nanodrop and got good concentrations.

Vienna & Nia ran a restriction digest on the LShahii Cas13a-Ddx6 plasmid.

SATURDAY, 7/1/2017

Tinna & Zoe transformed the LacZ Full & LacZ DR plasmids into competent E. Coli cells.

After getting the sequencing resuts from gene wiz, Nia & Vienna decided to re-run digests of all pENTR_LshCas13a plasmids, using PvuI restriction enzyme along with the Cas13a--ddx6 plasmid.

SUNDAY, 7/2/2017

Liz picked colonies from the LacZ Full & LacZ DR cells and grew them overnight.

MONDAY, 7/3/2017

The transformation did not go well. There were no colonies on the positive control plate and only white colonies on the golden gate plates.

Tinna decided to retransform the golden gate LacZ plasmids she and Molly made on Thursday as well as run another golden gate and transformation on the LacZ Full & LacZ DR.

TUESDAY, 7/4/2017

Ronit annealed the rest of the oligos in the fridge, except for one that had not been resuspended yet.

Today, the transformations Tinna did yesterday went slightly better than the ones on Friday. There was growth on all of the control plates. None of the plates except for one of the LacZ DR GG plates (# 2) had any blue colonies. LacZ DR GG # 2 only had two blue colonies so Molly & Tinna decided to run these reactions again to see if they could get better results. Molly & Tinna retransformed the LacZ Full and LacZ DR constructs using golden gate reactions # 1 and # 2. They also grew more mKate Intronic FF4 & U6 plasmids.

Molly ran a PCR to extract Exon 1 out of the mKate Intronic FF4 and then, did gel electrophoresis with it. Zoe performed gel extraction on the mKate FF4 Exon 1 gel product. She analyzed the gel extraction product on the nanodrop and got good concentrations, so we will most likely proceed with golden gate tomorrow.

Nia and Vienna ran the restriction enzyme digest on LS Cas13a, LS Cas13a-DDX6, Ms2, and Ms2-DDX6. The bands for Cas13a plasmids were faint but correct, so they were prepared for sequencing. Ms2 plasmids, on the other hand, had bands that looked like they were all at around 3.0 kb, including the pDONR, which could signify that the product was unsuccessful. Before concluding incorrect plasmids, we tried again with enzyme PvuI, and will run a gel tomorrow.

The LS Cas13a plasmid [179.9 ng/ul] was sent in for sequencing. There wasn't enough LS Cas13a-DDX6 [43.3 ng/ul] to be sent for sequencing, so Vienna and Nia transformed E.Coli to get more. Colonies should be ready to be picked and cultured tomorrow.

WEDNESDAY, 7/5/2017

Sequencing results for LS Cas13a came back and they were great! First successfully verified plasmid!

Nia and Vienna ran Ms2 and Ms2-DDX6 restriction digest. The Ms2 looked good, and was sent for sequencing. We concluded that the plasmids that were supposed to have Ms2-DDX6 didn't have the DDX6 included for some reason, so another PCR reaction was run to get Ms2-DDX6. The PCR product was confirmed in a gel, and prepped for gel extraction tomorrow (Wednesday). Nia and Vienna also made an overnight culture for the LS Cas13a-DDX6, and transformed E.Coli with the verified LS Cas13a plasmid to get more plasmid (this process will be repeated for all verified plasmids).

LacZ DR is too small a plasmid to do an accurate restriction enzyme digest, so it was directly sent to Genewiz to be sequenced.

Tinna miniprepped the LacZ DR overnight colonies. It had a good high concentration on the nanodrop. Because the LacZ DR is so short, we sent it off for sequencing directly because a restriction digest was not possible.

Tinna picked colonies from the transformation she did yesterday for the LacZ Full & LacZ DR construct and grew them overnight. Tinna also did a site directed mutagenesis of the HEPN2 region of Cas13a to create the deactivated version. She also picked colonies from the mKate FF4, mKate FF4 Exon 2, and pBDP3.2 hU6 plates to grow overnight.

THURSDAY, 7/6/2017

Sequencing for Ms2 verified!

The dCas13a SDM did not show any colonies on both the positive control and actual plate. So, Nia and Tinna are going to do the SDM again.

Tinna miniprepped the fifteen colonies grown overnight from the mKate Intronic FF4, mKate Intronic FF4 Exon 2, LacZ Direct Repeat, LacZ Full, and pBDP3.2 (hU6) plate. One of the colonies was discarded because it fell onto the floor open during the Lysis step of Miniprep, so we actually ended with fourteen colonies.

Nia started the Site Directed Mutagenesis process again for the HEPN2 region of the LS Cas13a gene because both the positive control and transformation plates had no colonies when Tinna looked at them this morning. Nia did the PCR process but there was not enough time to do the HDL treatment and transformation steps, so we will do those tomorrow.

Nia also miniprepped the LS Cas13a-DDX6, all of which was sent it off for sequencing. Furthermore, she extracted Ms2-DDX6 from the gel that ran yesterday, and ran a golden gate. The finished product which will be transformed tomorrow.

Adil & Ronit golden gated the oligos into the U6:LacZ DR & U6:LacZ Full backbones.

FRIDAY, 7/7/2017

Sequencing for LS Cas13a-DDX6 was verified! LacZ DR and LacZ Full was sent for sequencing.

Adil, Nia & Tinna transformed all of the sixteen oligos Adil & Ronit golden gated yesterday, along with the dCas13a (to finish the SDM), the Ms2, the Ms2-Ddx6 (golden gate), Cas13a-Ddx6 with three positive control tubes.

Nia grew overnight cultures of the L. Shahii Cas13a for an eventual glycerol stock/midiprep. She also took the remaining culture for the LS Cas13a-DDX6 and "re-cultured" it for another miniprep.

Sequencing for LS Cas13a-DDX6 was verified! LacZ DR and LacZ Full was sent for sequencing.

SATURDAY, 7/8/2017

Molly and Tinna miniprepped the LS Cas13a (GG2) #1, LS Cas13a (GG2) #2, and LS Cas13a-DDX6 (GG3). Nia and Tinna then created 5 fmol/ul dilutions of these.

Molly extracted some DNA from the HEK cells.

Nia & Tinna set up the PCR machine for the site-directed mutagenesis of the HEPN2 region of Cas13a. They also finished up the LR Molly started on Thursday.

Nia, Dennis, Sannidhi & Tinna also picked colonies to grow overnight from the sixteen oligos Adil & Ronit golden gated yesterday.

SUNDAY, 7/9/2017

Molly, Adil, & Vienna miniprepped as listed in the lab notebook. They did the proteinase K kill step to some LR's that Molly started yesterday and then stored them in the -20C freezer.

MONDAY, 7/10/2017

Ronit & Tinna miniprepped the colonies Adil picked yesterday (These were the golden gates that yielded low concentrations on the nanodrop.

Ronit performed an LR reaction on mKate Intronic FF4 to insert the hEF1a promoter.

Nia ran a genomic PCR on the HEK cells to extract the HBG Intron. Then, Nia & Tinna ran the PCR products on a gel. Bright bands showed up at the correct length, so all four bands were extracted from the gel and measured on the Nanodrop.

TUESDAY, 7/11/2017

Nia prepared all of the oligos (U6:ASOs guides 1-4 [except ASO guide 1+] and Guides 1-5). Because none of the Genewiz universal primers that could bind were available to us, a sequencing primer was created and put in the To-Order folder. The prepped tubes are being stored in the -20 C freezer. Nia and Dennis also ran a RE digest on the Ms2-DDX6 in preparation for sequencing.

Tinna did the site-directed mutagenesis of dCas13a for the fourth time. She ran three PCR reactions, treated them with KLD, and transformed them on three separate plates. She also transformed all of the LR reactions that Molly & Ronit started last week.

Molly & Tinna golden gated the oligos targeting the beta globin intron.

WEDNESDAY, 7/12/2017

Nia sent all 16 ASO oligos and guides for sequencing using the Genewiz U6 primer (The engineered primer made yesterday was disregarded). She also sent in the HBG Intron 2 PCR product (#2) to be sequenced using the PCR primers, and the mKate FF4 construct with the SDM Exon 2 with the respective primers.

Nia ran the restriction enzyme digest on Ms2-DDX6, and found that once again the plasmid appears to only contain Ms2, and lacks the DDX6. This issue will be discussed at stand-up tomorrow.

Tinna ran the two SDMs to make dCas13 on pENTR_LSh-GS-Ddx6 & L. Sh pC001-huLsh-C2C2-MBP to make more for future use however, there were issues with the transformation step so she will redo them tomorrow.

Nia & Tinna resuspended and made working stock for the ASO Guide 4 targeting the human beta globin (HBB) intron.

THURSDAY, 7/13/2017

Tinna annealed the HBB Intron 1 G4 forward & reverse oligos. Adil then golden gated the annealed oligo into a U6:LacZ DR backbone.

In the morning, Tinna miniprepped the overnight colonies of the dCas13a SDMs. These SDMs were two colonies from each plate totaling to six. The concentrations were a little low, too low for sequencing, so they will grow more tomorrow.

Tinna also did PCR to extract the mutated Exon 2 (ran three reactions) out of the verified plasmid. She ran the three PCR products on a gel and they all appeared at the expected length. So, she did gel extraction on all three gel products and nanodropped them. They all had good concentrations so she will use these in the golden gate tomorrow.

Adil, Ashley, & Sannidhi transformed twenty four plates yesterday: the seven LR reactions, the two sets of dCas13s (SDM), the antisense oligonucleotides targeting the HBB Intron 2, and the guides targeting the HBB Intron 2.

Molly ran some LR reactions: TRE L. Shahii Cas13a, TRE Ms2, hEF1a rtTA3.

Nia re-sent clones of the oligos and guides that weren't successful for sequencing.

FRIDAY, 7/14/2017

Tinna golden gated mKateExon1_HBBIntron2_mKateExon2MUT into a pDONR_L1_L2 backbone.

Adil, Ali, & Sannidhi picked colonies yesterday from the twenty four plates they transformed on Thursda: the two sets of SDMs, the antisenseoligonucleotides targeting the HBB Intron 2, and the guides targeting the HBB Intron 2. All of the LR plates had no growth.

Tinna retransformed the LR reactions that did not grow when transformed (TRE-Ms2, TRE-L. Shahii Cas13a, hEF1a Ms2, TRE rtTAe3). She also retransformed the 3 pENTR_L. Shahii dCas13 SDMs because the first time she miniprepped them, the concentration was not high enough to be sequenced.

Tinna started two LR reactions for hEF1a-L. Shahii Ddx6 & TRE-Tight L. Shahii Ddx6 because when we tried to retransorm them, we were out of them.

Nia regrew dCas13a for sequencing (since it requires a lot of DNA) and Ms2-DDX6 in case the DNA that had been sent for sequencing earlier today is successful.

SATURDAY, 7/15/2017

Tinna came in early and picked colonies from the pENTR (L1/L2)_mKateE1_HBBInt2_mKateE2MUT plate and the three SDM plates. They were grown in PDM (Fast-growth Media) at about 7:30 am.

Adil, Molly, & Tinna miniprepped all of the plates that were transformed yesterday. Molly miniprepped these colonies later that day at about 4:00 pm.

Nia transformed the 2 LR reactions that were started yesterday by Tinna.

Molly picked two colonies each from the LR plates Tinna transformed yesterday.

Nia made a glycerol stock for the verified pENTRs: LS Cas13a, LS Cas13a-DDX6, and Ms2. She also sent the dCas13a, dCas13a-DDX6. and mKate-HBG constructs that were miniprepped by Molly and Tinna in to be sequenced. Because Nia discovered that one of the tubes labeled LacZ Full was actually a LacZ DR sequence, she sent in all of the tubes labeled LacZ Full & LacZ DR to be sequenced in order to root out any mislabeling.

SUNDAY, 7/16/2017

Molly miniprepped the overnights from Friday (mainly LR's, also A1+ [for mK-FF4]).

Molly picked colonies from the hEF1a L. Sh. Cas13-Ddx6 & TRE L. Sh Cas13-Ddx6.

Nia ran PCR on a pDONR L1/L2 and on the original LS Cas13a plasmid using primers that came in this past week. She also transformed some of the ASOs and Guides targeting the mKate FF4 construct, specifically, ASO 0, 1, 3+, and Guide mKate (This was done due to a lac of Amp +Xgal plates, and we wanted to prioritize the guides/ASOs that were needed).

To create space for these transformations, Nia aspirated the overnight cultures containing the incorrect guides/ASOs (Guides 1-5, mKate; ASOs 0, 1, 2, 3+)

Nia miniprepped some extra pENTR LS dCas13a and Ms2-DDX6 that were re-grown from overnight culture in case more plasmid was needed.

MONDAY, 7/17/2017

Tinna ran yesterday's PCR products on a gel. The bands were very faint but they all (except for the fifth lane) appeared at the expected lengths. After the gel was done, Tinna did gel extraction on the three PCR products.

Tinna also miniprepped the four colonies Molly picked yesterday from the hEF1a L. Sh. Cas13-Ddx6 & TRE L. Sh Cas13-Ddx6.

Tinna picked colonies from yesterday's ASO transformations (ASO 0, ASO 1, ASO 3+, Guide mKate).

Tinna also picked fresh colonies from the plates of ASOs and guides that have been verified (ASO 0+Ms2, ASO 3, ASO 2+Ms2, ASO 3+Ms2, ASO 4+Ms2) in preparation for midiprep tomorrow.

TUESDAY, 7/18/2017

Tinna miniprepped the colonies she picked from Saturday's ASO transformations.

Nia ran restriction digests on completed pEXPR constructs (see LR notebook). Following a gel analysis, the hEF1a:LSCas13a and TRE:LSCas13a were sent for sequencing. The other LRs' digests will run on a gel, and be sent for sequencing tomorrow.

Nia sent in the miniprepped guides and ASOs to be sequenced,, including an ASO mKate FF4 1+ that somehow didn't get sequenced. There was also a mKate HBG junk guide (GJ) and Ms2DDX6 (round 2 #3) that was sent in.

Zoe made 8 gels! Ali & Dennis made Amp + XGal plates. Zoe & Tinna transformed the rest of the U6:ASO/U6:Guides golden gates (7 total). Tinna made fresh 5 mL cultures for midiprep from the verified cultures in the 4 C fridge.

WEDNESDAY, 7/19/2017

Tinna picked colonies from the seven plates Zoe transformed yesterday and grew them up in High Growth Media (PDM). (Brian also helped us make two 500 mL bottles of PDM.) Molly miniprepped the fourteen tubes later that day.

Nia sent in the following to be sequenced:

- mKate HBG guides and ASOs
- pEXPR constructs (see LR notebook)
- a different clone of dCas13a-DDX6 (first clone sent didn't have mutation)
- remaining mKate FF4 guides and ASOs that didn't work the first time

After sequencing confirmation, pENTR LS dCas13a was LR-ed with a hEF1a or a TREtight promoter and pDEST R2 R4. More pC001 (addgene Cas13a plasmid) that had been SDM-ed was grown to be sent off for sequencing.

Nia ran a PCR reaction was using some of our new primers on the original addgene plasmid. (DNA box 5 in -20 C). This was done to get Cas13a with nuclear localization, and to eventually add other domains.

Tinna made 50 mL cultures from the 5 mL cultures she made on Monday, so that she can midiprep them tomorrow. Tinna grew fresh 5 mL cultures of the SDMs done on the original L. Shahii pC001-huLshC2C2-MBP plasmid because we want better concentrations in order to be sent out for sequencing.

THURSDAY, 7/20/2017

Nia looked over all the sequencing results tha came back. The guides for both the mKate FF4 and HBG were unsuccessful, and tomorrow, we will ask Brian what the problem may be.

Tinna miniprepped the SDMs this morning and gave those to Nia to be sequenced.

Nia sent in the original add gene Cas13a with SDM in for sequencing, as well as a couple more pEXPRs and mKate intronic FF4 to test if it is correct since we've had some issues with PCR and amplifying Exon 1.

Vienna & Tinna finished the two LR reactions that were started yesterday. Vienna & Tinna also transformed all the golden gates Molly did earlier today (inserting Guides 1 - 5 targeting the HBB Intron into U6:LacZ DR backbones). Vienna & Tinna also prepared fresh 5 mL cultures of all verified U6:ASO constructs and other LRs in preparation for midiprep tomorrow.

FRIDAY, 7/21/2017

Nia sent out the remaining clones of dCas-DDX6 to see if the SDM was successful. She and Ali also made an overnight culture of hef1a: dCas13a, and re-cultured TRE:Cas13a, both of which can be miniprepped tomorrow.

Tinna & Nia midiprepped verified pEXPR vectors.

Molly miniprepped guides and pEXPR vectors. Molly set up golden gates for the U6:G4 & U6:G2 (redos for transformations that didn't grow), two sets of Lac Constructs (Lac Lw DR & Lac Lw SMut), and the NL-L. Sh. Cas etc.

Ali & Nia transformed the LRs (heF1a:dCas13-Ddx6 & TRE-Tight:dCas13-Ddx6) and the golden gates done earlier today.

Brian helped us out and ran the Exon 1 PCR for us (since we ran it eight times and kept on finding schmears on the gel). He ran the PCR product on a gel and there was no schmear! We will miniprep it tomorrow.

SATURDAY, 7/22/2017

Nia logged most of the DNA in the -20 C freezer on a spreadsheet for future use.

SUNDAY, 7/23/2017

Tinna ran the NL-LshCas13-NL product on a gel and nothing showed up, when she looked at it under the blue light transluminator and imaged it on the gel dock.

She also transformed the pENTR_NL-Lsh-NL product.

She also sent off the five constructs Molly made yesterday (U6:LwDR, U6:LwSMut, pENTR_mKateExon1_HBBInt2_mKateExon2MUT).

MONDAY, 7/24/2017

Brian showed Molly, Ronit, Vienna, and Tinna around the tissue culture room. He showed us how to make cell media for the HEK cell line. And we also thawed some HEK cells.

Nia and Vienna resuspended the new guides and primers that came in. Vienna made overnight cultures for some of the pEXPR vectors and pC001 SDM. Nia and Adil set up LR reactions for hEF1a and TRE: mKate FF4 as well as hEF1a and TRE: Ms2 (these need to be proteinase killed and transformed tomorrow).

Tinna reran the NL-Lsh-NL PCR. Tinna miniprepped the four TRE:Lsh constructs and sent them off for sequencing.

TUESDAY, 7/25/2017

Tinna ran the NL-Lsh-NL PCR Product from yesterday on a gel but there was a schmear. So, Tinna reran the NL-Lsh-NL PCR and ran it on a gel, but there was a schmear again on the gel.

Adil & Vienna miniprepped the overnight cultures from yesterday. Unfortunately, most of the cultures did not have high enough concentrations for sequencing, so Nia and Vienna prepared fresh 5 mL cultures to be miniprepped on Wednesday. Tinna sent out the pENTR:mKateExon1_HBBIntron2_mKateExon2MUT and pENTR_Ms2-Ddx6 constructs out for sequencing.

Nia transformed the LRs and will pick colonies for tomorrow. She also made overnight cultures for TRE:Cas13a and TRE:dCas13a to be midiprepped tomorrow in addition to the DNA that was regrown for sequencing (see above).

WEDNESDAY, 7/26/2017

Tinna picked colonies from yesterday's transformations.

THURSDAY, 7/27/2017

Overnight cultures were made of some of the verified pEXPRs

Nia and Molly sent in mutated pC001 and pENTR dCas-DDX6 for sequencing, as well as some pEXPR constructs to be re-sequenced and double checked for verification.

FRIDAY, 7/28/2017

Wangui midipreppred our verified pEXPRs for us.
Sequencing results showed that pC001 dCas plate 1 #1 had the correct mutation, and the reporter pEXPR were correct. SDM will have to be done on Cas-DDx6 again, and talk about TRE:eYFP since primers haven't bound twice.
To test that the promoter is in the plamid, Nia ran a RE digest on hef1a:mate FF4 #1 since both times it's been sequenced, one of the primers didn't prime.
Nia also sent in pENTR mKate HBG #1 to be resequenced after accidentally using the wrong primers on Thursday.

SATURDAY, 7/29/2017

Adil redid the transformation of the original pDEST mCherry for reference of the part we are improving. It turned out better. Also, attempted some painting/ bioart. Results were questionable at best, but hey anything is art right?? hahahaha ok I should probably be more serious, but I don't think anyone actually is going to read this. applesauce.

SUNDAY, 7/30/2017

Tissue culture stuff
Nia came in to do some maintenance work. This included cleaning out the fridge of unnecessary old cultures and making glycerol stocks of verified pEXPRs. She also:

- made another LR reaction of hEF1a:Cas13a, since we have yet to make a successful plasmid
- made an overnight culutre of another colony of pENTR mkate HBG to see if it has the correct sequence
- sent the second colony of hEF1a:mkate FF4 to be sequenced in case hEF1a: mkate FF4 #1 is invalid after analyzing RE digest.

MONDAY, 7/31/2017

Nia re-ran an SDM on the verified pENTR LS Cas13a-DDX6 plasmid and transformed it. She also finished the LR reaction for hEF1a LS Cas13a and plated that as well.
Another colony of pENTR mKate HBG INT2 was picked yesterday and miniprepped today and sent for sequencing using the new, specified sequencing primers.
hEF1a mKate FF4 #2 is being grown overnight for midiprep tomorrow

Adil and Ali resuspended a bunch of ASO, ASO+, and guide for the REST sequence as well as primers for RNA synthesis kit. We did PCR for the new pDEST mCherry with the scel cut site.

TUESDAY, 8/1/2017

Some of our HEK cells were infected! :(
Nia and Molly set up golden gate reactions for the following constructs, which were then transformed later along with pDONR (table/measurements in LS Cas13a notebook).
- pENTR mKate HBG
- ASO 3+
- Guide mKate
- NL-LS Cas-NL
- NL-LS dCas-NL

Nia also midiprepped pEXPR hEF1a:mKate FF4 #2 or transfections, and picked colonies from pENTR dCas-DDX6 and pEXPR hEF1a: LS Cas13a

WEDNESDAY, 8/2/2017

Nia miniprepped pENTR dCas13a-DDX6 and pEXPR hEF1a: LS Cas13a and sent them off for sequencing. She also transformed pDEST (2.5 ng/ul tube) to get more since we're running low.
Molly set up overnight cultures for the Golden Gates that were transformed yestersay as well as pENTR dCas13a-DDX6 1 and 2 to have more plasmid since the plasmids are so large.

THURSDAY, 8/3/2017

Nia made a glycerol stock of verified plasmids: hEF1a: LS Cas13a #1 (#2 was also correct) and pENTR dCas13a-DDX6 #1. She then miniprepped the golden gates that were transformed on Tuesday, and sent them in for sequencing. Later that day, Nia also set up overnight cultures for pDEST, hEF1a: LS Cas13a (for midiprep), and dCas13a-DDX6 #1 to grow more.
Molly transformed more pENTR mKate-HBG from the frozen golden gate reaction stored since Tuesday. This was done because very few white colonies were on the corresponding plate.
Nia, Adil, and Zoe also began the iGEM 2017 Interlab study, and transformed the DNA in the Distribution Kit. Detail notes are recorded in the notebook called "2017 Interlab Study".

TUESDAY, 8/8/2017

Midiprepped pEXPR hEF1a:mKate FF4 #2
Picked colonies of pENTR dCas13a-DDX6 and pEXPR hEF1a:LS Cas13a
Golden gated and transformed various constructs: pENTR mKate HBG, ASO 3, Guide mKate, and NL-LSh-NL #1 and #2 as well as pDONR.

WEDNESDAY, 8/9/2017

Miniprepped and sequenced the overnight cultures made yesterday. Also picked colonies of the golden gates and pDONR.
Transformed pDEST to make more plasmid.

THURSDAY, 8/10/2017

Made a glycerol stock of the verified hEF1a: LS Cas13a #1 and pENTR dCas13a-DDX6
Miniprepped the golden gates that were transformed on Tuesday 8/8/17.
Picked colonies for pDEST, hEF1a: LS Cas13a #1 for midiprepping, and pENTR dCas13a-DDX6 #1.

FRIDAY, 8/11/2017

Miniprepped the pDEST and pENTR dCas13a-DDX6 #1.
Midiprepped hEF1a: LS Cas13a #1, and also gave 25 ul of our 226,9 ng.ul miniprep of hEF1a: LS Cas13a #1 to grad student.
Transformed hEF1a: LS dCas13a-DDX6 and TRE: LS dCas13a-DDX6.
The sequencing for the NL-Cas-NL and NL-dCas-NL went well, so these were Gatewayed with hEF1a and TRE-tight, and also made into glycerol stock.
U6: A3+ looked alright (see sequencing prep for better description) and regrown for midiprep.
Overnight culture was also made for a re-run transformation of pENTR mKate HBG Int2

SATURDAY, 8/12/2017

Miniprepped pENTR mKate HBG re-runs and sent them in for sequencing
Midiprepped ASO3+ Afternoon #1
Proteinase K killed the LRs made yesterday, but since there were not enough competent cells, these were not transformed, and are stored in DNA Box5.
Made overnight cultures for hEF1aL LS dCas13-DDX6 and TRE: LS dCas13a-DDX6

SUNDAY, 8/13/2017

Miniprepped and sequenced the hEF1aL LS dCas13-DDX6 and TRE: LS dCas13a-DDX6
For Interlab, the test devices, postive, and negative controls colonies were picked and grown overnight.

MONDAY, 8/14/2017

Tinna miniprepped the U6:Guide (targeting mKate) & sent it off for sequencing.
Ali transformed the expression vectors with NL-LS Cas-NL and NL-dCas-NL.
Nia ran another golden gate for the pENTR mKate HBG. Prior to this, Nia consulted with Brian about trouble shooting for this construct, and decided to extend the number of cycles to the maximum allowed on the PCR machine (99).
Nia and Adil followed the OD600 Calibration and Fluorescein protocols for Interlab, followed by the cell measurement for the plate reader.

TUESDAY, 8/15/2017

Midiprepped hEF1a: LS dCas13a-DDX6 and TRE: LS dCas13a-DDX6, as well as pDONR (junk guide) and hEF1a: BFP.

RE-transformed the expression vectors containing NI-LS Cas-NL and NL-dCas-NL. since we got them out too late and they over grew. They were now plated with 100 ul of outgrowth instead of 200 ul. Two troubleshoot versions of the pENTR mKate-HBG were transformed: 1: 99 cycles, and 2: high concentration T4 ligase. #3 will be transformed tomorrow
Transformed some of Ali and Adil's work for updating a part.
Made overnights for TRE:Cas13a and TRE:dCas13a since those were left out for probably a week at room temperature OTL

WEDNESDAY, 8/16/2017

The improved part was successful! See the respective notebook for more details.
The overnights for TRE: Cas13a and TRE: dCas13a didn't grow, but Brian says they should be fine. To be safe, Nia streaked some of the respective glycerol stock onto plates to grow, and eventually be picked and midiprepped again.
Transformed pENTR mKate HBG int2 #1, #2, and #3 (#1 and #2 that were transformed yesterday were on plates w/o Xgal, so these needed to be redone).
Overnights were made for the expression vectors containing NL-Cas-NL and NL-dCas-NL, as well as TRE:eYFP.
Vienna and Tinna transfected 17 out of the 30 cell plates because we ran out of Viafect. Cammie ordered more for us.
Vienna made some cell media but it looks a little weird. Color is not red like it should be. They realized that one of the reagents was incorrect.
Ran golden gate for U6: Guide mKate

THURSDAY, 8/17/2017

Vienna, and Tinna analyzed the transfections from Tuesday in the flow cytometer using Brian's help. The Viafect arrived today!
Vienna & Tinna added DOX to the cells they transfected yesterday. They also made some new cell media because the cell media from yesterday used a wrong reagent.

Picked, grew in fast growth media, miniprepped, nd sequenced pENTR mKate HBG int2 reporter constructs #1, #2, and #3.
Miniprepped the NL-Cas-NL and NL-dCas-NL expression vectors, then realized the backbone in which they lie is incorrect. These need to be re-Gatewayed.
Midipreped TRE:eYFP for later transfections. Overnight cultures for the streaked TRE:Cas and TRE:dCas were made for midiprep tomorrow.
Transformed U6:Guide mKate construct

FRIDAY, 8/18/2017

Picked colonies, grew in fast growth media, miniprepped and seqeunced U6:Guide mKate.
Midiprepped more TRE:Cas13a and TRE:dCas13a
Ran a PCR screening on 12 colonies from the pENTR mKate HBG int2 #3 plate to see if the correct plasmid was there.
Unfortunately, there failed to be any colonies that had the correct construct. Brian will run the golden gate himself from the beginning.

FRIDAY, 9/8/2017

Annealed oligos for REST guides and ASO guides as well as an ASO 3 w/ T7, ASO 3+ w/ T7, and Lsh Guide 4 w/ T7

SATURDAY, 9/9/2017

Golden gated

WEDNESDAY, 9/13/2017

Midiprepped ASO 0,2,J and Guides 3 and J

THURSDAY, 9/14/2017

FRIDAY, 9/15/2017

SATURDAY, 9/16/2017

SUNDAY, 9/17/2017

MONDAY, 9/18/2017

TUESDAY, 9/19/2017

Midi-prep of

Table1

	A
1	mKate-FF4 Guide 1 [MIDI]
2	mKate-FF4 Guide 2 [MIDI]
3	mKate-FF4 Guide 4 [MIDI]
4	mKate-FF4 Guide 5 [MIDI]
5	mKate-FF4 ASO 1 [MIDI]
6	mKate-FF4 ASO 1+ [MIDI]

WEDNESDAY, 9/20/2017

- Transformed Golden Gates for REST Lw Guides 1-6
- Split H82 Line

THURSDAY, 9/21/2017

- Split HEK
- Made overnights for REST Lw Guides

FRIDAY, 9/22/2017

Miniprep of REST Guides

Table2

	A	B
1	Lw REST Guide 1 #1	62.5
2	Lw REST Guide 1 #2	61.0
3	Lw REST Guide 2 #1	68.4
4	Lw REST Guide 2 #2	63.2
5	Lw REST Guide 3 #1	67.7
6	Lw REST Guide 3 #2	65.9
7	Lw REST Guide 4 #1	64.0
8	Lw REST Guide 4 #2	54.5
9	Lw REST Guide 5 #1	59.9
10	Lw REST Guide 5 #2	64.2
11	Lw REST Guide 6 #1	65.4
12	Lw REST Guide 6 #2	61.3