Boot Camp	PUF Group 1 (Angela & Isiah)	PUF Group 2 (Anthony & Asha)	PUF Group 3 (Uros)	PHAT Group	Add groups if necesary														
5/21/2012																			
5/22/2012																			
5/23/2012																			
5/24/2012																			
5/25/2012																			
Week 1																			
5/29/2012	Due to incident, lab work in Biocomplexity was called off for the IGEM team by Courtney.																		
5/30/2012	Designed 9 primers for LacI. LacZ. and eYFP. Prepared LB.	Inoculation of B0014. J23100.wtPUF. B0030. Q04121	Transformed the psB1C3 biobrick into DH5a cells	Prof Ho will ship vectors from Korea on Fri. Should receive them Mon 6/4/12. Found more literature on why C4H doesn't express well in E.coli as well as investigating a protocol for expressing the BM3s in E.coli. Prof Koffas will ship us pUCo-VvSTS-AtCL as one piece if the MTA goes through. Mark Langston has offered to buy some trans-resveratrol for us. p-Courmaric acid is approx $60/25g at TCI America.															
5/31/2012		Transformed C0012, I732005, E0030, I732017. Also transformed PSB1A3, PSB1AK3, PSB1K3.	Inoculated LB media with a psB1C3 colony from the plate grown the day before. Met with Prof. Bhalerao to talk about PUF application and project direction (RNA scaffolding)	Group reading over literature. Revised BM3 primer design	Cara mini preped 5 cultures: B0030, B0014, Q04121, J23100, WT PUF and put gDNA in -20 temp box	Divya started work on blog, uploaded some pictures, started work on brochure, finished basic descriptions.													
6/1/2012	short-term goal changed: make one testing construct, [rbs-puf-eYFP] cloned into P.protet plasmid with a ptet promoter and terminator	Began inoculation of C0012, I732005, E0030, I732017, PSB1A3, PSB1AK3, PSB1K3. Was incubated for 9 hours, then put in 4 degree room	Inoculated again due to the round-bottom tube being misplaced. Mini-prepped the inoculation	Group reading over literature. Revised BM3 primer design again		Divya edited brochure more, however will have to change group description of projects after what was discussed at adviser meeting.													
6/2-3/2012	cultured [PUF+PIN], E0030, and P. protet. designed check primers for [rbs-puf-eYFP]		Inoculated with a psB1C3 colony in order to prepare a glycerol stock of the strain																
6/4/2012		Threw out old, incomplete inoculations from Friday. Transformed I732005, R0010. Miniprepped Venus and E0030 and put gDNA in the -20 temp box. Did new inoculations on C0012, E0030, I732017, PSB1A3, PSB1AK3, PSB1K3.	Made a glycerol stock of the psB1C3 strain. Helped Bob transform DH5a cells with the BBa_K590025 Petrobrick and BBa_B0015 Double Terminator. Reviewed and edited Divya's project description for the brochure.	Received BM3 vectors from Prof. Ho as dried samples. Submitted primer order to Courtney for ordering from IDT. Reading more literature. Will plan to extract vector from well-plate.		Brochure was completed													
6/5/2012		Made glycerol stocks of C0012, E0030, I732017, PSB1A3, PSB1AK3, PSB1K3 and put them in the -80 freezer. Transformation of I732005 failed, so replated with rest of culture. Transformed J04500 and PSB3T5. Minipreped PUF Amp and protet CM20 (spilled 1 tube of this) and put gDNA in the -20 degree in the temp box. Inoculated R0010.	Directed the project of University of Washing Petrobrick characterization to Divya, Isiah, and Bob. Met with Dr. Jin to see how the characterization should be done. Inoculated with a bacterial K12 strain from a glycerol stock (provided by one of Dr. Jin's grad students). Minipreped and quantified pCDF and pACYC.	Ordered the part BBa_K122012 from the parts registry. This is 4CL:STS with yeast promoter and terminator. Researched and found that in the Kim paper, the BM3s are under the control of an IPTG promoter. Talked to Rao about making a protocol for testing the BM3s once they are in an expression vector. Rao wants PHAT group to lyse the cells and try an in vitro assay on the BM3 enzymes first. Also found the ATCC company sells Rhodobacter capsulatus gDNA which could be useful for PCR of TAL gene. Parts registry does not have a TAL gene for us to order.		Isiah, Bob, Divya innoculated transformed cell cultures from University of Washington project; talked to Dr. Jin about characterization ideas; decided that we will change the sugars used in the pathway.												
6/6/2012			Made a glycerol stock of bacterial K12 strain for University of Washington petrobrick characterization. Bleached and washed conical tubes for reuse. Inoculated BL21 cells.	Read literature about TLC plates. Learned that we can get the plates from the chem store room.	Divya and Isiah did a miniprep of PetroBrick, the double terminator (B0015), and K12, and did an inoculation of K12 to make competent cells out of them, in which it is planned to transform the petrobrick into them. 1 Liter of LB Media was made.													
Notebook	Restructured	Beyond	This	Point															
6/7/2012	Divya + Isiah	Uros	Angela + Asha	Adi	Cara	Bob	Anthony												
6/8/2012	Received K12 cells from Dr. Jin, made a glycerol stock	Set goal to create RNA scaffold by 6/15			Received feedback from Dr. Jin at the advisers meeting. Will investigate whether TLC plates can separate piceatannol from resveratrol.														
6/11/2012	Inoculated K12	Transformed split-GFP constructs BBa_K157005 and BBa_K157006 into DH5a cells. Constructed the first RNA scaffold draft.	Inoculated both parts of split GFP, as well as J04500.			Helped Cara transform BM3 into cells													
6/12/2012	Miniprepped K12 cells,	Worked with Tod to plan out the RNA scaffold design and timeline. The design will most likely be synthesized through IDT's gBlock option. The block will include two scaffolds which need to stored into one plasmid and then ligated into separate plasmids.	Made glycerol stock of both split GFP parts, J04500, and miniprepped both split GFP.			Inoculated BM3 WT, #10, and #13. Two 4mL of each													

6/13/2012	Transformed Petrobrick into K12 cells	Constructed the first draft of the gBlock to be synthesized. Needs to be reviewed and a second, unique scaffold to be designed.				Miniprepped BM3 WT, #10, and #13. Two 50uL of each. Also note that initial pellet of mutants after centrifuging from media had a slightly grey tint, opposed to the lack of this color in WT.													
6/14/2012	Plated transformations; inoculated cell growth using CM resistance	Finalized an updated version of the scaffold based on new data on PUF binding. Final modifications need to be made before sent for synthesis.	Performed PCR with Angela. 2 50 uL tubes of control (no PUF binding site) and 2 50 uL tubes with the PUF binding site. Used WT PUF as a template and phusion as the polymerase. Ran gel and saw a result of all primer dimers. Contacted Dr. Amos about getting access to DCL labs. It will take up to 2 weeks for lab access to be approved.															
6/15/2012	Made plan to digest cells in order to see whether or not petrobrick was transformed into K12.	Final modifications made on the design, the construct is ready for synthesis. Sponsorship from IDT needs to be updated.	Cleaned cuvettes left by Angela. second PCR. Did a temperature gradient of 55-63-71 degrees Celsius. used the WT PUF as template DNA. Gel results show only primer dimers, the faintest of faint lines at around 700 base pairs, but it's not a positive result. Miniprepped pTET is in the temp box of the -20.															
6/18/2012	Made NEB Buffer 2	Talked with Courtney about IDT funds, it remains unknown. RNA scaffold ready for synthesis.	PCR attempt 3: using hotstart mix at 63 degrees, using protet as the template DNA and including a blank tube that contained no template DNA as a control. Gel reveals another failed PCR with all primer dimers. Discussed PCR troubleshooting with Todd, Anthony, and will consult Angela tomorrow.															
6/19/2012	Made Digestions of Petrobrick and K12 cells using EcoRI as restriction enzyme; Ran a gel; brightest band was at 5 kb mark. Made more 1x TAE buffer	Designed primers for BBa_K157006 restriction enzyme site (KpnI/XhoI) incorporation in order to put into pETDuet-1 along with BBa_K157005.	Anthony calculated the concentration of the primers to be 30 mM (ideal concentration of 10 mM). Skyped with Angela. Created a plan to subclone WT PUF into pBAD (inducible by IPTG) and eYFP into protet. Also designed primers for the WT PUF with Angela, who submitted them to Courtney. Inoculated E0030 from glycerol stock for the PCR tomorrow as well as MC4100 from a plate Angela prepared.			Plated Resveratrol BioBrick from Agar stab on CM plate. Since we don't have inoculation rings, used glass plating rod. 1) Put glass rod into stab. 2) Spun rod a little. 3) Small chunk as well as residue on glass rod spread onto plate as if plating a liquid. Placed in 37 degree room for 16+ hrs.										
6/20/2012	Dr. Jin asked us to prepare a presentation for the advisor's meeting regarding the progress of Characterizing the WashU project. Divya has begun typing the information.	Primers and RNA Scaffold are ordered. Todd will put in a pETDuet-1 subculture later today so I can miniprep the plasmid and start digestion/ligation tomorrow of BBa_K157005. IDT responded saying the RNA scaffold can't be synthesized through gBlock synthesis. They suggested synthesis through Ultramer Oligos. A decision needs to be made by tomorrow.	Miniprepped MC4100 and E0030 (stored both in the temp box of the -20). Performed PCR with E0030 (eYFP) as the template to make 2 tubes of control (no PUF binding site) and 2 tubes with the PUF binding site. Used the hotstart mix and diluted the primers from 30 mM to 10 mM. Gel bands of 700-800 base pairs confirmed a successful PCR. PCR cleanup was performed, and the resulting DNA put into the tem pbox of the -20. Digestion cannot be completed without HindIII. Will talk to Courtney about it tomorrow. Also, SORF approved funding for us to travel to the high school jamboree. Will also discuss this with Courtney tomorrow.			No colonies on CM plate. Cara and I plated Agar stab on different plates, K, Amp, CM, and Tet following same procedure as before. Put in 37 degree room.										
6/21/2012	Worked on Presentation; no lab work	Miniprepped the pETDuet-1 overnight received from Todd. Digested both pETDuet-1 and BBa_K157005 with EcoRI and PstI in EcoRI buffer. Talked with Todd what the best option would be for synthesis of the RNA scaffold and it seems the miniGene option would be suitable. Emailed Dr. Silver about how they synthesized their scaffold and will base my decision upon her reply. Preliminary split-GFP/PUF tethering conceptualization done.	Got 20 uL of HindIII from Kori. Digested the 2 minipreps of control eYFP, the 2 minipreps of PUF eYFP, pBAD, and ptet. The NEB website recommends that EcoRI and HindIII be used in sequential digest because the buffers aren't compatible. However, online testimonies suggest that using EcoRI buffer for both enzymes will be sufficient. Ran digest and will view gel tomorrow.			Met with Dr. Bhalerao and got remote access for Ubuntu server. Must familiarize self with SSH and Linux terminal so that we may host website on a server we own. We always have the option of paying for a hosting service for ~100$.										

6/22/2012	Presented to Jin, received feedback for procedures. Discussed various ways to characterize, including various sugars and alternate carbon sources. The plan is to start growing cells in TB by Monday or Tuesday so that we can prepare M9 with Glucose. However, will need to test procedures with blank K12 and then with petrobrick-K12 so that we have a control	Double digested BBa_K157005 and pETDuet-1 with EcoRI and PstI in NEB EcoRI buffer. Asha ran my two samples on the gel along with hers.	Ran out of low melt agarose, use normal high melt agarose to run gel purification of digest. ran 2 gels of 1 kb ladder, pET duet and RID0015 (both for Uros) and pBAD with ptet. The second gel was a 1 kb ladder with control eYFP 1, control eYFP 1, PUF eYFP 1, PUF eYFP 2. Got the procedure to purify regular melt gel from Cara (Brad's procedure				Miniprepped 4CL: STS fusion. Nanodropped, 45-50 ng/ml.													
6/25/2012	Divya started making TB media. Added tryptone, yeast, glycerol to make media, autoclaved. Now, we need to add a 100 mL filter of .17 M KH2PO4 and . 72 M K2HPO4. After that, we will need to grow 5 mL of the cells (both normal and petrobrick-transformed) in the TB along with 1: 1000 ratio of antibiotic (CM) and grow it overnight (hopefully tomorrow)	Asha extracted BBa_K157005 and pETDuet-1 from the gel. pETDuet-1 had a very faint band. The wrong ladder was used for BBa_K157005 so the band was not located. Digests were ran again with NEB Buffer 3 and 2 uL of enzymes were used to cut the pETDuet-1 vector. Scaffold sent for synthesis through IDT's miniGene option.	Ran gel to see if digests worked, all were successful, but not Uros' pET duet. Ran digests through the nanodrop and got low numbers for all. Went ahead and did ligation to make the following: pBAD+control eYFP, pBAD+PUF eYFP, ptet+control eYFP, ptet+PUF eYFP. transformed the ligations into both DH5a and BL21.				Helped do some lab work for Asha. Nanodropped pBAD, ptet, p1, p2, c1 ,c2. Exploring Ubuntu server, problems with SSH. Will relocate server and equip with hardware.													
6/26/2012	Isiah finished making the TB media. Divya prepared 2.5 mL cultures of K12-Petrobrick in the TB. Also, an additional two innoculations of normal K12 was prepared to grow over night. We heard back from UW's team advisor from last year, Ingrid Swanson-Pultz, and she replied back with what strains of E. Coli they used. We will be fine using K12 cells because she said there wasn't much difference from the alkane production with the different cells, so we will not need to order any more. However, we need to order C15 Alkanes to inject in one of the production cultures to ensure alkane production	Digestion of BBa_K157005 failed again with no presence of bands. Digestion of pETDuet-1 worked so the BBa_K157005 plasmid was ran on a gel to confirm viability. The plasmid was still viable so double digestion was done again with EcoRI-HF/PstI-HF in NEB Buffer 4 with BSA and Alkaline Phosphatase.	Transformations of DH5a and BL21 resulted in 3 lawns and no growth. A replate was necessary, and they were streaked out. Divya is making new amp plates. Talked with Angela about future PUF plans. Performed additional transformation of all the following into DH5a: pBAD+control eYFP, pBAD+PUF eYFP, ptet+control eYFP, ptet+PUF eYFP.				Aquired hardware, relocated to IGB. Anthony's monitor works well. Data from previous teams backed up onto external hard drive. Reinstallation going smoothly of Ubuntu 12.04													
6/27/2012	Both the TB cultures were successful. However, one of the normal K12 innoculations did not grow. Therefore, Divya made two more TB cultures using just the K12 innoculation which worked. In addition, Isiah and Divya prepared CM and Amp plates for future use.	Absent	Replates of pBAD with control/PUF eYFP in both DH5a and BL21 all had growth. However, BL21 cells looked translucent and flat. Inoculated all of the plates. No growth on transformations of the DH5a pBAD/ptet YFP constructs, so replated all of them. Checked resistance of ptet, and it's Cm, not Amp.				Anthony started biobricking PUF WT. Ran two PCR reactions with hotstart mix at 61 and 63 degrees C. Ran gel of PUF PCR samples done in May, bands looked to be the correct size. Gel extracted all five samples. Inoculated PSB1C3 overnight culture.													

6/28/2012	Today, Isiah made the M9 Media. The TB cultures from the K12 innoculations were successful. Currently, all cultures that we plan to use are in the 4 degree room. We will not take further action on the production protocol until we have received the C15 alkanes and once Dr. Jin is back. We have all the materials to start the production media culture, but we will need Dr. Jin's guidance on the extraction method as well as GCSM analysis as the last steps of the protocol happen rather immediately. We will wait until next week to finish it up.	Digestions of BBa_K157005 and pETDuet-1 seemed to have worked. However, the gel showed the split-gfp band at 300bp even though it was expected to be ~250bp.	Overnight inoculations: the DH5a inoculations of pBAD with control and PUF eYFP both grew. However, both of the BL21 inoculations didn't grow at all. Also, the replates of the YFP constructs didn't grow at all. So retransformed BL21 with WT PUF + control eYFP and WT PUF + PUF eYFP. Also retransformed DH5a with pBAD that had both control and PUF eYFP as well as the same in ptet.			Set up wired connection and talked to computing department about running a server. They offered to host a website for us on their machines. Spoke to David Slater in the gatehouse, said there may be complications if we decide to do Drupal/PHP/Apache stuff.	Anthony digested the PSB1C3 culture made 6/27/12. Digested the PSB1C3, then ran a gel of the 3 total PUF PCR samples and 2 PSB1C3 miniprepped sample. Both PSB1C3 showed two bands, which was not expected (one at about 2kb and one a 1kb). Will have to run a control uncut PBSB1C3 with digested PSB1C3 to check what the problem is, could be that the strain used for inoculation had an insert in it. The PUF PCR digests looked about right. Gel extracted all 3 PUF PCR samples and the one band from the PSB1C3 that was at the right size. Started an overnight culture of PSB1C3.												
6/29/2012	Today more M9 media was made as we were unaware that it is not suppose to be autoclaved. Isiah did a miniprep of pSB1C3 for Anthony and things were washed.		None of the eYFP constructs in DH5a grew. Conclude that neither the control eYFP of the PUF eYFP constructs were properly ligated within either pBAD or ptet. While the retransformations of BL21 grew some odd looking colonies, it is almost certain that they only contain the WT PUF plasmid and not a correct eYFP plasmid. Attempt a second ligation, used same old digest (because the bands on that were correct). Ran the ligation overnight. Inoculated the successful but weird looking and dubious BL21 plates.			Melissa sent me an e-mail about how previous teams were successful with a seperate website and advised against making one this year.	Vacation										
7/2/2012	Not much work in lab was done today, as we are still waiting on C15 alkanes to arrive so we can inject it into our first round of alkane media-culture	Vacation	Over the weekend: checked inoculations and had no growth on the control eYFP, so reinoculated it. It proceded to fail again. Ran a 24 hour ligation of pBAD/ptet YFP constructs. Plated them, they failed again. Emailed Angela about adjusting the PUF plan and tips of ligations troubleshooting. On Monday, checked transformations of DH5a with ptet+control/PUF eYFP. Had a few small colonies! so inoculated. Did another ligation using Angela's new procedure. Also did inoculation of 10 tubes of DH5a for making competent cells.				Vacation											
7/3/2012	Divya ran a gel for Asha's PCR samples (control and PUFiYFP). Again, we are still waiting on C15 alkanes to arrive.	Vacation	Bought 1.5 mL centrifuge tubes and 50 mL conical tubes from RAL storeroom. Inoculations of YFP in protet grew, so made glycerol stock, miniprepped, and stored in the temp box in the -20. Also nandropped them. Did a colony PCR of the colonies with protet and control YFP or protet and PUF binding site YFP. diluted the primers for this from 30 mM to 10 mM. Colony PCR fails. Digested PSB1C3 with EcoRI and PstI in buffer 2 for Anthony. Digested for 2 hours.				Vacation											
7/4/2012	Vacation	Vacation				Vacation	Vacation												
7/5/2012	Divya helped Asha finish preparing competent cells for DH5a; Talked to Jin about UW project; decided not to order C15 alkanes and instead start on the alkane production ASAP	Vacation	Made 10 tubes of DH5a competent cells. Streaked out Control YFP and PUF binding site YFP from glycerol stock.			Vacation	Anthony looked at the gel that Asha ran from the 7/2/12 digestions of PSB1C3. There were two bands again. Both PSB1C3 glycerol stocks have the same problem. The stocks could have been incorrectly prepared. Perhaps there was an insert in the stocks. Will likely just use linearized PSB1C3 from biobrick kit for ligation. Made 1L LB and autoclaved some more 1.5mL microfuge tubes.											

7/6/2012	Meeting with Angela & Uros; discussed deadlines for project, planned more publicity ideas (Divya)	Tested the gel stored from last weeks' digestion to see if the DNA bands are still viable. The bands were too dilute so double digestion of BBa_K157005/pETDuet was ran again with EcoRI-HF and PstI-HF in NEB Buffer 4 with BSA for 4 hours. Digestion failed as neither the pETDuet-1 was present, nor was there presence of the split-CFP band. Digestion was ran again with creation of a supermix in order to assure correct pipetting under the same conditions except for 3 hours. Digestion failed once more, no presence of pETDuet-1 and extremely faint split-CFP band. pETDuet-1 and BBa_K157005 were both inoculated from glycerol stocks in order to miniprep more plasmid for further digestion. Digestion will be tried once more with vector concentrations of 500ng and 1000ng along with addition of Alkaline Phosphatase to see if there is any increase in yield. BBa_K157007 (split-cYFP) was transformed as well in order to be used if digestion of the BBa_K157005 part doesn't succeed.	Streaked out plates all grew successfully. Performed colony PCR on them, but had an annealing time of 30 sec instead of 1 minute. Got primers for WT PUF in pBAD, diluted to 2.5 *10^-5. Did PCR of the WT PUF for insertion into pBAD. Used hotstart mix, DMSO, and 2 minute annealing step. Ran colony PCR and WT PUF PCR on the same gel, got all primer dimers in every lane. Need to redo both.			Vacation	PCR'ed two more WT PUF samples. Started the following overnight cultures: 2 of the ISB 179 PSB1C3, 2 of the 6/4/12 prep PSB1C3, 1 of the Protet, 1 of the E0030 (YFP), two WT PUF. Transformed PSB1C3 from the biobrick kit 1 into DH5a, plated it as well.										
7/7/2012		Inoculated BBa_K157005 and pETDuet-1 to make new volume stocks of plasmids. Concurrently plated BBa_K157005 and pETDuet-1 in case the inoculations from glycerol stocks don't work and so I can inoculate from colonies if needed. Plated BBa_K157007 (the split-cYFP part) in case it works better than the BBa_K157005 split-cCFP part. However, colonies did not grow even after plated two plates as a backup. Miniprepped BBa_K157005 and pETDuet-1 inoculations put in last night and got very low, unusable concentrations (perhaps due to inadequate incubation time). Miniprepped pETDuet-1 and BBa_K157005 from this morning and got higher concentrations. Double digested BBa_K157005/pETDuet with EcoRI-HF/PstI-HF with BSA and Alkaline Phosphatase for 3 hours (BBa_K157005 was digested both at 500ng and 1000ng vector quantity). Gel purified both bands which seemed to appear better than previous attempts. Concentrations after purification were still fairly low so chances of successful ligation might be compromised.	Redid PCR of WT PUF with a temp gradient of 59-63-67-71. Used the supermix. Redid colony PCR with a temp gradient of 59-63-67-71 and the colony PCR supermix. Inoculated BBa_157005 for Uros. Ran a gel of the WT PUF for pBAD. Only the 2 lanes showed any bands at all - the ladder and the 59 degree lane (had the right band!) Redid another gel with more dye, but still saw nothing else. Ran a gel of the colony PCR and got all primer dimers. PCR cleanup on the successful 59 degree lane for WT PUF, yielded a nanodrop of 63.7 ng/uL. Digested the pBAD miniprep and the WT PUF from the PCR. Ran a gel of the digestion with high melt gel. Got bands of the right length, but WT PUF was very faint. Used Cara's procedure for gel extraction, yielded very low nanodrop! Did ligation anyways				PCR'ed four samples of WT PUF for subcloning into pBAD. Ran a gel of the PCR products. Only the 59 and 71 degrees samples worked (63 and 67 didn't). PCR cleanup of the two samples that worked, and digested with HindIII and EcorI. Gel extracted the two bands, then ligated over night										

7/8/2012		Asha inoculated BBa_K157005 and pETDuet-1 for me from bacterial cultures grown the day before. Ligated BBa_K157005 with the digested pETDuet-1 plasmid with a 10:1 ratio of insert to plasmid along with a no insert control. Miniprepped inoculations of BBa_K157005 and pETDuet-1 grown from bacterial cultures and received high concentrations (it seems inoculation from bacterial colonies is best). The ligations were then transformed into DH5a cells with 5uL of vector and the transformations were plated after 6.5 hours of incubation. Inoculations were made of BBa_K157005 and pETDuet-1 from bacterial cultures in order to prepare for more samples if needed. However, work with the split-CFP construct will be suspended due to limitations of time.	Made glycerol stock of pBAD 30 inoculations, stored in green box in neg 80. miniprepped 2 tubes of pBAD and nanodropped. Stopped ligation after 8 hours and did 20 min inactivation at 80 degrees in the PCR machine. Transformed the ligations, so WT PUF in pBAD in DH5a on Amp plates. Inoculated pETDUET and K175005 for Uros.				Asha helped me transform the ligations, and plated as well.											
7/9/2012	Isiah started growing the K12-petrobrick cultures in the M9 media for Alkane Production	Decided to suspend work on the split-CFP integration into pETDuet-1 and focus on sending a PUF-CFP sequence out for synthesis.	Tried to made plates (LB, Amp, Amp/Cm) but didn't have enough Agar in the mix, so they didn't solidify. =(Need a recipe other than that in notes. Starting from scratch for YFP by redoing the PCR. Used DMSO and supermix.. Failed, getting ghost bands that are very faint and at the incorrect length.				PCR'ed three more samples of WT PUF for subcloning into pBAD											
7/10/2012	Incubation day; waiting on C15 alkanes to arrive. Divya made Amp, CM, and Amp-CM plates for Asha	Read the UNC paper again to receive more insight as to how PUF was tethered. Need the supporting information and figures to find all the details.	Transformed last ligation of PUF. miniprepped inoculations of pBAD30. Yesterday the PCR of YFP failed, so redid with different temps on melting and annealing temps. used E0030 as new template, and no hotstart polymerase. Tried both the control site and PUF binding site at GC buffer and HF buffer. Ran a gel, all worked! Performed PCR cleanup, got good nanodrop numbers. Digested all PCRs and protet with EcoRI and HindIII. Gel of digestion shows success. Performed gel purification, but got concentrations all under 6 ng/uL. Tried several ligations of control and PUf binding site YFP into protet anyways. Used 10:1 insert to vector ratios. Running ligations overnight.			Helped Cara with TLC of Resveratrol and Piceatannol. 5mg/mL dilution of powder in EtOH. Piceatannol used up. 95 mg of Resveratrol left. Used solution of 85 parts Chloroform : 15 parts MeOH : 3 parts AcOH for 257.7 mL of Normal mobile phase. 30 parts MeOH : 70 parts water : 0.1 parts AcOH for 250.25 mL of Reverse. Clear results, Resveratrol and Piceatannol easily separated in Normal phase. Reverse more difficult to read. Will use Normal phase in the future.	Did PCR cleanup on the three samples from yestereday, digested with EcorI and HindIII. Digested pBAD as well. Ran gel of the digests, only one of the PCR's worked, the digest of pBAD didn't work. Extracted the PCR sample digest that worked, and ligated it with a previously digested pBAD sample											
7/11/2012	Incubation for alkane production finished; Isiah took out the culture from the chromotography tubes; Not sure whether we extracted sample using Ethyl Acetate or not. Divya Talked to Dr. Jin about GCMS; He directed us to Dr. Lee. He also mentioned that before we do anything we GCMS, the C15 alkanes have to be injected in M9 media to test for a standard curve	Received the complete information of the UNC paper from Angela. Proceeded to investigate what split-GFP sequence is best to use and which sequence is reputable. Found two sources which provided the sequence, however their credibility is questionable. E-mailed a graduate student from the Silver lab about the split-GFP sequence he used in the same RNA scaffold paper. E-mailed Dr. Broude, an investigator who researched split-GFP and it's uses, which was also mentioned in the Silver paper. Asked for either the sequence or the actual part if possible. E-mailed Todd to further discuss the possibility of cloning this construct ourselves and the best new direction.	Transformed ligations, and plated on CM plates.			Ran gels for revised 4CL:STS PCR Cara did. Results somewhat inconclusive. Best bet of target segment was found in 4D. Plated 4 of Asha's pTET transformations, placed back in 37 degree room. Poured LB Divya made into 50mL conical tubes in sterile hood in MMG since somehow previous LB got contaminated. Updated lab notebook section of Wiki.	Transformed and plated the ligation reaction											

7/12/2012	We Talked to Nawei about the GCSM, and she told us that we needed to find specific conditions for the alkanes. I contacted Ingrid again, and she responded back with an exact GCMS protocol the team used last year.	Talked with Todd about whether to synthesize PUF-CFP or go ahead and tether the proteins ourselves. Todd believes we could tether the proteins together before August, mainly through the primer extension method. I have decided to continue to produce the construct. Been designing primers for cCFP amplification, PUF-CFP tethering of both N and C termini, and with the help of Angela, creating check primers for MSC1 and MSC2 of pETDuet-1 in order to sequence the constructs.	Transformations from yesterday failed, so replated all of them. Started a second ligation from the digests, now using a 6:1 insert:vector ratio instead of a 10:1.				There were no colonies, replated two more plates from the 7/10/12 digest. Also transformed and plated two plates using a ligation from 7/7/12											
7/13/2012	Helped around lab; waiting for C15 alkanes	Talked with Todd in more detail about how to exactly go about tethering PUF-CFP through primer extension. Also discussed possible solutions on how to construct the split-CFP pETDuet-1 construct (higher pETDuet-1 concentration, amplifying split-cCFP, using CPEC). Designed more primers for the construct.					Found many colonies, however the number of colonies seems to be too many. Difficult to isolate individual colonies.												
7/16/2012	Updated blog, twitter, flickr.	Ordered 16 primers for construction of pETDuet with split-CFP parts and the tethering of PUF-CFP (primer extension). 4 primers were constructed by Angela for the sequencing of MSC1 and MSC2 of pETDuet-1.					Inoculated two cultures from the transformation plate (one each from the 7/7 ligation and 7/10 ligation. The culture did not grow. Reinoculated four cultures (same as before except duplicates of each with no ampicillin). Also streaked competent cells on an amp plate. Did this to see if it is problem regarding ampicillin concentration. Also started two new digestions of PUF and pBad for and construct.											
7/17/2012	Today, we ran a PCR of wtPUF and a gel to go along with it. We will contact Sigma-Aldrich to see when the C15 alkanes are arriving and plan for it. We should also transform pSB1C3 into dH5a.	Corrected a primer for PUF-cCFP tethering and re-submitted the order. Made a detailed plan of the cloning that needs to be done for PUF-CFP tethering and split-CFP construction. Bob inoculated pETDuet-1 and K157005 for me as well as transformed K157006 into DH5a cells. I plated the transformation on an ampicillin plate afterword.	Made new primer sequences for PUF project. Modify YFP construct using Venus gene (proven it worked) instead of biobrick: E0030. A set of CPEC primers are also designed. Transformed the 6:1 ligation of YFP. Also inoculated more protet. For YFP florescence testing will need a negative and a positive control....				Ran gel of digested pBAD and PUF for construct. Only found band for PUF. Second time in a row where digested pBAD showed no bands.Gel extracted the PUF band. Also started three new digestions of pBAD. Checked inoculations, the two without ampicillin grew, but the other two with ampicillin did not grow. Also checked streaked plate of competent cells on amp plate. Found a lawn. Concluded that the amp plates do not have enough ampicillin. Therefore the transformation plates were not reliable.											
7/18/2012	Isiah did a gel extraction as well as ran a gel of Divya's PCRed wtPUF. The PCR was successful as the bands were around 1.6 kb, close to 1.7 kb.	Received the split-GFP sequences used by Mrs. Valencia-Burton in her paper for characterizing an RNA scaffold! The final PUF-GFP sequence or synthesis can be subsequently made to have as backup if tethering doesn't work.					Did PCR cleanup on the three pBAD digests (no gel extraction in order to increase yield). Concentrations were still extremely low. Used pBAD digest with 7/10 and 7/16 PUF digests for two ligation reactions. Also started a digestion of PUF construct PCR and two digestions of pBAD. Increased the DNA mass for the digestions so that concentrations could be high enough after PCR cleanup for different ligation ratios.											

7/19/2012	Isiah did a PCR of wtPUF according to a different protocol. He PCRed five samples. Divya ran a gel of those samples in the afternoon; they were unsuccessful. We shall repeat the pcr tomorrow.	Did PCR of cCFP in order to amplify the band before digestion. PCR proved to work after Bob ran a gel of it. Made a final draft of PUF(WT)-aGFP ready for synthesis in case tethering doesn't work. Made two inoculations of K157006 in preparation for tethering and cloning tomorrow.	Angela miniprepped three plasmids: pBAD30, P. protet. E, and the plasmid encoding venus. A glycerol stock of pBAD30 was made. Ligation of several testing constructs (6 YFP test constructs and 1 PUF binding site and YFP test construct) were completed and sit overnight.					Transformed and plated the ligation reactions from yesterday. Plated two plates for each ligation reaction (100 ul and 200 ul) for a total of four plates. Also did PCR cleanup for the three digestions from yesterday. Yields were a bit better due to greatly increased DNA mass used, but loss of total DNA mass after cleanup/extraction was still severe. Started two ligations with this set of digestions using a 1:3, 15 ul reaction and a 1:6, 20 ul reaction.											
7/20/2012	Ran the gel for wtPUF PCR for PCR clean up. The gel's bands for wtPUF and ladder were squiggly, although it does appear that the bands matched up. As a precaution another wtPUF pcr will be ran. pSB1C3 as well.	Adi miniprepped and nanodropped two tubes of K157006 as well as a tube of K157005 and pETDuet-1 for me. Bob and Adi then ran digestion of cCFP and pETDuet-1 at 1500ng with EcoRI/PstI in NEB Buffer 3 with BSA for 4 hours. I ran a gel of the digestions and extracted the proper bands. Ran gel purification and then let the ligation run overnight with 5:1, 3:1, and No Insert reactions stored in the 4oC.						Checked plates from yesterday, non of the plates had colonies											
7/23/2012	Alkanes arrived from Sigma-Aldrich. Isiah plated K12-Petrobrick cells on a CM plate (from glycerol stock) to grow more colonies. Divya made 4 innoculations of K12 cells (one of them accidentally has more than the required cells in it).	Bob did PCR of 3 reactions of cCFP and I continued with PCR cleanup once it was done. I also amplified the two parts for PUF (WT)-cCFP tethering through PCR. After PCR was done I performed PCR cleanup. Made a plan for Bob to take over Split-CFP cloning which includes bypassing gel purification due to low concentrations of vector/insert.						Transformed ligation reactions from 7/19/12 to 65 ul DH5a. Unfortunately electroporation of the 1:3 reaction arced. Transformed using heat shock for both 1:3 and 1:6 ligation reaction. Plated transformations. Started two new reactions using the same volumes as the 7/19 ligations (a 1:3 in 15 ul and a 1:6 in 20 ul)											
7/24/2012	The K12 innoculations did not grow because Divya accidentally grew them with CM. Isiah took out the K12-Petrobrick plated cells; they grew successfully. TB media was made, and the filter solution was prepared as well (needs to be autoclaved, however). Divya re-did innoculations of K12, and prepared innoculations of K12-Petrobrick.	Performed PCR in order to tether PUF (WT)-cCFP with GC buffer and the PCR failed, presence of primer dimers. PCR of the amplification of cCFP and PUF for tethering seemed to have worked although there was low cCFP product. The order of PCR for tethering needs to be reversed and optimized tomorrow. Inoculated two tubes of pETDuet-1 for further cloning work.						Desalted the ligations from yesterday and transformed them into DH5a. Plated the transformations. Started two new digestions of pBAD and a digestion of PUF PCR.											
7/25/2012	Isiah made minipreps of both K12 and K12-Petrobrick innoculations. We prepared 2 tubes of K12 TB cultures to grow overnight.	Did primer extension in order to tether PUF-cCFP with HF buffer and PCR didn't work, presence of primer dimers. Performed PCR of PUF-cCFP under a gradient of temperatures 50/55/60/65/70oC with an annealing time of 1 minute instead of 30 seconds. PCR was done with WT-PUF, cCFP, and primers PUF-cCFP tether 1F/1R.						Found no colonies on the plates. Also ran a gel of an uncut miniprep of pBAD with one of the pBAD digestions from yesterday. No bands for either.											
7/26/2012	11 reactions for wtPuf was made for PCR, 7 was ran today the rest will run tomorrow. Starting over on pSB1C3 tomorrow due to strange results. Divya accidentally put miniprep in TB cultures, so she redid TB culture prep (2 tubes K12; 2 tubes K12-Petrobrick) and set them to grow overnight	Presence of primer dimers under all temperatures of the PCR done yesterday. Ran another PCR for PUF(+)-cCFP tethering with PUF(+) including primers PUF-cCFP tether 1R/PUF-cCFP 1F and cCFP with primers PUF-cCFP tether 1F/PUF-cCFP 1R in order to amplify the parts before tethering. Made 4 inoculations of pETDuet-1 with Bob.																	

7/27/2012	Found pSB1C3 vectors, ran a gel of pCR and digest of pSB1C3; BOTH WORKED! Took TB cultures out of the 37 degree; EVERYTHING GREW!	Ran a gel of yesterdays PCR due to taken gel not being clear. The PCR for both PUF(+) and cCFP amplification resulted in primer dimers. Ran PCR cleanup for Bob's digestion of pETDuet-1 and ligated with a 3:1 and No Insert at 15fmole concentrations and left it to incubate overnight.																	
7/28/2012	Made M9 media, resuspended k12 TB cultures in M9 media and set to grow overnight (1 tube /6 ml) and also injected 75 mg C15 alkanes. Digested wtPUF; Did a gel purification of both wtPUF and pSB1C3. started ligation																		
7/29/2012	Finished ligating wtPUF and pSB1C3, transformed construct in dH5a, plated cells on CM plate. Resuspended 1 tube of k12-petrobrick tb culture in m9 media; left other tube out in room temp overnight to increase OD for optimization																		
7/30/2012	Colonies grew, but we have to replate transformations because we lost colonies due to Colony PCR we weren't supposed to do. Ran a gel anyways; A BAND WORKED! (L1 - 3). Replated more L1, L2 Transformations to use for colony PCR tomorrow.	Vacation					Started two new digestions of pBAD miniprep from angela and another digestion of PUF PCR. Used newly bought nuclease free water for these reactions.												
7/31/2012	Isiah ran a colony PCR and restreaked colonies on a gridded plate. Divya ran the gel; no bands showed. Talked to Dr. Jin about GCMS; have to regrow cultures in order to have enough samples for a standard curve. Restreaked two colonies from original transformation plates on a gridded plate, innoculated 2 colonies from Asha/Anthony's PUF-YFP/protet colony plates, transformed remaining ligation (L3) and streaked onto a separate plate, replated remaining L1/L2 transformations. Isiah made 5 K12 TB cultures.	Vacation					Talked to Kori as well as my graduate student about test expression of PUF. Finished test expression plan details. Received Arabinose from Kori.												
8/1/2012	The L3 transformation did not grow any colonies; Isiah ran colony PCR on all the remaining plates, but no colonies grew. Fortunately, he streaked colony 3 from the original L1 plate on to a new grid. Divya made 3 innoculations of that colony in 0 CM, CM10, and CM20. The K12 TB cultures were resuspended in M9 media and put for incubation. Divya added CM20 to 5 K12-Petrobrick TB cultures and put them in the 37 degree to grow overnight. Divya made 4 different dilutions of C15 alkanes and injected in the control K12 M9 cultures (1 mg/L, 10 mg/L, 50 mg/L, and 100 mg/L).	Vacation					Inoculated 6 cultures (3 for WT PUF and 3 for PUF in pBAD). Allow for 16 hours of growth												

8/2/2012	Isiah made glycerol stocks and minipreps of the innoculated L1 3 colony and prepared the sequencing bag. Everything was sent for sequencing. Divya took out K12-Petrobrick TB cultures, spun them down and resuspended in M9 media to grow for 48 hours.	Vacation					Sucultured 50ul of each PUF culture into 5mL of LB. Grow for 2 hrs for PUF in pBAD, and 3 hrs for WT PUF. Induced with Arabinose and IPTG for corresponding cultures. Grow for 16 hours												
8/3/2012	Divya took out K12-M9 cultures which had been injected with alkanes and centrifuged them. Ethyl Acetate was added to extract 200 uL of the top layer after centrifuging it. The samples were collected in 4 1.5 mL Eppendorf tubes. Dr. Jin said to talk to Na Wei on Monday to make time to run the samples through GCMS	Vacation					Pellet cells, resuspend in 700ul Binding buffer. Lyse cells by sonication. Run gel of pellets and lysates (12 samples). Stain and destain gel. Protein gel does not show clear expression of PUF in any of the samples.												
8/4/2012	Divya came back at night to take out the K12-Petrobrick M9 cultures. She centrifuged the samples after adding .7 mL of Ethyl Acetate and noticed that one sample had a particularly high amount of cell debris when compared to the other. 2 extractions of 200 uL each was made of the tubes, respectively. Pictures were taken on her phone. These samples, along with the control samples, will be run on the GCMS on Monday	Vacation					Cotransformed minipps of PUF in pBAD and YFP-control/YFP-PBS into DH5a cells using 3 different ratios of PUF:YFP. Plate cells on AMP+CM plates. Also transformed PUF in pBAD into YFP-control/YFP-PBS overnight cultures (wash cells in glycerol first). Plate cells on AMP+CM plates.												
8/5/2012		Vacation					Found colonies on Yesterday's plates (cotransformation only). Started digestions for cloning YFP-control and YFP-PBS into pET-duet. Subcultured yesterdays PUF overnights (1/100 dilutions again). Grew PUF in pBAD for 2 hrs before inducing Arabinose. Grew WT PUF for 3 hrs before inducing with IPTG. Allow to express for 16 hrs (in 20 degrees this time).												
8/6/2012		Vacation					Pellet cells, resuspend in 700ul Binding buffer. Lyse cells by sonication. Run gel of pellets and lysates (12 samples). Stain and destain gel. Protein gel does not show clear expression of PUF in any of the samples. Growing in 20 degrees seemed to reduce expression of all proteins.												
8/7/2012		Made a plan for further RNA scaffold work.					Run colony PCR on Asha's cotransformations. Only one colony (Had YFP-contol band) showed a band. Tested amp and CM stocks on DH5a to ensure quality. All tested ones worked.												
8/8/2012		Designed and ordered primers for the RNA scaffold. An addition of EcoRI will be made at the 5' site in order to clone into a T7 promoter vector (pETDuet-1). Sent a draft of PUF(+)-(a)GFP for synthesis to Brad in order to receive a final review before sending out.					Made m9 minimal media (with glucose) as well as m9 media plates. Autoclaved 6 boxes of p1000 tips, 6 boxes of p10 tips, 4 boxes of p200 tips. Made 500 mL of LB media. Inoculated 4 cultures of PUF in BL21 for test expression.												

8/9/2012		Received an improved version of the PUF(+)-(a)GFP draft from Brad, the 6x His Tag was changed. Sent the updated and final sequence to Courtney in order to get a quote and purchase.					Made Amp+Cm plates. Made new stock of Cm 34mg/ml and Amp 100 mg/ml. Missed inducing time, will need to start inoculations again. Inoculated 6 cultures of PUF WT in BL21.												
8/10/2012			Performed quality control on M9 liquid media (grow Dh5a), M9 plate (grew PUF in pBAD, DH5a, YFP-control, colony 3), Cm20 plate (grew DH5a)				Subcultured cells (1/100) in 5 mL, induced at OD600 of 0.6-0.8 with 6 different IPTG concentrations. Performed quality control on M9 liquid media (grow Dh5a), M9 plate (grew PUF in pBAD, DH5a, YFP-control, colony 3), Cm20 plate (grew DH5a)												
8/11/2012			re-inoculate biobrick. Get grid plate and innoculate. Remake M9 media using Ahmet protocol. Miniprep and test colony 3 by running gel. Transform mutant PUF. Inoculate pKRSTc or streak a plate. Make tet12 and Kan40 plate. Buy DH5a comp cells. Minipret WT PUF and PUF biobrick fo sequencing. Prepare strong RBS SOE. Make more YFP (control/PBS). Kitplate 1 well 16P, tetR repression transformation. Plate 2 (3K)				Pellet, resuspend cells, sonicate, run gel, stain and destain gel. There seems to be a band the corresponds to the molecular weight of PUF but it is still relatively feint and is present in every lane.												
8/12/2012							Co transformed minipreps of: 1) protet (empty) + PUF in pBAD 2) YFP-control + PUF in pBAD 3) YFP-PBS + PUF in pBAD. Inoculated colony 3, PUF biobrick, and PUF in pBAD												
8/13/2012		Prepared 20 plates of Kanamycinand 20 plates of Kanamycin + Tetracycline					Found colonies in all plates, did colony PCR of colonies. Inoculated E0030 and PUF biobrick. Transformed mutant PUF into BL21 DE3, plate on Kan plate.												
8/14/2012		Made the final draft of PUF(6-2/7-2)-BGFP and sent to Brad in order to confirm before synthesis. Made a wetlab plan that spans till the regional competition.					Ran gel of colony PCR, found 2 colonies that worked. Transformed TetR repressor into DH5a, plate on kan plate. Inoculate tubes for YFP fluorescence test: DH5a, Protet, YFP-control, YFP-PBS, Protet+PUF (pBAD), YFP-control+PUF (pBAD), YFP-PBS+PUF (pBAD).												
8/15/2012		Prepared IDTE buffer for resuspension of the RNA scaffold.					Subcultured YFP test cultures into 2 sets (37 and 24 degrees expression) as well as separate induced/uninduced for the appropriate tubes (20 tubes total, 10 tubes per set). Induced the appropriate tubes after 2 hrs, and moved tubes into their respective expression temperatures. Measured fluorescence of each culture after 6 hrs using plate reader. Inoculated WT PUF and mutant PUF for first test expression set. Inoculated pBAD PUF, YFP-control+PUF (pBAD), YFP-PBS+PUF (pBAD) for second set of test expression.												

8/16/2012		Sent out PUF(6-2/7-2)-(B)GFP to get a quote from GenScript. Studied the in-vitro transcription protocol and followed a graduate student perform the procedure.					Ran day 2 test expression of mutant PUF. Subcultured and induced at various IPTG concentrations. Grow cultures overnight, one set at 24 degrees and another at 37 degrees. Ran day 2 test expression for YFP constructs as well. subcutured and induced the appropriate tubes with 50ul 20% arabinose. Grew at room temperature for 6 hrs. Pelleted, resuspended, sonicated, ran gel, stain/destain gel.											
8/17/2012		Sent Courtney the quote received from GenScript					Ran day 3 test expression for mutant PUF. Pellet, resuspend cells, sonicate, run gel, stain and destain gel. There is a clear indication that mutant PUF is expressed											
8/20/2012		Diluted the RNA scaffold in IDTE buffer to make a final concentration of 50ng/uL. Transformed the scaffold into DH5a cells and plated on an ampicillin plate.																
8/21/2012																		
8/22/2012		Inoculated four tubes of 5mL LB with DH5a RNA Scaffold cells. Miniprepped the four tubes and received low concentrations so inoculated a 500mL flask with 20mL of LB.																
8/23/2012		Miniprepped 10mL of the flask inoculated last night and received very low concentrations. Miniprepped the remainder of the inoculation using N3 buffer instead of P3 buffer and received 650ng/uL.																
8/24/2012		Digested 6.5ug of the miniprepped RNA scaffold with PstI for 7 hours. Ran a gel of the digest to gel purify and received a large smear in a range of unexpected lengths. Ran a gel to confirm plasmid integrity and showed expected structure therefore digestion will be ran for less due to star activity. Inoculated 50mL of LB with DH5a RNA scaffold transformed cells.																
8/25/2012		Miniprepped 10mL of the 50mL inoculated flask and received 72ng/uL. Miniprepped in RAL and received a similar concentration of 82ng/uL. The concentrations are too low to get 6ug of DNA so made a 20mL inoculation of the RNA scaffold cells for tomorrow.																
8/26/2012		Miniprepped 10mL of the 20mL inoculation made last night, received 100ng/uL. Decided to try digestion with 3ug of DNA using PstI-HF for 3 hours + Alkaline Phosphatase. Gel purified the band, which showed to be correct, and received very low concentrations/low integrity DNA.																
8/27/2012		Miniprepped 10mL of the remainder of the 20mL inoculation and received 210ng/uL. Digested 4.8ug of DNA with PstI-HF for 4 hours with addition of CIP after 1 hour of incubation. Proceeded to gel purify the band which was at the expected length and received 30ng/uL. The concentration was low, but started in-vitro transcription with an overnight incubation to ensure product formation.																

8/28/2012		Eluted the RNA transcripts from the reaction and cleaved DNA in order to get pure samples. Quantified the RNA and received very low concentrations of 5ng/uL and an abnormal A260/A280 number (which is probably due to the plate reader not assessing properly at low concentrations). Tried to troubleshoot the experiment, but both the protocol and procedure look correct with the exception of low starting DNA template. Started another run of in-vitro transcription overnight with a minor change in substrates added.					Inoculate for fluorescence test: DH5a, protet, YFP-C, YFP-PBS, YFP-C+pBAD, YFP-PBS+pBAD, YFP-C+PUF, YFP-PBS+PUF											
8/29/2012		Eluted RNA from the in-vitro transcription ran overnight following a more extensive and detailed protocol. Quantified the RNA and received decent concentrations of approximately 30ng/uL in a 30uL stock. The A260/280 values were abnormal, however, since there is presence of RNA, a denaturing gel should be done in order to check the length in order to assess desired purity. Inoculated a 250mL flask with the RNA scaffold in 50mL of LB if more DNA template is needed to further isolate RNA.					Reinoculated the same cultures as yesterday since subculure time was missed. Transformed LacZ-WTPUF and LacZ-mutant PUF into DH5a and plated on amp plates.											
8/30/2012		To further test the RNA purified from the previous two in-vitro transcriptions a 10% denaturing acrylamide gel was cast. The two products were ran with RNAase free loading dye and NEB ssRNA Low Range Ladder. The two products were pure, one containing a higher concentration of RNA, although greater presence of degraded parts. The ladder shows the products are approximately 150bp, however, the ladder was not heated before loading into the gel therefore presence of secondary structure would account for the lower length seen.					Subcultured yesterday's cultures (1/100). Induced with arabinose after 2 hrs. Read fluorescence after 4 hrs. Inoculate the same tubes as yesterday for another trial. Inoculated one tube of BL21 DE3, LacZ+WTPUF from transformation plate, and LacZ+MutantPUF from transformation plate.											
8/31/2012		Constructed a plan for further scaffold work which mainly consists of purifying PUF(+/-) proteins in order to run further gel-shift assays.					Subcultured yesterday's cultures (1/100). Induced with arabinose after 2 hrs. Read fluorescence after 4 hrs. Make glycerol stocks of Asha's cotransformation of mutantPUF+YFP-PBS/control. Made new BL21 DE3 and DH5a competent cells											
9/3/2012																			
9/4/2012																			
9/5/2012																			
9/6/2012		Reviewed papers for insight into how PUF was purified. Looked through past protocols and planed a rough outline for purification.																	
9/7/2012		Consulted Brad about purification and need for supplies. Researched further into PUF purification. Confirmed with Dr. Jin that he might have proper purification supplies.																	
9/8/2012		Performed PCR cleanup and quantified tubes for Angela.																	
9/9/2012		Transformed and inoculated for Angela. Plated WT PUF-PIN on an Amp plate and mutant PUF-PIN on a Kan plate after Angela transformed the plasmids (pET4.3) into BL21 cells.																	

9/10/2012		Inoculated 25mL of WT PUF-PIN and 6-2/7-2 PUF-PIN for protein purification experiments. Concurrently, prepared two separate 1L LB medias for purification of each protein and another 1L of LB for general iGEM use. Inoculated 3mL of WT PUF-PIN in pBAD for Divya.																	
9/11/2012																			
9/12/2012		Inoculated two 1L bottles of LB media with 5mL of WT PUF-PIN overnight in one and 5mL of 6-2/7-2 PUF-PIN overnight in the other after adding 1mL of Ampicillin to each media at 3:30PM. Incubated at 37oC and checked OD levels at 4:00PM when there was no change in OD of the cultures from standard LB media. Checked the OD levels again at 5:12PM and received 0.0419 for the WT PUF-PIN. It was at this time that it was observed that the 6-2/7-2 culture wasn't growing like the WT PUF-PIN culture was. This was due to Ampicillin being added to the culture rather than Kanamycin. Checked OD levels at 5:38PM and received 0.0902. Checked again at 7:07PM and received a value of 0.5558, at which time 2mM of IPTG was added. After 2 hours of incubation, the cells were spun down at 10000g for 15 minutes. The pellet was then resuspended and centrifuged multiple times in one 50mL conical tube at 6000g for 5 minutes until there was a single pellet.																	
9/13/2012		Inoculated 1L of LB media with 5mL of 6-2/7-2 PUF-PIN overnight after adding 1mL of Kanamycin at 12:15PM. After incubating at 37oC, checked OD levels at 3:00 and received a measure of 0.0275. Checked again at 5:25PM and received 0.3509. Checked once more at 6:07PM and received a value of 0.5427 and proceeded to add 2mM of IPTG. Incubated the media for 2 hours and then spun down cells at 10000g for 15min. Resuspended the cells and kept transferring culture into one 50mL conical tube until one pellet was formed. Autoclaved water and other glassware in order to prepare buffers for protein purification.																	
9/14/2012		Prepared buffers for protein purification which includes 500 mL of 25mM Tris-Cl at pH8, 500 mL of 0.5 M NaCl, and 50 mL of 0.1% Triton-X. Autoclaved numerous 100 mL bottles in order to serve as containers for a gradient of wash buffers and a final elution buffer (25, 70, 115, 160, 205, and 250 mM concentrations). Transformed WT PUF-aGFP into both DH5a and BL21 and plated on Ampicillin plates which were left to incubate.																	
9/16/2012		Inoculated 25 mLs of LB media with WT PUF-aGFP in BL21 and left to incubate overnight.																	

9/17/2012		Prepared the final wash and elution buffers for protein purification with a gradient of concentrations 20, 40, 60, 80, 100, and 500 mM imidazole. Prepared the lysis buffer and stored all buffers in the 4oC room. Due to absence of literature for purification of PUF-a/BGFP, three vials of 200 mL LB media were inoculated with 2 mL of overnight and set for incubation at different induction times (2 hr., 6hr., and overnight). Inoculated at 1:00PM and checked the OD levels at 2:40PM and received a value of 0.0916 nm. Checked again at 4:43PM and received a value of 1.0831 nm. At 4:55PM the vials were induced with 2 mM IPTG. Once the 2 hr. and 6 hr. induction was done it was spun down into a pellet and stored in the freezer.																	
9/18/2012		Spun down the WT PUF-aGFP inoculation which was induced for 31.5hrs. into a pellet by 4 cycles of 4000g for 5 minutes at 4oC.																	
9/19/2012		Resuspended all pellets by using 13 mLs of lysis buffer and sonicated for 6 intervals of 30 sec. at setting 3. Then spun down the suspension at 35000g for 30min. at 4oC. Meanwhile, cast a 6% acrylamide gel which will be used for SDS-PAGE to assess production of protein in each pellet. Heated 5 uL of lysate sample with 5 uL of SDS in 60oC water for 15 minutes and then ran the samples with 2 uL of BioRad Kaleidoscope Protein Plus Ladder diluted in 8 uL of H20 for 40 minutes at 240V. Stained the gel for 20 minutes with a coomassie stain and destained with H20 for 1 hour.																	
9/20/2012																			

9/21/2012		Thawed frozen lysates of WT PUF-PIN and 6-2/7-2 PUF-PIN. Proceeded to cast a 6% 15 well acrylamide gel to test for protein purity after purification. Added 1 mL of Ni-NTA to the WT PUF-PIN lysate and attached it to the vortex to spin for 1 hour in the 4oC room. Then the protein purification stand was set up and the lysate poured into the purification column. A cap was placed over the column and a syringe used to push the lysate through. After the lysate passed, 20 mL of wash buffers of gradients 20, 40, 60, 80, and 100 mM imidazole were used to wash away unspecified proteins all the while using the syringe and collecting the fractions in 1.5 mL centrifuge tubes. Finally, 20 mL of 500 mM imidazole were used to wash the his-tagged protein and the entire volume was collected in centrifuge tubes. Then 5 uL of 1x SDS loading dye were added to 5 uL of each fraction and let to incubate in 60oC water for 20 min. Meanwhile, the cast gel was pre-run for 20 min. on 240V before samples were added. Once the gel was prepped, the samples were added and ran for 40 min. at 240V. The gel was then stained with coomassie stain for 1 hr. and destained with H20 for 1 hr. After the gel was finished, it was imaged on GelDoc XR along with the SDS-PAGE gel of lysates from 9/19. While the gel was running, 1 mL of used Ni-NTA were added to 6-2/7-2 PUF-PIN and left to shake in the 4oC room. The same procedure for purification was followed and another SDS-PAGE gel was ran to test for purity of the protein collected.																	
9/22/2012																			
9/23/2012		Inoculated 1 L of LB media with 5 mL of WT PUF-aGFP with 1 mL of Ampicillin and incubated at 37oC at 2:30PM to prep for protein purification. Checked the OD levels at 6:20PM and received a reading of 0.5530nm. Decided to incubate for longer and checked the OD at 6:50PM where it was at 0.6814nm and induction with 2 mM IPTG was done at 6:55PM. The culture was left to incubate in the 37oC room overnight.																
9/24/2012		After 22 hours of induction, the culture was spun down in 500 mL flasks at 10000g, 4oC, for 15 min. The pellets were then resuspended and spun down again in 50 mL conical tubes at 4000g, 4oC for 5 minutes until one pellet was formed.																

9/25/2012		A 10% denaturing acrylamide gel was cast in preparation for the endonuclease assay. Due to literature referencing PIN working best with Mn2+ ions, different ion concentrations were prepared (3mM concentrations of MnO2, MnCl2*4H20, and MgCl2 were made). While the gel was casting, the reactions were prepared. There were 12 reactions, with three sets of 4 different conditions (RNA control, RNA+WT PUF-PIN, RNA+6-2/7-2 PUF-PIN, and RNA+WT PUF-PIN+6-2/7-2 PUF-PIN) with the three sets comprised of the three different ions used. The amounts of each reagent include 5 uL of each protein (or H20 when not included), 2 uL of RNA, 2 uL of loading dye, and 1 uL of ions ~ 15 uL reaction. The reactions were incubated in 37oC for 30 minutes and then loaded into the gel once the gel was pre-ran. The gel was ran for 40 min. at 240V with 1X TBE buffer. Once the gel was done, it was stained in 10 ug/mL EtBr for 20 min. and destained in H20 for 20 min. whereupon it was imaged with GelDoc XR. The gel image showed no signs of RNA so new a new 10 ug/mL EtBr stain was prepared and the gel was stained again for 20 min. followed by a 20 min. destain with H20. The gel was imaged again and there was were no signs of RNA.																	
9/26/2012																			
9/27/2012		Due to the endonuclease assay failing, the RNA and protein preparations were tested. A 15 well 6% SDS-PAGE was ran for testing WT PUF-PIN and 6-2/7-2 PUF-PIN. In order to test the RNA viability a 10% urea denaturing gel was prepared.																	
9/28/2012		The protein SDS-PAGE gel was stained with coomassie blue for 45 min. and destained overnight.																	
9/29/2012																			
9/30/2012																			
10/1/2012		A 10% Denaturing 10 well acrylamide gel was cast. A 30mM MnCl2*4H20 pH 8.25 buffer was made. 1 uL of RNA was added to 5 uL of RNAse free H20 and 1 uL of annealing buffer (**EDTA, Tris). The RNA was then denatured at 95oC for 3 min. and let to fold for 5 min. at 4oC. Then 2 uL of protein was added (WT, 6-2/7-2 PUF-PIN) to the respective tubes followed with 1 uL of either 30 mM MnCl2*4H20 or 30 mM MgCl2. The tubes were then incubated at 37oC for 30 min. Afterward, 10 uL of 80% formamide, EDTA/Tris were added to the tubes. The tubes were let to sit for another 30 min. while the gel was pre-run at 120V for 25 min. The samples were then loaded and the gel was let to run at 120V for 55 min. The gel was then stained in 2 ug/mL EtBr for 20 min. and destained in H20 for 20 min. Imaged using **																	
10/2/2012																			
10/3/2012																			
10/4/2012																			
10/5/2012																			