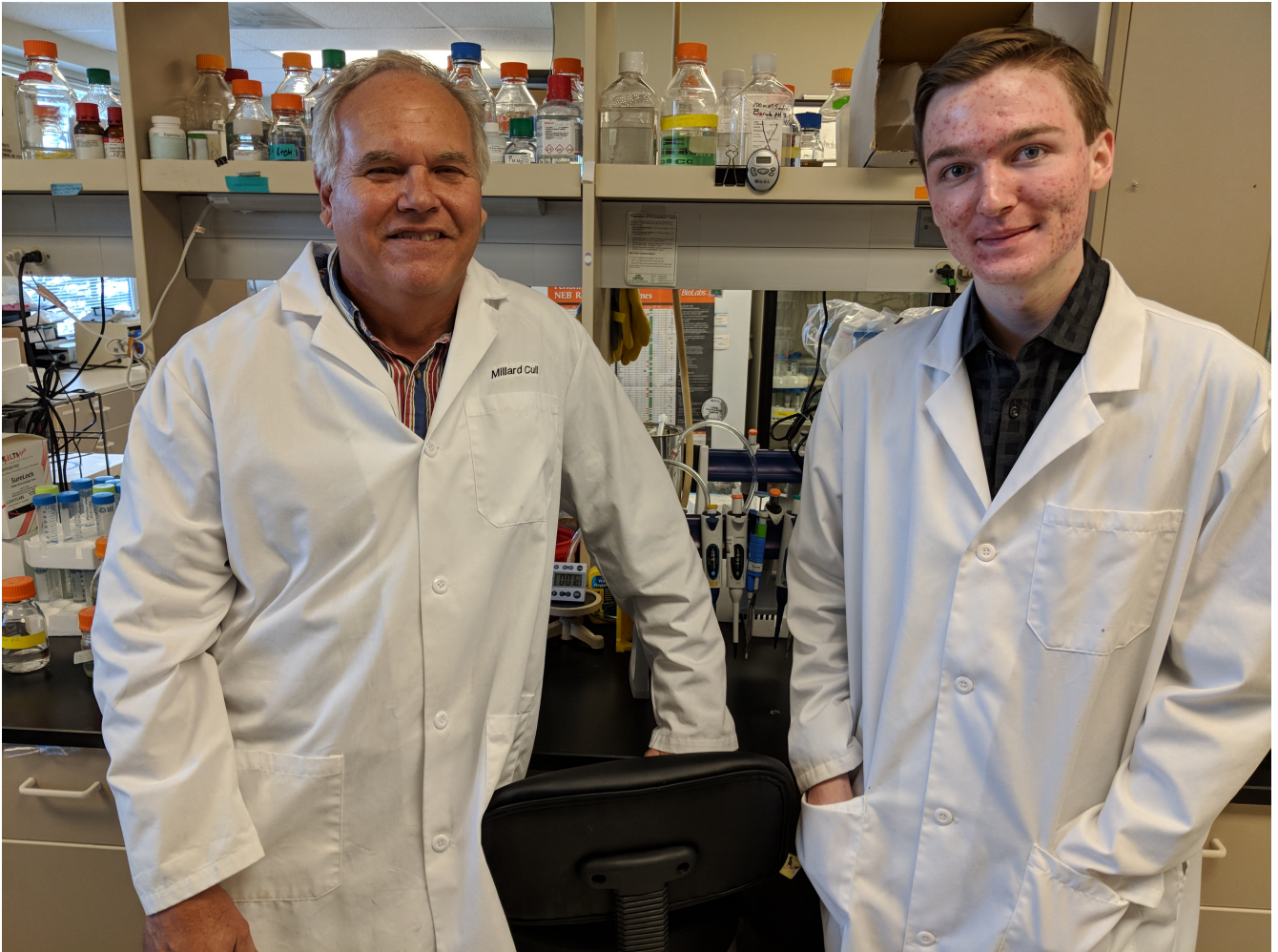


Senior Capstone Portfolio



Academic Research in the Field of Biotechnology

Brooks J Rady – Class of 2018

Table of Contents

Curriculum Vitae.....	1
The Pursuit of Pansophy (College Essay).....	4
Sheffield Scholarship Essay.....	6
Online Presence.....	9
Capstone Journal.....	10
18 October 2017 — Planning and Research.....	10
24 October 2017 — Planning and Research.....	11
30 December 2017 — Planning and Research.....	12
31 December 2017 — Planning and Research.....	13
2 January 2018 — Planning and Research.....	14
4 January 2018 — Planning and Research.....	17
13 February 2018 — Planning and Research.....	18
14 February 2018 — Planning and Research.....	19
Mentorship Hours (Contact Log).....	20
Mentor Evaluation.....	21
Acknowledgements.....	22

Curriculum Vitae

Brooks J Rady
High School Senior
720-394-0259
b.j.rady@gmail.com

OBJECTIVE

Seeking a research position in Bioengineering or Microbiology. Looking to contribute in either a dry or wet-lab environment and to pick up new skills by working closely with a professional in the field.

EDUCATION AND WORKSHOPS

Prospect Ridge Academy HS (2014-Present)

GPA: 4.766

Completed STEM Courses:

- ◆ AP Calculus BC
- ◆ AP Biology
- ◆ AP Computer Science
- ◆ AP Chemistry
- ◆ AP Physics C

4 Years of English

4 Years of Social Studies

3 Years of French

Workshops at Denver Biolabs

Covered basic biochemistry, the central dogma, and gene structure

Designed basic generic circuits using BioBricks

Practised sterile lab techniques and basic lab procedures

Deep dive into CRISPR and its applications

Speaker on clinical microbiology and microbe culturing

HONOURS AND AWARDS

National Honor Society (2017)

Prospect Ridge Academy High Honor Roll (2015-2017)

Design award and Finalist Alliance at FTC State Championship (2017 & 2018)

Won 2nd in Junior Energy and Transportation at CSEF (2014)

Won 1st in Alternative Fuels at Denver Metro Science Fair (2014)

EXPERIENCE AND WORK

Avidity LLC (2016-2017)

Designed unique DNA tether and bridge sequences for use in biosensors

Directed evolution panning for peptides binding to a DNA-PNA hybrid target

Extensive work with sterile technique, solution calculations, and *E. Coli* culturing

Performed *E. Coli* transformation via electroporation and antibiotic based selection

Expressed in *P. Pastoris* and subsequently purified a mutant Gaussia Luciferase protein

Assessed Gaussia Luciferase activity using a Luminometer

Running protein gels to assess the purity of a protein sample

Designed a lateral flow assay on an aluminium surface and chemically altered the hydrophilicity of the surfaces in order to achieve the desired flow rate and fluid flattening / spread across the detection zone of the device.

SKILLS

Biology:

- ◆ Biotech lab procedures (PCR, Electrophoresis, Restriction Enzyme Digests, etc.)
- ◆ Chemical calculations and reagent preparation
- ◆ Bacterial plasmid design
- ◆ DNA primer / tether design
- ◆ Sterile Technique
- ◆ In vivo expression of foreign proteins
- ◆ Bacterial and Yeast transformation

Computing:

- ◆ Extensive experience with Linux and Windows operating systems
- ◆ Fluent in LATEX, R, Haskell, Rust, Elixir, LISP, Java, HTML, CSS, and JavaScript
- ◆ Full Stack Web, Data Processing, and Machine Learning experience

- ◆ Worked with digital biotechnology tools such as Benchling, SnapGene, Thermo Fisher Multiple Primer Analyzer, and IDT OligoAnalyzer
- ◆ Extensive experience with VCS and contributing to open source

Writing / Language:

- ◆ Experience with reading scientific papers
- ◆ Essay and report writing experience
- ◆ Basic French language skills (listening, speaking, reading, and writing)
- ◆ Public speaking and presentation experience

PROJECTS, PRESENTATIONS, AND PAPERS

Honours Physics “Build a Planet” Project

Link - <http://bit.ly/2IA9f5F>

FTC_HTTP: An Application for Easily Programming FTC Robots

Link - http://bit.ly/ftc_http

Link - http://bit.ly/ftc_http_video

The Regicide of the Fisher King

Link - <http://bit.ly/2FHoYSy>

Pokéstats — What Type Of Pokemon Is The Match For You?

Link - <http://bit.ly/2FVjMqh>

The Effect of Varying Lamp Emission Spectra on the Rate of Photosynthesis

Link - <http://bit.ly/2HCx2QU>

How I Learned to Stop Worrying and Embrace the Absurd

Link - <http://bit.ly/2Gwqkg2>

EXTRACURRICULARS AND SERVICE

Founding member of the Prospect Ridge Academy Robotics Club

Parted-out and built around ten computers for personal use or for family and friends

Designed and maintained a web application for managing student activities and clubs at
Prospect Ridge Academy

Reverse-engineered a web-based programming interface and developed a tool for wirelessly
updating robot code for the FTC competition

During my final year of robotics I created and taught a curriculum on robot programming
that was designed to prepare the underclassmen to lead in the seniors' absences

The Pursuit of Pansophy (College Essay)

If I could have one superpower, it would be the ability to rip the universe apart — bit by bit, instant by instant, atom by atom. I would explode the very fabric of existence and run it through my fingers, analysing it with a violent curiosity. No secret of science, life, or logic would be spared. It is the pursuit of this power that makes me tick.

It is this hunt for knowledge and understanding that has cultivated my fascination with the sciences. I was first hooked by computer science in the seventh grade. I thought that programming was beautiful and I was amazed by its power to take my ideas and bring them to life. As my passion for computers flourished, so too did my passion for the other sciences. Physics awed me with its predictions about the motions of projectiles and planets, chemistry with its power to transform matter, and biology with its ability to cast chaos into living, breathing order. For a while, I struggled to reconcile my varied scientific interests, but after interning at a biotechnology lab, it all clicked. I spent my time genetically modifying *E. Coli* and realized that biology combined aspects of both physics and chemistry with the logic and abstraction of computers: biology was essentially programmable chemistry. Over the past couple of years, I have reaffirmed my interest in bioengineering by going back for a second summer to the biotechnology lab, attending several meetings at Denver Biolabs (a local DIY synthetic biology group), and working on a senior Capstone project that uses Cas9 to teach yeast cells how to recognize specific segments of viral DNA. Bioengineering provides me with the perfect opportunity to draw together my interdisciplinary talents and satisfy my curiosity by pioneering a relatively new field of science. I hope to be able to expose the hackable nature of biology by understanding it from the bottom up — repurposing life to help us live longer, maintain a cleaner planet, and lift billions from poverty and famine.

My quest to self-learn computer science has helped me hone my problem solving skills, my ability to read and write technical documents, and to leverage computers to automate monotonous tasks. My wet-lab experience has helped me acquire myriad practical and technical skills from sterile technique, to proper measurement, to keeping an organized lab notebook. My work in the lab over the past two summers and my courses in chemistry and calculus have kept my math skills sharp, particularly in regard to chemical calculations. When working in the sciences, it is important to communicate findings in an unambiguous and understandable way. Because of this, I have developed a flexible set of presentation skills — both written and oral. Throughout the maturation of these presentation skills, I have gained familiarity with tools like LaTeX for report authoring and formula formatting, R for the creation of informative graphics and statistics, and HTML5 for the interactive and beautiful presentation of concepts and data.

Outside of academics, I spend my time participating in robotics, playing video games, and reading. During my first year of high school I founded a robotics team that I have played an active role in leading over the past four years. Since its founding, the team has grown significantly and placed in several tournaments.

The UK is a world leader when it comes to bioengineering, and the focus on depth of education, rather than breadth, aligns perfectly with my style of learning. The allure of academic rigour, in conjunction with an emphasis on independent research, is hard to resist: the UK draws an array of brilliant minds from around the world. I want to see the world from as many angles as possible so that I can craft a worldview that is truly my own. I want to feed my mind not with homogeneity, but with multiformity. Such insatiable curiosity requires an intellectually challenging environment in which it can grow and mature — the familiar and comfortable simply will not do.

Sheffield Scholarship Essay

Prompt: This is an opportunity to demonstrate your outstanding academic, personal or professional achievement or potential. This statement may include the following information:

- Academic and other relevant professional achievements
- How you expect to benefit from your chosen course of study
- How you have the potential to make an exceptional contribution to the University, and to society after you graduate

~ ~ ~ ~

From time to time, something curious piques my interest and I find the inspiration for a project that both takes advantage of the occasion and teaches me something new. Of my projects and accomplishments of which I am most proud, all are somehow products of my insatiable curiosity. When gearing up for a project, I often aim to approach it in a way that is alien and challenging so that I am forced to add new skills to my repertoire. Over my academic and professional career, I have cultivated diverse skills and, perhaps more importantly, developed the ability to quickly identify the holes in my knowledge. This allows me to efficiently fill those holes and complete the project while concurrently developing a new and useful skill.

Throughout my scholastic career, I have produced a number of projects that I feel aptly reflect my unique skill set. For example, in physics, I was tasked with designing a fictional planet and performing a number of calculations that would provide a glimpse into what life on that planet would be like. I decided to put together a website that would present all of the calculations elegantly and show the orbit and scale of my planet (relative to our Earth and Sun) in an interactive JavaScript simulation (<http://bit.ly/2IA9f5F>). This project showcases skills in applied maths and physics, web programming, LaTeX equation formatting, and engaging report authoring.

A more recent project, for which I studied Pokémon, provided me with the opportunity to teach myself the basics of performing statistical analysis using R (<http://bit.ly/2FVjMqh>). This further developed my digital presentation skills via the report's numerous graphics, and my ability to leverage digital processing to handle large datasets and draw reasonable, real-world conclusions from the numbers — an essential skill in any scientific discipline.

Outside of the strictly scientific, I was tasked in my AP English class with comparing a poem and a painting from the Modernist era and decided to be ambitious and analyse T.S. Eliot's "The Waste Land". This challenged my analytical and critical thinking skills and ultimately led to an intensely researched and meticulously crafted essay (<http://bit.ly/2FHoYSy>). I feel that this essay displays powerful writing skills and a highly developed understanding of an exquisitely sophisticated text — skills that are applicable throughout academia. The research and synthesis skills I gleaned over the course of this essay are just as important to bioengineering as they are to literary analysis.

When it comes to giving back, during the last season of FTC (a high-school robotics competition), I developed a piece of software that filled a need within countless robotics teams across the country. In years past, teams had to choose between writing robot code in the bloated Android Studio or in the woefully under-featured web editor. FTC_HTTP is a reverse-engineering effort that allows the user to write code on a local machine and make the best of both approaches (http://bit.ly/ftc_http). Because this project was designed as a boon to the community, I also produced an accompanying tutorial video and spread word of the application on forums and during competitions (http://bit.ly/ftc_http_video). This project is an enquiry into Rust programming and reverse-engineering techniques that also showcases contribution to open source and the community.

Because I am passionate about a wide variety of subjects, I sought an interdisciplinary degree and I have found that in Bioengineering. I can incorporate computer science, physics, and chemistry all into my biological studies. Developing my bioengineering skills has been difficult to do on my own, as I do not have easy access to a lab in which to experiment and learn. During my course at Sheffield, I

will finally have the facilities with which to pursue my interest in biological engineering. My course at Sheffield will not only further my general engineering knowledge through a variety of STEM courses, but will also teach me to synthesize that knowledge and apply it to solving biological problems.

During my time at Sheffield, I aspire to research and study concurrently and I am strongly considering pursuing a long-term position at the University. I always aspire to help where there is a need, and I plan on working with others at the University to address issues both on and off campus. I have been heavily influenced by the open-source culture of the internet and aspire to share the fruits of my research and study with the world so that everyone can benefit from the cutting edge work of the University and its alumni.

For more, see: <http://thelostlambda.xyz>

Online Presence

For my online presence I am developing the website: <http://thelostlambda.xyz>. It is going to be a running project and I will update it with links to all of my projects going forward. I also intend to turn it into a blog of sorts. It will serve as a way for me to get my work out to the public and will help me build an online identity for my professional self. I'm coding the website from scratch and hosting it on my own server for mainly didactic reasons, but also because it allows me much more flexibility and creative freedom for when I have time to really add to it. So far it just has a couple of links and there are a lot more on the way. Check it out!

Capstone Journal

18 October 2017 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

Objective: Begin designing the POC test for the DNA activated gene expression. More specifically, the following components need to be designed and integrated onto a plasmid:

- ◆ Decide on a selection gene (likely an antibiotic resistance gene).
- ◆ Pick an origin of replication
- ◆ Decide on a promoter for the expression of the signal gene
- ◆ Design a Cas9-Repressor fusion
- ◆ Design the genetic circuit that keeps the signal gene just barely repressed. Somehow introduce a significant delay between signal production and re-repression.

Objective 1 — Decide on a selection gene:

In beginning to design my plasmid, I decided that I would use Addgene's *Plasmids 101* reference as a stepping off point. For this particular objective, I focused on the "Antibiotic Resistance Genes" section.

I have decided to use a bactericidal and rather stable antibiotic Kanamycin so that I won't need to worry about satellite colonies or the other issues that come with working with Ampicillin.

After searching the NCBI gene database for KanR (the Kanamycin resistance gene), I decided on Gene ID: 7872406 as it was the only KanR gene for *E. Coli* in the database. While I have seen other similar sequences, this particular one is published by NCBI and has been recently updated so I have ample reason to trust this particular sequence.

Objective 2 — Pick an origin of replication:

I also sought out a high-copy-number origin of replication (so that there are more opportunities for the trigger DNA to set off the signal and keep the signal strong. The origin obviously also needed to be compatible with *E. Coli*. As opposed to piecing together my own origin, I decided that I would borrow one from the pUC118 plasmid. This particular origin is known to have a high-copy-number and wide bacterial compatibility and has been around for a long time.

24 October 2017 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

Objective: General upkeep of the plasmid. Pick a promoter for the antibiotic gene and add terminators at the end of operons.

Picking a promoter for the antibiotic resistance gene:

When picking the promoter for the antibiotic gene it was important that it was expressed but not to the point that it becomes a metabolic burden on the cell. I looked for my promoter in the iGEM database and found a set of bacterial promoters from Berkeley's 2006 iGEM team. They developed several promoters of various strengths, and I selected J23106 because that promoter showed an intermediate amount of activity — perfect for an antibiotic resistance gene. One question I still have about antibiotic resistance genes is whether or not they need a promoter, or if most sequences have one integrated. Just to be safe, I added this one of my own, which shouldn't mess things up even if there is an integrated promoter.

Adding a terminator for the antibiotic resistance gene:

Here I kept my terminator selection simple. I picked the T0 terminator which is short (only 52 bp long) and has a high termination efficiency of 97%. The specific sequence used was taken from the iGEM part K864600.

30 December 2017 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

Today I am shifting my approach a little. I am adding too many variables by building a plasmid from scratch right now. As a proof of concept, I should take something that is already working, like pdCas9 and modify it. It looks like I should just be able to slap in a new guide RNA plus the signalling gene and be golden. I should also check that this plasmid includes a repressor fusion. I am assuming that it does, based on the paper it came from, but I realized that I didn't actually see that in the plasmid map.

Okay, I have learned that this plasmid does *not* have a fused repressor, but I have also been reminded that in prokaryotes, this is not necessary! The dCas9, if bound to the promoter region of a gene, blocks the RNAP from binding the gene and transcription is repressed.

Additionally, I have decided that my indicator gene will be a mutant RFP from the BioBrick database. It is visible with the naked eye and appears red. Here is the link to this part:

http://parts.igem.org/wiki/index.php/Part:BBa_E1010

I thought about my circuit design for this detector, and I have come up with the following: LacO <1> -> RFP -> crRNA Leader + repeat + spacer (2) + repeat -> TERM TERM

And: Constitutive <2> -> crRNA Leader + repeat + spacer (1) + repeat -> TERM TERM

And: Constitutive -> LacI

The reporter gene, RFP, is behind a Lac Repressor so it can be repressed by either LacI or the dCas9 guided by the target 1.

I need to express LacI somewhere! Here is an RBS: http://parts.igem.org/Part:BBa_B0030

And here is the LacI: http://parts.igem.org/Part:BBa_C0012

And the LacO: http://parts.igem.org/wiki/index.php/Part:BBa_R0010

Okay, so the overall plan is as follows:

The “default” guide RNAs that are constitutively promoted will target and repress the reporter gene. This will be an imperfect match, so the repression will be susceptible to competition. When external DNA comes in and competes off this weakly bound dCas9 and allolactose or IPTG is present, the RFP reporter gene will be activated and the cell will turn red. In addition, this reporter gene will produce new guide RNAs that direct the dCas9 towards the other CRISPR region. This will stop the repression of the signal even after the DNA that competed it off in the beginning is gone. This will ensure a strong signal. The cell can then be reset by removing the allolactose analog. The LacI represses the reporter again and the RFP and second CRISPR region are no longer expressed. This allows the first set of guide RNAs to direct the dCas9 back at the RFP codon and resets the cell to its initial state. Once the allolactose is re-introduced, the cell is re-armed and is ready to produce another signal if the dCas9 is competed off.

31 December 2017 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

Today I am working on taking the detailed plan in the last entry and placing it into a plasmid. I will be doing this on Benchling.

As it turns out, there was no DNA sequence for that LacI before, so I chose a part that includes an RBS and that same LacI with the LVA degradation tag. Here is the part I used:

http://parts.igem.org/Part:BBa_I14002

In my plasmid, I placed this immediately after the CmR gene, under the same promoter. Note that I had to reverse the sequence from the part registry because CmR is expressed in the reverse direction.

I also just added a double T0 terminator after the CRISPR regions.

I have finished the plasmid. I will send it off to Millard tomorrow.

Here is the Benchling link: <https://benchling.com/s/seq-GGJVMkAhHqaGdgjo45sh>

2 January 2018 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

Today I will be working on creating mutants of my plasmid that differ in the guide RNA region of the first CRISPR cluster. This is the one that is supposed to target the RFP promoter. I will make mutations that will hopefully make its binding to the promoter progressively weaker. I will do this to make it possible for the dCas9 to be competed off by external DNA. I will create an array of different mismatches and test experimentally which, if any, displays the desired behaviour. I will also create a plasmid that just has the original BSAI cloning site between the spacers. This should be a control plasmid that should always express red. Because of the cloning sites, however, I can add in any of the other guide RNAs that I want to. This way we only need to make one plasmid, and all of the mutants that vary in guide RNA sequences can be cloned into this one plasmid.

Here are the plasmids:

1. pdCas9-M: This base plasmid has no CRISPR guide in the locus after the Cas9 coding region. This should always express RFP (in the presence of an allolactose analog).
 - `tgagaccagtctcggaagctcaaaggctc`
2. pdCas9-MA: The A mutant has a CRISPR guide that perfectly matches the RFP promoter. This should result in no RFP ever being expressed.
 - `tatgctccggctcgtatgttg`
3. pdCas9-MB: The B mutant has a CRISPR guide that has a single point mutation directly next to the PAM.
 - `tatgctccggctcgtatgtg`
4. pdCas9-MC: The C mutant has a CRISPR guide that has a single point deletion directly next to the PAM.
 - `tatgctccggctcgtatgt`
5. pdCas9-MD: The D mutant has a CRISPR guide that has a single point mutation 5 nucleotides from the PAM.
 - `tatgctccggctcgtatattg`
6. pdCas9-ME: The E mutant has a CRISPR guide that has a single point deletion 5 nucleotides from the PAM.
 - `tatgctccggctcgtatttg`
7. pdCas9-MF: The F mutant has a CRISPR guide that has a single point mutation 10 nucleotides from the PAM.

- tatgcttccggctgtatgttg
- 8. pdCas9-MG: The G mutant has a CRISPR guide that has a single point deletion 10 nucleotides from the PAM.
 - tatgcttccggctgtatgttg
- 9. pdCas9-MH: The H mutant has a CRISPR guide that has a single point mutation 15 nucleotides from the PAM.
 - tatgcttcaggctcgtatgttg
- 10. pdCas9-MI: The I mutant has a CRISPR guide that has a single point deletion 15 nucleotides from the PAM.
 - tatgcttccggctcgtatgttg
- 11. pdCas9-MJ: The J mutant has a CRISPR guide that contains a single mismatching base on the tail end of the spacer (the end farthest from the PAM).
 - aatgcttccggctcgtatgttg
- 12. pdCas9-MK: The K mutant has a CRISPR guide that contains two mismatching bases on the tail end of the spacer (the end farthest from the PAM).
 - attgcttccggctcgtatgttg
- 13. pdCas9-ML: The L mutant has a CRISPR guide that contains three mismatching bases on the tail end of the spacer (the end farthest from the PAM).
 - atagcttccggctcgtatgttg
- 14. pdCas9-MM: The M mutant has a CRISPR guide that contains four mismatching bases on the tail end of the spacer (the end farthest from the PAM).
 - ataccttccggctcgtatgttg
- 15. pdCas9-MN: The N mutant has a CRISPR guide that contains five mismatching bases on the tail end of the spacer (the end farthest from the PAM).
 - atacgttccggctcgtatgttg
- 16. pdCas9-MO: The O mutant has a CRISPR guide that contains six mismatching bases on the tail end of the spacer (the end farthest from the PAM).
 - atacgatccggctcgtatgttg

As can be seen here, there are a lot of different mutations to the CRISPR spacer that I would like to test. Including the two controls, pdCas9-M and pdCas9-MA, there are 16 different sequences. At this point it seems quite impractical to order that many different plasmids when they are all so similar. Because of this, it is my plan to just have the pdCas9-M plasmid synthesized and then to synthesize the

other guide RNAs + BsaI sites as oligos. I could then clone those oligos into my pdCas9-M plasmid to form all of my other plasmids.

NOTE: After doing some digging, it appears that the orientation of the spacer within the CRISPR region is unimportant, so that makes cloning a bit easier. I downloaded a procedure, but it looks like I can just digest my dpCas9-M plasmid with BsaI and gel purify it so that I am only left with the large plasmid and no longer have the short cutout. I then ligate it with my guide RNA sequences.

I would need two oligos for each guide RNA insert (5'-3'):

1. AAAC – (gRNA) – G
2. AAAAC – (RC gRNA)

This means that my oligos would all be between 29 and 28 base pairs in length, which seems reasonable.

NOTE: I have gone back and changed my LacO sequence a little bit. I am now only using bases 130-200 from the original source, leaving out the tail end of the LacI sequence and the CAP binding region. This shortens and simplifies things a bit and also means that glucose won't catabolically repress the gene. This way the cells can be raised in SOC media post-electroporation and I don't have to worry about the glucose in the SOC being an issue.

4 January 2018 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

According to OpenWetWare (https://openwetware.org/wiki/Escherichia_coli/Vectors), my pdCas9-M, with an origin of p15A, has a copy number of 10-12. This means that if I use pUC57-Kan, with a copy number of 500-700 and put my perfectly matching sequences in here, I should be able to compete the dCas9 off of my pdCas9-M plasmids.

I have just created the pUC57-Kan-M plasmid. This plasmid carries the very high copy number pUC origin and Kanamycin resistance. I have also added a region with the following sequence taken from the spacer cloning site in pdCas9: tgagaccagtctcggaagctcaaaggctc and have added PAM regions on either side of this cloning region.

This will allow me to insert my guide sequence with a PAM into the E Coli cell at a high copy number, which should compete off the dCas9 repressor from the RFP gene on the pdCas9-M plasmids where it matches incompletely.

At 23:20 I have emailed Millard my procedure, links to the plasmids, and a list of all the the required oligos. I'm calling it a day.

13 February 2018 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

Okay, so a good while back, Millard told me that he could not find my plasmids I had designed anywhere. Of course not, I had made them totally digitally! It is too expensive to make these plasmids from scratch, (something that I figured might be the case), so now I have to come up with a new way to make my plasmids. Hopefully this won't be too awful... Let's jump into Benchling!

Okay, I have made two of the 3 inserts. I can now fully clone the pdCas9-M plasmid. I just need to make one more insert that drops some BsaI sites into my high copy-number plasmid.

Here are the Benchling links:

LacI Insert – <https://benchling.com/s/seq-GNchUCgjQbV3Q5vzG2q0>

Signal + Multiplier Insert – <https://benchling.com/s/seq-QkLjd0JOHnJzT0XG1aK>

Combined, these sequences are ~2100bp. This is way down from 11755. Only 17% of the original size and cost.

14 February 2018 — Planning and Research

Living Viral Assay: POC Plasmid in *E. Coli*

Okay, I think that I have everything that I need. I have my final adapter sequence done.

Adapter Insert – <https://benchling.com/s/seq-YFD2nS2QyVp1pTjL5CXx>

Here is the new plasmid situation:

I'll need to order two plasmids from Addgene, pdCas9 and pUK21

- <https://www.addgene.org/46569/>
- <https://www.addgene.org/49788/>

Here are the inserts that need synthesizing

- <https://benchling.com/s/seq-GNchUCgjQbV3Q5vzG2q0>
- <https://benchling.com/s/seq-QkLjd0JOHnJzT0XG1aK>
- <https://benchling.com/s/seq-YFD2nS2QyVp1pTjL5CXx>

I'll need the following restriction enzymes:

- BsaI
- PciI
- KasI
- EcoNI
- BciVI
- BaeGI
- PflFI

Mentorship Hours (Contact Log)

January 18, 2018



A V I D I T Y , L L C

To whom it may concern:

Brooks worked in my laboratory for eight weeks in each of the last two summers for 640 hours' worth of work. Brooks completed a number of projects, including a bioinformatics project to develop efficiently hybridizing oligonucleotides to allow attachment of oligonucleotides and proteins to surfaces, a directed evolution project to find peptides that bind PNA/DNA duplex molecules, and development of a lateral flow platform using aluminum as the target capture surface.

Sincerely,



Millard Cull, CEO
Avidity, LLC
millard@avidity.com

Mentor Evaluation



March 14, 2018

A V I D I T Y . L L C

Brooks Rady worked in my lab for two summers. The first summer, Brooks worked on a Directed Evolution project to select 12-mer peptides that can bind PNA/DNA heteroduplex molecules, and also a bioinformatics project to select oligonucleotides optimized for hybridization to surface-bound complementary nucleotides.

The next summer, Brooks worked on a lateral flow design adapted for luminescent detection of target nucleic acids that can be used in diagnostic applications. At this time, Brooks developed a fascination with “biological machines” (synthetic biology) and conceived his Capstone Project, “In Vivo Detection and Signaling of Arbitrary DNA Sequences”. We are currently working towards a demonstration of his ideas and hope to complete his project this summer (2018).

Millard Cull, CEO
Avidity, LLC

Acknowledgements

Thanks, first and foremost, to **Millard Cull** for allowing me to work in his lab for not one, but two summers. I learned countless things during my two summers in the lab and Millard was always there to answer questions and provide guidance whenever it was needed. Interning at the lab gave me the opportunity to acquire many of the real world skills that I'll be using throughout my career and I'm incredibly thankful for that opportunity. After my internship at the lab and when I'd moved on to my Capstone project, Millard helped me to refine and realize my ideas in addition to generously offering to supply all of the materials and equipment I would need to complete my project.

Thank you also to **Dr. Michael Bennett** who, when I was faced with the challenge of obtaining the plasmids I needed from Addgene (who only sells to non-profit and academic institutions), stepped in and offered to order the plasmids through the school. Without Dr. Bennett's help, Millard and I would have been hard-pressed to acquire the genetic material needed for my project.