Project no. 043338

Project acronym: EMERGENCE

Project title: A foundation for Synthetic Biology in Europe

Instrument: NEST Pathfinder

Thematic Priority: Synthetic Biology

Deliverable 2.1 "Report documenting the Synthetic Biology Summer Courses"

Due date of deliverable: 32
Actual submission date: 32

Start date of project: 1.12.2006 Duration: 36 months

Organisation name of lead contractor for this deliverable: ETHZ

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

In this deliverable, we have collected the following informations:

On the iGEM Europe course 2009:

- A brief document by Randy Rettberg on the course itself
- The course schedule
- The course participants
- The documentation of an introductory presentation
- The documentation of how to use the MIT Registry of Standard Biological Parts
- A tried instruction for team training courses

As the 2009 built upon the experiences of the 2008 course and was substantially expanded, we submit the material from this course.

For the 2008 course, we submit only the organizational document by Randy Rettberg and the schedule.

European Synthetic Biology Workshop London
June 20-21, 2009

Four instructors from iGEM Headquarters at MIT held a synthetic biology workshop at Imperial College, London over two days, June 20-21, 2009. About 60 instructors and students from European schools attended the workshop. The topics included how to develop synthetic biology projects, how to use the Registry of Standard Biological Parts at MIT, how to make and standardize biological parts, how to submit them to the Registry, and how to assembly systems from parts. Much of this training was done in the form of an example iGEM project, "iGEM in a day (or two)", that the participants joined, designed, and submitted to the Registry. A group dinner was provided the evening of the first day.

The instructors were Randy Rettberg, Director iGEM, Biological Engineering and Meagan Lizarazo, Assistant Director, iGEM, both from MIT. Also, Dr. Reshma Shetty and Dr. Barry Canton, recent graduates from the Endy Lab at MIT participated under contract from Ginkgo Bioworks. These instructors held a preparatory meeting at Imperial College on Friday before the workshops.

In addition, Randy Rettberg held meetings with Prof. Alfonso Jaramillo and Francois Kepes of Genopole and Dr. Ariel Linder of Paris Descartes University. He also met with Victor de la Torre at Imperial College, London, regarding software for WP4 and with Prof. Richard Kitney regarding possible European regional activities.

Agenda for the European Workshop in London, June 20-21, 2009

Saturday

Time	Duration	Event
10:30 AM	0:15	**Welcome**
10:45 AM	0:45	**Synthetic biology based on parts**
11:30 AM	0:15	**LUNCH (working)**
11:45 AM	2:00	**Team Introductions**
		iGEM in a day (or two):
		Coming up w a project
1:45 PM	0:30	ideas, navigating literature
		Navigating registry
2:15 PM	0:20	search
2:35 PM	0:45	catalog/curation/categories/tables
3:20 PM	0:10	registry stars
		Finding parts
3:30 PM	0:20	dna distribution
3:50 PM	0:20	QC information
4:10 PM	0:20	**Making parts**
4:30 PM	0:30	**BREAK**
		Assembling of parts
5:00 PM	0:20	standard assembly
5:20 PM	0:20	plasmids
5:40 PM	0:20	assembly schemes
6:00 PM	0:30	**Proteins**
6:30 PM		**End**

Sunday

Time	Duration	Event
		iGEM in a day (or two) continued:
9:00 AM	0:20	**Promoters**
9:20 AM	0:30	**Devices**
9:50 AM	0:20	**Measuring Parts**
		Sending parts
10:10 AM	0:20	adding a part
10:30 AM	0:20	favorites
10:50 AM	0:20	shipping parts
11:10 AM	0:20	sequencing
11:30 AM	0:15	**LUNCH (working)**
		iGEM 2009:
11:45 AM	0:30	requirements
12:15 PM	0:30	changes
12:45 PM	0:30	safety
1:15 PM	0:30	judging/awards
1:45 PM	0:15	partner offers
2:00 PM	0:15	software tools track
2:15 PM	0:30	**Questions/Discussion**
2:45 PM	0:15	**BREAK**
3:00 PM	1:30	**Mathworks workshop**
4:30 PM		**End**

Partial list of attendees for the European Workshop, London, June 20-21, 2009

Last Name	First Name	Country	Company
Micklem	Gos	GB	University of Cambridge
Vanwalleghem	Gilles	BE	ULB-Brussels
French	Chris	GB	University of Edinburgh
Carlsen	Simon Carlsen	DK	Technical University, Denmark
Viollet	Sébastien	FR	ESBS
renault	renaud	FR	ESBS
Brune	Karl	DE	University of Uppsala, Sweden
Fracchia	Charles	GB	Imperial College London
Hugenholtz	Jeroen		University of Amsterdam
Tsaneva	Krasimira	GB	University of Bristol
Hallinan	Jennifer	GB	Newcastle University
Albiol	Joan	ES	Universitat Autònoma deBarcelona
Poppinga	Wilfred	NL	University of Groningen
Van de Gronde	Jasper	NL	University of Groningen
Deeg	Christoph	DE	University of Freiburg
Hagen	Sven	DE	University of Freiburg
Browne	Gavin	FR	SupBiotech Paris
Ougen	Pierre	FR	SupBiotech Paris
Chabbert	Christophe	FR	Mines Paristech
Le Fevre	François	FR	Genoscope, CEA
Nystrom	Axel	GB	UCL
Rademacher	Anne		University of Heidelberg
Krämer	Stephen	DE	University of Heidelberg
Camsund	Daniel	SE	Uppsala University
Gubelmann	Carine		EPFL
Fournier	Heidi		EPFL
Visco	Ilaria	DE	Biotec-TU Dresden
Jiang	Lei	CN	Shanghai Jiaotong University
Magni	Paolo		Università degli Studi di Pavia
Genee	Hans Jasper		
de la Torre	Victor		
Can	Tolga		
Solís Escalante	Daniel		
Cockerton	Caitlin		
Keienburg	Jens		
İŞ	Özkan		
tastan	cihan		
Monsefi	MohammadNaser		
Tiezheng	Mao		
Nikerel	Emrah		
Martinez	Genaro J.		
Stansfield	Ian		
Lower	Michał		
Krawczyk	Paweł		
Bellomo	Domenico		
Thijs	Inge		
Tao	Yixin		

iGEM 2009

IGEM Workshops 2009

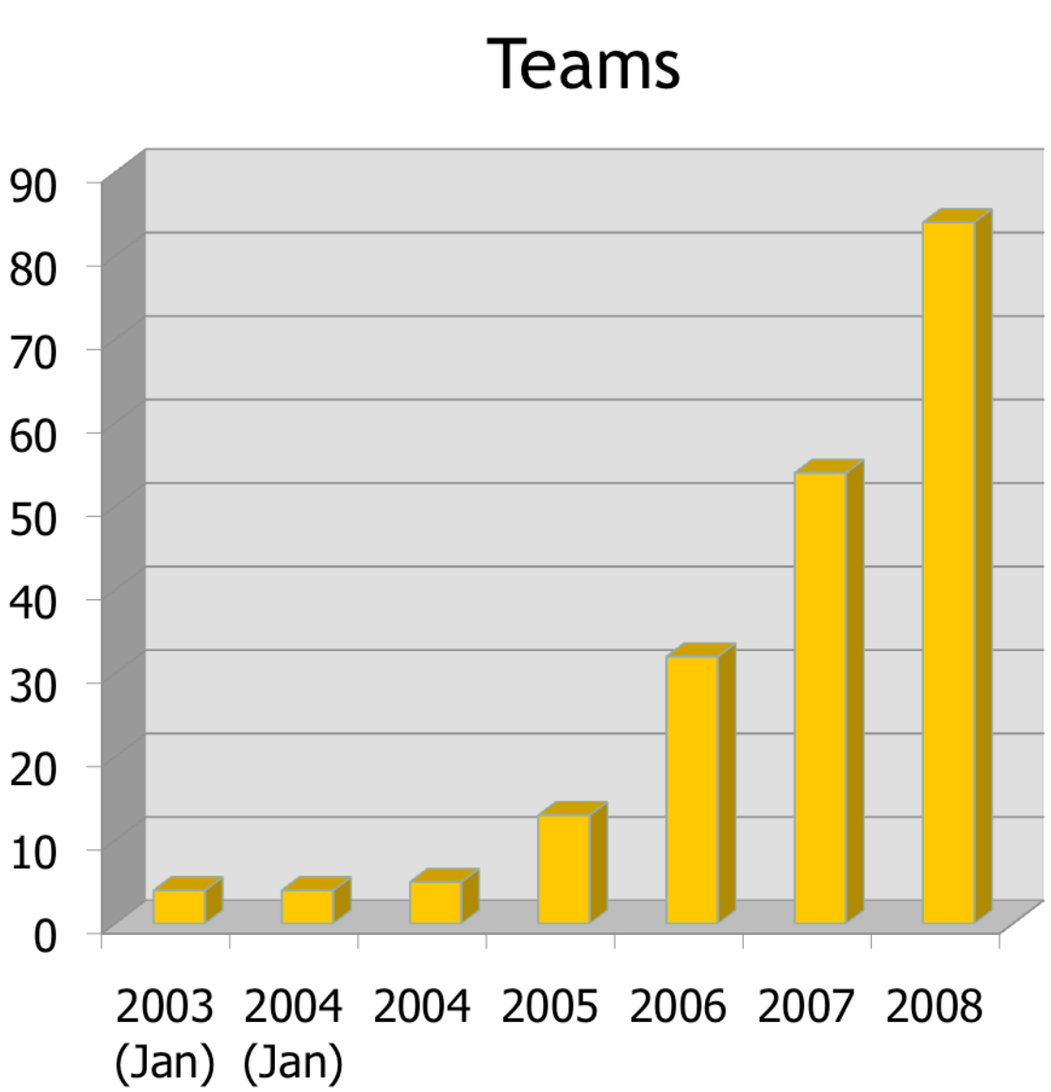
Randy Rettberg
hq@igem.org
igem.org

Synthetic Biology Question

Can simple biological systems be built from standard, interchangeable parts and operated in living cells?

Or, is biology so complex that each case is unique?

iGEM Growth and Scale



iGEM Scale and Growth

Year	Teams	Jamboree	Total
IAP	4	20	20
2004	5	70	70
2005	13	120	150
2006	32	360	400
2007	54	570	750
2008	84	825	1180
2009	112	1100	1650
2010	180	1800	2700
2011	270	2700	4000

Or Not !

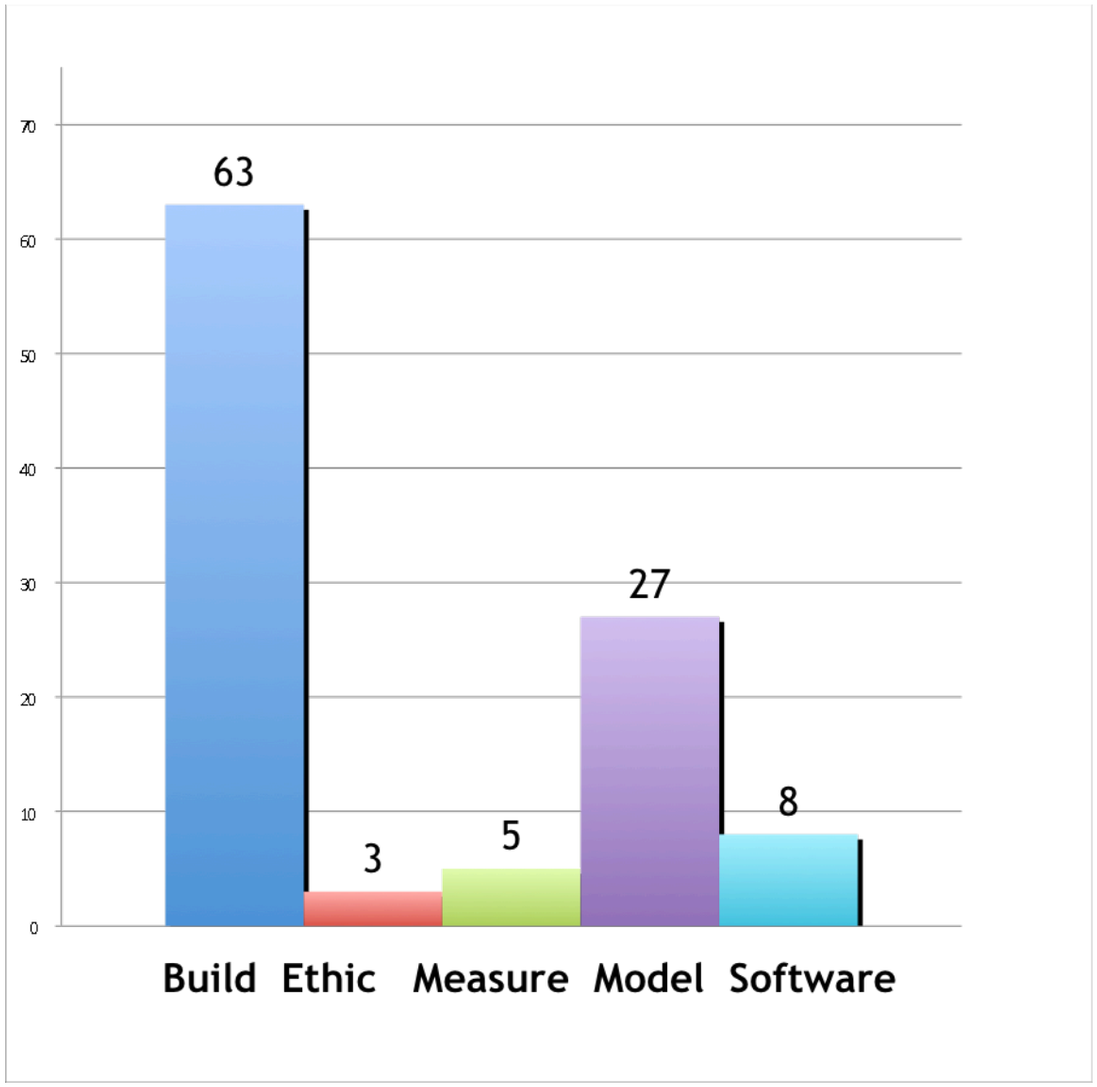
World-Wide Participation 2009

- 37 USA
- 30 Europe
- 11 Canada
- 28 Asia
- 5 South and Central America

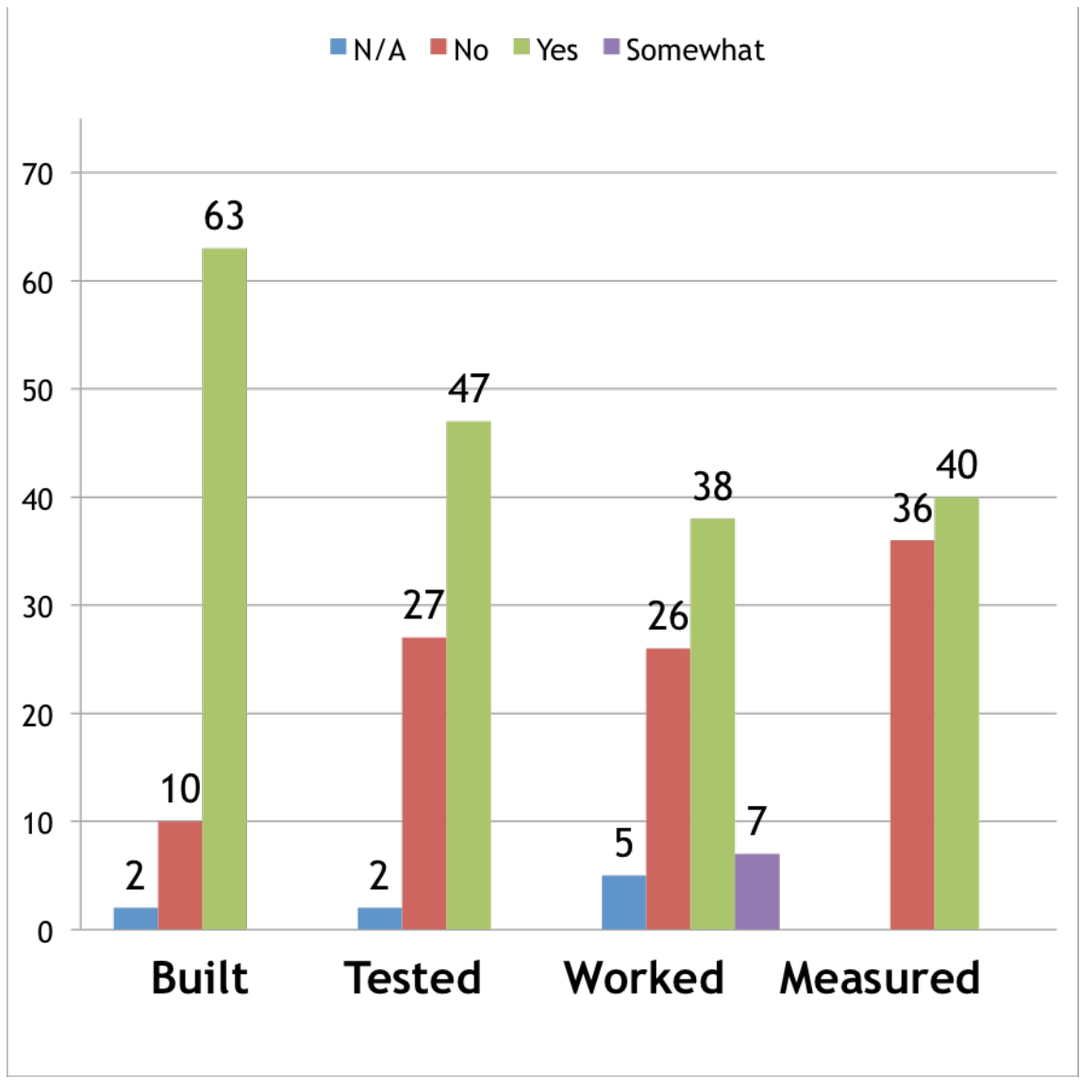
iGEM 2008 Activity and Success

igem.org

Activities



Successes



What Does iGEM Cost?

Small sample of team budgets (2007)

$20K8

$30K8

$40K1

$50K4

>$50K 2

iGEM 2008 Projects

igem.org

- RNA Logic, Biological Memory,
- Turing Patterns, Voltage Output,
- Magnetic Bacteria,
- Polutants
- GFP Modification, Biomacromolecule
- Fuel, bio-removal
- Betacarotine production
- Quorum sensing
- Counter in yeast
- Game of life
- GM Symbiots
- Cyanobacteria
- Slot machine
- ‘Stress kit’, transcription factors
- Yeast biosensor
- Bioprinter
- Starch-producing E.coli
- Yeast sex detector
- Bacterial drug delivery system
- Ligase independent cloning
- Counter, time bomb
- Ligain peroxide degrading
- Biogurt
- Repressilator
- “Bug Busters”
- Kill bad E.coli O157:H7
- Bacter O’Clock
- Hormone biosensor
- Bacterial UV sensor
- “Redhawk: Search and destroy”
- Viral amplification
- Biobeer
- Chemotaxis
- Thermometer
- Lysophonix - sound sensitive bacteria
- Clotho (biocad)
- Multiplexor/Demultiplexor
- Singing bacteria

And 40 more . . .

iGEM 2008

igem.org

iGEM Philosophy

iGEM Philosophy: Get and Give

Teams are expected to use the parts, ideas, and experience of teams in previous years.

Teams are expected to contribute their parts, ideas, and experiences.

Registry of Standard Biological Parts

Go
Search

page discussion view source history Log in / create account

The Registry of Standard Biological Parts has moved from parts.mit.edu to partsregistry.org.
References to the Registry at parts.mit.edu will be automatically redirected to the new site.

- **Browse Parts by Type**

Featured Parts

Help & Documentation

Users & Groups

Apply here for a Registry account

Registry News

- We are considering releasing the Registry's DNA Repository and Library system to the Registry labs and IGEM teams. This is the system we use to keep track of parts in our freezer boxes and plates. Please check it out and let us know what you think. - June 2, 2008
- A bug that kept Internet Explorer users from seeing the Part menu on Part pages has been fixed. Now, if you go to a part, you will see menu choices for hard information and physical location. - June 2, 2008
- The sequence and features for all parts are available through DAS, the Distributed Annotation System. Learn more here - May 26, 2008
- Changes to the Registry software are underway. Check it out!
- We have a new tutorial for starting teams in the Help section
- We are starting an editorial board for promoting well-defined and useful parts to BioBrick™ part status. To join this effort check the BioBrick™ Part Program
- There is a problem with using primers VR and VF2 to PCR parts containing B0015 or B0010.
- News archive...

Report any bugs here | Request new features here | See new features here | See old bugs,requests, and features here

Registry Tools

- Add a part
- Search Parts
- More...
- Measurement
- DNA Repositories
- Sequence Analysis
- Send Parts to the Registry

Parts From Groups

- 2008 iGEM Teams
- 2007 iGEM Teams
- 2006 iGEM Teams
- 2005 iGEM Teams
- Parts by Lab

Registry Community

Frequently Asked Questions

partsregistry.org

Plasmids / Assembly

igem.org

page discussion edit history delete move protect watch Randy My account Log out

Plasmid backbones/Assembly

< Back to Plasmid backbones

Part assembly | System operation | Help! | Protein expression | Assembly of protein fusions | Part measurement | Screening of part libraries | Building BioBrick vectors | BioBrick friendly | Non-BioBrick | Archive

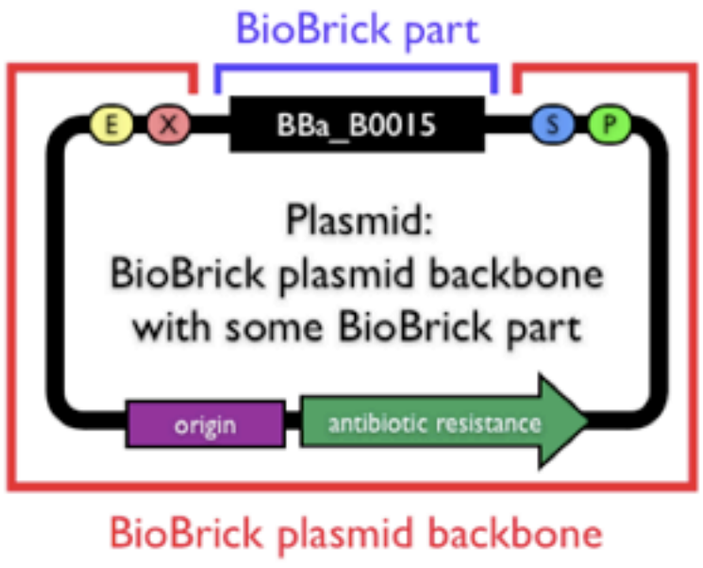
High copy plasmid backbones · Low or medium copy plasmid backbones · Inducible copy number plasmid backbones

Plasmids are circular, double-stranded DNA molecules typically containing a few thousand base pairs that replicate within the cell independently of the chromosomal DNA. Plasmid DNA is easily purified from cells, manipulated using common lab techniques and incorporated into cells. Most BioBrick parts in the Registry are maintained and propagated on plasmids. Thus, construction of BioBrick parts, devices and systems usually requires working with plasmids.

Note: In the Registry, plasmids are made up of two distinct components:

1. the BioBrick part, device or system that is located in the BioBrick cloning site, between *(and excluding)* the BioBrick prefix and suffix.
2. the plasmid backbone which propagates the BioBrick part. The plasmid backbone is defined as the sequence beginning with the BioBrick suffix, including the replication origin and antibiotic resistance marker, and ending with the BioBrick prefix. [Note that the plasmid backbone itself can be composed of BioBrick parts.]

Many BioBrick parts in the Registry are maintained on more than one plasmid backbone!



One of the most common tasks that biological engineers do is to assemble two parts together using BioBrick® standard assembly. To make the process of assembling two BioBrick® parts together easier, there are several kinds of assembly plasmid backbones available via the Registry.

High copy number assembly plasmid backbones

Registry Contents 1/2009

3500 Parts Available as DNA

(Includes 1300 from iGEM 2008)

- Promoters

- Protein Coding

- Reporters

- RNA

- Terminators

- Signaling

- Many project parts

DNA Repository

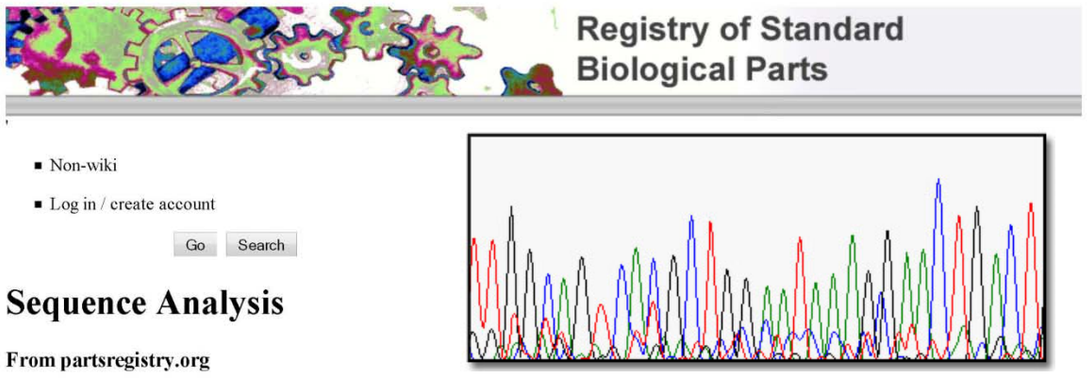
igem.org

Quality Control

igem.org

Sequence verification

igem.org



Registry of Standard Biological Parts

- Non-wiki
- Log in / create account

Go Search

Sequence Analysis

From partsregistry.org

This tool is used to organize and analyze a set of DNA sequencing runs by comparing DNA sequences against parts in the Registry.
Use Blast at NCBI to compare sequences with a large number of genomes.
The BioBrick Blast database was last updated on Thu Sep 4 10:03:31 2008. (Update now)

Current Sequence Analysis

Source Plate 1000, Well 1A, Lib QC08	randy	2008-03-16
Target part: BBa_B0011 (length: 46bp)	Linked to part info page	

B0011

Sequence 1623 (QC08_P620_W39493_VF)

VF Length: 1283bp Blast against: BBa_B0011 Basic Parts All Parts
Get machine files: *(Sequence)(Trace)* Get Phred files: *(Sequence)(Quality)(Trace)*
BB Prefix found at 79 BB Suffix found at 147
Use inside sequence (46bp)

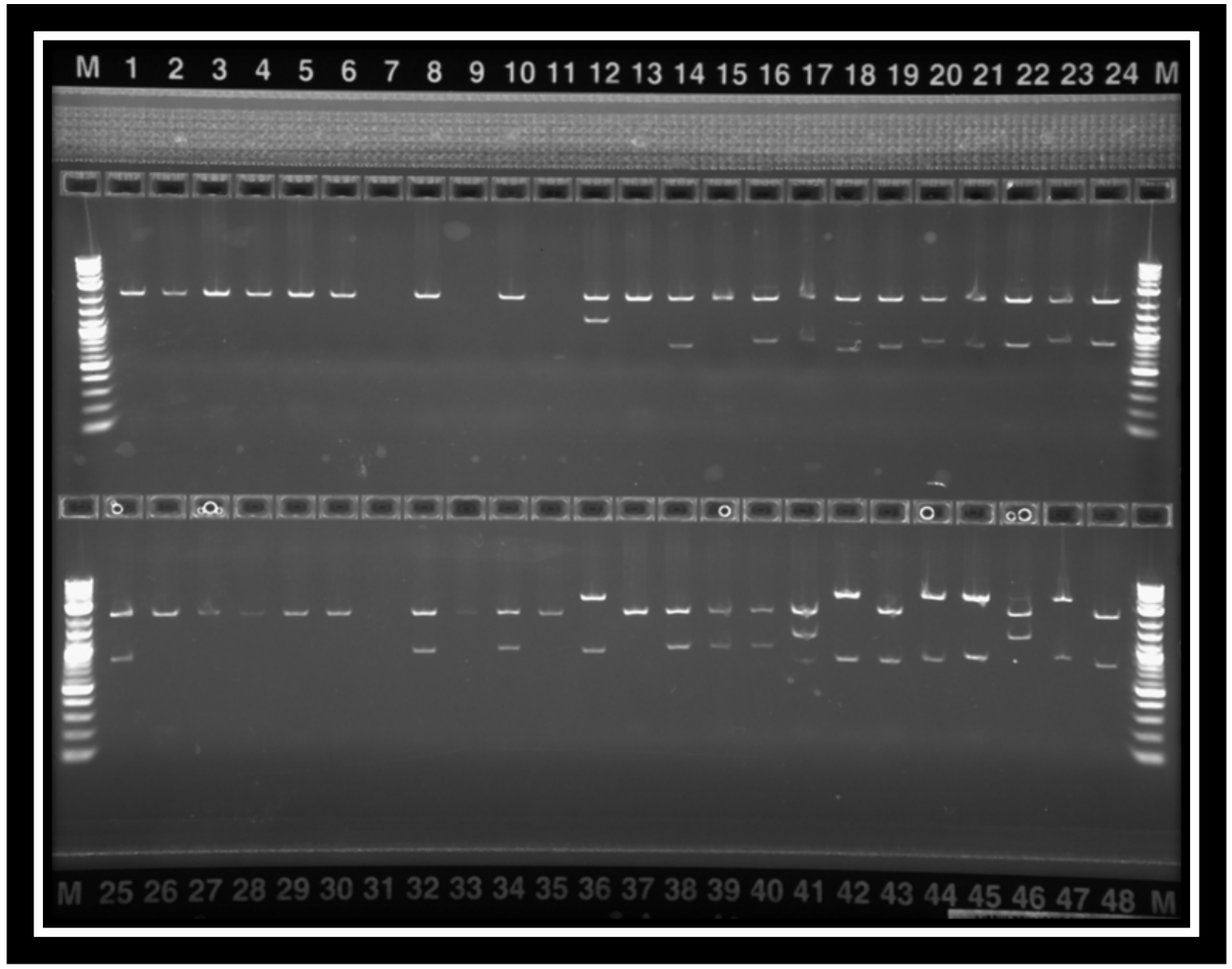
Sequence 1655 (QC08_P620_W39493_VR)

VR Length: 1387bp Blast against: BBa_B0011 Basic Parts All Parts
Get machine files: *(Sequence)(Trace)* Get Phred files: *(Sequence)(Quality)* *(Trace)*
BB Prefix found at 1258 BB Suffix found at 1326
Use inside sequence (46bp)

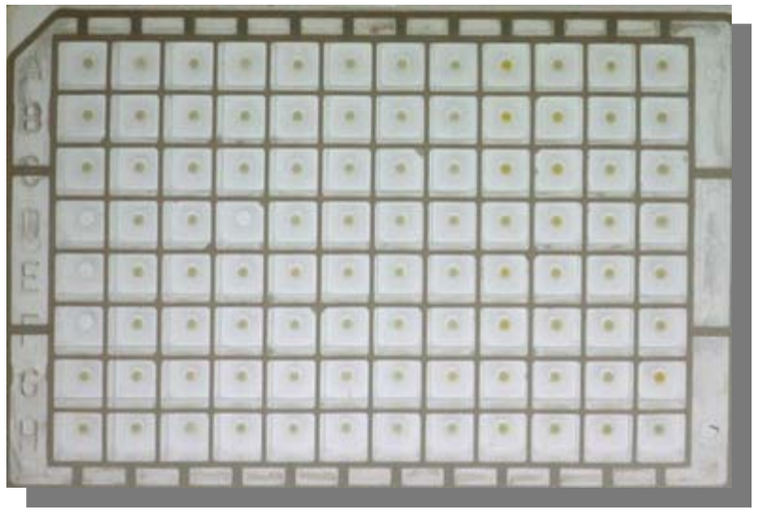
Automatic Alignment

Confirmed Confirmed Good: 46, Bad: 0, Not clear: 0, Not covered: 0

Part
1623
1655



Source Plate 1000 Image Antibiotic A



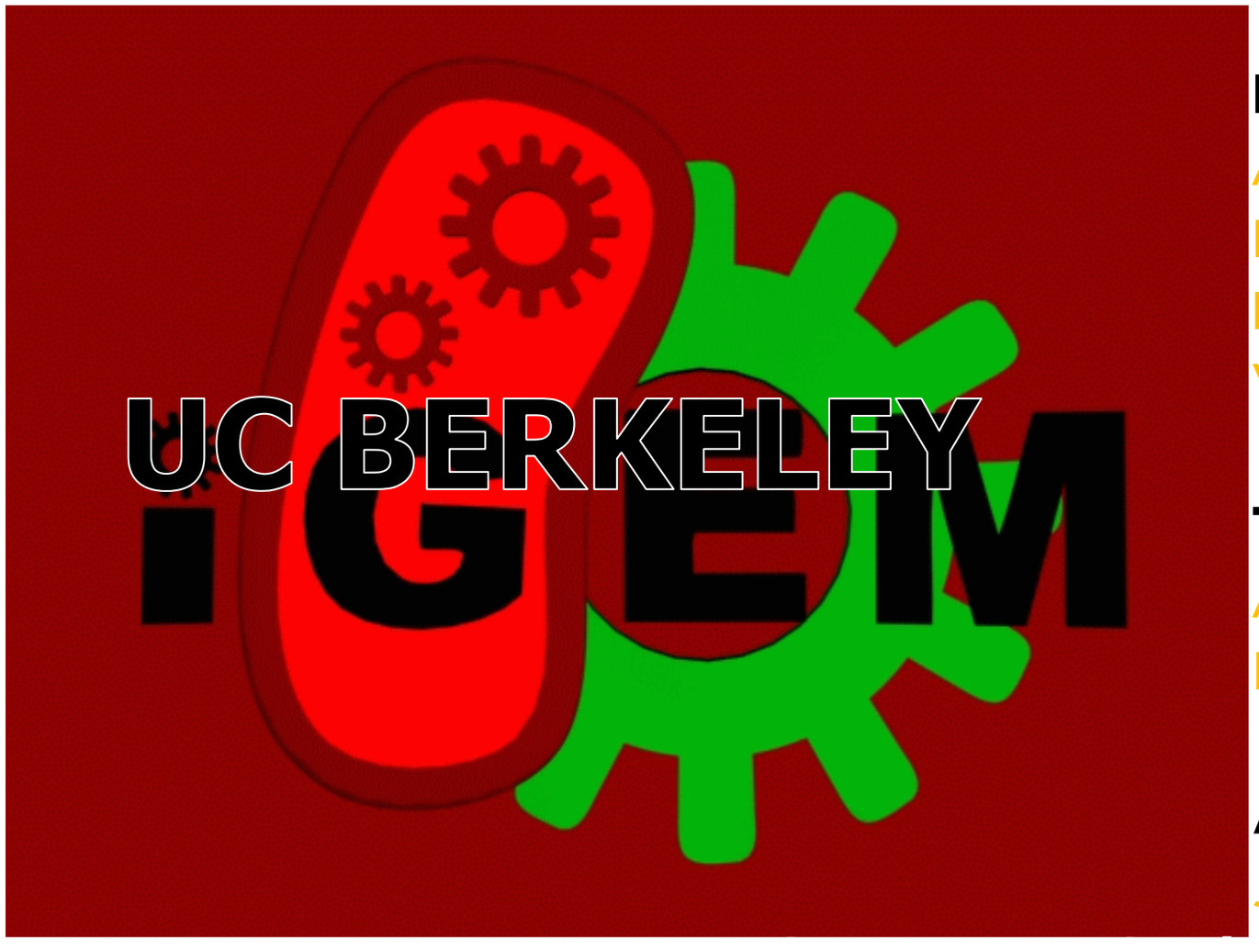
Impact of iGEM

- Students
  - Making future synthetic biologists
  - Teaching entrepreneurial competition
- Instructors
  - Opportunities for junior faculty
  - New programs - new ideas
- Schools
  - Synthetic biology entering curriculum
  - Energize research programs
- Synthetic Biology
  - Examples, parts, successes, testimonials
  - Academic research projects – SynBERC
- A task worth the effort

Commercial Applications

igem.org

BACTOBLOOD



Researchers

Arthur Yu • Austin Day • David Tulga • Hannah Cole • Kristin Doan • Kristin Fuller • Nhu Nguyen • Samantha Liang • Vaibhavi Umesh • Vincent Parker

Teaching Assistants

Amin Hajimorad • Farnaz Nowroozi • Rickey Bonds

Advisors

John Dueber • Christopher Anderson • Adam Arkin • Jay Keasling

Save the World - Arsenic Detector

A test tube could contain all the necessary components: Freeze dried bacteria, growth medium, indicator powder, Ampicillin salt, etc...

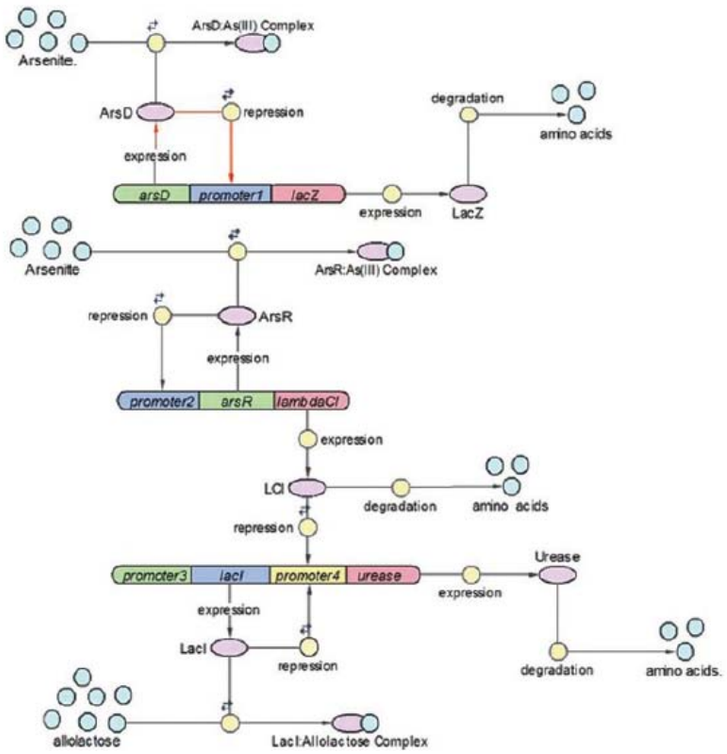
- These tubes could then be given to local villagers to monitor their own water quality themselves
- A good alternative to the widely used Gutzeit method

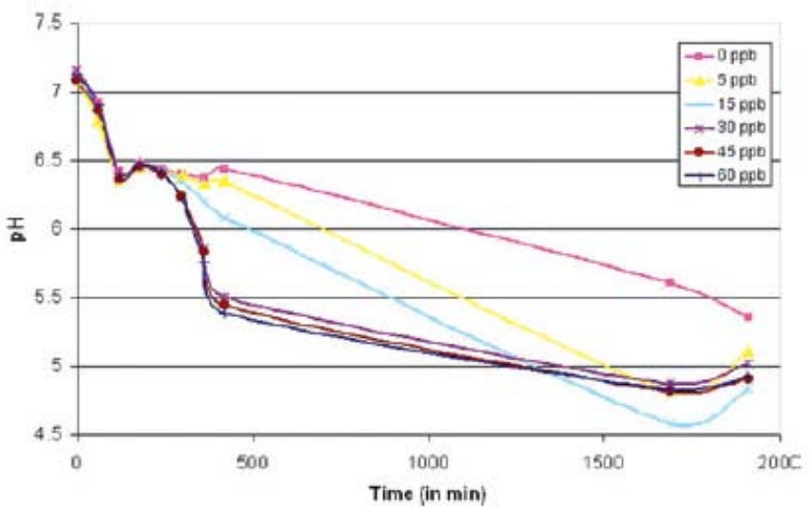
www.Macteria.co.uk

iGEM After iGEM – PublishYour Work

Development of a novel biosensor for the detection of arsenic in drinking water

J. Aleksic, F. Bizzari, Y. Cai, B. Davidson, K. de Mora, S. Ivakhno, S.L. Seshasayee, J. Nicholson, J. Wilson, A. Elfick, C. French, L. Kozma-Bognar, H. Ma and A. Millar



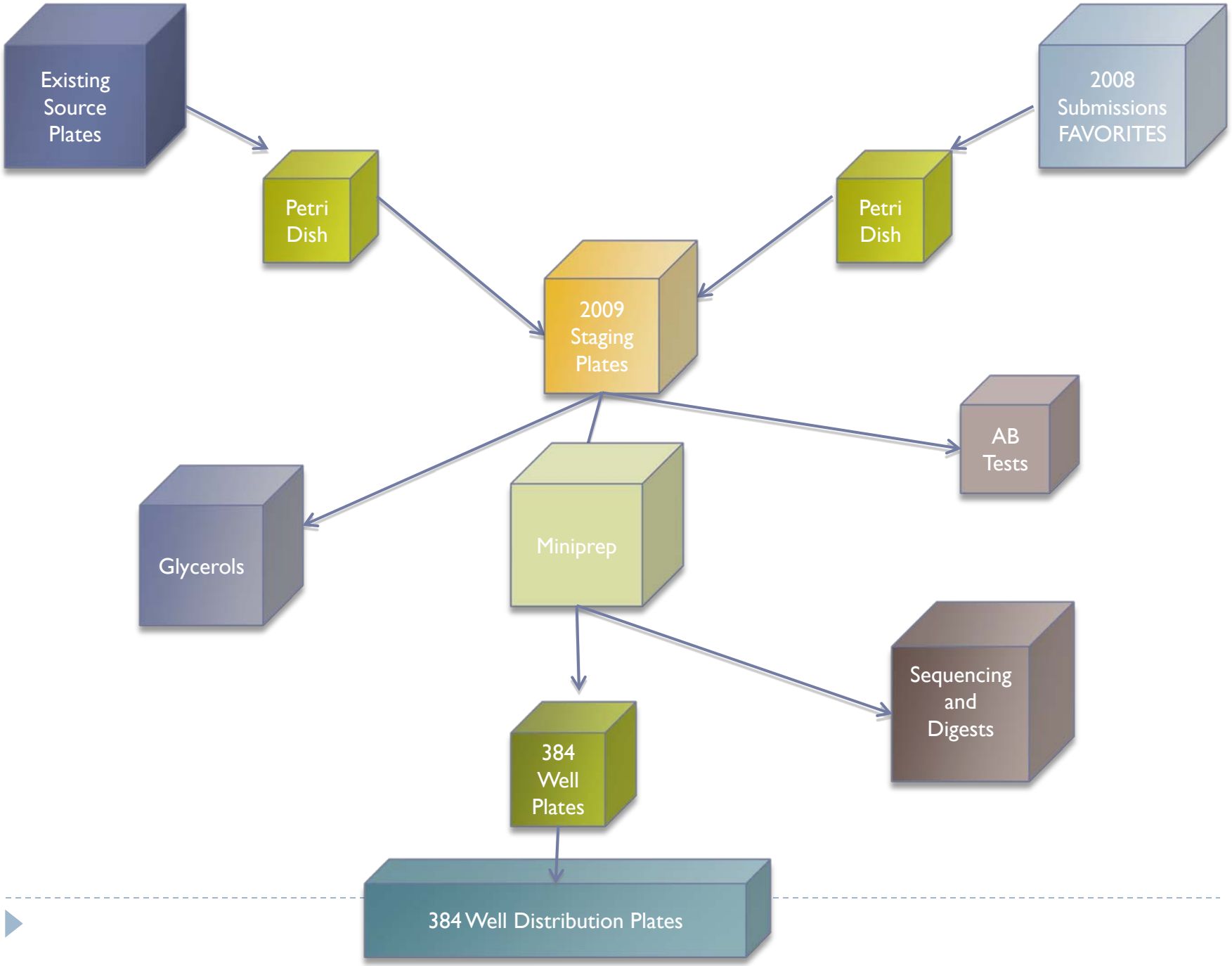


Sponsors

- NSF / SynBERC
- MIT
- Microsoft (2005-2006)
- MathWorks
- GeneArt

DNA Distribution

generating the spring 2009 distribution


Existing Source Plates
Petri Dish
2008 Submissions FAVORITES
Petri Dish
2009 Staging Plates
AB Tests
Glycerols
Miniprep
Sequencing and Digests
384 Well Plates
384 Well Distribution Plates


iGEM

Using the Registry

searching for parts and quality control information

DNA distribution Help:

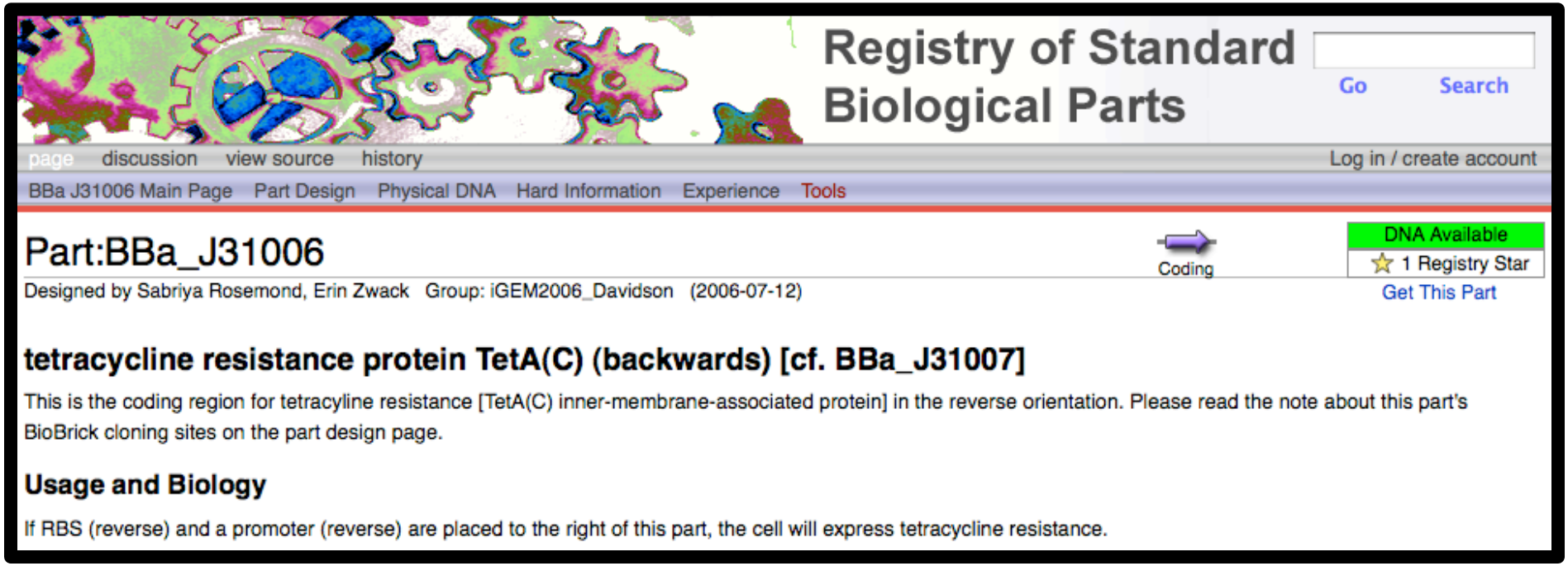
http://partsregistry.org/Help:IGEM_09_DNA_distribution

DNA Part Repositories

- Information about the 2009 Parts Kit
- **NEW:** Plasmid Backbone Availability 2009
- Transforming Competent Cells
- Automatic Sequencing Algorithm
- How to check if a part is correct
  - How to read sequence analyses
  - How to read gel images
- How to request and recieve stabs
- More...

Searching for Parts: Specific Search

1. When you are on any page in the Registry, use the search box on the upper right and enter the part number that you are interested in.
2. At the part's main page, you can find a detailed description of the part and its features. Click on the ***Get This Part*** link at the top right. Please note that we only have physical DNA for parts whose part status reads ***Available***.



Searching for Parts: Browsing

Catalog of parts & devices

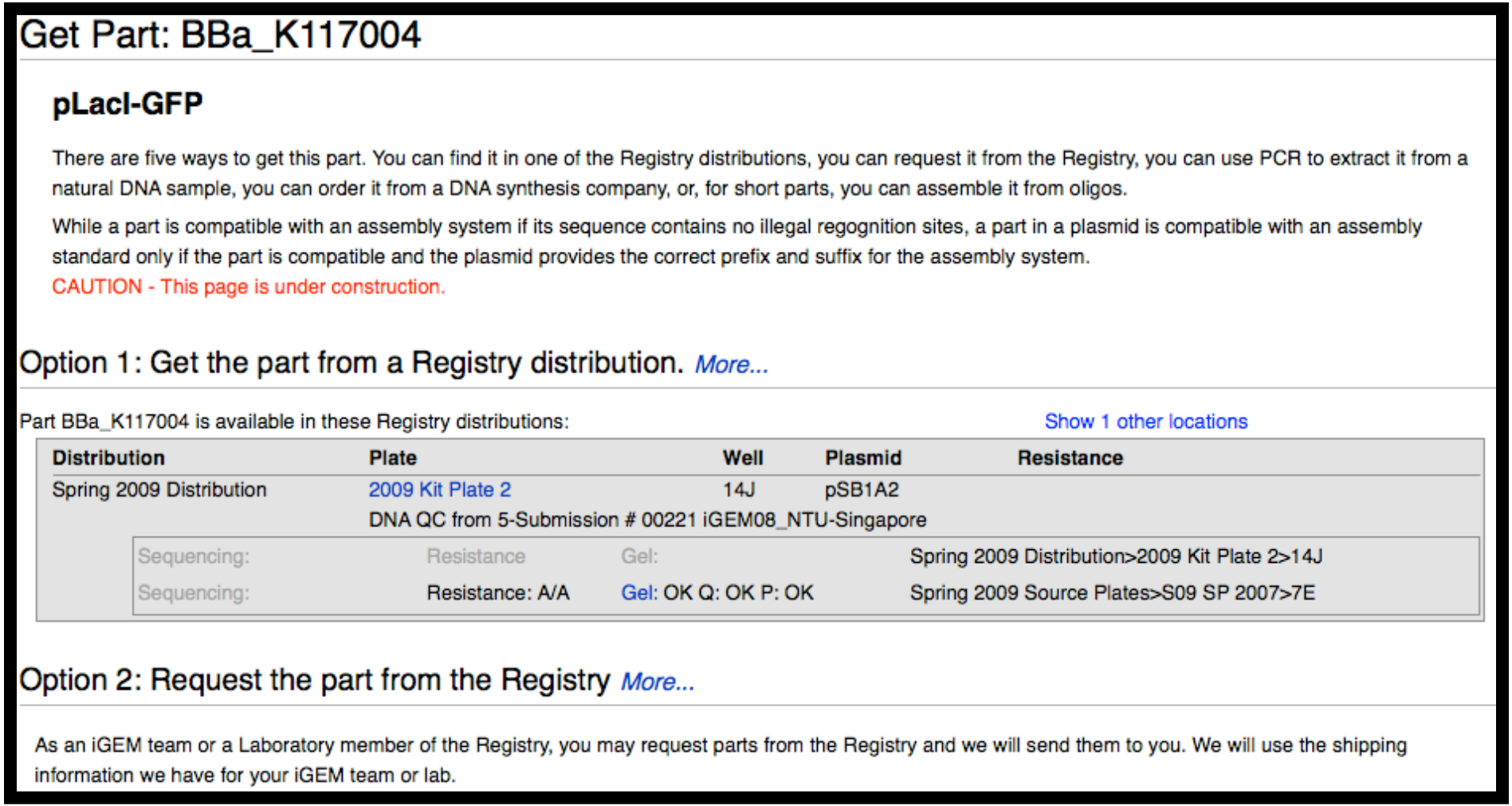
1. At the Registry main page click on ***Catalog of parts & devices.***
2. You can choose from a particular family of parts in the ***Catalog***
3. From there you can enter the catalog for that family of parts, and narrow down the search by attributes such as ***function*** and ***family***.
4. You will arrive at a detailed list of parts that are within your search parameters. Parts that are ***available*** through the registry will have an "***A***" in the leftmost cell. Parts that are in the 2009 DNA distribution have a Registry star.

-?-	Name	Description	Promoter Sequence	Positive Regulators	Negative Regulators
1☆	BBa_I721001	Lead Promoter	. . . gaaaaccttgtcaatgaagagcgatctatg		
	BBa_I731004	FecA promoter	. . . ttctcgttcgactcatagctgaacacaaca		
	BBa_I760005	Cu-sensitive promoter	atgacaaaattgtcat		
A	BBa_I765000	Fe promoter	. . . accaatgctgggaacggccagggcacctaa		
A	BBa_I765007	Fe and UV promoters	. . . ctgaaagcgcataccgctatggaggggggtt		
1☆	BBa_J3902	PrFe (PI + PII rus operon)	. . . tagatatgcctgaaagcgcataccgctatg		

5. Selecting the part name will take you to the part's main page. Click on the ***Get This Part*** link at the top right.

Get This Part

- Whether you are browsing for a part or searching specifically for one, you will go to the ***Get This Part*** page. This page outlines the options you have in obtaining the part (distribution location, requesting, etc.), as well as giving you an overview of the part's QC information.



Get Part: BBa_K117004

pLacI-GFP

There are five ways to get this part. You can find it in one of the Registry distributions, you can request it from the Registry, you can use PCR to extract it from a natural DNA sample, you can order it from a DNA synthesis company, or, for short parts, you can assemble it from oligos.

While a part is compatible with an assembly system if its sequence contains no illegal regognition sites, a part in a plasmid is compatible with an assembly standard only if the part is compatible and the plasmid provides the correct prefix and suffix for the assembly system.

CAUTION - This page is under construction.

Option 1: Get the part from a Registry distribution. *More...*

Part BBa_K117004 is available in these Registry distributions: Show 1 other locations

Distribution	Plate	Well	Plasmid	Resistance
Spring 2009 Distribution	2009 Kit Plate 2	14J	pSB1A2	
	DNA QC from 5-Submission # 00221 iGEM08_NTU-Singapore			
Sequencing:	Resistance	Gel:		Spring 2009 Distribution>2009 Kit Plate 2>14J
Sequencing:	Resistance: A/A	Gel: OK Q: OK P: OK		Spring 2009 Source Plates>S09 SP 2007>7E

Option 2: Request the part from the Registry *More...*

As an iGEM team or a Laboratory member of the Registry, you may request parts from the Registry and we will send them to you. We will use the shipping information we have for your iGEM team or lab.

Requesting a Part

- If you find a part that is listed as ***Available*** but is not part of your distribution kit, feel free to request the part from us.
- Currently the best way to request a part would be to email us at hq@igem.org with the part name, the institution you represent, and your mailing address.

Special case: Plasmid backbones

- Plasmid backbone availability chart
  - Two versions of plasmids – BBa_P1010 (ccdB cell death gene) and BBa_J04450 (RFP)
  - If a version doesn't work, we have suggested alternate locations
    - Should PCR this alternate version!

http://partsregistry.org/Help:Plasmid_Backbone_Availability_2009

Where to find QC information: Overview

If you would like an **overview** of the QC info for your part…

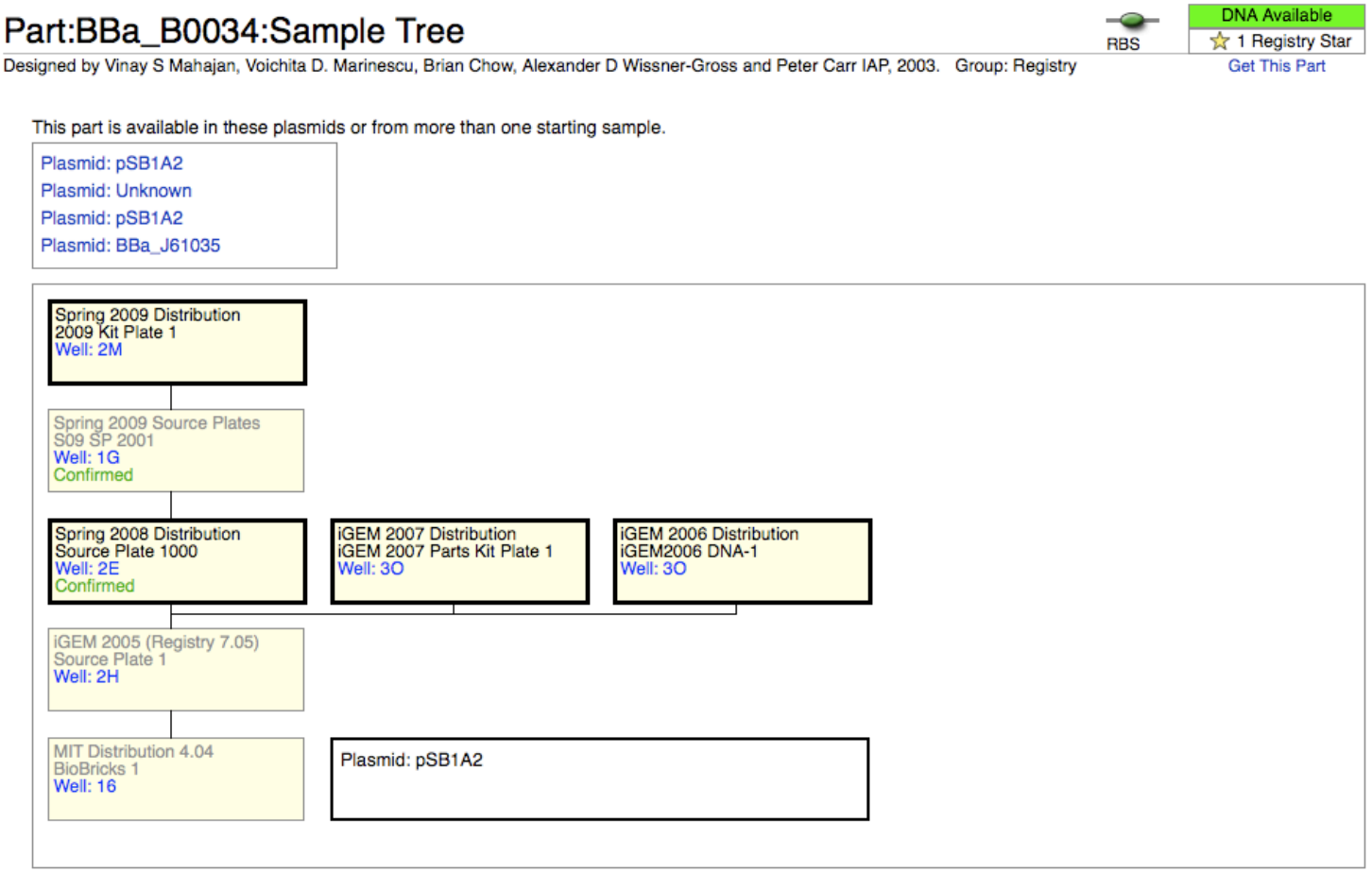
1. When you are at the main page for your part, click on ***Get This Part***.
2. On the ***Get Part*** page look at ***Option 1***, to see which distribution the part is available in.

- In addition to showing the location of the part in the distribution kits and source plates, this section gives our evaluation of the sequencing, AB resistance, and restriction digests.

Distribution	Plate	Well	Plasmid	Resistance
Spring 2009 Distribution	2009 Kit Plate 1	13F	pSB1A2	
	DNA QC from 7A-Spring 2008 -> Source Plate 1004			

Sequencing:	Resistance	Gel:	Spring 2009 Distribution>2009 Kit Plate 1>13F
Sequencing:	Resistance:	Gel:	Spring 2009 Source Plates>S09 SP 2002>7C
Sequencing: Confirmed	Resistance: A	Gel: OK Q: OK P: OK	Spring 2008>Source Plate 1004>7A

Where to find QC information: Tree view



Where to find QC information: Details

For more detailed quality control info, and to evaluate the results yourself…

1. When you are on the Registry's main page, visit ***DNA Repositories***
2. Click on ***Spring 2009 Source Plates***
3. And choose the source plate of interest

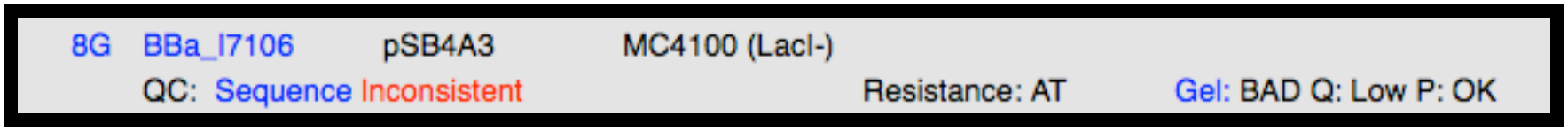
- From this page you can evaluate the gel results, sequencing and the AB Test Plates.
- This step is particularly useful if you find your part to be questionable from the overview. You should keep in mind the location of your part in the 2009 Source Plates according to the ***Get This Part*** page.

Sequencing

- Using the epMotion, we created three 384 well sequencing plates, each of which reflects a plate of the distribution kit.

- The plates are being sequenced externally through Genewiz, and the results will be uploaded soon to the DNA repository.

- The sequences are then compared with their target sequence through software, and are given the following qualitative values:
  - ***Confirmed***
  - ***Partially Confirmed***
  - ***Long Part*** – *we don't know whether the entire part is confirmed, but the sequence ends are*
  - ***Inconsistent***
  - ***Bad Sequence*** – *usually caused by low DNA concentration or incorrect primers*
  - ***User Confirmed*** – *we manually reevaluate the inconsistent sequences and look at the trace files to see if a simple shift of the sequence will confirm it*



Sequencing Results

- For more in-depth analysis, when reviewing quality control information for a part, just click on ***Sequence***.

8G BBa_I7106 pSB4A3 MC4100 (LacI-)
QC: Sequence Inconsistent Resistance: AT Gel: BAD Q: Low P: OK

- Every user can look at all the results we get back from Genewiz: including the trace files, quality scores, and sequence reads.

Sequence Analysis

This tool is used to organize and analyze a set of DNA sequencing runs by comparing DNA sequences against parts in the Registry.
Use Blast at NCBI to compare sequences with a large number of genomes.
The BioBrick Blast database was last updated on Fri May 8 01:29:06 2009. (Update now)

Current Sequence Analysis

Source Plate 1004, Well 8G, Lib QC08 meagan 2008-05-16
Target part: BBa_I7106 (length: 941bp) Linked to part info page

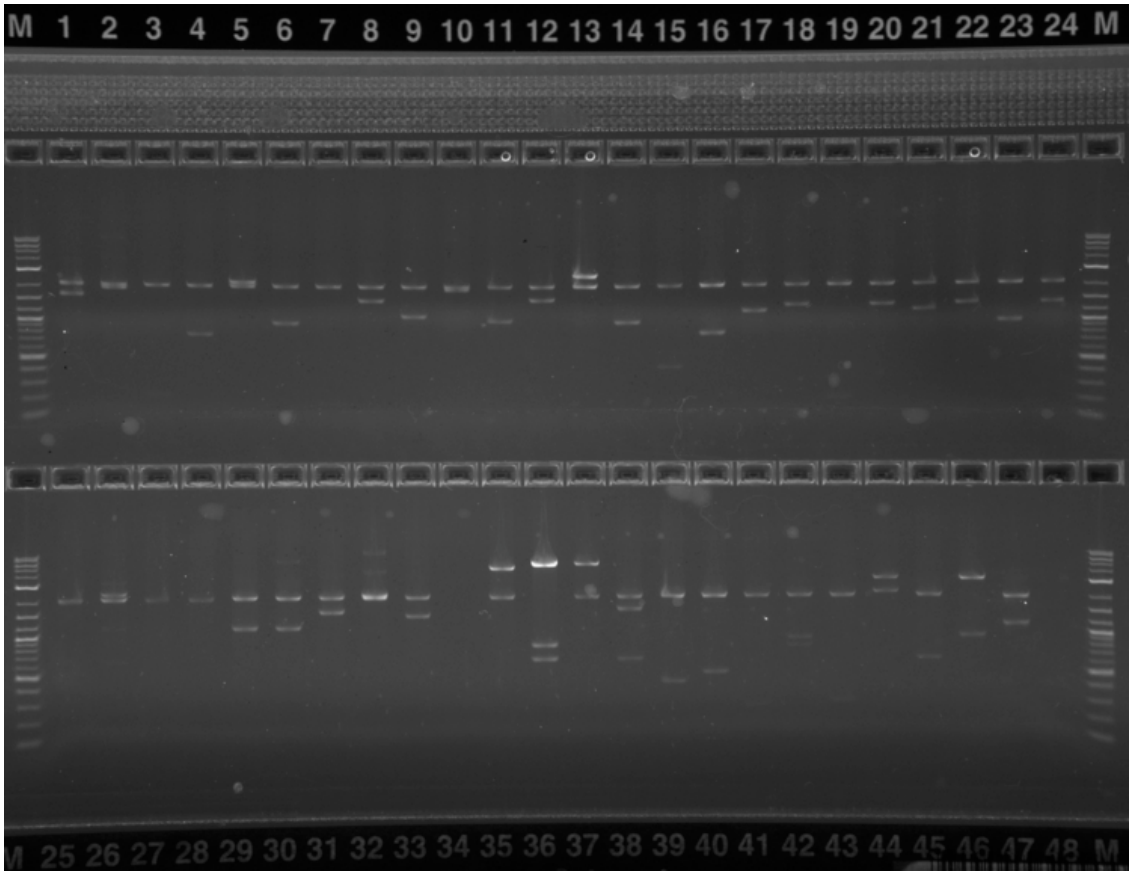
Sequence 2788 (QC08_P628_W39939_VF)

VF Length: 1489bp Blast against: BBa_I7106 Basic Parts All Parts
Get machine files: (Sequence) (Trace) Get Phred files: (Sequence) (Quality) (Trace)

BB Prefix found at 91
☑ Use inside sequence (1376bp)

Restriction Digests

- We did restriction digests on parts using EcoR1 and Pst1 as our restriction enzymes. Afterwards we ran the digests on an e-gel and then imaged the gel using standardized parameters.



Gel Results

- The gel image was then uploaded to the DNA repository. We evaluated each lane, using a set of qualitative statements for plasmid length (**P**), plasmid quantity (**Q**), and insert length (**Gel**).
  - Plasmid Quantity **(P)**: None / LOW / OK / HIGH
  - Plasmid Length/Quality **(Q)**: OK / BAD / ???
  - Insert Length/Quality **(Gel)**: OK / BAD / ???

Distribution	Plate	Well	Plasmid	Resistance
Spring 2009 Distribution	2009 Kit Plate 1	13F	pSB1A2	
	DNA QC from 7A-Spring 2008 -> Source Plate 1004			

Sequencing:	Resistance	Gel:	Spring 2009 Distribution>2009 Kit Plate 1>13F
Sequencing:	Resistance:	Gel:	Spring 2009 Source Plates>S09 SP 2002>7C
Sequencing: Confirmed	Resistance: A	Gel: OK Q: OK P: OK	Spring 2008>Source Plate 1004>7A

- If you have any question as to the quality of the part you can view the gel yourself by clicking on the ***Gel Images and Results*** at the Source Plate.

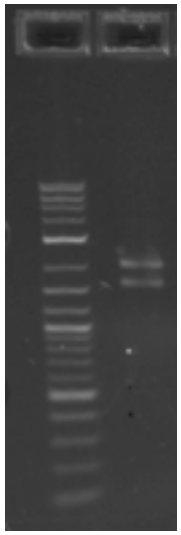
Get antibiotic files for this plate
Get an Excel file for this plate

Gel Images and Results
Wells 1A thru 6H
Wells 7A thru 12H

Sequencing and Results
Go to sequencing

Plate Images and Results
Image 00162: Antibiotic A
Image 00161: Antibiotic C
Image 00164: Antibiotic K
Image 00150: Growth other
Image 00151: Growth other
Image 00152: Growth other
Image 00163: Antibiotic T

Evaluating the Gel Results



- On the ***Gel Images and Results*** page, you can find the expected length of the part (insert) and the plasmid, as well as the gel image. This allows you to compare the expected lengths of the part and plasmid with their gel bands.

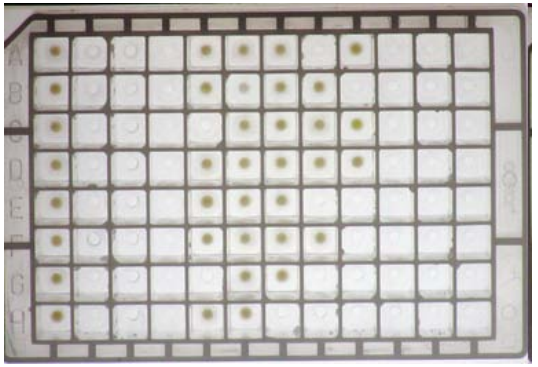
				Lengths (As cut)		Insert	Plasmid	
Lane	Well	Insert	Plasmid	Insert	Plasmid	Result	Quantity	Quality
1	7A	K081003	pSB1A2	1594	2058	OK	OK	OK

- If you find that the gel results for your part do not match with their expected lengths then you may first want to take a look at the part's Main Page to find out its restriction sites. Some parts and plasmids may have more than one or no EcoR1 and Pst1 cut sites; which will of course differ from the expected banding on the gel.
- When evaluating the parts we made sure to take these exceptions into consideration; if the gel results matched the band length calculations, the part was described as OK.

Antibiotic Test Plates

- Each of the four AB test plates contained LB broth with a different antibiotic: Amp, Cm, Tet, or Kan. After inoculation with the pintool, the plates were then incubated on a shaker at 37 degrees overnight. The plates were spun down, the media was drained, and the pellets were photographed.

Amp



Kan

- The AB Test plate images were then uploaded to the registry.

Get antibiotic files for this plate
Get an Excel file for this plate

Gel Images and Results
Wells 1A thru 6H
Wells 7A thru 12H

Sequencing and Results
Go to sequencing

Plate Images and Results
Image 00162: Antibiotic A
Image 00161: Antibiotic C
Image 00164: Antibiotic K
Image 00150: Growth other
Image 00151: Growth other
Image 00152: Growth other
Image 00163: Antibiotic T

What do the AB test plates tell you?

- Whether a part grew up in the AB broth that its plasmid was resistant to.
- Whether the part's plasmid is resistant to more than one AB.

If there are discrepancies between the plasmid AB description and the AB test…

- Take a look at the gel results, to find out if the plasmid quality (**P**) was bad.
  - If the quality (P) was BAD, the plasmid that the part is in may be incorrect. If so, check the insert length to see if the part is wrong as well.
  - If the quality was OK for the plasmid, as well as the insert length, and the sequencing, then it is likely that the AB Test Plate(s) may have had an insufficient antibiotic concentration.

8G	BBa_I7106	pSB4A3	MC4100 (LacI-)		
	QC: Sequence Inconsistent			Resistance: AT	Gel: BAD Q: Low P: OK

QC Information: The Take Away

- All the quality control information on the Registry is there so that you can make the best possible decision when it comes to choosing a part. We strongly encourage that you evaluate the results for your part, as it is particularly important when an aspect of a part's QC appears questionable.

DNA distribution Help:

http://partsregistry.org/Help:IGEM_09_DNA_distribution

DNA Part Repositories

- Information about the 2009 Parts Kit
- **NEW:** Plasmid Backbone Availability 2009
- Transforming Competent Cells
- Automatic Sequencing Algorithm
- How to check if a part is correct
  - How to read sequence analyses
  - How to read gel images
- How to request and recieve stabs
- More...

Notes:
- Registry: partsregistry.org
- always have the tutorial open in a window.
- you can go to a NEW TAB by right clicking on the link → open link in new tab (*nice to do it this way so that everyone can get a visual and follow along*)

Design the system (vanillin synthesizer)

1. NEW TAB: "PubMed"
    a. Search "vanilla e.coli"
    b. Click on the first hit (paper by Barghini)
    c. Click on "free full text"
    d. Scroll down to the paragraph just above the RESULTS section
    e. Find (APPLE+F) *Genbank*
2. NEW TAB: "Genbank AJ536325"
    a. Find (APPLE+F) *ech*
        i. 2nd result: gene = "ech"
    b. Click on "CDS"
    c. KEEP THIS TAB OPEN

Making a part

3. NEW TAB: "Registry"
    a. Click on "add a part"
    b. Click on "add a basic part now"
        i. You might have to log in
            1. Username: Stephen
            2. Pwd: igem
        ii. Click on return to: partsregistry.org/Add_a_Part…
        iii. Click on "add a basic part now"
    c. Check box for iGEM09_Example
    d. In part name box type BBa_K294999
    e. **Part type:** Coding
    f. **Short description:** feruloyl-CoA hydratase for vanillin biosynthesis (ech)
    g. **Long description:** This is one of the genes needed to synthesize vanillin. It is one of two steps in the bioconversion of ferulic acid to vanillin. The authors of the paper have shown that it works in E. coli. It requires both ech and fcs for vanillin biosynthesis.
    h. **Source of the part:** P. fluorescens, Genbank # AJ536325
    i. **Design considerations:** Change stop codon from TGA to TAATAA to conform with BioBrick standards.
    j. Click to proceed
        i. KEEP THIS TAB OPEN
4. SWITCH to Genbank entry page
    a. In DISPLAY pull down menu choose FASTA
    b. Copy all sequence (NOT THE HEADER LINE)
5. SWITCH to Add a Part tab
    a. Paste sequence
    b. Change TGA stop codon at end of sequence to TAATAA
    c. Click Save
    d. Click "add a feature"
        i. 1 to end (831bp), type: cds, label: ech

** Important to remember that the part DOES NOT include the prefix and suffix.

Make a device

6. GO BACK to tutorial tab
7. NEW TAB: "Registry"
8. NEW TAB: "browse part types"
    a. Click "regulatory" (for promoters)
    b. Close tab, return to REGISTRY tab
9. Click on "add a part"
    a. "Add a composite part"

b. Check box for iGEM08_Example
c. In part name box type BBa_ K294998
d. **Part type:** Reporter
e. **Short description:** RFP coding device
f. **Long description:** Constitutive RFP device. The colonies are red in color under natural light. Smaller colonies are visibly red under UV. The RFP part does not contain a degradation tag and the RBS is strong.
g. **Source of the part:** composite part made from Registry distribution 2008
h. **Design considerations:** None.
i. **Subparts:** R0010 B0034 E1010 B0015
j. Click to enter info.

Favorites (and reviewing part)

10. Partsregistry.org
11. Catalog
    a. By contributor
    b. iGEM 2009
    c. Team Example
    d. Click on a part
        i. Hard Information
            1. Edit
            2. Favorites → Yes
            3. Save

Shipping a part

12. Partsregistry.org
13. Send parts to the Registry
    a. Read **Detailed Instructions**
        i. Send DNA
        ii. Important to look at the format requirements
            1. PCR tubes in a 50ml tube
            2. PCR tube strips in a 50ml tube
            3. 96-well microtiter plate
            4. filter paper grid
            5. NOT standard 1.5ml eppendorf tubes – volume too small
14. Start a new DNA submission
    a. Fill in information about you and your submission
    b. Fill in sample information
        i. BBa_ K294998
        ii. Plasmid – if you are not using a Registry supported plasmid (usually indicated by a pSB number) you need to:
            1. Request a variance for the assembly system that you are proposing (IN ADVANCE – see calendar)
            2. Add your plasmid as a part and document to the level that Registry supported plasmids are documented
        iii. Resistance should be automatically filled-in if you are specifying a Registry supported plasmid
        iv. IF sending plasmid not part you should send with RFP (BBa_J04450) and BBa_P1010.

Notes about cell strains

- Registry has moved to using NEB 10B cells that are T1-phage resistant
    - Please do your work in T1-phage resistant cells as well, especially prior to miniprepping DNA to send to the Registry
    - If you don't want to keep purchasing the cells there are instructions on OpenWetWare on how to make your own competent cells which you can follow for NEB10B cells as well

European Synthetic Biology Workshop Paris
May 31, 2008

Three instructors from iGEM Headquarters and MIT held a synthetic biology workshop at Paris Descartes Medical Facility in Paris May 31, 2008. About 20 instructors and students from European schools attended the workshop. The topics included Synthetic Biology Based on Standard Parts, parameter measurement, how to use the Registry of Standard Biological Parts at MIT, quality control, software tools and information about the iGEM competition. The participants went through the normal processes of designing a system, finding and specifying parts, and submitting their results to the Registry as an "iGEM in 2 Hours" exercise. A group dinner was held the evening of the first day.

The instructors were Randy Rettberg, Director iGEM, Biological Engineering ,Meagan Lizarazo, Assistant Director, iGEM, and Dr. Tom Knight, CSAIL, all from MIT.

Costs for the European Synthetic Biology Workshop Paris 5/31/2009

(This workshop was partially supported by the Howard Hughes Medical Institute, HHMI)

Travel M. Lizarazo	Supported by HHMI
Travel R. Rettberg	$1,633.56
Worhshop Dinner (50 persons)	$278.25
Travel T. Knight	$1,433.30
TOTAL	**$3,345.11**

Agenda for the European Workshop in Paris, 5/31/2008

Time	Duration	Session	Speakers
8:30a	0:30	Registration & Coffee/Rolls	
9:00a	0:30	Introduction to iGEM 2008	Randy Rettberg
9:30a	1:00	Synthetic Biology Based on Standard Parts	Tom Knight
10:30a	0:30	Break	
11:00a	0:30	Parameter Measurement	
11:30a	0:30	Parts, Plasmids, Assembly, Quality Control	Meagan Lizarazo Randy Rettberg
12:00p	1:30	Lunch combined with Team Introduction Activity	
1:30p	1:00	iGEM in 2 Hours - Part 1	Meagan Lizarazo
2:30p	0:30	Break	
3:00p	1:00	iGEM in 2 Hours - Part 2	Meagan Lizarazo
4:00p	0:30	iGEM 2008 Details and Software Tool Track	Randy Rettberg
4:30p	0:30	Awards and Safety	Drew Endy
5:00p	0:30	Discussion	All
5:30p		End of Session	

Attendees for the European Workshop in Paris 5/31/2008

Angeliki Tsokou	TU Munchen
Arnau Montagud	Valencia
Bart Deplancke	EPF-Lausanne
Bastiaan van den Berg	TUDelft
Domenico Bellomo	TUDelft
Emilio Navarro	Valencia
Geoff Baldwin	Imperial
Inge Thijs	KULeuven
Irwin Zaid	TUMunchen
Jean-Louis Giavitto	Paris
Jennifer Hallinan	Newcastle
Jens Keienburg	Heidelberg
Mario di Bernardo	BCCS-Bristol
Osbaldo Resendis-Antonio	LCG-UNAM-Mexico
Oscar Kuipers	Groningen
Paolo Magni	UNIPV-Pavia
Sebastian Maerkl	EPF-Lausanne