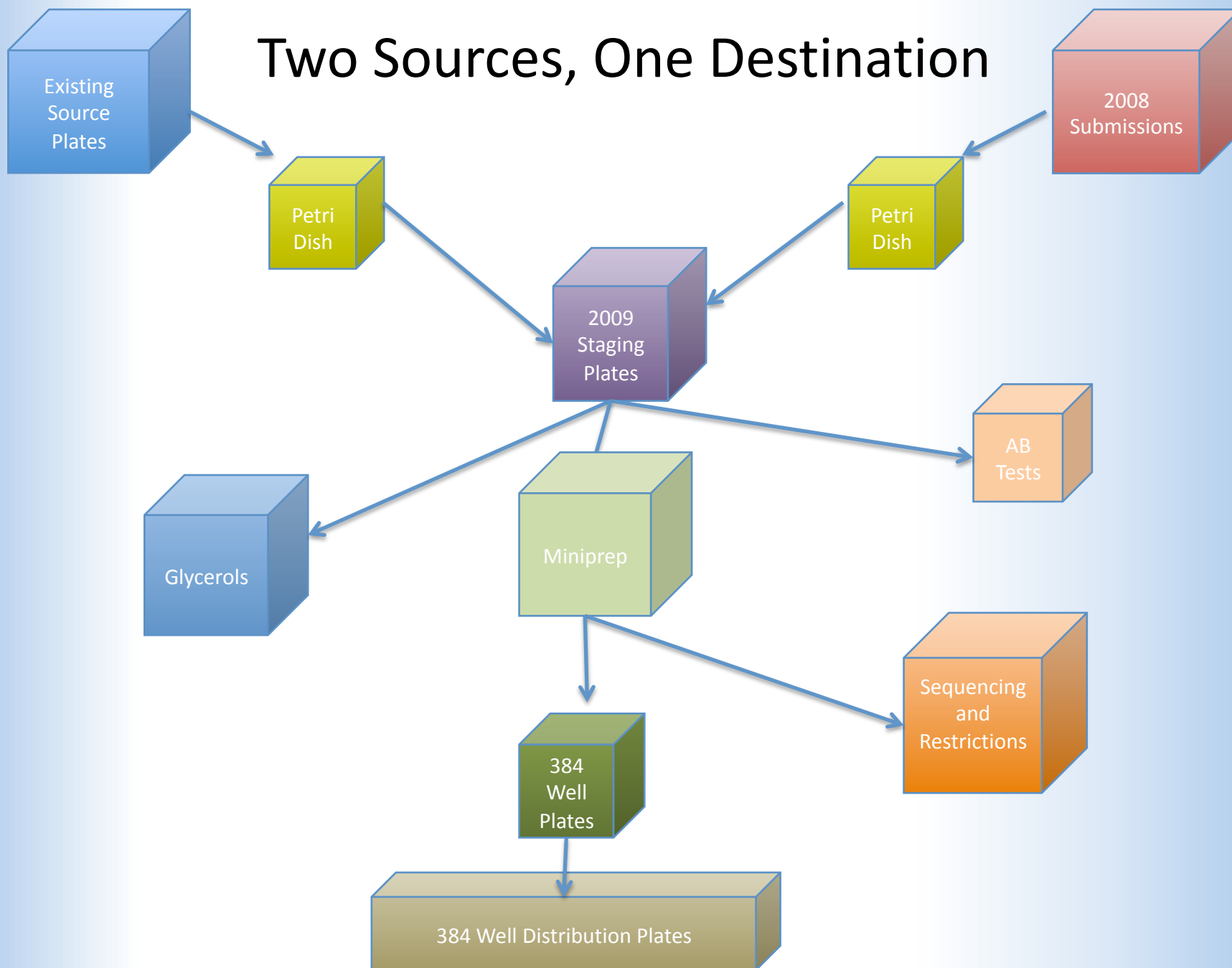


# Two Sources, One Destination

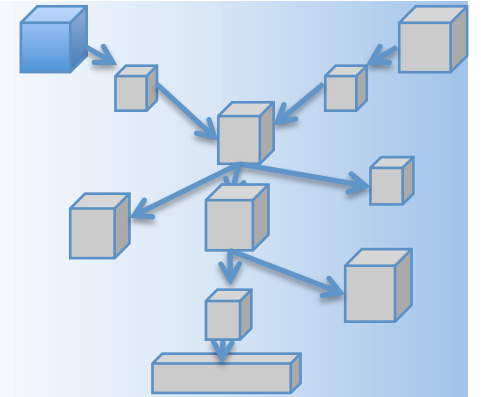


## Existing Source Plates

Parts come in two varieties: those that have been distributed in previous years (and so have been QC'd) and those received from last years teams (which have not been QC'd).

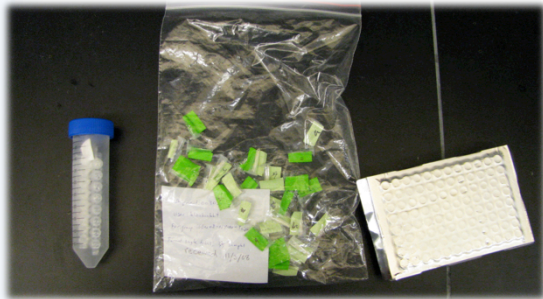


- Previously distributed parts are stored within transformed bacteria and suspended in a 10% glycerol solution in our -80 degree freezer.
- Parts are scraped from these 96 well plates and transferred to Petri dishes for growth.

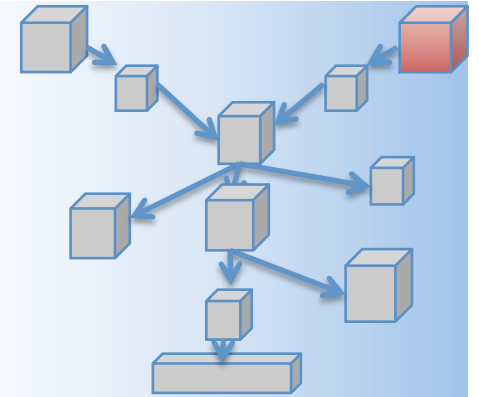


## 2008 Team Submissions

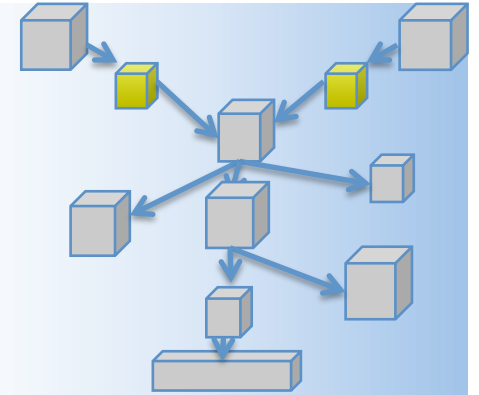
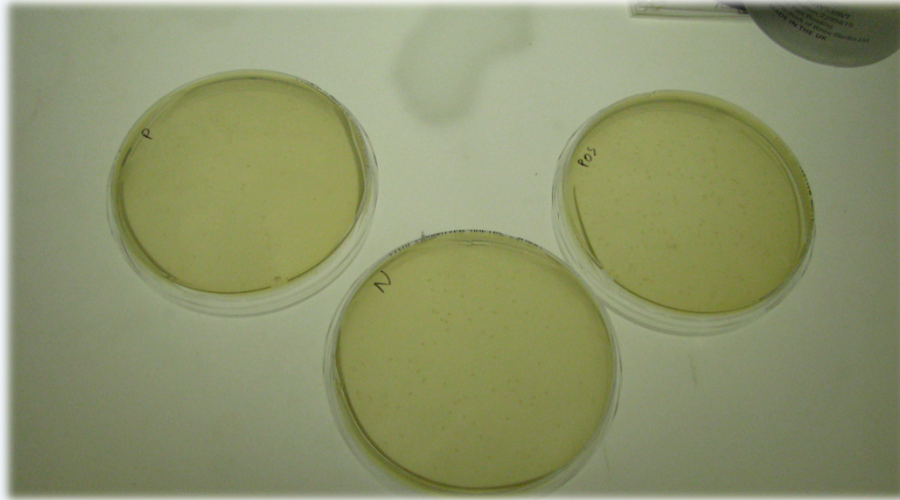
- Parts submitted by teams in last year's competition have been kept as raw DNA in storage at iGEM headquarters.
- Those parts which were selected as favorites by the submitters are being included in this year's distribution.



- These parts were used to transform NEB 10 competent cells in LB broth on 96 well plates.
- After being grown overnight, they were placed on Petri dishes for colony formation.



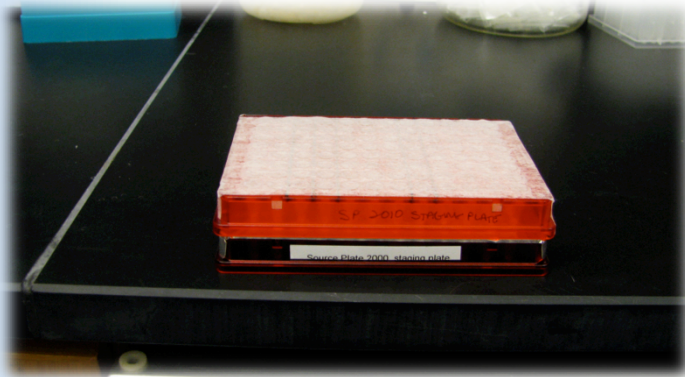
## Colony Growth on Petri Dishes



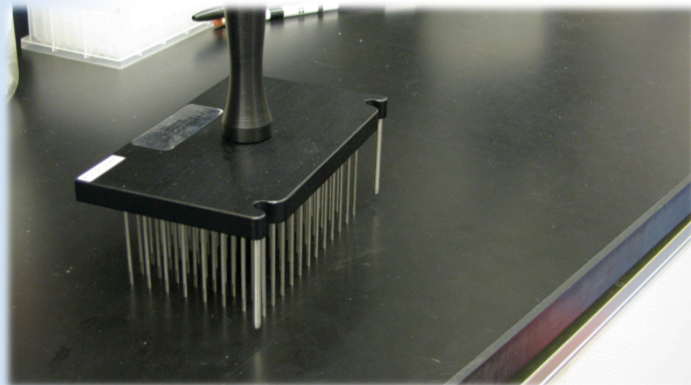
- Regardless of whether parts came from the registry or were submitted by last year's teams, the transformed cells are plated on Petri dishes.
- These Petri dishes are inoculated with one or more antibiotics for which the plasmids containing the parts have resistance.
- Once the e.coli has been allowed to grow overnight at 37 degrees, individual colonies are picked and used to inoculate individual wells filled with LB broth in a 96 well plate.

## Staging Plates

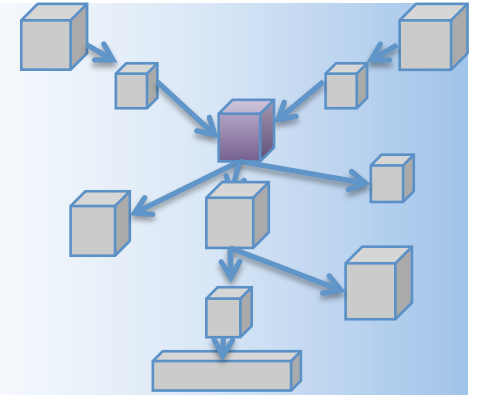
- Once successful colony growth has been observed, single colonies are placed in LB broth on a staging plate.



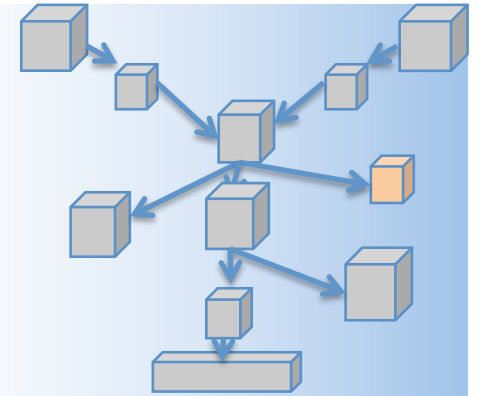
- A 5  $\mu$ L pin tool is then used to transfer transformed bacteria from the staging plate to several 96 well plates with identical well layouts.



- All parts end up in two miniprep plates and two glycerol plates. Additionally, the parts submitted by teams in last year's competition as well as existing parts that have not previously passed QC also end up on antibiotic plates for QC checks.



## Antibiotic Test Plates



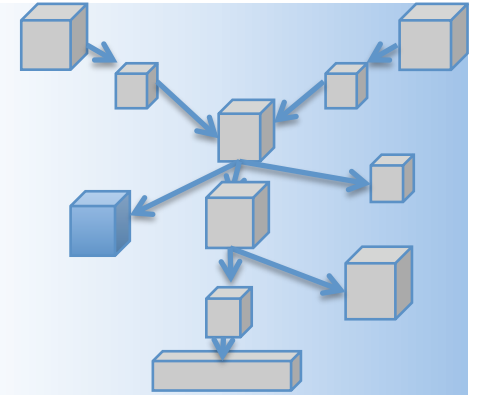
- The antibiotic test plates serve to check the antibiotic resistance of the parts relative to four different antibiotics. These plates are used only for parts that have previously passed QC.
- The antibiotic test plates are part of the quality control process and as such will be discussed in greater detail later.

## Glycerol Plates

The glycerol plates are long term storage for registry parts. After being stamped out with the pin tool, they are left to incubate for 10 hours.

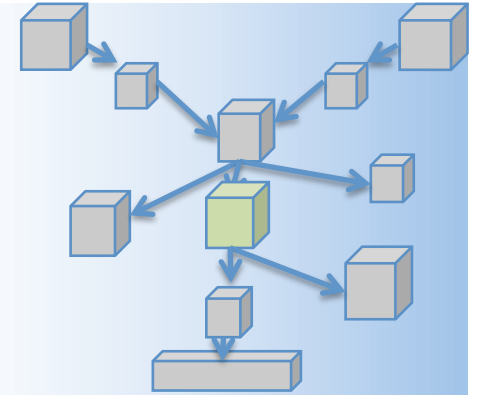


- These 96 wells plates are kept in the minus 80 for future registry use and iGEM competitions, for which they are source plates.
- Two copies of each plate are made to ensure preservation of parts.



## Miniprep Plates

Miniprep plates contain transformed bacteria suspended in 1.8 mL of LB broth- each staging plate produces two identical miniprep plates. After incubating overnight at 37 degrees, the plates underwent automated miniprepping via the Autogen.

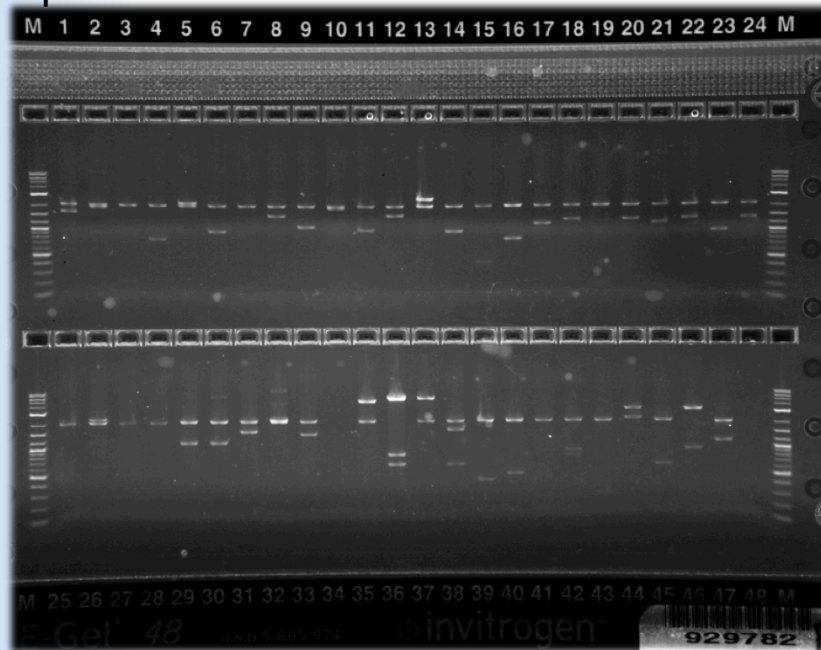


After the miniprep process is completed, the purified DNA is resuspended in TE buffer, and identical plates are combined. 20 uL are put aside for QC purposes, while the remaining 80 proceeds towards eventual distribution.

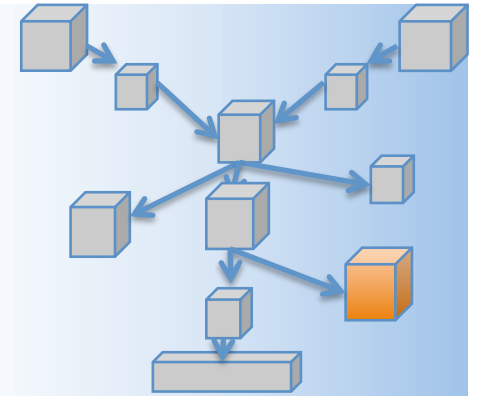


## Sequencing and Restriction Digests

- Two quality control processes using the remaining DNA left over from the miniprep stage.
- Parts are being sequenced externally by Genewiz to confirm they match expected nucleotide sequence. This requires 50 ng of purified DNA.

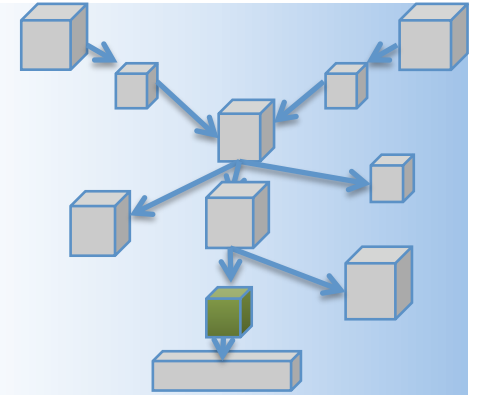


- Parts not used in previous years, and those which had not been previously QC'd, have also undergone restriction enzyme digests to confirm part sizes are accurate and that DNA is at sufficient concentration.



## Consolidation of 384 Well Plates

The bulk of the DNA solution is diluted in TE with Cresol Red dye.



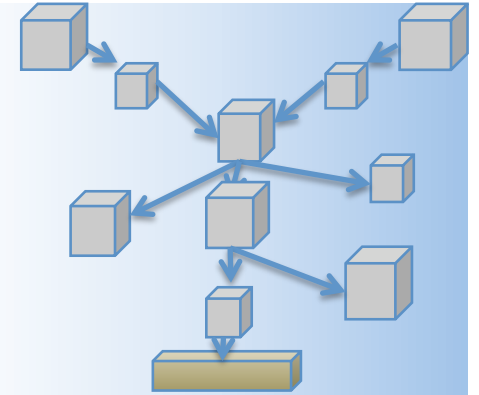
- This diluted DNA is distributed into four identical copies of the 384 well distribution plate by the epMotion 5075 robot.
- After this, the parts are in their final configuration for distribution, and all that remains is to mass-produce the plates and mail them out.

## 384 Well Distribution Plates

The distribution plates have been mass produced (~200x each) by use of the PlateMate Plus.

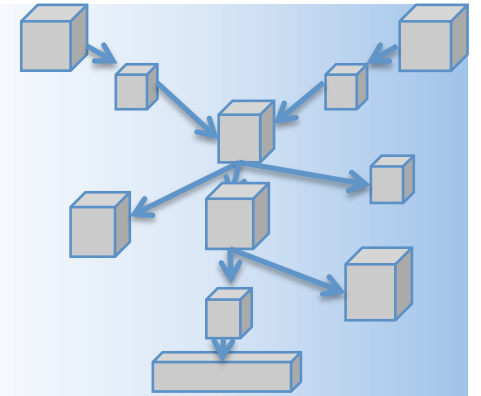


- The PlateMate Plus aspirates DNA from the 384 deep well plates produced by the ep Motion and dispenses it into each well of the plates to be distributed.
- These plates are then dried down for distribution.



## Distribution and Wrapping up

With all the labwork finished, all that remained was to box up the parts (along with some iGEM swag) and mail them out.



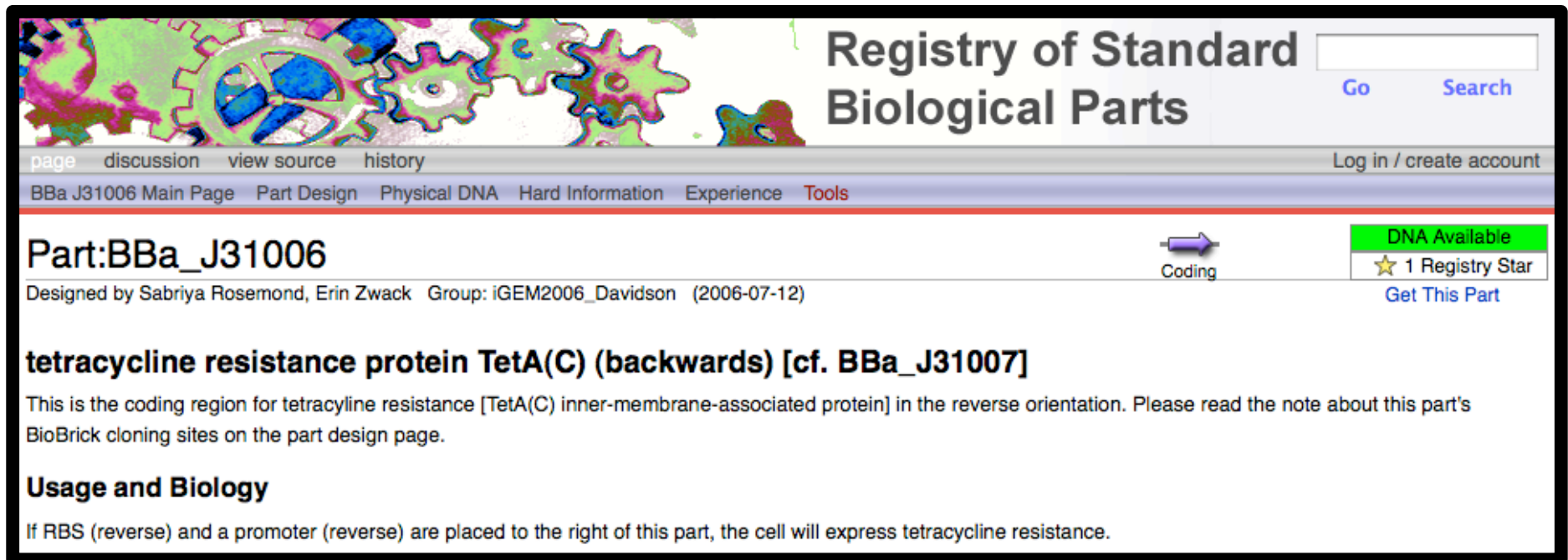
The 2009 parts have now been sent out to this years teams!

# Using the Registry

searching for parts and quality control information

# 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**.



The screenshot shows the Registry of Standard Biological Parts website. At the top, there is a search box with "Go" and "Search" buttons. Below the search box, there are navigation links: "page", "discussion", "view source", and "history". On the right side, there is a "Log in / create account" link. The main content area displays "Part:BBa\_J31006" with a "Coding" icon (a purple arrow) and a "DNA Available" status (a green box). Below this, there is a "★ 1 Registry Star" and a "Get This Part" link. The description of the part is "tetracycline resistance protein TetA(C) (backwards) [cf. BBa\_J31007]". The text below the description reads: "This is the coding region for tetracycline resistance [TetA(C) inner-membrane-associated protein] in the reverse orientation. Please read the note about this part's BioBrick cloning sites on the part design page." The "Usage and Biology" section states: "If RBS (reverse) and a promoter (reverse) are placed to the right of this part, the cell will express tetracycline resistance."

# 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.

-?-	Name	Description	Promoter Sequence	Positive Regulators	Negative Regulators
1★	<a href="#">BBa_I721001</a>	Lead Promoter	. . . gaaaacctgtcaatgaagagcgatctatg		
	<a href="#">BBa_I731004</a>	FecA promoter	. . . ttctcggtcgactcatagctgaacacaaca		
	<a href="#">BBa_I760005</a>	Cu-sensitive promoter	atgacaaaattgcat		
A	<a href="#">BBa_I765000</a>	Fe promoter	. . . accaatgctgggaacggccagggcacctaa		
A	<a href="#">BBa_I765007</a>	Fe and UV promoters	. . . ctgaaagcgataccgctatggagggggtt		
1★	<a href="#">BBa_J3902</a>	PrFe (PI + PII rus operon)	. . . tagatatgcctgaaagcgataccgctatg		

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 eventually arrive at 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 recognition 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	<a href="#">2009 Kit Plate 2</a>	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](mailto:hq@igem.org) with the part name, the institution you represent, and your mailing address.



# Where to find QC information: Overview

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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	<a href="#">2009 Kit Plate 1</a>	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	
<a href="#">Sequencing: Confirmed</a>	Resistance: A	<a href="#">Gel: OK Q: OK P: OK</a>	Spring 2008>Source Plate 1004>7A	



# Where to find QC information: Details

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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

<a href="#">Get antibiotic files for this plate</a> <a href="#">Get an Excel file for this plate</a>	<b>Gel Images and Results</b> <a href="#">Wells 1A thru 6H</a> <a href="#">Wells 7A thru 12H</a> <b>Sequencing and Results</b> <a href="#">Go to sequencing</a>	<b>Plate Images and Results</b> <a href="#">Image 00162: Antibiotic A</a> <a href="#">Image 00161: Antibiotic C</a> <a href="#">Image 00164: Antibiotic K</a> <a href="#">Image 00150: Growth other</a> <a href="#">Image 00151: Growth other</a> <a href="#">Image 00152: Growth other</a> <a href="#">Image 00163: Antibiotic T</a>
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- ▶ 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

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- ▶ 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

8G BBa\_I7106 pSB4A3 MC4100 (LacI-)

QC: Sequence Inconsistent

Resistance: AT

Gel: BAD Q: Low P: OK

# Sequencing Results

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

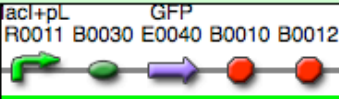
8G	BBa_I7106	pSB4A3	MC4100 (LacI-)	Resistance: AT	Gel: BAD Q: Low P: OK
QC: <a href="#">Sequence</a> <b>Inconsistent</b>					

- ▶ 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: <a href="#">BBa_I7106</a> (length: 941bp)	Linked to part info page	
lacI+pL GFP R0011 B0030 E0040 B0010 B0012		
		

Sequence 2788 (QC08\_P628\_W39939\_VF)

VF	Length: 1489bp	Blast against:	<a href="#">BBa_I7106</a>	<a href="#">Basic Parts</a>	<a href="#">All Parts</a>
Get machine files: ( <a href="#">Sequence</a> ) ( <a href="#">Trace</a> )		Get Phred files: ( <a href="#">Sequence</a> ) ( <a href="#">Quality</a> ) ( <a href="#">Trace</a> )			
BB Prefix found at 91					
<input checked="" type="checkbox"/> Use inside sequence (1376bp)					

# Restriction Digests

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- ▶ We did restriction digests on parts using EcoRI and PstI 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 / ???

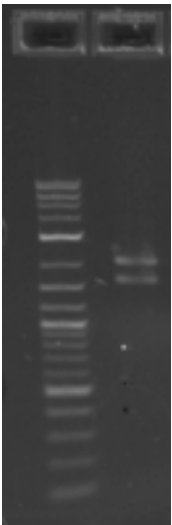
Distribution	Plate	Well	Plasmid	Resistance
Spring 2009 Distribution	2009 Kit Plate 1	13F	pSB1A2	
	DNA QC from 7A-Spring 2008 -> Source Plate 1004			
Sequencing:	Resistance	Gel:	Spring 2009 Distribution>2009 Kit Plate 1>13F	
Sequencing:	Resistance:	Gel:	Spring 2009 Source Plates>S09 SP 2002>7C	
Sequencing: <b>Confirmed</b>	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.

<a href="#">Get antibiotic files for this plate</a> <a href="#">Get an Excel file for this plate</a>	<b>Gel Images and Results</b> <a href="#">Wells 1A thru 6H</a> <a href="#">Wells 7A thru 12H</a> <b>Sequencing and Results</b> <a href="#">Go to sequencing</a>	<b>Plate Images and Results</b> <a href="#">Image 00162: Antibiotic A</a> <a href="#">Image 00161: Antibiotic C</a> <a href="#">Image 00164: Antibiotic K</a> <a href="#">Image 00150: Growth other</a> <a href="#">Image 00151: Growth other</a> <a href="#">Image 00152: Growth other</a> <a href="#">Image 00163: Antibiotic T</a>
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# Evaluating the Gel Results

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- ▶ 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.

Lane	Well	Insert	Plasmid	Lengths (As cut)		Insert Result	Plasmid	
				Insert	Plasmid		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 EcoRI and PstI 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.



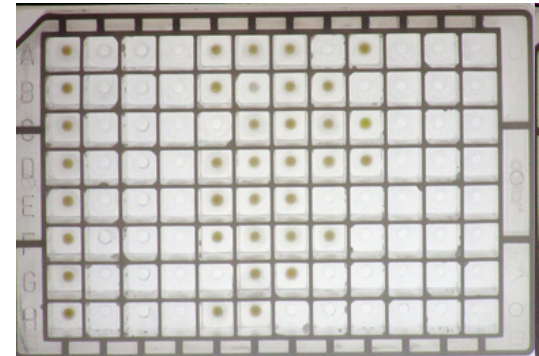


# Antibiotic Test Plates

- ▶ 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?

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- ▶ 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

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- ▶ 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.

