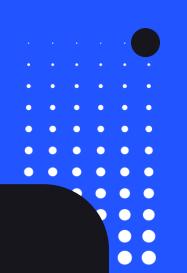
Exploring the iGEM Distribution







Overview



Getting Started

Introduction, information and structure

Data Exploration & Analysis

Diving deep into the Registry, analyzing how the distribution is used by teams

Suggestions

Files and software demo

Discussion

IGEM wiki tools search PRODUCTION 2017 SERVER Registry of Standard Biological Parts tools catalog repository assembly protocols help search **DNA Repository Plates and Boxes** Physical DNA is held in tubes in freezer boxes or multi-well plates. This program manages the contents of boxes and plates. Label: 2021 Kit Plate 1 ID: 6411 Description: Spring 2021 DNA Distribution Kit Plate 1 384-Well Plate 2021-07-02 10:59:48 Location: Substance: DNA OK Plate Status: Aliquot: Checked if this plate contains unprocessed samples from its source plate. Get antibiotic files for this plate Gel Images and Results Plate Images and Results Get an Excel file for this plate Wells 1A thru 6H Add a plate image Get a detailed Excel file for this plate Wells 7A thru 12H Sequencing and Results Go to sequencing Contents Resist. Cell Well Part Plasmid Comments Quality control information: Sequencing, Antibiotics, Restriction Digests 1A BBa K314110 pSB1C3 E. coli strain NEB 10-beta 1B BBa K731722 pSB1C3 E. coli strain NEB 10-beta E. coli strain NEB 10-beta#1 - 520 ng 1C BBa K398326 pSB1C3 1D BBa_K731722 pSB1C3 E. coli strain NEB 10-betal was not able to remove this line from the list. It is the same sample as #21 1E BBa_K398331 pSB1C3 E. coli strain NEB 10-beta#2 - 990 ng E. coli strain NEB 10-beta may be toxic for gramm negativ bacteria due to lipase activity if its expressed into 1F BBa_K808025 pSB1C3 perplasma or surface expressed 1G BBa K314100 pSB1C3 E. coli strain NEB 10-beta 1H BBa K808001 pSB1C3 E. coli strain NEB 10-beta 11 BBa K314101 pSB1C3 E. coli strain NEB 10-beta 1J BBa_K808003 pSB1C3 E. coli strain NEB 10-beta 1K BBa K314201 pSB1C3 E coli strain NEB 10-beta 1L BBa_K808010 pSB1C3 E. coli strain NEB 10-beta 1M BBa_K314202 pSB1C3 E. coli strain NEB 10-beta 1N BBa K808011 pSB1C3 E. coli strain NEB 10-beta 10 BBa_K346002 pSB1C3 E. coli strain NEB 10-beta PmerT promoter 1P BBa_K808013 pSB1C3 E. coli strain NEB 10-beta 2A BBa K548000 pSB1C3 E. coli strain NEB 10-beta

E. coli strain NEB 10-beta

2B BBa_K325210 pSB1C3

Parsing the data

Files are not Excel but CSV files

Some software applications like Excel and Pandas have problems parsing the file even though the file seems to use RFC4180

Deleting the space between commas and double quotes can easily fix this problem

```
---> 1 test df = pd.read CSV("old formating.CSV")
c:\users\cedkb\appdata\local\programs\python\python37\lil
p, delimiter, header, names, index col, usecols, squeeze
se values, skipinitialspace, skiprows, skipfooter, nrows
se dates, infer datetime format, keep date col, date par
s, decimal, lineterminator, quotechar, quoting, doubleque
lines, delim whitespace, low memory, memory map, float pr
    687
--> 688
            return read(filepath or buffer, kwds)
    689
    690
c:\users\cedkb\appdata\local\programs\python\python37\lil
    458
    459
            try:
                data = parser.read(nrows)
--> 460
    461
            finally:
    462
                parser.close()
c:\users\cedkb\appdata\local\programs\python\python37\lil
            def read(self, nrows=None):
   1196
                nrows = validate integer("nrows", nrows
   1197
-> 1198
                ret = self._engine.read(nrows)
   1199
                # May alter columns / col dict
   1200
c:\users\cedkb\appdata\local\programs\python\python37\lil
   2155
            def read(self, nrows=None):
   2156
-> 2157
                    data = self. reader.read(nrows)
                except StopIteration:
   2158
                    if self. first chunk:
   2159
pandas\ libs\parsers.pyx in pandas. libs.parsers.TextRead
pandas\ libs\parsers.pyx in pandas. libs.parsers.raise pa
ParserError: Error tokenizing data. C error: Expected 17
```

All ccdb parts have been withdrawn from the Registry. Samples of parts containing the ccdB gene cannot be requested. - iGEM HQ

Do the csv files match the data on the website?

They do in fact differ slightly. The csv file contains more entries than the website

The parts in question are:

BBa_K581008

BBa_K145151

BBa_K805013

BBa_K658001

All of these include the ccdB gene which was removed from the registry due to patent issues

CSV overview

Duplicate Columns:

Plasmid

Empty Columns:

Resistance, Gel Overall, Quantity, Seq Comment

Just one unique value:

Sequencing, Well Status

Columns

Plate

Well

Part

Plasmid

Well Status

Comments

Type

Subparts

Source

Gel Overall

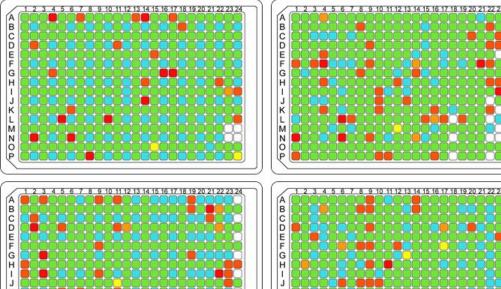
Quantity

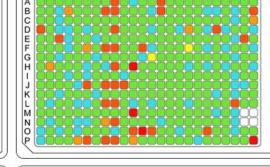
Plasmid

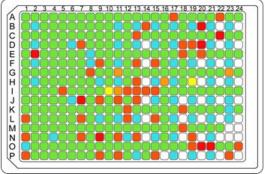
Sequencing

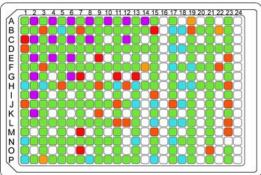
Seq Comment

Short Description









Sequencing





- Long Part length of sequence reads are insufficient to cover the middle of the part
- Inconsistent part does not match its
- target sequence, may have a single bp mutation or not match at all
 - Bad Sequence usually caused by low
 - DNA concentration or incorrect primers
- Single Error
 - No Sequencing Information

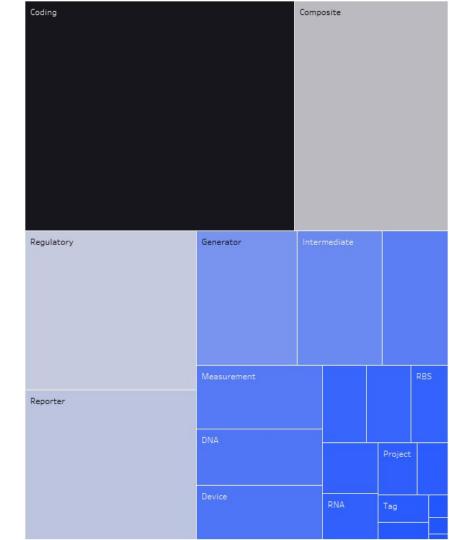
GH

Sequencing II

Differentiate between confirmed and all others categories

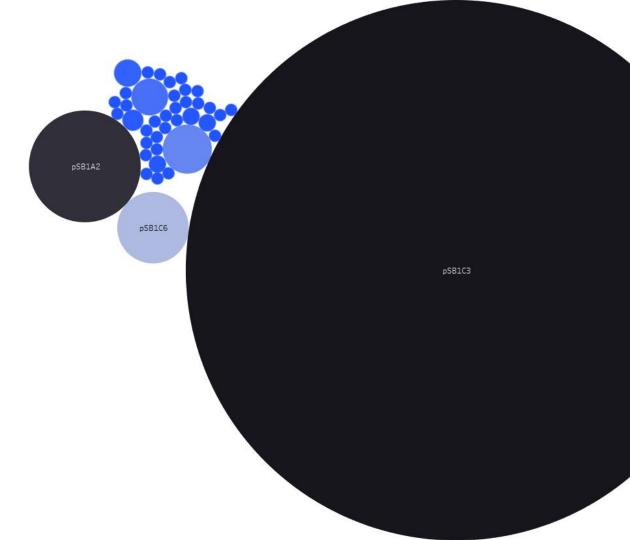
Type of parts

Coding Composite Regulatory Reporter Generator Intermediate Translational_Unit Measurement DNA Device Terminator Signalling RBS Protein_Domain RNA Project Other Tag Inverter Conjugation T7	577 329 254 239 126 106 82 76 66 63 32 27 26 24 19 15 13 8 4
Temporary	1



Plasmid

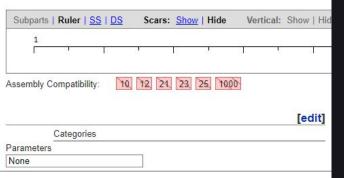
pSB1C3	1936
pSB1A2	83
pSB1C6	34
BBa_J61002	16
pSB1C00	9
pSB3K3	5
pSB1A3	3
pSB1AK3	2
BBa_J63009	2
pSB1AK8	2
BBa_P10501	1
pSB2K3	1
pSB1K01	1
BBa_P10506	1
pSB4K5	1
pSB1K3	1
pSB3C04	1
pSB1K04	1
pSB6A1	1
pSB3C02	1
pSB1AT3	1
pSB1K03	1
pSB3C01	1
BBa_J63010	1
BBa_P10503	1
BBa_P10504	1
BBa_P10507	1
pSB1K02	1
pSB1T3	1
pSB1AC3	1
BBa_P10502	1
pSB4C5	1
pSB3C5	1
pSB1A10	1
pSB3T5	1
BBa_P10505	1
pSB4A5	1
BBa_J23006	1
BBa_P10508	1
pSB3C03	1



GoldenBraid destination vector pDGB3 alpha1R

More information to come

Sequence and Features



Note: The sequence below is incorrect because the backbone that the SP device was inserted into was psb1A2 rather than psb1A3, so there is a terminator (not annotated) in the sequence below at position 1044-1121 that isn't actually present in the DNA of psb1A10. Updating this will take some time because I need to change all the feature locations by hand - but the Vector NTI file is correct.

Some of the plasmids that are used lack basic data

Some of the plasmids used in the distribution plate lack either:

- General information
- Information about the resistance
- Sequence annotations

Or everything of the above.

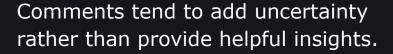
Additionally, some of the information may also be incorrect

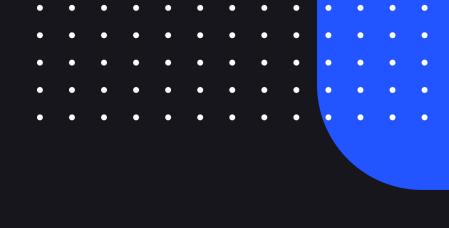
IGEM PRODUCTION 2017 SERVER Registry of Standard Biological Parts tools catalog repository assembly protocols help search BBa_ Repository: Eppendorf Files for Broth Plates Show all plates Label: 2021 Kit Plate 1 ID: 6411 Spring 2021 DNA Distribution Kit Plate 1 384-Well Plate Description: Location: 2021-07-02 10:59:48 Substance: DNA Well Part Plasmid Cell Unexpected Plasmid: pSB1C3 in this well: E. coli strain NEB 1A BBa K314110 pSB1C3 10-beta Unexpected Plasmid: pSB1C3 in this well: E. coli strain NEB 1B BBa K731722 pSB1C3 Unexpected Plasmid: pSB1C3 in this well: E. coli strain NEB #1 - 520 ng 1C BBa K398326 pSB1C3 10-beta Unexpected Plasmid: pSB1C3 in this well: E. coli strain NEB I was not able to remove this line from the list. It is the same sample as #21 1D BBa K731722 pSB1C3 Unexpected Plasmid: pSB1C3 in this well: E. coli strain NEB #2 - 990 ng 1E BBa K398331 pSB1C3 Unexpected Plasmid: pSB1C3 in this well E. coli strain NEB may be toxic for gramm negativ bacteria due to lipase activity if its expressed into perplasma or 1F BBa K808025 pSB1C3 10-beta surface expressed Unexpected Plasmid: pSB1C3 in this well E. coli strain NEB 1G BBa K314100 pSB1C3 10-beta Unexpected Plasmid: pSB1C3 in this well E. coli strain NEB 1H BBa_K808001 pSB1C3 10-beta Unexpected Plasmid: pSB1C3 in this well E. coli strain NEB 1I BBa_K314101 pSB1C3 Unexpected Plasmid: pSB1C3 in this well E. coli strain NEB 1J BBa_K808003 pSB1C3 10-beta Unexpected Plasmid: pSB1C3 in this well: E. coli strain NEB 1K BBa_K314201 pSB1C3 Unexpected Plasmid: pSB1C3 in this well:

E. coli strain NEB

Schrödinger Parts

Some of the comments suggest that the part is in a different plasmid than officially stated.





10H	BBa_E0020	pSB1C00	pSB1C5 is a Type IIS Plasmid for Level 0 parts
10J	BBa_E0030	pSB1C00	pSB1C5 is a Type IIS Plasmid for Level 0 parts
10L	BBa_J97004	pSB1C00	pSB1C5 is a Type IIS Plasmid for Level 0 parts
10N	BBa_J97005	pSB1C00	pSB1C5 is a Type IIS Plasmid for Level 0 parts
10P	BBa_J97006	pSB1C00	pSB1C5 is a Type IIS Plasmid for Level 0 parts

15D	BBa_I14044	pSB1C3	E. coli strain NEB 10-beta Plasmid unknown, AmpR
15E	BBa_K863020	pSB1C3	E. coli strain NEB 10-beta
15F	BBa_I13013	pSB1C3	E. coli strain NEB 10-beta Plasmid unknown, AmpR

Sorry for the Plasmid Backbone being pSB1A2 because of having no time to transfer.

404

File not found

What about files?

A *in silico* version of the distribution (i.e. GenBank files) would be helpful for both exploring as well as cloning parts of interest

Sadly, iGEM does not offer the possibility to download such files

Furthermore, the linked part tool with which one could download a part as a genbank file is no longer accessible

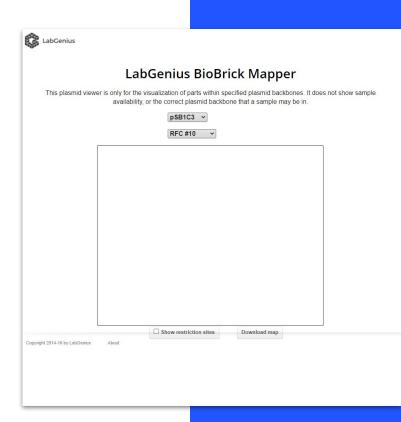
Plasmid Mapper

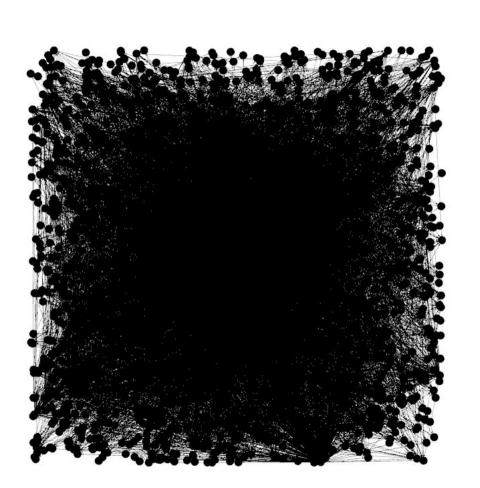
Although the registry links to a plasmid mapper, it is not a substitute for raw data

It doesn't feature the actual plasmid maps, it only creates a custom in silico cloning of the part

Contains less than half the plasmids used in the distribution

It's currently broken for all major browsers





Relationship between parts in the distribution

Embedded all of the parts into a directed graph

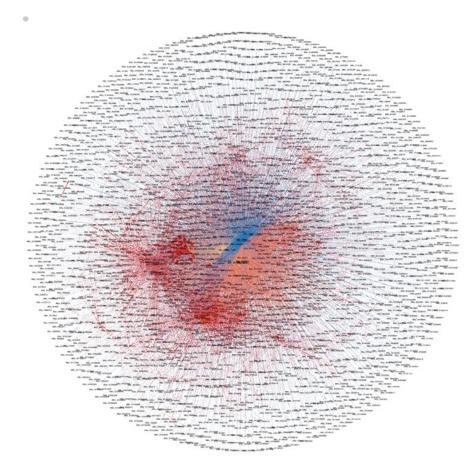
The resulting graph consists of 1,985 edges and 10,236 vertices

Graph

To better visualize the graph we utilize the force-directed graph algorithm from Fruchterman & Reingold

The resulting graph is surprisingly connected

One part in particular stands out, it seems to be included in every other part



Mysterious Part

A rather specialized part without defined uses

Only consist of 1bp... A

This false sequence occurrence both on the registry site as well as the registry database dump

Part:BBa K1497024

Designed by: Sascha Hein, Rene Sahm Group: iGEM14_TU_Darmstadt (2014-10-07)

GBD-Domain

The GBD domain and its ligand (BBa_K771106) are suitable tools for protein colocalizaion. Initially, the domain was a part of the N-WASP protein (GTPase binding domain) in *Rattus rattus*. The GBD domain is used as a binding unit of the so-called protein scaffold published by Dueber et al. in 2009. The scaffold (BBa_K1497033) is composed of different binding units, which enable the assembly of multiple target proteins. The Domain was initially edited by Team BioX-Shanghai 2012 (BBa_K771105). The iGEM Team TU Darmstadt 2014 modified this BioBrick by adding a Bglll and BamHI restriction site in front of and behind the previously constructed domain sequence and codon optimized it for expression in *E. coli*.

Now, different binding units of the scaffold protein can be fused together without the introduction of restriction sites. This allows the easy construction of BioBricks of different permutations of the scaffold protein domains.

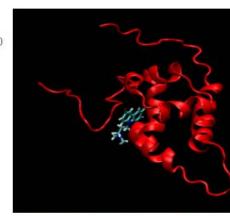


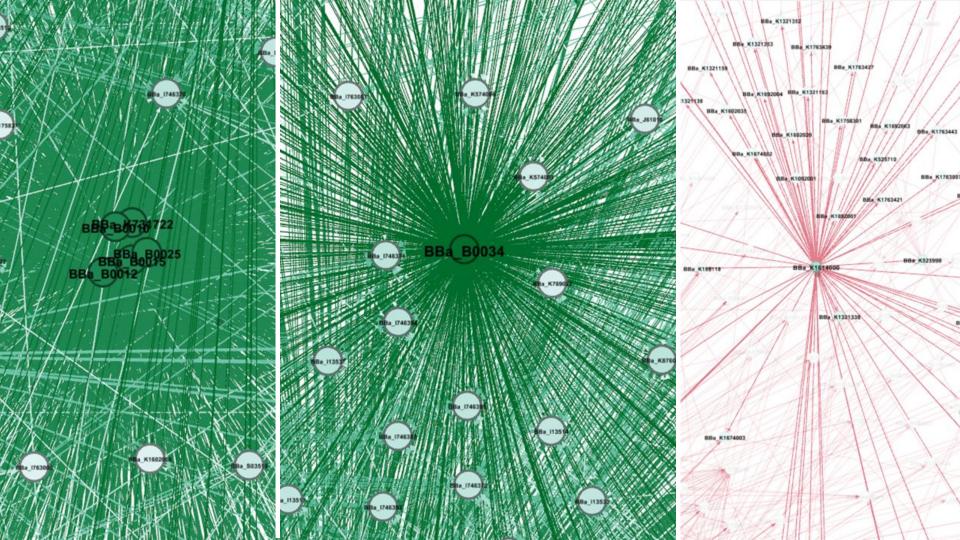
Figure 1 Crystal structure of the GBD domain from the protein (red) of Rattus rattus binding the small molecule (1-(3,6-Dibromo-carbazol-9-yl)-3-dimethylamino-propan GBD domain is locked by Wiskostatin in its autoinhibited conformation (Peterson et al. 2004). PDB entry 1T84.

Sul	bparts [Ruler SS DS	Scar	s: Show Hide	Ver	tical: Show	Hide	Length: 1 bp	
1	1 a	11	21	31	41	51	61	71	81
	00000								

Assembly Compatibility:

Duplicate Wells

<u>Part</u>	<u>Count</u>	<u>Name</u>	<u>Uses</u>
BBa_J04450	40	RFP Coding Device	<mark>69</mark>
BBa_E0240	5	GFP Generator	293
BBa_R0040	4	TetR Repressible Promoter	1081
BBa_P10599	4	GoldenBraid destination vector alpha insert	0
BBa_P10598	4	GoldenBraid destination vector omega insert	0
BBa_J04455	4	RFP Selection Device for Even Level Loop Type IIS Parts	0
BBa_J04454	4	RFP Selection Device for Odd Level Loop Type IIS Parts	0
BBa_E0020	3	Cyan Fluorescent Protein	95
BBa_I20270	3	Promoter MeasKit	2
BBa_J364000	3	Test Device 1 for the iGEM InterLab Study	8



Parts w/ same sequence but different name

```
amilCP blue/purple chromoprotein (incl RBS)
['BBa K592025', 'BBa K1033930']
Screening plasmid intermediate
['BBa_K1357010', 'BBa_I13507']
Promoter (luxR & HSL regulated -- lux pL)
['BBa R0063', 'BBa K783024']
green fluorescent protein derived from jellyfish
['BBa K895006', 'BBa E0040']
CFP
['BBa E0020', 'BBa K1418040']
RBS30
['BBa B0030', 'BBa K1789002']
RFP Selection Device for Level 0 Type IIS Parts
['BBa J04450', 'BBa J04452', 'BBa J04455',
'BBa J04454']
dCBD with N-terminal linker in RFC 25
['BBa K1321340', 'BBa K1321347']
```

```
MoClo format of BBa J23112 with AB fusion
['BBa K1114003', 'BBa K1114014']
pTetR-lasR-Term-Term
['BBa K876015', 'BBa K876057']
pSB-AraC-pBAD
['BBa I0500', 'BBa K1321333']
constitutive promoter family member
['BBa K823007', 'BBa J23103', 'BBa J23112']
['BBa K823010', 'BBa_J23113']
['BBa K823013', 'BBa J23117']
['BBa K823006', 'BBa J23102']
['BBa K823004', 'BBa J23100']
['BBa_K823014', 'BBa_J23118']
'BBa K823005', 'BBa_J23101']
['BBa K823008', 'BBa J23106']
['BBa K783032', 'BBa J23115']
['BBa K823011', 'BBa K783031', 'BBa J23114']
```

Twin Parts

"Two or more parts are twins if they have the same DNA sequence"

Although twin parts should generally be avoided, there are parts that have over 50 twins

This makes it more difficult to collect all of the necessary data

```
▼ <twins>
   <twin>BBa J34805</twin>
   <twin>BBa_J70612</twin>
   <twin>BBa J72049</twin>
   <twin>BBa K783020</twin>
   <twin>BBa M36912</twin>
   <twin>BBa K1045009</twin>
   <twin>BBa T6000</twin>
   <twin>BBa K1520029</twin>
   <twin>BBa K1685000</twin>
   <twin>BBa K2042020</twin>
   <twin>BBa K1972011</twin>
   <twin>BBa K2023000</twin>
   <twin>BBa S05347</twin>
   <twin>BBa_K1903000</twin>
   <twin>BBa K2175002</twin>
   <twin>BBa K2101000</twin>
   <twin>BBa K2406215</twin>
   <twin>BBa_K2560035</twin>
   <twin>BBa K3136005</twin>
   <twin>BBa K3228005</twin>
   <twin>BBa K3296007</twin>
   <twin>BBa K3158004</twin>
   <twin>BBa K3135007</twin>
   <twin>BBa K2969993</twin>
 </twins>
```

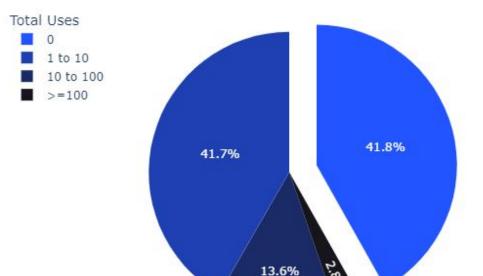
```
T7 Promoter
    T7 promoter (strong promoter from T7 bacteriophage)
          UNS 3 Sequence, from Torella et al., 2013
                           hPCD
                          flvPCD
                          fshPCD
          UNS 2 Sequence, from Torella et al., 2013
                   yeast GAL1 promoter
                 Medium strength T7.2 RBS
                 T7 consensus -10 and rest
              QPI (B0034.C0051.B0015.R0051)
              QPI (B0034.C0040.B0015.R0040)
             TetR repressed POPS/RIPS generator
                         WM Pad1
                         WM Pad2
                      Strong T7.2 RBS
             ADH1 terminator from S. cerevisiae
                     Linker b (BamH I )
              QPI (B0034.C0012.B0015.R0011)
                    SH3 domain + Linker
                   PDZ domain + Linker
                          Linker h
       ECK120029600 - Escherichia coli K-12 terminator
                          His tag
                  attB1 recombination site
                           sfGFP
                           VP64
           Terminator (artificial, small, %T~=85%)
                     MoClo RBS B0034
                   [rnpB-T1] Terminator
N-terminal start overhang (T)(A)-(G)ATG=RBS+Start RFC[105] A
 Linker e( cutting site of Prescission Protease)
 hixC binding site for Salmonella typhimurium Hin recombinase
                   T7 R0.3 RNAseIII site
              Transcription Terminator (Strong)
           scaffold of sqRNA in CRISPR/Cas system
         Linker a( Nde I and Nhe I)
                         Sumo tag
                      Terminator (His)
```

Stop codon free GFP in RFC[23] standard hU6 Promoter

Most used parts that are not in the distribution

Analyzed the usage of parts in both the distribution and the entire registry

While some of the most commonly used parts are missing, others are included in the registry under a different name which can further complicate their use



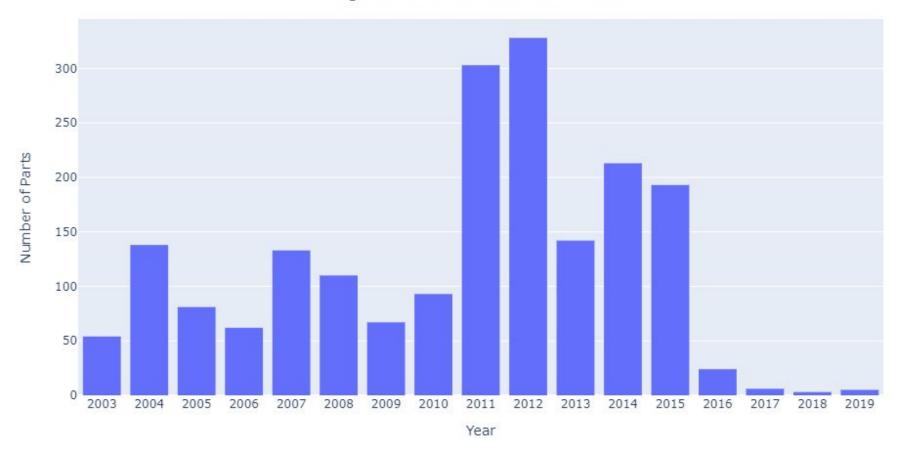
Most parts are barely used

Including all of the twin parts, the majority of parts in the distribution are rarely used

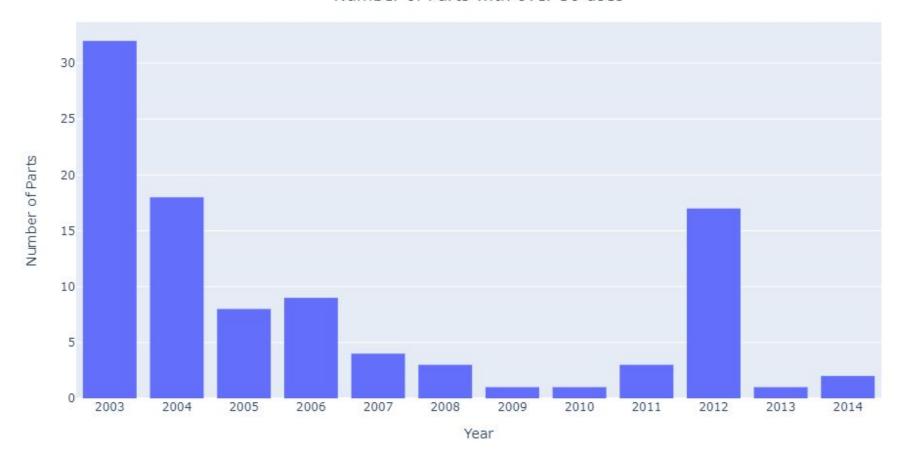
818 (41.8%) haven't been used at all

1633 (83.5%) have been used less than 10 times

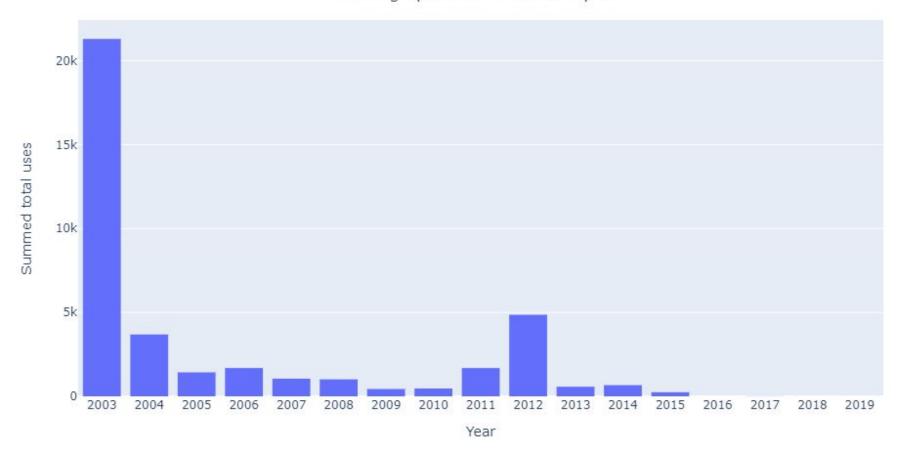
Age of the Parts in the distribution

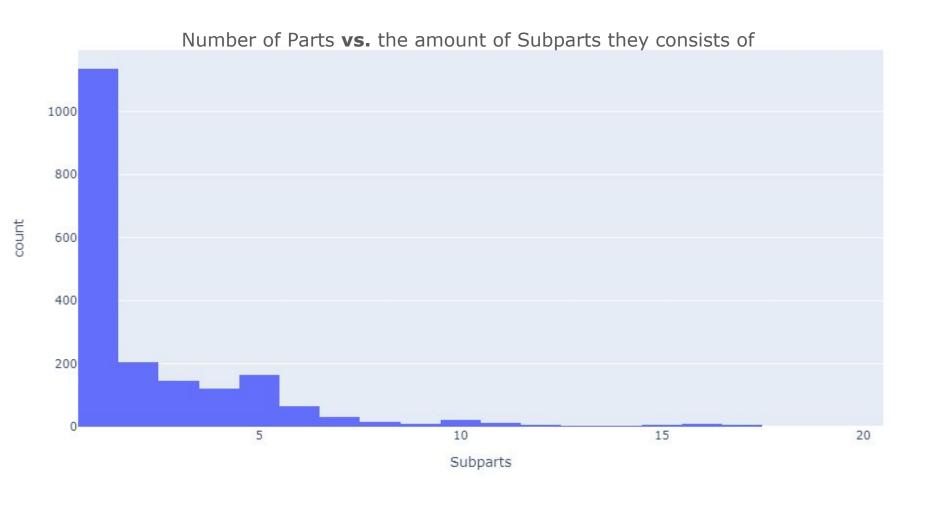


Number of Parts with over 50 uses



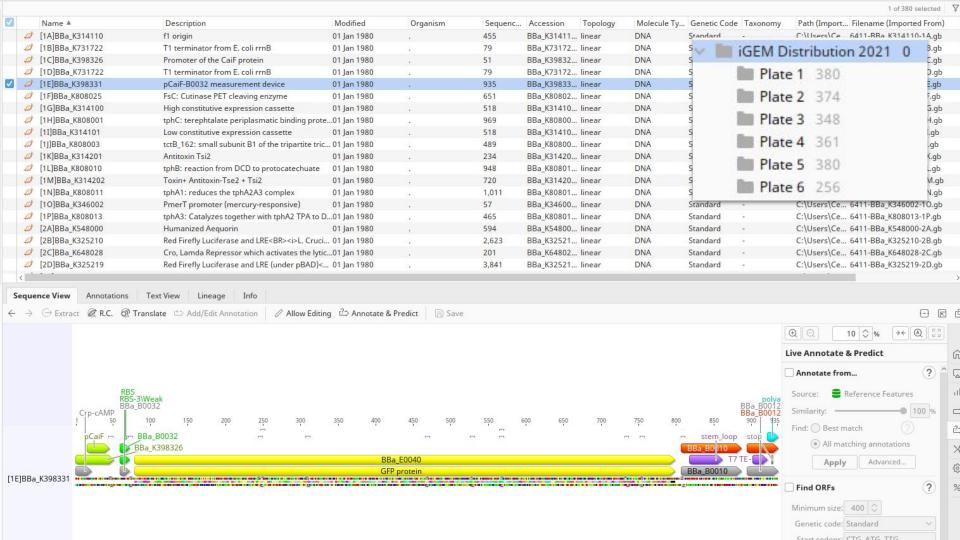
Adding up the uses for each year





Number of Parts with over 10 uses **vs.** Subparts Subparts

Examples



GFP

ar...

O 14. pSB1A3

O 15. pSB1A10

AmpR

ori

mRFP1

Show Description

Show sequence numbers

v J=

Discussion