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DIVA Design instructions SOP (User's section)

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LBNL (ABF) Agile BioFoundry


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ABSTRACT

This is a "how to" step-by-step instruction for creating *Projects* and *Designs* in DIVA for automated design of oligos, plasmids and build instructions. User can either build plasmids on their own or submit the final Design for review/PI approval and third party (e.g., DIVA team) construction pipeline. DIVA also allows for tracking Design progress and is linked to ICE (a repository for parts, plasmids and strains used as Design input and/or construction output in DIVA).

EXTERNAL LINK

http://diva.agilebiofoundry.org

PROTOCOL CITATION

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BEFORE STARTING

1. Define backbone(s), insert(s), and assembly strategy.
   a.Guided by Target/Host expert (e.g., expression of a gene cluster) and/or Learn (e.g., design of experiments), compile all ICE entries (i.e., DNA parts linked to Strains **with samples**) and/or annotated files of **sequence-verified** DNA/protein sequences (e.g., genbank) to be used.
      i.Synthetic fragments will not require physical template pre-available.
      ii.Synthetic fragments must be ≤5kb and verified for synthesis constraints (e.g., https://boost.jgi.doe.gov/index.html)
      iii.Optional: Codon juggle, polish and/or partition synthetic fragments
2. Resource *Crop data* and *Relevant milestone* (contact PI for reference)
3. You will be asked to select a Project from a self-complete list. If you don't have a Project, create one first. Although Projects can be added later to each Design from the Design page, you must have a Project linked to your Design to submit it for review.

DIVA access

1 Go to DIVA server at https://diva.agilebiofoundry.org/ and login by using your LBNL LDAP/affiliate username and password.

You may need access to the ABF ICE parts registry. To create an account/login go to: https://registry.agilebiofoundry.org/

Define Project

2 Click the New Project button.

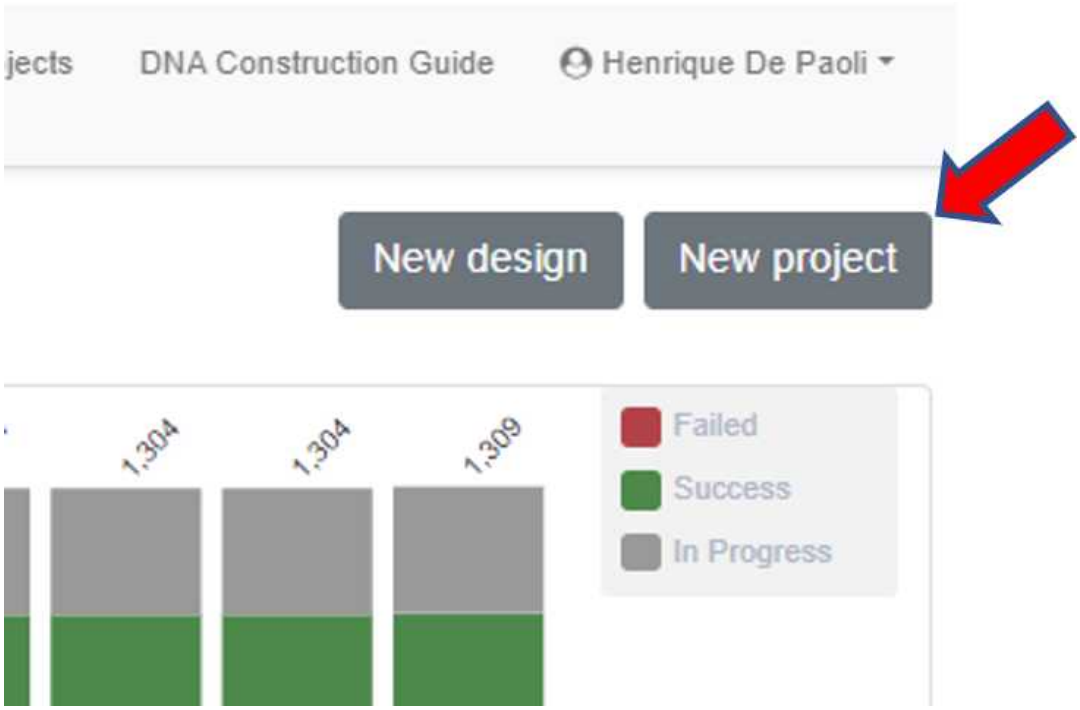


Fig.1

You can skip to step 3 if you already have a Project created that you want to use for your designs.

2.1 Input a *Project Name*, *Description* and define the DIVA Team who will review your design.

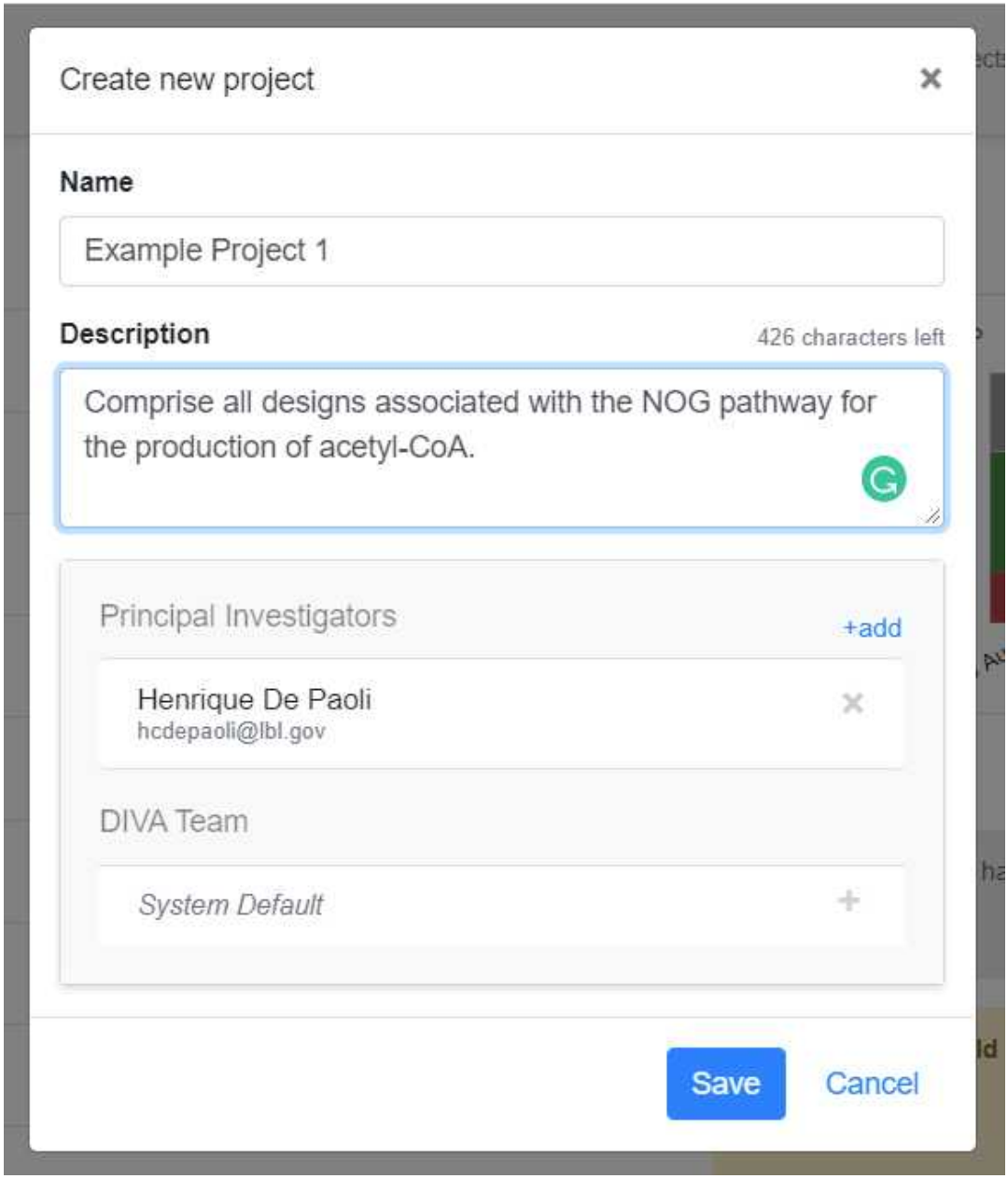


Fig.2

The System Default is the LBNL ESE DIVA Team. If you are not using the ESE DIVA team for your designs, create a Group (with desired people) within your Profile (top right corner) so the group appears in the self-complete field.

2.2 Add your Principal Investigator/Supervisor's Name, who is responsible for approving your expenses, then save the project.

Create Designs

3 Click the New Design button.

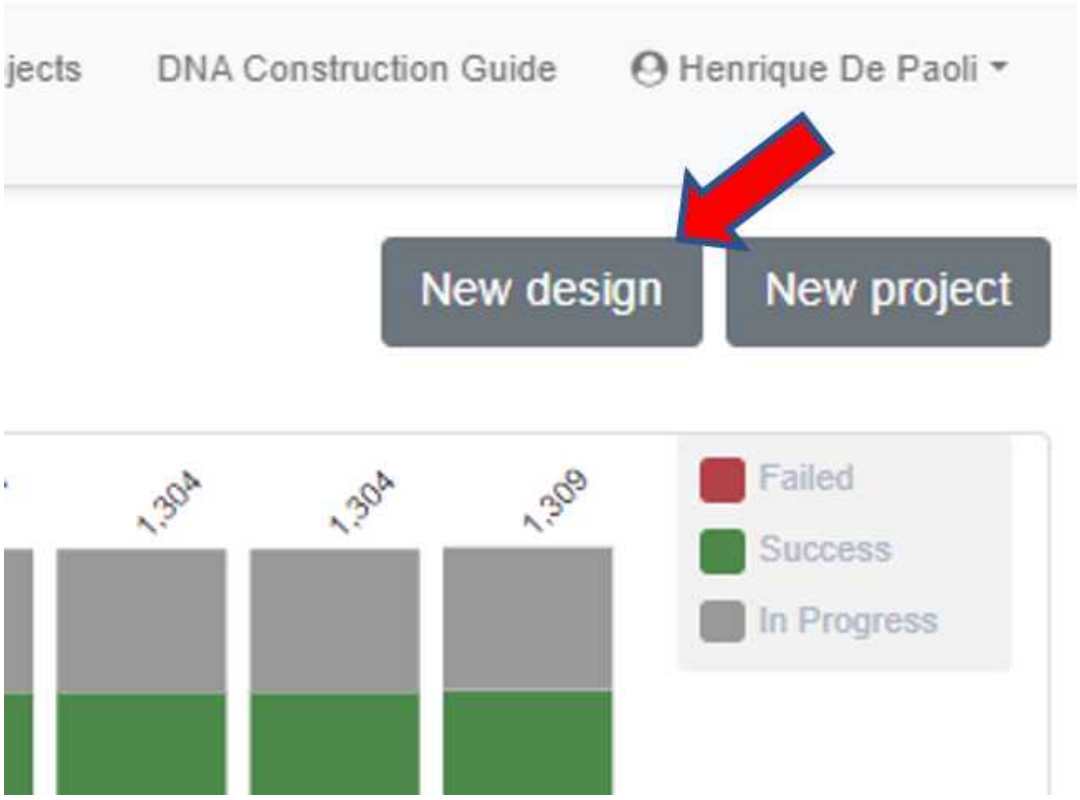


Fig.3

4 Input *Design Name*, *Description*(with *Crop data*, e.g., 11-2-TRY_M1, and *Relevant milestone*, e.g., FY21Q4_DBTL_AS2) and *Project*. Hit Create (it will take you directly to *Device Editor*).

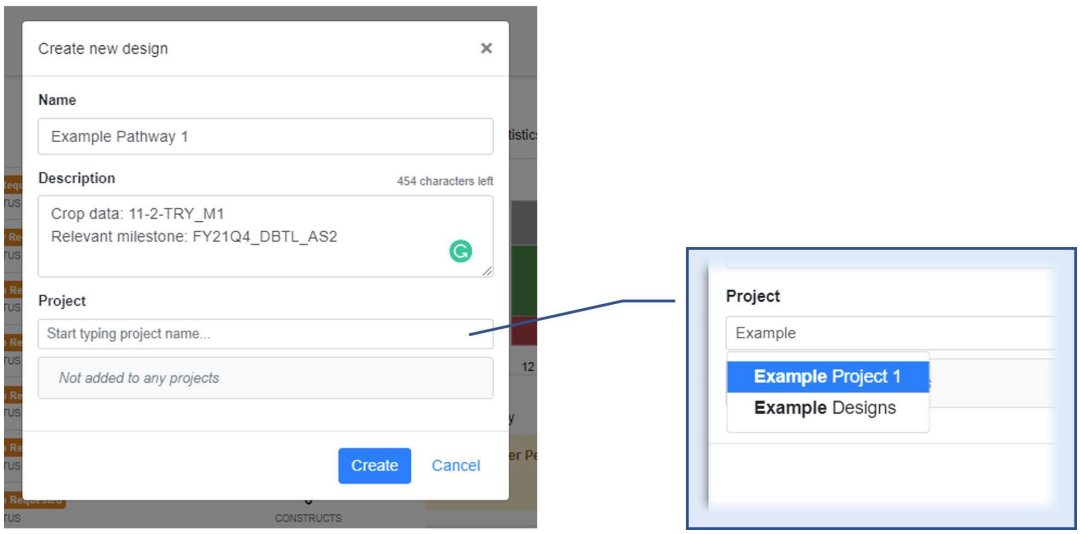


Fig.4

5 Input desired sequences in each cell using 'right click' and collecting from the appropriate source (e.g., Search for Part, Fig.5B; insert from ICE, Fig.5C-D, e.g., ABF_009895). Use left-most column for plasmid backbone.

First-time Users: follow links of Mock Parts to win a hands-on experience!!!

- You will design 2 variants, 1 insert each, and Run j5 as SLIC/Gibson/CPEC.

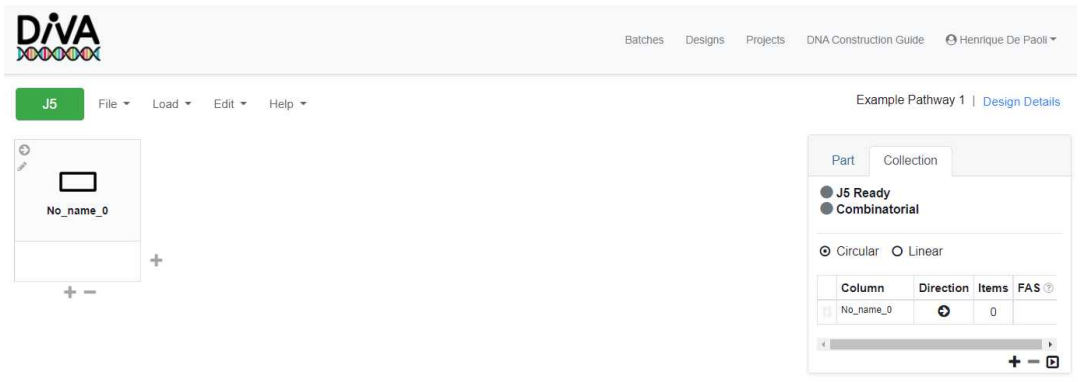


Fig.5A.

Pro tip: Change the SynBol icon to help you with the design structure.

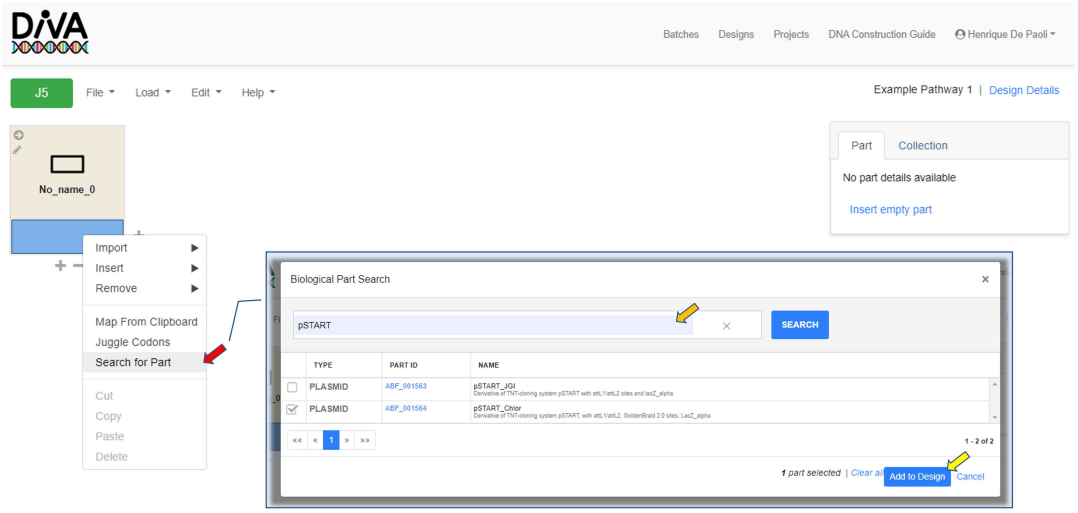


Fig.5B
Pro tip: Arrows reading order follows the rainbow spectrum (red, orange, yellow, green, blue, cyan and violet, when applicable throughout the Protocol).

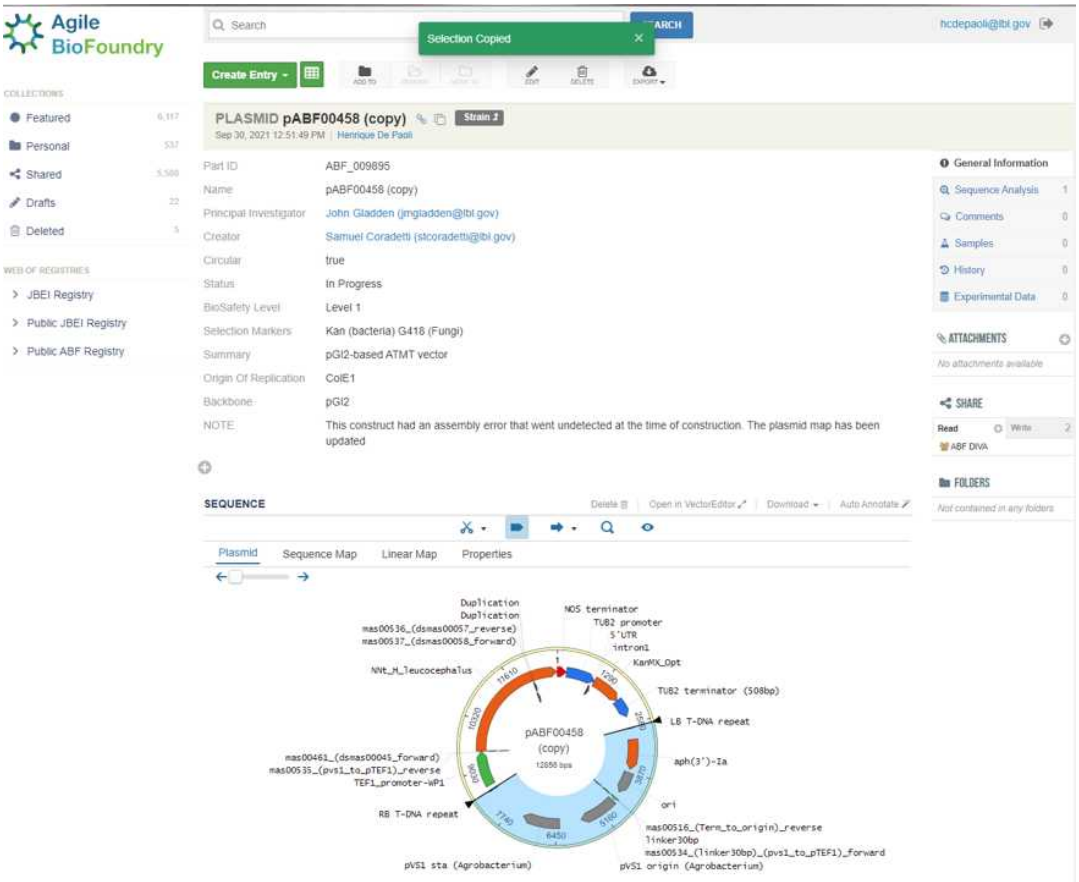


Fig.5C

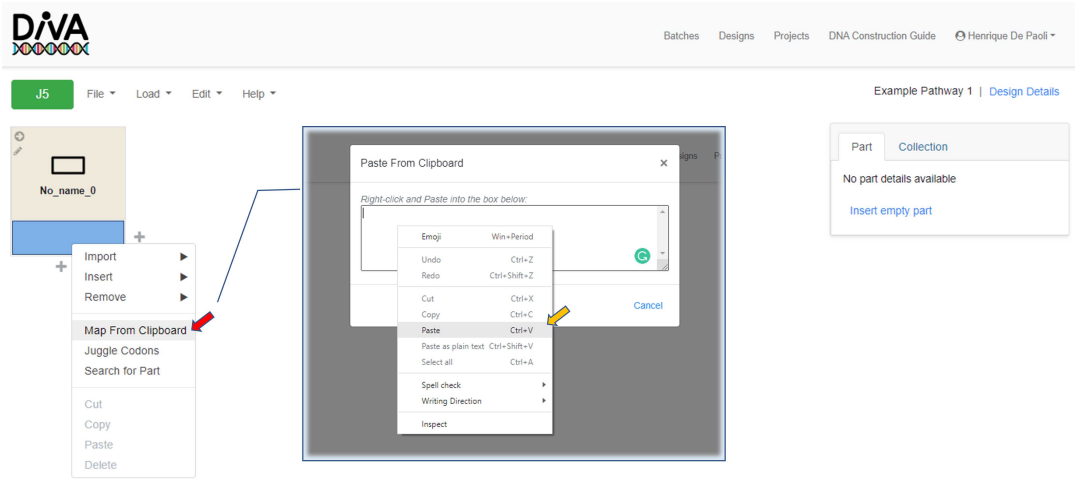


Fig.5D

6 Define *Part Source*, *Name* (with '_marker' and '_enzyme', e.g., bb_tDNA_kan and ABF_009842_EcoRI_amp), and *Start BP* and *End BP*. If using gDNA, use '_gDNA' instead of '_marker'.

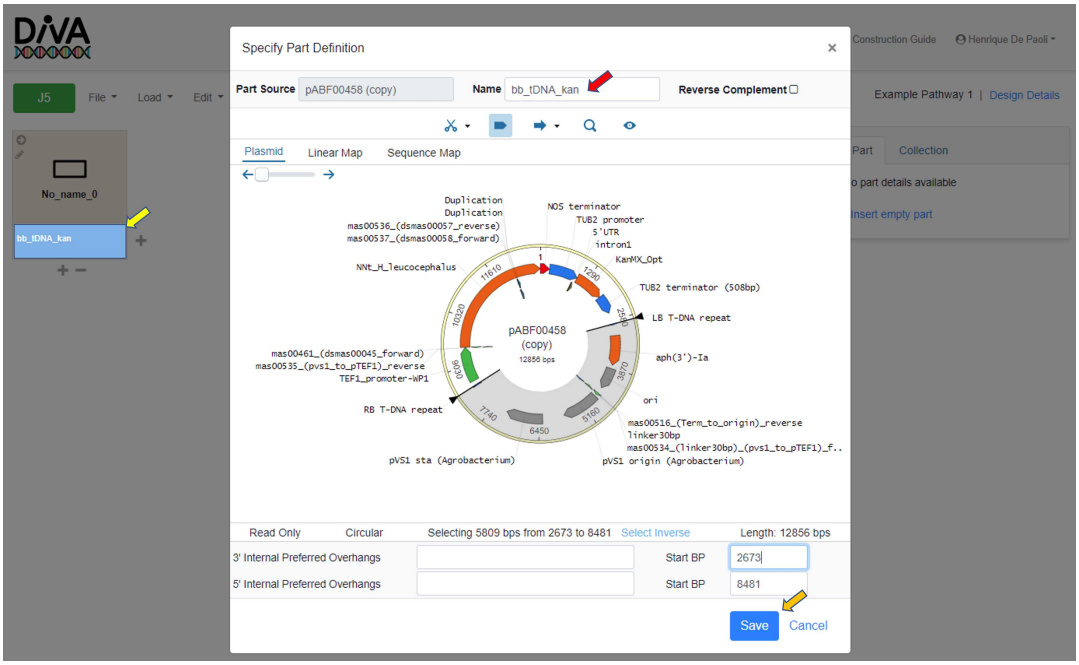


Fig.6
Pro tip: Part source parameters can also be defined by going under *Part* tab, change part definition and/or 'double clicking' on desired part.

7 Add columns and rows as needed for the remaining parts (e.g., Insert 1 ABF_009896, Insert 2 ABF_009569).

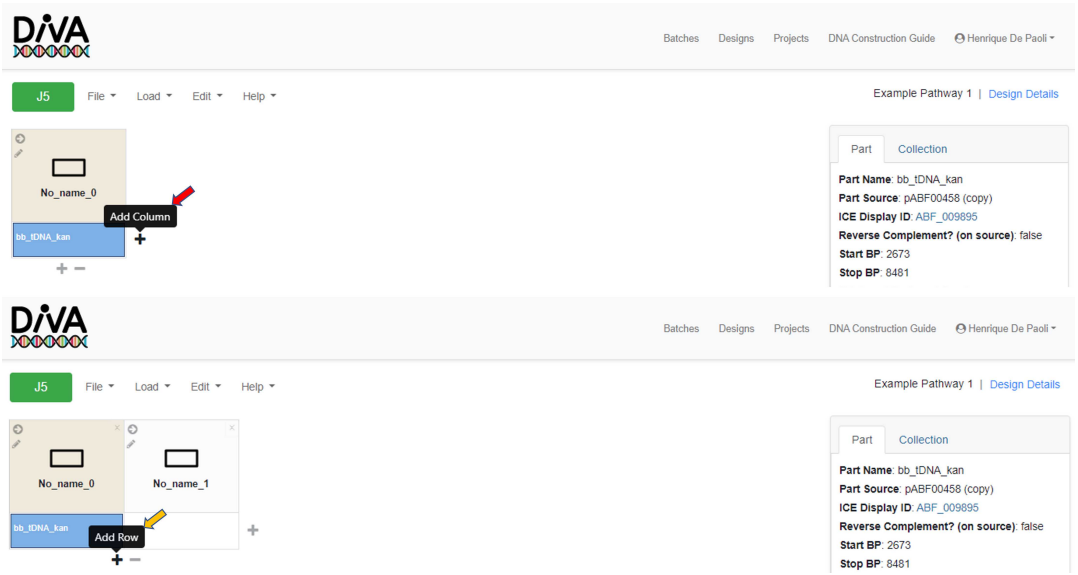


Fig.7

8 Under the Part tab, define Forced Assembly Strategy for each part individually (if left blank, PCR will be automatically adopted by j5). Avoid PCRs larger than 5.1kb.
- Optional: define Eugene rules.

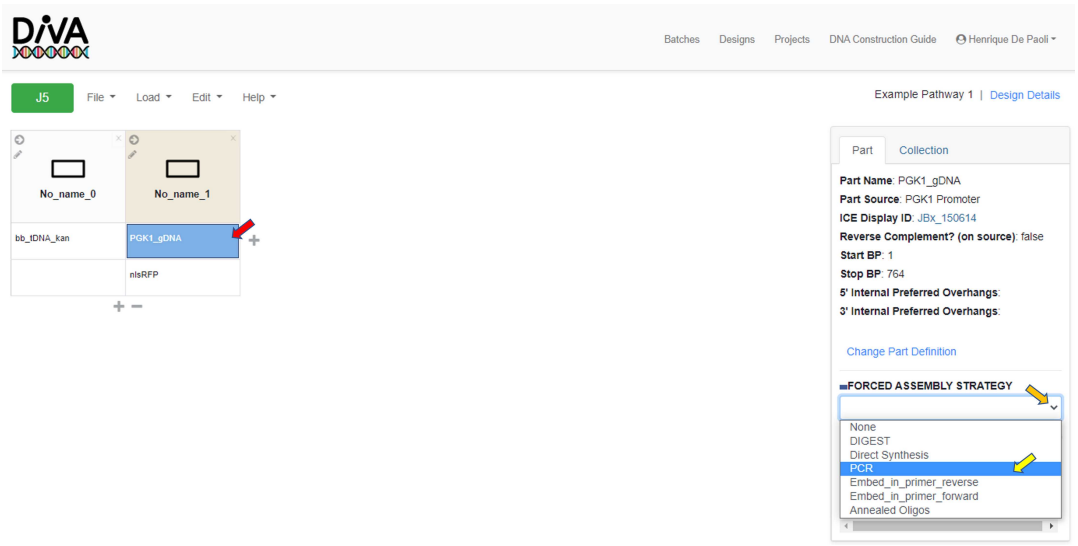


Fig.8A

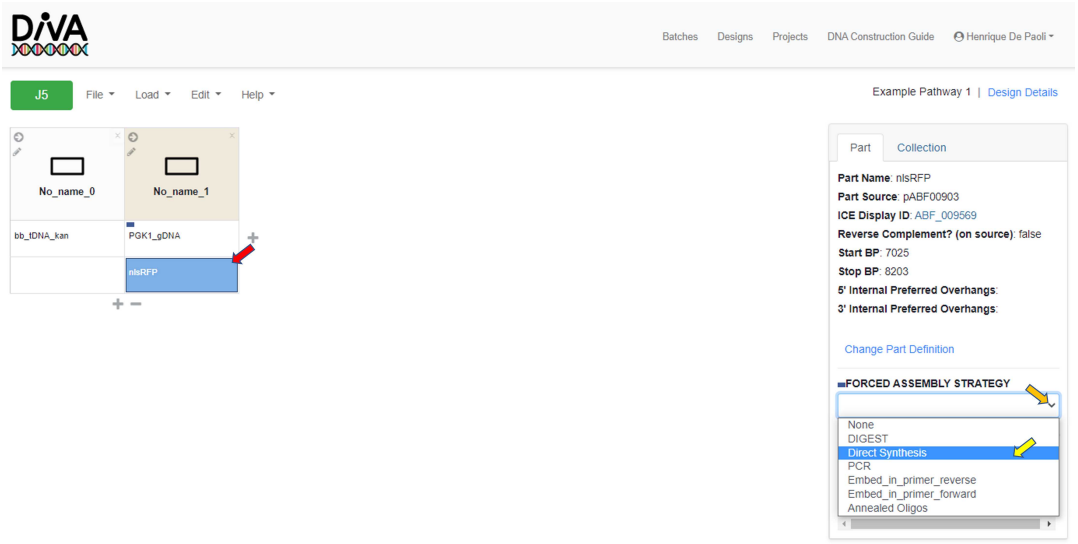


Fig.8B

9 Under the Collection tab, define Columns names (double click on e.g., 'No_name_0'). Also, define combinatorial (set by default) vs 'one per row' (must have all rows filled to be toggled).
a. Optional: select cells and insert empty part as needed
b. Optional: edit DNA assembly directives and parameters
c. Optional: define Column Direction (must toggle in the Device Editor area, not in the table).

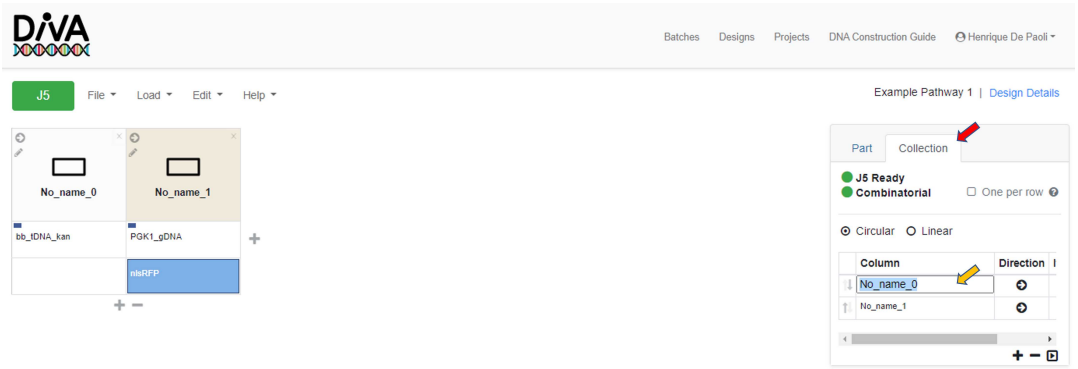


Fig.9

Run j5

10 Press the J5 (green) button to open the 'j5 controls' and select Preview assembly (this is simply putting sequences together). Inspect output visually for desired output (e.g., number of variants) and adjust as needed.

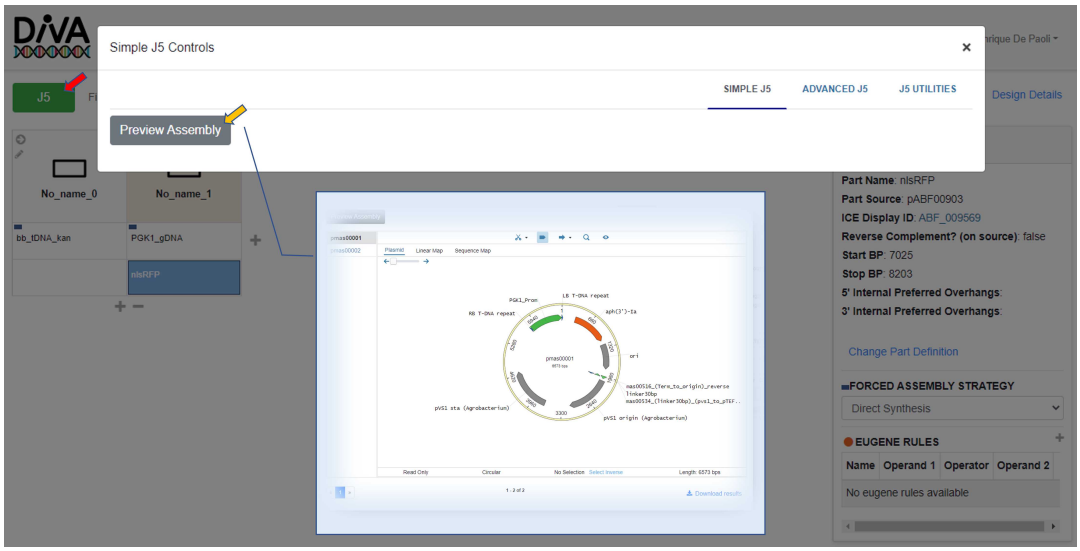


Fig.10

11 While in the pop-up section, select the Advanced j5 tab for more options.
a. Optional: Edit j5 parameters
b. Define master lists

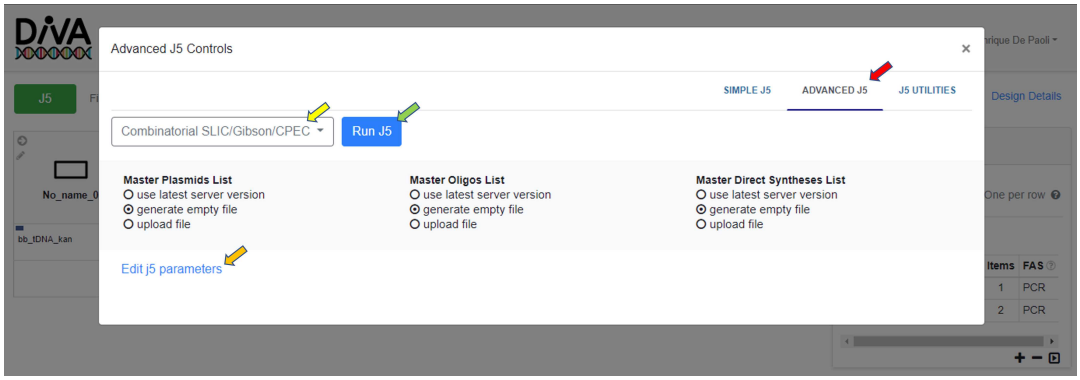


Fig.11
Also serves Step 12.

12 Select desired assembly from the drop-down menu.

You must select SLIC/Gibson/CPEC or Golden Gate (i.e., non-mock assembly; checks for assembly feasibility, designs oligos and output warnings and Build instructions) to be able to submit design for review.

Select the 'Run j5' button.

Note: If j5 returns an error message, check the j5 User's manual from Help tab in Device Editor.

13 Download results and open the combinatorial file (e.g., *pmas00001_combinatorial.csv,* or simply *pmas00001.csv* if making just one construct). Must be free of critical warnings (e.g., mispriming, incompatibilities between assembly pieces, homologous sequence repeat, etc).
Examples of "non-critical" warnings:
- Primer3 could not find a reasonable set of primers.
- The master plasmids/oligos list file is empty.

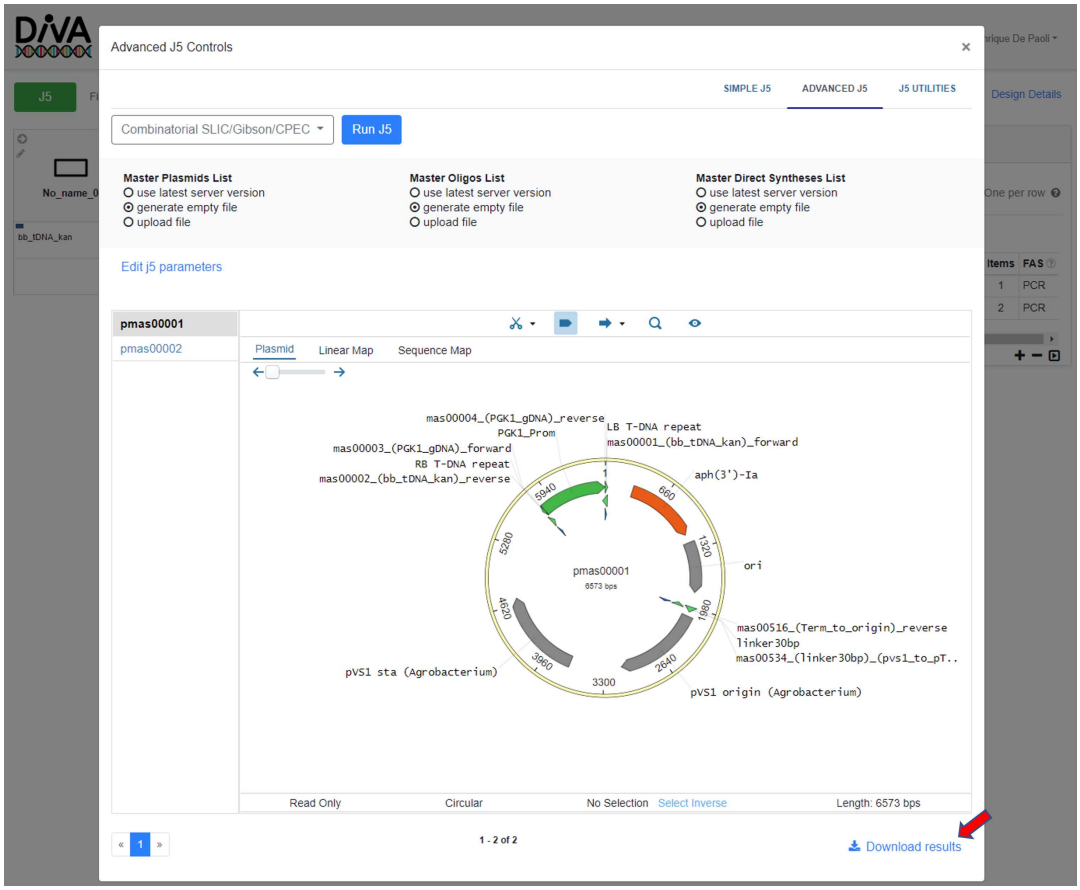


Fig.13A

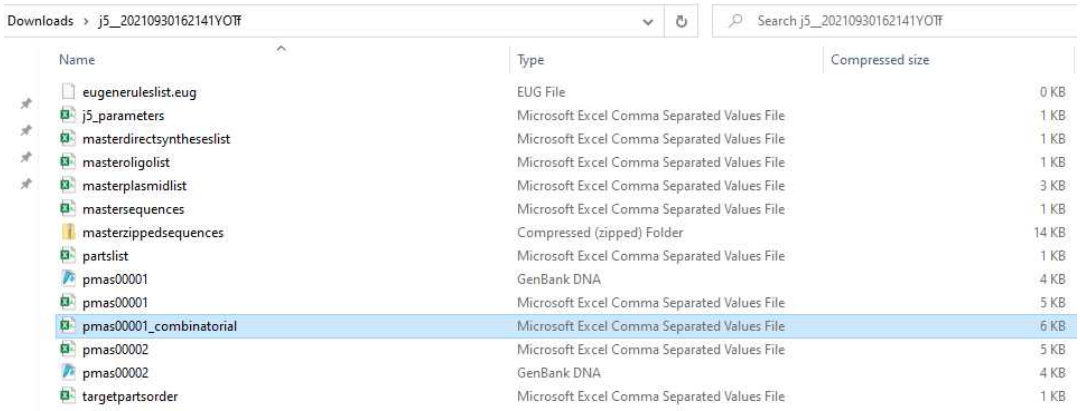


Fig.13B

Non-degenerate Part IDs and Sources								
Part		Location						
ID Number	Name	Source Plas	Reverse Co	Start (bp)	End (bp)	Size (bp)	Sequence	
0	bb_tDNA_k	pABF00458	FALSE	2673	8481	5809	GTTTACACCACAATATATCCTGCCACCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAA	
1	PGK1_gDN	PGK1 Prom	FALSE	1	764	764	GACTTCAACTCAAGACGCACAGATATTATAACATCTGCATAATAGGCATTTGCAAGAATTACTCG	
2	nlsRFP	pABF00903	FALSE	7025	8203	1179	ATGCCAAAGAAAAAGAGAAAAGTTACCGGTATGGTTTCCAAGGGCGAAGAAGATAACATGGCT	
Target Bin Selected Relative Overlap Positions and Extra 5'/3' CPEC bps:								
ID Number	Name	Relative Ov	Extra 5' CPI	Extra 3' CPEC bps				
0	No_name_	-13	0	0				
1	No_name_	13	0	5				
Direct Synthesis								
ID Number	Name	Length	Cost	Sequence				
0	dsmas0000	1236	482.04	GGTTTACCCGCCAATATATCCTGTCAATGCCAAAGAAAAAGAGAAAAGTTACCGGTATGGTTTCCAAGGGCGAAGAAGATAACATGGCT				
Oligo Synthesis								
ID Number	Name	Length	Tm	Tm (3' only	Cost	Sequence	Sequence (3' only)	
0	mas00001_	31	68.796	68.796	3.1	GTTTACAC(	GTTTACACCACAATATATCCTGCCACCAGCC	
1	mas00002_	26	64.463	64.463	2.6	TGACAGGA	TGACAGGATATATTGGCGGGTAAACC	
2	mas00003_	48	68.791	61.812	4.8	GGTTTACC(	GACTTCAACTCAAGACGCACAG	
3	mas00004_	65	59.06	57.328	46.5	GGCTGGTC	TGTTTTATATTTGTTGTAAAAAGTAGATAATTAC	
4	mas00005_	31	68.882	68.882	3.1	GGTTTACC(	GGTTTACCCGCCAATATATCCTGTCAATGCC	
5	mas00006_	24	65.346	65.346	2.4	GGCTGGTC	GGCTGGTGGCAGGATATATTGTGG	
PCR Reactions			Forward Oligo		Reverse Oligo			
ID Number	Primary Tei	Alternate T	ID Number	Name	ID Number	Name	Note	
0	pABF00458	(copy)	0	mas00001_	1	mas00002_	PCR	
1	PGK1 Promoter		2	mas00003_	3	mas00004_	PCR	
2	dsmas00001		4	mas00005_	5	mas00006_	Direct Synt	
Assembly Pieces (SLIC/Gibson/CPEC)								
ID Number	Type	Type ID Nu	Part(s)	Relative Ov	Extra 5' CPI	Extra 3' CPI	CPEC Tm N	
0	PCR	0	(bb_tDNA_	-13	0	0	64.463	
1	PCR	1	(PGK1_gDN	13	0	5	68.796	
2	Direct Synt	2	(nlsRFP)	13	0	5	68.796	
Combinations of Assembly Pieces								
Variant			Bin 0		Bin 1			
Number	Name	Assembly N	Part(s)	Assembly P	Part(s)	Assembly Piece ID Number		
0	pmas00001	SLIC/Gibsor	(bb_tDNA_	0	(PGK1_gDN	1		
1	pmas00002	SLIC/Gibsor	(bb_tDNA_	0	(nlsRFP)	2		

Overlap wit	Overlap wit	Overlap wit	Sequence L	Sequence									
66.6295	4.333	66.6295	4.333	5809	GTTTACACCACAAT								
63.9255	9.731	59.57	4.484	821	GGTTTACCCGCCA								
67.114	3.536	67.114	3.536	1236	GGTTTACCCGCCA								
Overlap wit	Overlap wit	Overlap wit	Sequence L	Sequence									
26	GGTTTACC(	TGACAGGA	5809	GTTTACACCACAATATATCCTG(									
31	GTTTACAC(	GGCTGGTG	821	GGTTTACCCGCCAATATATCCT(									
31	GTTTACAC(	GGCTGGTG	1236	GGTTTACCCGCCAATATATCCT(									

Fig.13C

14 If no changes are needed, you can close the Advanced j5 Controls pop up and select the link Design Details (top right corner) to go to the main Design page.

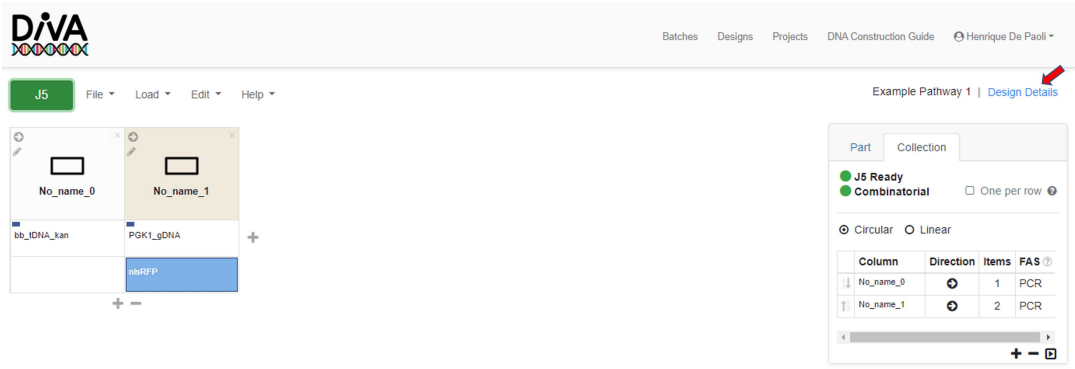
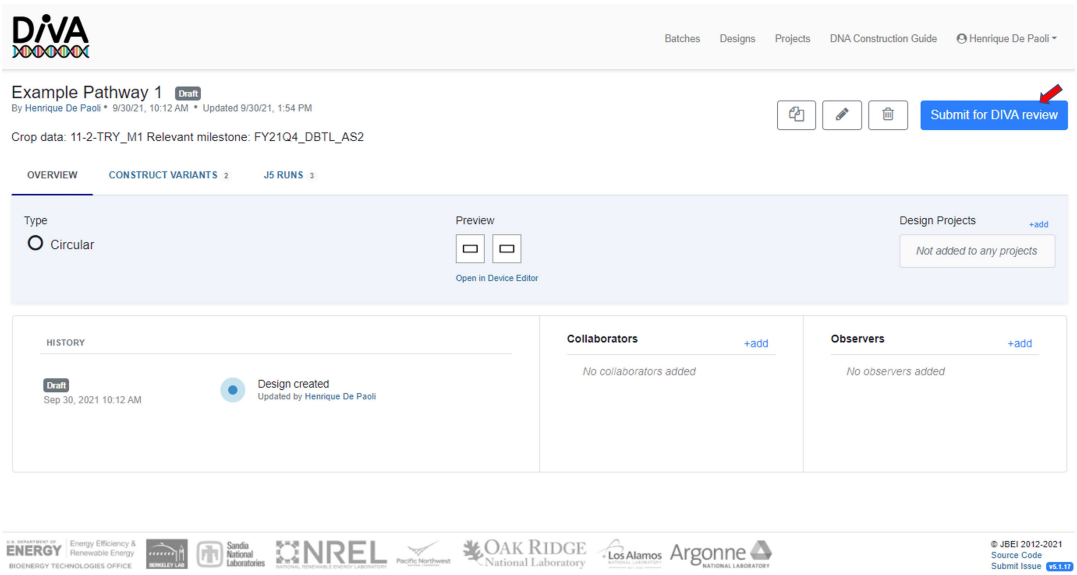


Fig.14

Submit for DIVA Review

15 You can now Submit for DIVA Review. Pay attention to prompted messages and (un)check boxes accordingly.



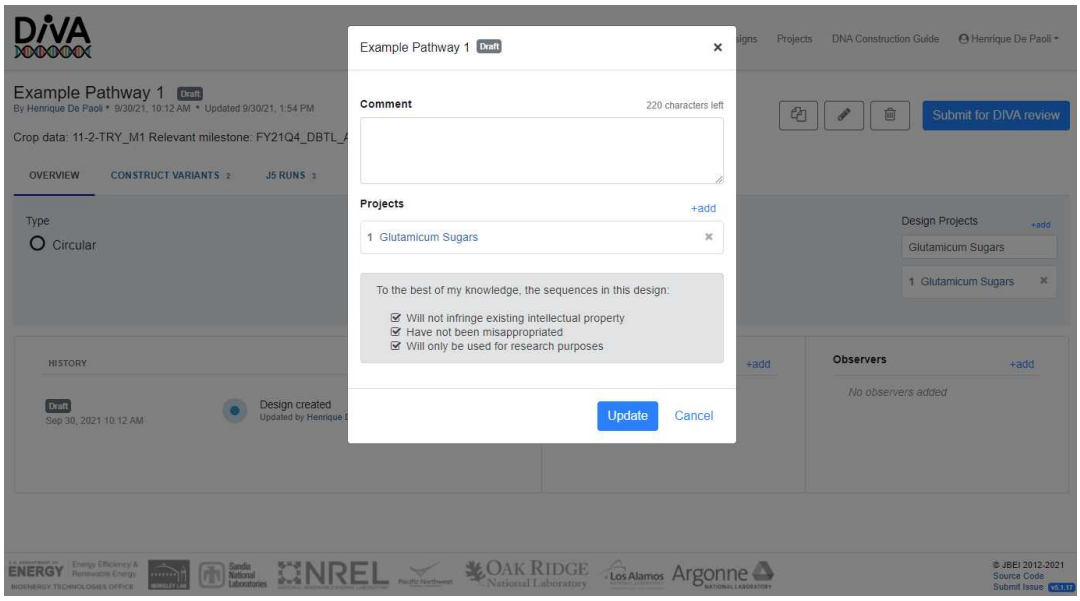


Fig.15

16 Wait for Reviewer feedback. Address Reviewer requests (if any) and re-submit for Review, or solicit PI budgetary approval (Submit to PI button).
- PI was defined while creating the Project you linked to the Design.

Next Steps: PI will receive an email to open Design in DIVA for reviewing expenses, input Project Charging code and activity ID, and submit design for DNA Build construction.