The Order of the MEO array

A DNA Foundry Adventure Game

This role-playing game by the Edinburgh Genome Foundry is meant to introduce molecular and computational biologists to the different challenges of running an academic DNA assembly platform. It is meant to be played at the EGF, with EGF members as non-player characters.

This document is available online !

This PDF and its sources can be consulted at this address:
github.com/Edinburgh-Genome-Foundry/egf-shared-documents

Scenario

Anna is a Post-Doc at the University of Edinburgh, where she studies the role of the MEO sequence pattern in kitten cancer. MEO is a 30-nucleotide sequence generally found as 10- or 20- tandem repeats upstream of important developmental genes across feline species. To better understand the regulatory role of MEO, Anna contacted the Genome Foundry with the following email.

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Hello Genome Foundry,

I am writing to enquire about costs and lead time for
a MEO array, which would consist of 40 times the MEO
sequence (below), with 20-nucleotide padding between
each consecutive repeats.
I would like the final sequence on a plasmid, with
flanking XbaI sites so I can easily clone it
elsewhere in the future.

MEO sequence: ATTGTGCGTGTAAATACGCATGTGTGCA

Thanks,

Anna E. Robik (anna.e.robik@gmail.com)
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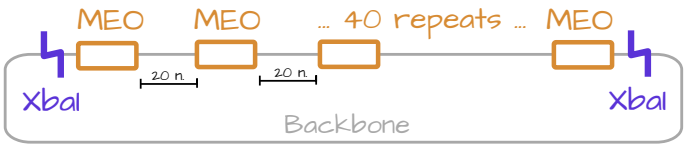


Awwwww

Will you help Anna save this poor kitten ?

You are members of the Edinburgh Genome Foundry (see characters sheet). Using the knowledge base (documents attached) and your own creativity, can you make Anna a happy customer ?

Characters

Project Owner

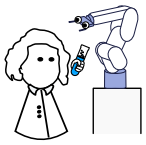
You communicate with your team and the customer to make sure the project will succesfully go through all assembly steps, will be validated by the customer, and will make the most of the Foundry's automation capabilities.

Sequence Designer

You communicate with your team and the customer to make sure the project will succesfully go through all assembly steps, will be validated by the customer, and will make the most of the Foundry's automation capabilities.

Supplies Officer

You specialize in finding the best DNA providers and listing everything needed for DNA assembly and verification.



Automation engineer

You specialize in planning operations, selecting the right robots for the right tasks, and writing picklists for the robots.

Quality Specialist

You are responsible for planning and conducting the Quality Control of the assemblies.

Non-player characters

Anna

Anna needs to publish as fast as possible, otherwise kitten will literally die. She can be contacted at any time at anna.e.robik@gmail.com

Advisory board

Played by the actual EGF staff, they are always available to answer your questions. Don't hesitate to reach out !

Short-overhangs assembly methods

This sheet describes two methods for assembling DNA parts via four-nucleotides sticky ends. In both methods, the idea is to mix together DNA fragments with four bare nucleotides on each end. Fragments with complementary ends will assemble together and form the desired construct (Figure 1a). However these methods differ slightly in their protocol and in the nature of the DNA fragments to order.

Overhang design. The sequences of the 4 terminal nucleotides (also called *overhangs*) must must be carefully chosen to ensure that the parts will assemble in the right order. If two overhangs are identical (or reverse-complementary), mis-annealing can occur between non-consecutive parts (Figure 1b). If an overhang is palyndromic, then the fragment will *self-anneal* with other copies of the fragment in the reaction mix (Figure 1c). Overhangs with no G/C or no A/T should also be avoided. Designing valid collections of valid overhangs can therefore be tricky, but software can help (see Software section).

Type 2S restriction assembly. In this method, the genetic parts to be assembled must each be flanked, on each side, by an arbitrary nucleotide followed by a BsmBI site (as shown on Figure 2). The parts are stored on a plasmid and kept in purified form in 96-well plates. Assemblies are carried out by mixing 13 femtomol of each part in a well containing 200 nanoliters of buffer and water to complement to 1 microliter. The buffer contains BsmBI restriction enzymes to produce the sticky fragments, and a ligase to assemble the fragments together. After incubation in a thermocycler, a few circular assemblies are (hopefully) formed, and they are amplified by the usual process (transformation, plating, colony picking, plasmid purification).

Oligo-based assembly. This method only differs from the restriction assembly method in that the parts are not originally on a backbone. Instead, they are formed by annealing (in a thermoclycler) two oligomers in order to form a double-stranded DNA sequence with sticky ends. The parts are then mixed together (this time no BsmBI is needed), ligated, and the rest of the protocol is the same.

Questions: what are the advantages and the limits of each method ? Could they work for Anna's project ?

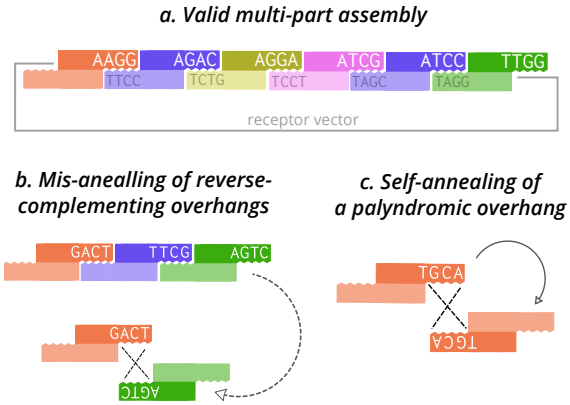


Figure 1: examples of valid and invalid overhang design.

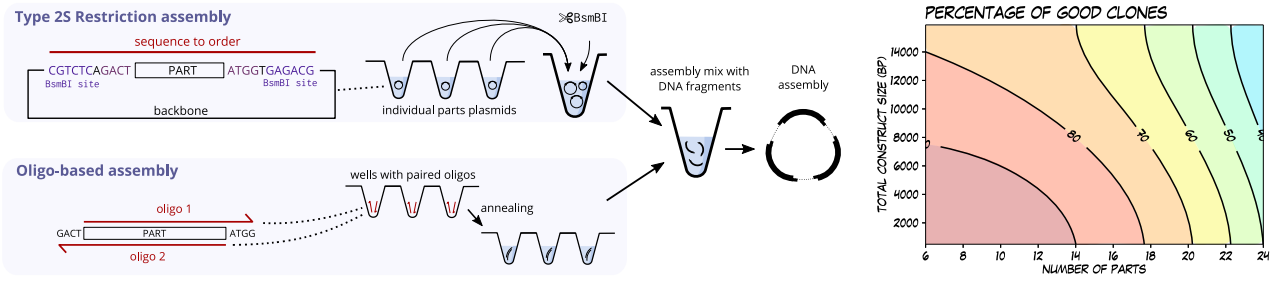


Figure 2: Implementation of Type 2S and oligo based assemblies. Left: part domestication and protocol for the two types of assembly discussed. Red annotations indicate the DNA sequence to synthetize in order to use the genetic part. **Right plot:** (idealized) graph of the percentage of good clones observed over various Type 2S restriction assembly experiments at the Foundry.

DNA Providers

Heliveroo

A fast and good-value DNA company. They assemble blocks from oligos.

Plasmid prep

Delivered as a 250ng of dry plasmid.

- $ 20c/bp (+ 30$ for custom backbone)
- 3 weeks
- 50-3000bp
- No BsaI, XbaI site. No repeats*

DNA string

250ng of dry double-stranded DNA.

- $ 10c/bp (+ 30$ for custom backbone)
- 50-1200bp
- No BsmBI site. No repeats*

Primerk

You only source for primers and oligos.

ssDNA oligo

Delivered on plate as 250ng of single-stranded DNA.

- $ 20c/bp
- 5 days
- 10-200bp
- No Repeats

ssDNA pre-mixes

Pre-mixed: 2 oligos per plate well

- $ 17c/bp (+ 30$ to include annealing)
- 5 days
- 10-200bp
- No Repeats*

Plasmibaba

Master cloners. They will make any sequence (by hand) as long as you pay.

Plasmid insert

Delivered as a 250ng of dry plasmid.

- $ $1/bp
- 6 weeks
- 50-20,000bp

Artificial Chromosome

Delivered as a 250ng of dry plasmid.

- $ $3/bp
- 10 weeks
- 20k-100k

Chramazon

#1 DNA retailers. They treat customers much better than their own employees.

Plasmid prep

Delivered as a 250ng of dry plasmid.

- $ 20c/bp (+ 30$ for custom backbone)
- 3 weeks
- 1000-3000bp
- No BsmBI site, no repeats*

*No repeats means that every 20bp subfragment is unique in the sequence.

Operations

Operation costs

Operation	£ / 96w run
Assembly ligation	68
Transformation/plating	170
Minipreping	110
Fragment Analysis	40
Sequencing	131

(Keep in mind that for a batch of 96 assemblies, you may need to do more than 96 minipreps, fragment analysis or Sanger sequencing operations)

Tecan

Details

Source plates	96w / 384w
Minimal volume / dispense	0.1 µL
Maximal volume / dispense	1 mL
Tips dead volume	0.1 µL
Resolution	0.1 µL

Example of protocol spreadsheet

Action	Plate	Well #	V (µL)
Aspire	Source	1	49
Dispense	Destination	2	49
Wash tubes			
Aspire	Source	23	10
Dispense	Destination	40	10
Wash tubes			

(The well number corresponds to the number of the well, column-wise, from left to right)

Echo

Details

Source plates	384w / 1536w
Minimal volume / dispense	2.5 nL
Maximal volume / dispense	500 nL
Resolution	2.5 nL

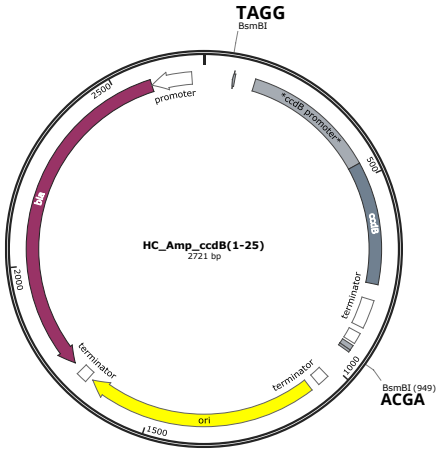
Example of protocol spreadsheet

Source	Destination	Volume (nL)
H13	A1	15
F5	A1	35
G11	A2	135

Plates

plate	capacity	dead volume (ECHO)	dead volume (TECAN)
96w	150 µL	-	10 µL
384w	49 µL	9 µL	10 µL
1536w	2 µL	0.5 µL	-

Backbone



Available Enzymes

This page summarizes the restriction enzymes available in your Foundry. For each enzyme it is indicated whether it is High Fidelity, its price for 200 units, and whether it is Methylation dependent. All enzymes work at 37C.

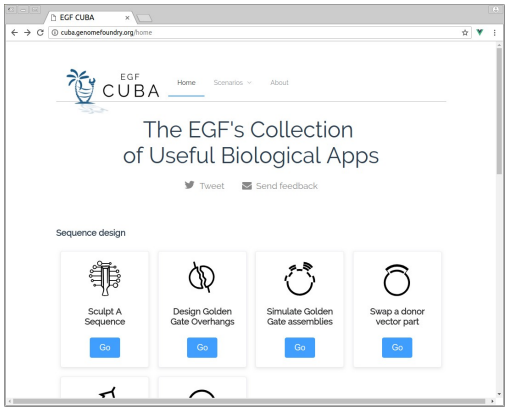
Enzyme	HF	$ / 200u	Meth.
AgeI	✔	50	
AseI		46	
AvaI		42	
BamHI	✔	36	
BanII		46	
ClaI		46	Dam
DraI		45	
EcoRI	✔	34	
EcoRV	✔	38	
HindIII	✔	34	
KpnI	✔	38	
MfeI	✔	48	
MfeI	✔	48	
NaeI		42	
NdeI		46	
NheI	✔	43	
NotI	✔	46	

Enzyme	HF	$ / 200u	Meth.
NspI		46	
PstI	✔	41	
PvuI	✔	46	
PvuI	✔	46	
SacI	✔	38	
SalI	✔	39	
ScaI	✔	43	
ScaI	✔	43	
SnaBI		42	
SpeI	✔	43	
SphI	✔	46	
StyI	✔	48	
XbaI		42	Dam
XhoI		42	

EGF Software

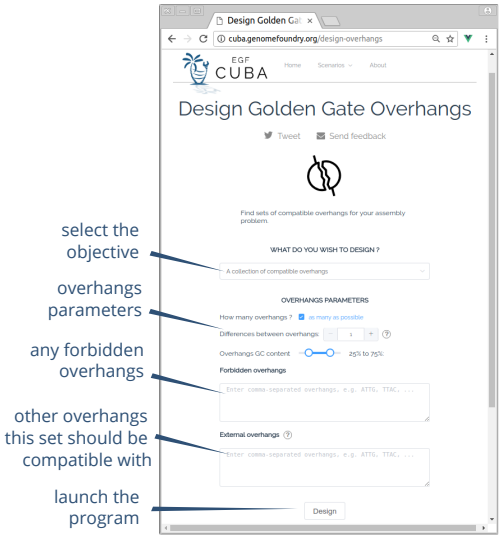
EGF CUBA

EGF CUBA gathers software wep applications for various tasks. In this section we focus on some of the most useful apps for our purpose, but feel free to use others as well.
It is available at is available at cuba.genomefoundry.org



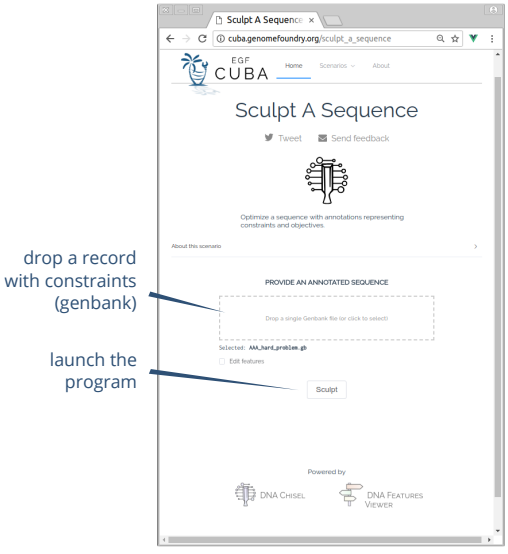
Design overhangs

This app generates collections of compatible overhangs under a set of specifications. It can also be used to fragment a sequence into fragments which can then be assembled using type-2S restriction assembly.



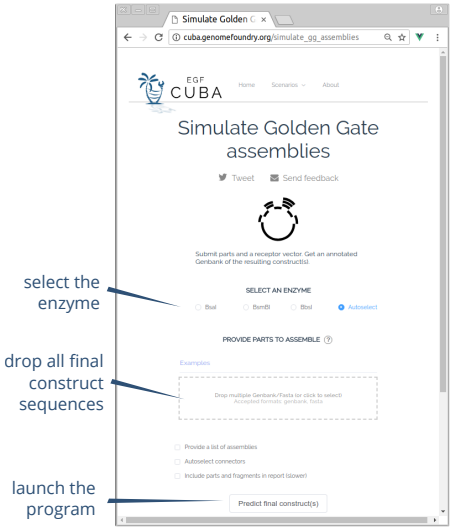
Sculpt a sequence

This app can optimize a sequence with respect to constraints and objectives, for instance to remove a restriction site, remove homologies between parts of a sequence, etc.



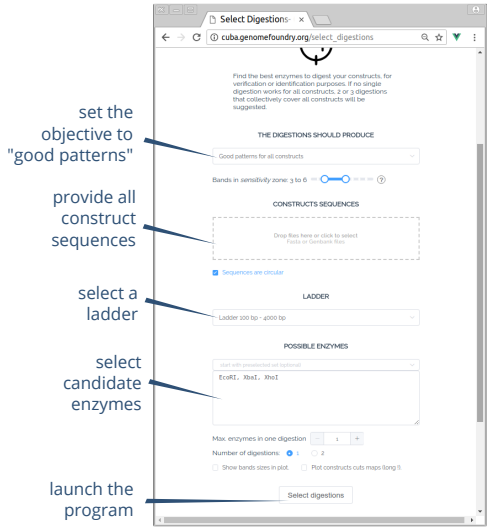
Simulate cloning

This app can predict whether several genetic parts will assemble correctly into a circular construct. It returns the final assembly sequence (required for quality control) and helps detect and troubleshoot design mistakes.



Select digestions

This app automatically finds enzymes (or sets of enzymes) that will produce informative digestion patterns for all genetic construct in a given set.



Select primers

This app can generate a Sanger sequencing plan to sequence a batch of construct. It takes advantage of homologies between constructs, and already-available primers, to minimize the number of new primers to buy.