



Biolab I: NCBI Introduction to the BioBrick concept DNA Atlas exercise

21.09.2021

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NCBI database

“The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.”

Let´s check some features of this database



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iGEM

The International Genetically Engineered Machine (iGEM) Foundation is an independent, non-profit organization dedicated to education and competition, the advancement of synthetic biology, and the development of an open community and collaboration.

iGEM runs three main programs

- the iGEM Competition - an international competition for students interested in the field of synthetic biology
- the Labs Program - a program for academic labs to use the same resources as the competition teams
- the Registry of Standard Biological Parts - a growing collection of genetic parts use for building biological devices and systems.



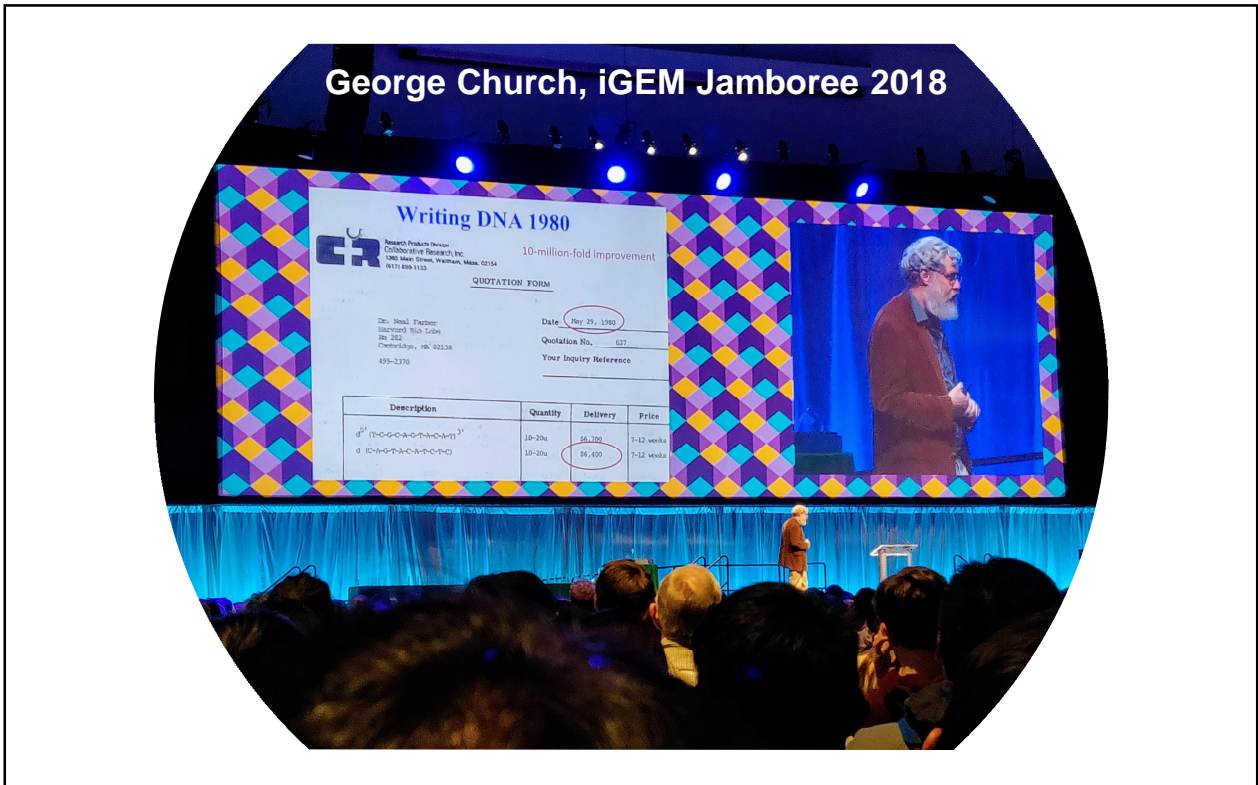
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Check: www.aaltohelsinki.com

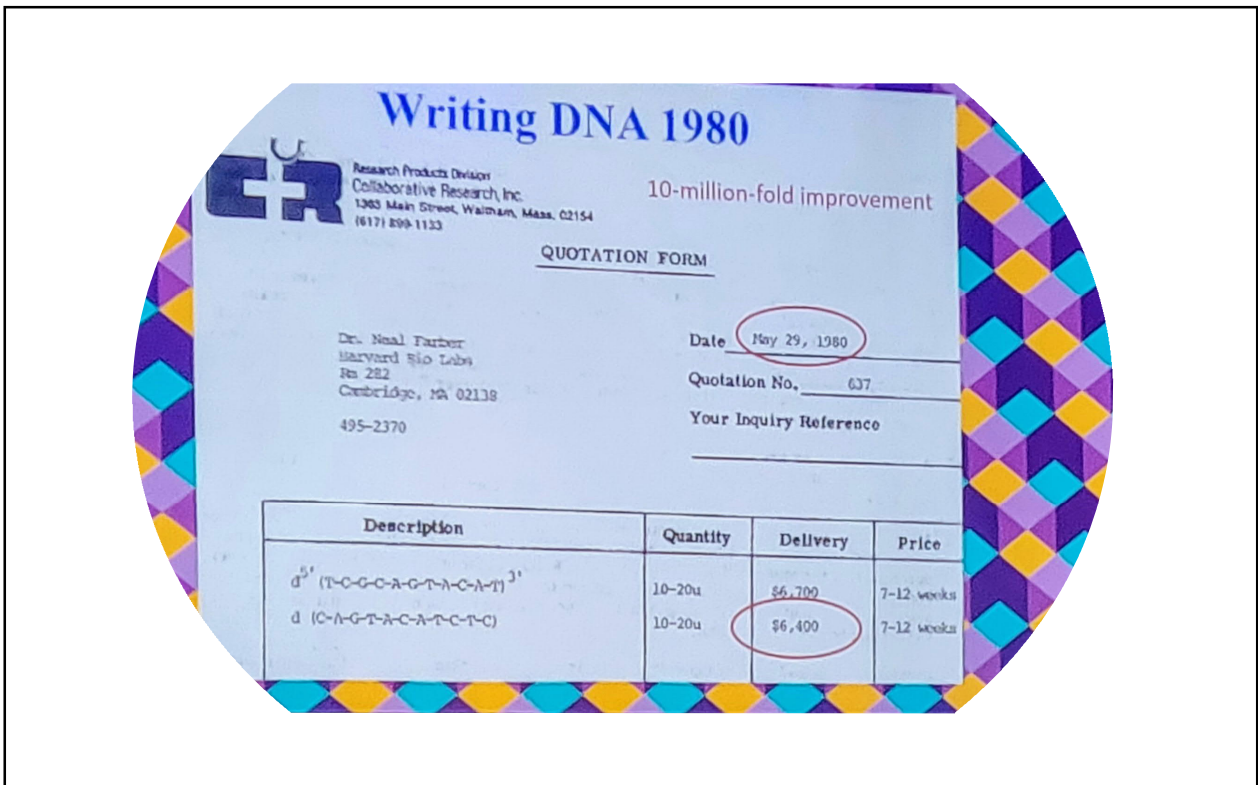
**Group of students joining an annual worldwide competition,
over 300 teams participating**

Note: recruiting annually!

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BioBricks

Register of standard biological parts – BioBricks™

- Promoters
- Ribosome Binding Sites
- Protein domains
- Protein coding sequences
- Translational units
- Terminators
- DNA
- Plasmid backbones
- Primers
- Protein generators
- Reporters
- Inverters
- Receivers and senders
- Measurement devices etc etc.

Part Number	Function	Notation
BBa_G00000	BioBrick cloning site prefix	
BBa_G00001	BioBrick cloning site suffix	
BBa_P1016	ccdB positive selection marker	
BBa_I50022	pUC19-derived high copy replication origin	
BBa_B0042	translational stop sequence	
BBa_B0053 & BBa_B0054	forward transcriptional terminator	
BBa_B0055 & BBa_B0062	reverse transcriptional terminator	
BBa_G00100	forward verification primer annealing site (VF2)	
BBa_G00102	reverse verification primer annealing site (VR)	
BBa_B0045	NheI restriction site	
BBa_P1006	ampicillin resistance marker (reverse orientation)	
BBa_P1002	ampicillin resistance marker	
BBa_P1003	kanamycin resistance marker	
BBa_P1004	chloramphenicol resistance marker	
BBa_P1005	tetracycline resistance marker	
BBa_I50042	pSC101 replication origin	
BBa_I50032	p15A replication origin	

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The BioBrick Concept



one reaction with the USER™ and a final *E.coli* transformation



The aim:
From "trial and error" approaches
to "plug and play" systems

In reality, not always this simple...



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BioBricks for assembling genetic constructs

- BioBricks can be used to learn what standardization in molecular biology means and how standardized genetic parts can be utilized to assemble new functional units
- You will create two recombinant plasmids *in silico* by using BioBrick principle and DNA Atlas software
- Gene of interest is GFP (green fluorescent protein)
- One plasmid with constitutive, one plasmid with inducible promoter
 - Constitutive = gene expressed all the time
 - Inducible = gene expressed only (or at least more efficiently) when inducer present (here IPTG)

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BioBrick parts

- Database: <http://parts.igem.org>
- Help pages: <http://parts.igem.org/Help>

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Digestion and Assembly, also done in DNA Atlas exercise

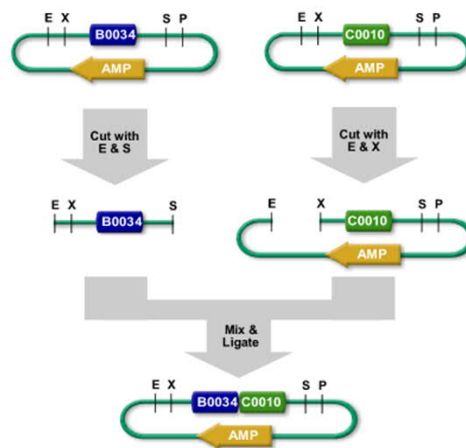
http://parts.igem.org/Assembly:Standard_assembly

E = EcoRI
 X = XbaI
 S = SpeI
 P = PstI

X and S have the same sticky ends -> digests can be joined together
 At the same time, the specific recognition sites for each enzyme are lost -> mixed site

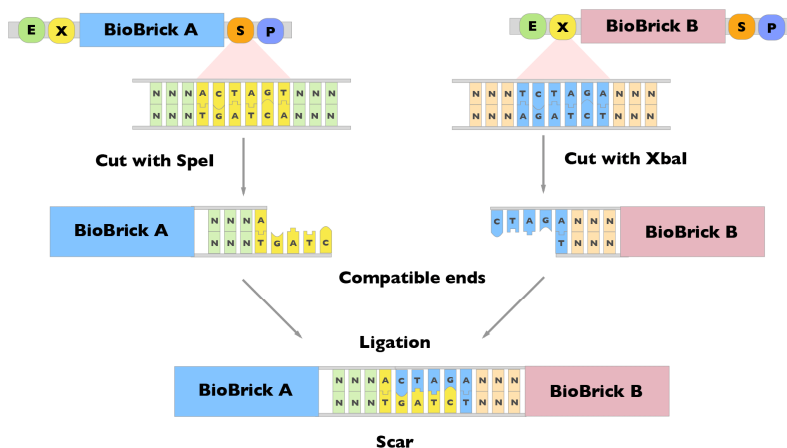
XbaI 5'...T[↓]CTAGA...3'
 3'...AGATC[↓]T...5'

SpeI 5'...A[↓]CTAGT...3'
 3'...TGATC[↓]A...5'



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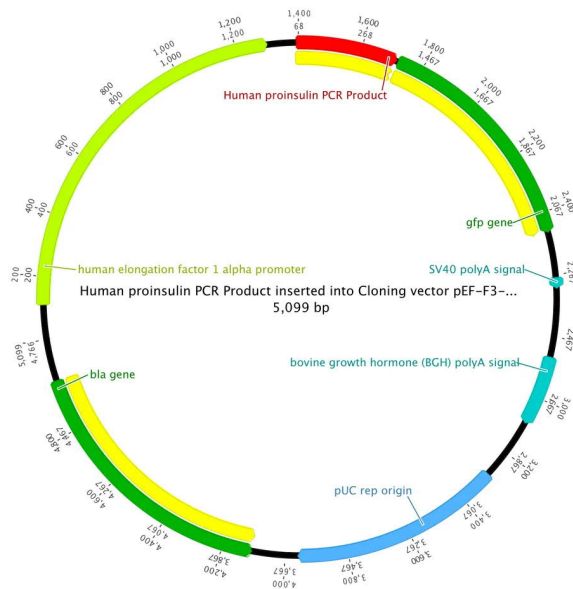
Mixed site, SpeI + XbaI



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An annotation example

“An annotation is extra information associated with a particular point in a document or other piece of information. It can be a note that includes a comment or explanation.”



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This week's remote work task

Watch the DNA Atlas tutorial, can be found in MyCo -> Materials

Also DNA sequences and general PCR primer sequences are in MyCo

Create two recombinant plasmids by using DNA Atlas; use some time to look around in the interphase, study the features etc.

When you're done submit the annotated plasmid maps as figures to MyCourses -> Assignments (DL 1.10.), Heli checks them

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Tomorrow Thu 23.9.

Heli will be in Zoom at 12.15 if you need assistance with the assignments

Next week

No lectures from Heli, Zoom sessions can still be arranged

Vera's starting session timing will be informed at the latest on Monday by e-mail