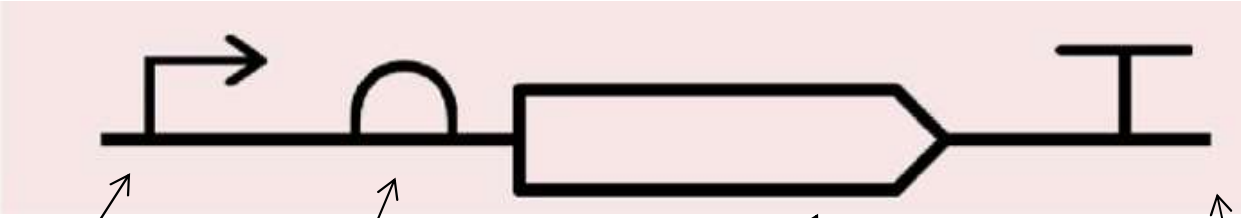
Part properties



Promoter (works in this organism?)
http://parts.igem.org/Promoters/Catalog
- Constitutive
- Inducible (check the repressor/ activator)

BRPOM:
http://www.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb

5'UTR
- Ribosome Binding Site (RBS)

https://www.denovodna.com/software/login

- Kozak Sequence [eukaryotes]
- IRES [eukaryotes]

Expression Noise?

Gene
- Start codon (**AUG, GUG, CUG**)
- Codon Usage (CAI, tAI)

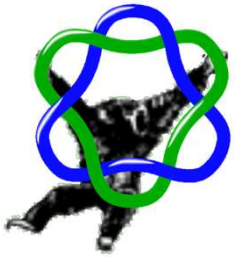
http://www.kazusa.or.jp/codon/
http://genomes.urv.es/CAIcal/
- Codon Harmonization

https://galaxyproject.org/use/codon-harmonizer/
- Self-cleaving tag (2A)
- Fusion tag (His, Myc, GST, HiBiT)
- Degradation tag (LAA, LVA)
- Stop codon (**TAA, TAG, TGA**)
- mRNA structure/ stability

Termination
- Intrinsic (Rho-independent)
- Rho-dependent
- Poly-A signal [eukaryotes]

FindTerm:
http://www.softberry.com/berry.phtml?topic=findterm&group=programs&subgroup=gfindb

Plasmid editing tools



ApE plasmid editor

Download:
https://jorgensen.biology.utah.edu/wayned/ape/

Tutorials:
https://www.youtube.com/channel/UC_-pObWrnUZRhsO8YblX6gQ

Benchling

Starting Tutorial:
https://benchling.com/tutorials/49/nav-redesign-overview

Molecular Biology:
https://www.youtube.com/watch?v=rhamB8IiWxA

SnapGene

€

Geneious

€

Vector NTI

€

Plasmid annotation: Key principles

1) ALL **relevant parts** should be annotated:
(promoter, RBS/ Kozak sequence/ UTR, CDS, terminator/ polyA site, replication origin/ recombination site, selection marker, any additional feature of interest)

2) Annotation should be **unambiguous**
(use known Accession numbers from standard repositories: NCBI, Biobrick)

3) Annotation should be **correct**
(correct symbol used, parts with accession number must match, reading frames must be correct)

4) Annotation should be **descriptive**
(say what the part is: promoter, gene, UTR, etc.)

Practical Exercise

Benchling

1) Assemble a GFP expression unit:
   a) Vector
   b) Constitutive Promoter (design your own?)
   c) RBS (design your own?)
   d) GFP
   e) Terminator

Starting Material (make your own copy)
tinyurl.com/BLmssb

2) Annotate existing plasmid (**DNAseq1**)
   a) Identify and annotate parts
   b) Sequence analysis
   c) Add primers
   d) Design new primers

Zip file
tinyurl.com/mssb-DNAseq1

Registry of Standard Biological Parts: http://parts.igem.org/Main_Page
NCBI BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi

Questions welcome.

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