



GenoCAD


Advanced Topics


Virginia Bioinformatics Institute

Computer-Assisted Design Software
for Synthetic Biology


Materials prepared by:
Mary E. Mangan PhD
www.openhelix.com/genocad2



National Science Foundation
WHERE DISCOVERIES BEGIN



Version 1




GenoCAD Advanced Agenda

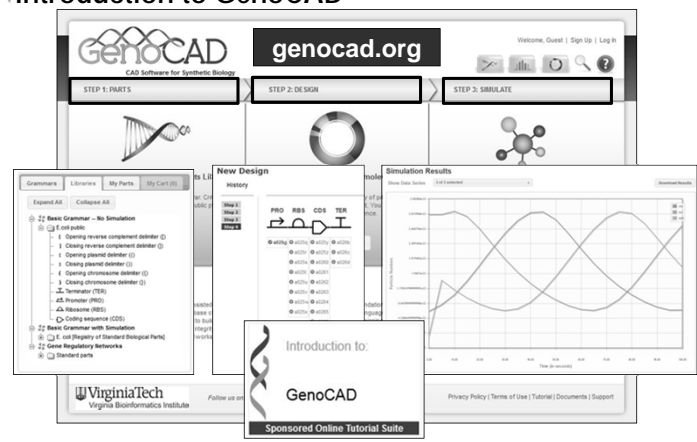
- Introduction and Review
- New Library with Advanced Searching
- Advanced Design
 - Copying and Reworking
 - Export GenBank format → other tools
- Grammar from Scratch
- Modifying (Editing) Grammar
- Summary
- Exercises

GenoCAD: <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent.



Introduction to GenoCAD



- Computer-assisted design software for synthetic biology

Copyright OpenHelix. No use or reproduction without express written consent.

Register and Log In at GenoCAD

Completed first tutorial

Features we explore here require saving to your workspace

<http://dx.doi.org/10.6084/m9.figshare.153827>

Copyright OpenHelix. No use or reproduction without express written consent.

Conceptual Framework for GenoCAD

Grammar rules are made of Parts of Speech

Sentence

Subject Verb

R2 NounPhrase

Rules make the framework, words make the sentence

The patent system adds the fuel of interest to the fire of genius

Intellectual property has the shelf life of a banana

~Abraham Lincoln

~Bill Gates

GenoCAD uses a similar set of rules to develop biological constructs.

New Design History

PRO RSS CDS TER

Aspects of DNA function explained with language metaphors: transcription, translation, code

GenoCAD lets you develop a language for DNA designs

Copyright OpenHelix. No use or reproduction without express written consent.

GenoCAD Advanced Agenda

- Introduction and Review
- New Library with Advanced Searching
- Advanced Design
 - Copying and Reworking
 - Export GenBank format → other tools
- Grammar from Scratch
- Modifying (Editing) Grammar
- Summary
- Exercises

GenoCAD: <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent.

New Project Library

Best practice: New Library for each project

Locate and reuse parts

Continue to use Training Set Grammar for now

Copyright OpenHelix. No use or reproduction without express written consent.

Add New Library in Training Set

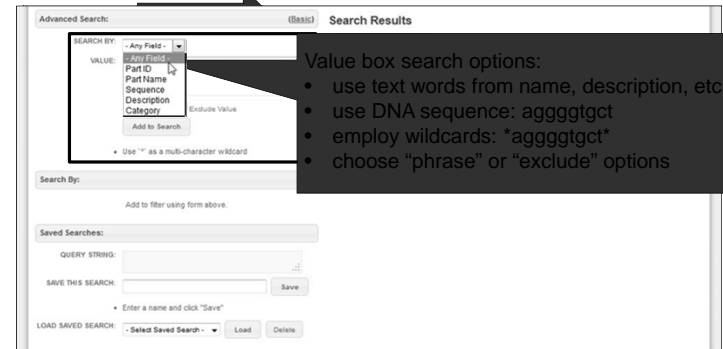


- In the Training Set, create a "new library"
- Name it "Fluors"

Copyright OpenHelix. No use or reproduction without express written consent

9

Search for Project Parts



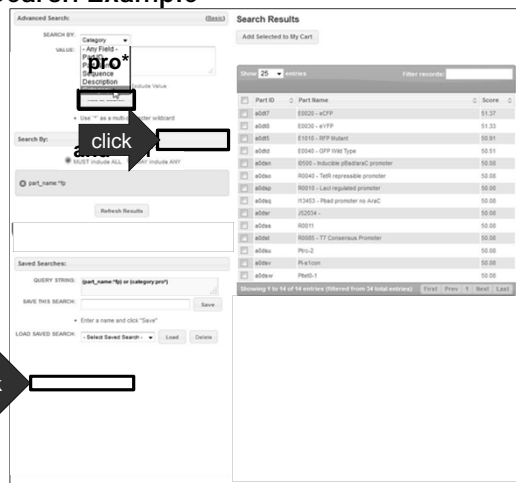
- Locate specific parts quickly with a search
- Advanced searches offer precision
- Build queries with multiple options

Copyright OpenHelix. No use or reproduction without express written consent

10

Advanced Search Example

- Set field to: **Part Name**
- Set value to: ***pro***
- Set field to: **Category**
- Set value to: **pro***
- Choose **MAY include**



Copyright OpenHelix. No use or reproduction without express written consent

11

Save a Search

- Save searches that you might use often



Copyright OpenHelix. No use or reproduction without express written consent

12

Saved Searches and My Cart

Basic Search: (Advanced)

QUERY: (part_name="fp") or (category="rbs")

Search Parts

Saved Searches:

SAVE THIS SEARCH: Enter a name and click "Save"

LOAD SAVED SEARCH: fluor search

Categories:

Show All Categories

Training Set E. Coli Grammar

Gene

Promoter

Basic Grammar - No Simulation

Coding sequence

Promoter

Gene Regulatory Networks

Search Results

There are now 5 parts in your Cart.

Add Selected to My Cart

Show 25 entries

Part ID	Part Name	Score
a0d7	E0020 - eCFP	57.19
a0d8	E0020 - eYFP	57.12
a0d5	E1010 - RFP Mutant	56.64
a0d4	E0040 - GFP Wild Type	56.17
a0d6	E0500 - Inducible pBadlacZ promoter	55.8
a0d9	R0040 - TetR repressible promoter	55.8
a0da	R0010 - LacI regulated promoter	55.8
a0db	I13453 - Pbad promoter no AraC	55.8
a0dc	J2034 -	55.8
a0dd	R0011	55.8
a0de	R0085 - T7 Consensus Promoter	55.8

- Load and run the saved search
- Highlight the "Training Set" category
- Then select the five parts shown
- Click to add to My Cart

Copyright OpenHelix. No use or reproduction without express written consent

13

My Cart

GenocAD

CAD Software for Synthetic Biology

Welcome, Researcher 1 | My Profile | Log Out

STEP 1: PARTS STEP 2: DESIGN STEP 3: SIMULATE

Grammars Libraries My Parts My Cart (5)

Expand All Collapse All Empty List

Training Set E. Coli Grammar

Promoter (PRC)

Gene (GEN)

My Cart

Select Library - New Library

Copy to Library Remove from My Cart After Copy? Export to: FASTA Tab-Delimited

Show 25 entries

Filter records:

Part ID	Part Name	Modified
a0d5	E1010 - RFP Mutant	07/25/2013
a0d7	E0020 - eCFP	07/25/2013
a0d8	E0020 - eYFP	07/25/2013
a0d4	E0040 - GFP Wild Type	07/25/2013
a0d6	E0500 - Inducible pBadlacZ promoter	07/25/2013

Showing 1 to 5 of 5 entries

First Prev 1 Next Last

- My Cart has my selections

Copyright OpenHelix. No use or reproduction without express written consent

14

Add More Parts

- We need other parts from Training grammar
- Find T7 terminator, and an RBS
- Key point: parts need to be compatible with the grammar you intend to use
- Choose ones compatible with Training Grammar

GenocAD

CAD Software for Synthetic Biology

Welcome, Researcher 1 | My Profile | Log Out

STEP 1: PARTS STEP 2: DESIGN STEP 3: SIMULATE

Advanced Search: (Basic)

SEARCH BY: Any Part

SEARCH RESULTS

Part ID Part Name Score

a0d7	E0020 - eCFP	57.19
a0d8	E0020 - eYFP	57.12
a0d5	E1010 - RFP Mutant	56.64
a0d4	E0040 - GFP Wild Type	56.17
a0d6	E0500 - Inducible pBadlacZ promoter	55.8
a0d9	R0040 - TetR repressible promoter	55.8
a0da	R0010 - LacI regulated promoter	55.8
a0db	I13453 - Pbad promoter no AraC	55.8
a0dc	J2034 -	55.8
a0dd	R0011	55.8
a0de	R0085 - T7 Consensus Promoter	55.8

Build a query

Part name: T7

Category: RBS

Join with "MAY Include"

click

Copyright OpenHelix. No use or reproduction without express written consent

15

Return to My Cart

GenocAD

CAD Software for Synthetic Biology

Welcome, Researcher 1 | My Profile | Log Out

STEP 1: PARTS STEP 2: DESIGN STEP 3: SIMULATE

Grammars Libraries My Parts My Cart (7)

Expand All Collapse All Empty List

Training Set E. Coli Grammar

Promoter (PRC)

Gene (GEN)

Terminator (TER)

My Cart

Select Library - New Library

Copy to Library Remove from My Cart After Copy? Export to: FASTA Tab-Delimited

Show 25 entries

Filter records:

Part ID	Part Name	Modified
a0d5	E1010 - RFP Mutant	07/25/2013
a0d7	E0020 - eCFP	07/25/2013
a0d8	E0020 - eYFP	07/25/2013
a0d4	E0040 - GFP Wild Type	07/25/2013
a0d6	E0500 - Inducible pBadlacZ promoter	07/25/2013
a0d9	R0040 - TetR repressible promoter	07/25/2013
a0da	R0010 - LacI regulated promoter	07/25/2013
a0db	I13453 - Pbad promoter no AraC	07/25/2013
a0dc	J2034 -	07/25/2013
a0dd	R0011	07/25/2013
a0de	R0085 - T7 Consensus Promoter	07/25/2013

Showing 1 to 7 of 7 entries

First Prev 1 Next Last

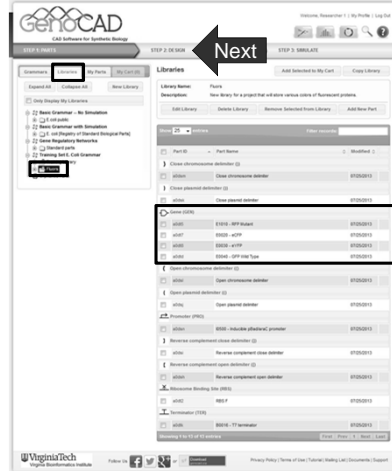
- Cart now has the items we need to design
- Select all, copy to Fluors library

Copyright OpenHelix. No use or reproduction without express written consent

16

Examine the Fluors Library

- Access the libraries tab
- Click the Fluors item
- All the parts are available
- Next let's design some color constructs



Copyright OpenHelix. No use or reproduction without express written consent

17

GenoCAD Advanced Agenda

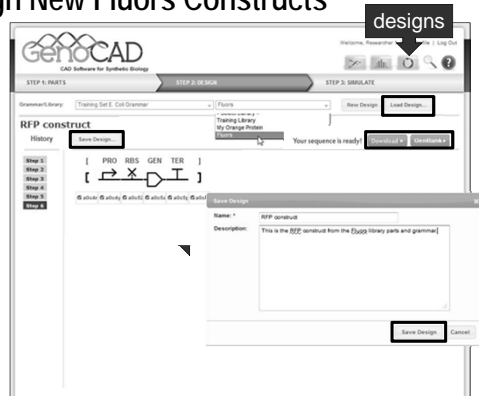
- Introduction and Review
- New Library with Advanced Searching
- Advanced Design
 - Copying and Reworking
 - Export GenBank format → other tools
- Grammar from Scratch
- Modifying (Editing) Grammar
- Summary
- Exercises

GenoCAD: <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent

18

Design New Fluors Constructs

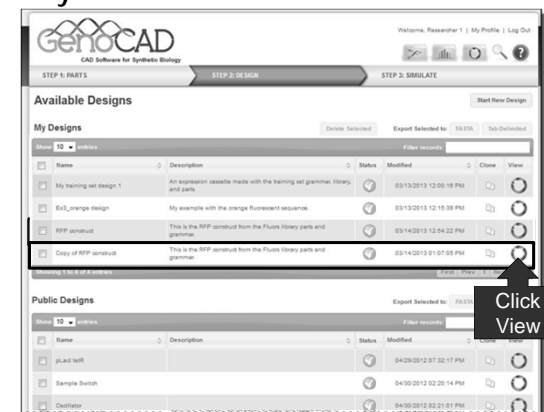


- Use the Training Set Grammar + Fluors Library
- This time let's reverse the orientation, use PCT-

Copyright OpenHelix. No use or reproduction without express written consent

19

Quickly Clone a Construct



- From Designs list you can quickly "clone" copies
- Click to "clone" the RFP construct

Copyright OpenHelix. No use or reproduction without express written consent

20

Work with a Cloned Design



- You can quickly swap out pieces
- Generate similar constructs easily

Copyright OpenHelix. No use or reproduction without express written consent

21

GenoCAD Advanced Agenda

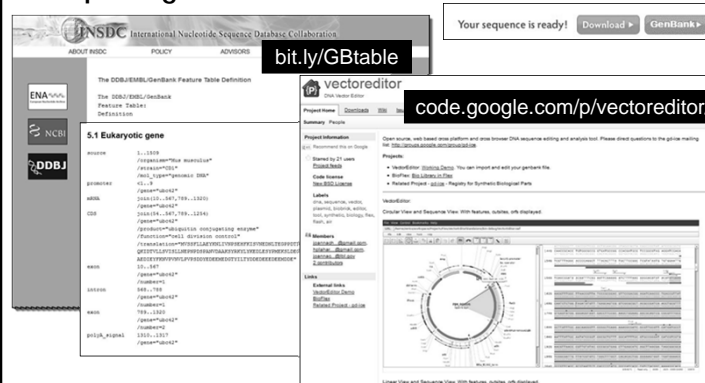
- Introduction and Review
- New Library with Advanced Searching
- Advanced Design
 - Copying and Reworking
 - Export GenBank format → other tools
- Grammar from Scratch
- Modifying (Editing) Grammar
- Summary
- Exercises

GenoCAD: <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent

22

Exporting GenBank Format

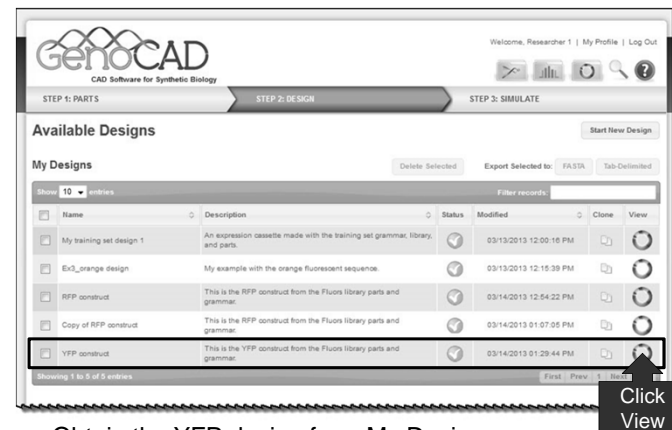


- Categories may be mapped to GenBank features
- Can use this to visualize features in GenBank-compliant tools
- Illustrated with VectorEditor Demo web tool

Copyright OpenHelix. No use or reproduction without express written consent

23

Obtain YFP GenBank Format File



- Obtain the YFP design from My Designs
- Click "View" to access GenBank output

Copyright OpenHelix. No use or reproduction without express written consent

24

Exporting with GenBank Features

The screenshot shows the GenoCAD interface with the 'Export' dialog box open. The 'GenBank' button is highlighted. The 'Features' area is visible, showing a list of features for the 'YFP construct'.

- Select the GenBank button
- Obtain your text file
- Examine the Features area

Copyright OpenHelix. No use or reproduction without express written consent

Upload the YFP File

The screenshot shows the VectorEditor interface with the 'Upload' dialog box open. The 'orientation' button is highlighted. The 'Features' area is visible, showing a list of features for the 'YFP construct'.

- Access VectorEditor <http://bit.ly/VectorEditor>
- Import the YFP construct file
- View the features

Copyright OpenHelix. No use or reproduction without express written consent

GenoCAD Advanced Agenda

- Introduction and Review
- New Library with Advanced Searching
- Advanced Design
 - Copying and Reworking
 - Export GenBank format → other tools
- Grammar from Scratch
- Modifying (Editing) Grammar
- Summary
- Exercises

GenoCAD: <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent

Grammar from Scratch

The screenshot shows the GenoCAD interface with the 'Create New Grammar' dialog box open. The 'Import Grammar' button is highlighted. The 'Features' area is visible, showing a list of features for the 'YFP construct'.

- Can create a new grammar to suit project needs
- Use them to create a construct with "Design" options

Copyright OpenHelix. No use or reproduction without express written consent

GenoCAD
CAD Software for Synthetic Biology

STEP 1: PARTS STEP 2: DESIGN STEP 3: SIMULATE

Create New Grammar

Back to Parts Browser

Create a new grammar by completing and submitting the following form:

Name: * test new grammar

Description: *

Image Set: * biobricks_icon_set

Preview click

Virginia Bioinformatics Institute

Twitter Facebook YouTube LinkedIn

Download Grammar

Browse Images

Browse Icon Set: biobricks_icon_set

Icon_1.png Icon_2.png Icon_3.png Icon_4.png

Icon_5.png Icon_6.png Icon_7.png Icon_8.png

Icon_9.png Icon_10.png Icon_11.png Icon_12.png

Icon_13.png Icon_14.png Icon_15.png Icon_16.png

Icon_17.png Icon_18.png Icon_19.png Icon_20.png

Select Close

- Name and describe a grammar, choose an icon set
- Brand new grammar is created

Copyright OpenHelix. No use or reproduction without express written consent

- Copyright OpenHelix. No use or reproduction without express written consent

2

The screenshot displays the GenCAD software interface, specifically the 'Manage Grammar' window. The window has a title bar with 'GenCAD' and 'CAD Software for Synthetic Biology'. Below the title bar, there's a 'STEP 1: INITIALS' and 'STEP 2: DESIGN' section. The 'Manage Grammar' window is divided into several sections: 'Name' (Researcher's new grammar), 'Description' (creating a grammar from scratch), 'Starting Category' (Start: Transcription unit (1)), and 'Icon Set' (transcription_unit). There's a 'Categories' section with a 'New Category' button highlighted. Below it, there's a list of categories: 'Expand All', 'Collapse All', 'Resuable Categories', 'Terminal Categories', and 'Non-Terminal Categories'. To the right, there's a 'Category Detail' window with a 'Letter' field, 'Description', 'Genbank Qualifier', and 'Icon'. Below this, there's a 'Category Rules' window with 'Add Rules', 'Edit Rules', and 'Delete Rules' buttons. The 'Add Rules' button is highlighted. At the bottom, there's a 'Save' and 'Cancel' button. The bottom of the screen shows the Virginia Tech logo and social media links.

- Copyright OpenHelix. No use or reproduction without express written consent.

30

The screenshot displays the GenCAD software interface. At the top, the GenCAD logo is visible on the left, and the user's profile and log out options are on the right. Below the header, there are three tabs: 'STEP 1: PARTS', 'STEP 2: DESIGN', and 'STEP 3: SIMULATE'. The 'Grammar Summary' section is active, showing a list of grammars on the left and a 'Manage Grammar' dialog box on the right. The dialog box is titled 'Basic Grammar - No Simulation' and contains the following information:

- Name:** Basic Grammar - No Simulation
- Description:** 1, 2 or 3 cassette(s), each with a promoter, a ribosome binding site, a coding sequence and a terminator
- Starting Category:** Start / Transcription unit (S)
- Non-Set:** ribo/..._3'_non_set

The 'Copy' button is highlighted in the dialog box. The background interface shows a sidebar with a tree view of grammars, including 'Public Grammars', 'Basic Grammar - No Simulation', 'Gene Regulatory Networks', 'User Grammars', 'Training Set E: Coli Grammar', and 'Researcher1 new grammar'. The main area displays the 'Manage Grammar' dialog box, which is a form for editing grammar details. The 'Copy' button is located in the top right corner of the dialog box, next to the 'Export' button. The 'Copy' button is highlighted with a red box. The 'Export' button is also highlighted with a red box. The 'Copy' button is located in the top right corner of the dialog box, next to the 'Export' button. The 'Copy' button is highlighted with a red box. The 'Export' button is also highlighted with a red box.

- Another way to create a new grammar: copy one
- It will then become editable to modify for your needs

Copyright © GenCoE. No use or reproduction without express written consent

- Copyright OpenHelix. No use or reproduction without express written consent

3

The screenshot shows the GenoCAD software interface. At the top, the logo 'GenoCAD' is displayed with the tagline 'CAD Software for Synthetic Biology'. The interface is divided into three main sections: 'STEP 1: PARTS', 'STEP 2: DESIGN', and 'STEP 3: SIMULATE'. The 'Grammar Summary' section is active, showing a list of grammars: 'Public Grammars', 'Basic Grammar -- No Simulation', 'Basic Grammar with Simulation', 'Regulatory Network', 'User Grammars', 'Training Set E. Coli Grammar', and 'Researcher's new grammar'. The 'Manage Grammar' dialog box is open, displaying details for the 'Basic Grammar -- No Simulation'. The dialog box has a tab 'Basic Grammar -- No Simulation' and a button 'Add / Import Grammar'. It contains fields for Name, Description, Starting Category, and Icon. The 'Export' button is highlighted. A secondary dialog box titled 'Export Grammar (Basic Grammar -- No Simulation)' is open, showing options to export the grammar as a file or a folder. The 'Export' button in this dialog is also highlighted.

- Another way to work with a grammar: export
- Any grammar can also be exported and shared

Copyright: OpenHelix. No use or reproduction without express written consent

- Copyright OpenHelix. No use or reproduction without express written consent

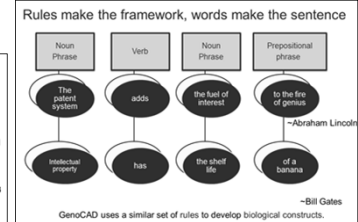
32

2

- GenoCAD:** <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent

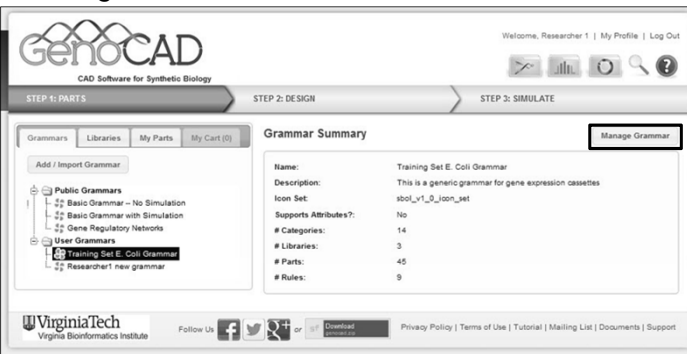
31



- Copyright OpenHelix. No use or reproduction without express written consent

34

2



- Copyright OpenHelix. No use or reproduction without express written consent

35

2



- Copyright OpenHelix. No use or reproduction without express written consent.

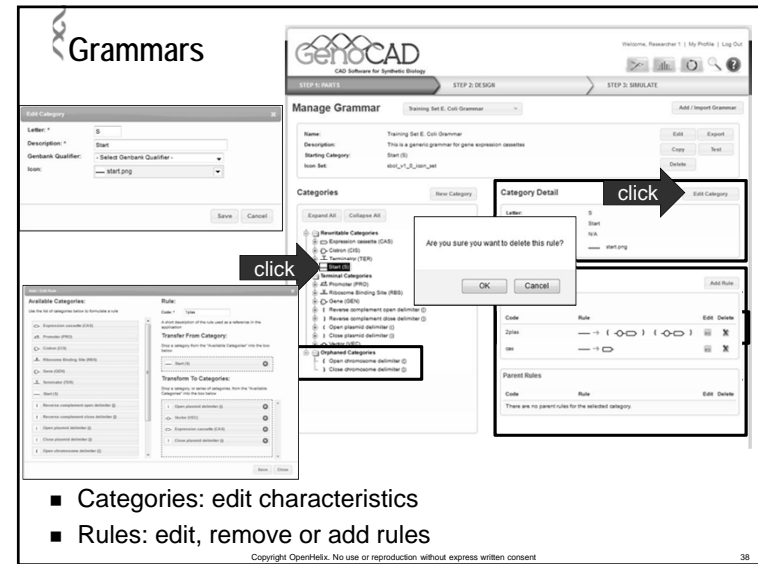
36

[illegible]

- Rewritable category items transform to another rule
- Parent \rightarrow child
- Child \rightarrow child
- Terminal category has no child rules

Copyright OpenHelix. No use or reproduction without express written consent

37



- Categories: edit characteristics
- Rules: edit, remove or add rules

Copyright OpenHelix. No use or reproduction without express written consent

38

Adding Custom Grammar Elements

A)

DNA encoding Protein

His-TAG

vector

ligation

DNA encoding Protein

His-TAG

vector

B)

DNA encoding Protein

His-TAG

Forward Primer

Reverse Primer

PCR

His-TAG

DNA encoding Protein

Antigens

Antigen

Antigen-binding site

Antibody

<http://en.wikipedia.org/wiki/File:His-tag.png>

A sample column for Ni^{2+} -affinity chromatography. The sample and elution buffers are manually poured into the column.

<http://en.wikipedia.org/wiki/Polyhistidine-tag>

Protein specific sequence

5' ATG cat cat cat cat cat cat CAT TNN TNN TNN ... 3'

↑

START codon

His-TAG

Protein specific sequence

5' ... TNN TNN TNN TNN TNN TNN TNN TAG 3'

↑

STOP codon

His-TAG

<http://en.wikipedia.org/wiki/File:His-tag-primers.png>

- Add new grammar items useful for your projects
- A commonly used strategy: protein tags
- We'll employ a his-tag, poly-histidine stretch

Copyright OpenHelix. No use or reproduction without express written consent

39

Add Category

- Add a new category to the Training Grammar
- Histidine tags
- Letter: HisTag
- GenBank Qualifier: misc_binding
- Icon: protein-stability-element.png

The screenshot shows the GenOCAD software interface. The main window is titled 'GenOCAD' and 'CAD Software for Synthetic Biology v2.2.1'. The 'STEP 3: DESIGN' tab is active. The 'Manage Grammar' section shows the 'Training Set 5: Cdk Grammar' selected. The 'Add Category' button is visible. The 'Categories' list shows 'Reversible Categories' (e.g., 1-10 Systemic categories (SAS), 1-10 Cation (SAS), 1-10 Anion (SAS), 1-10 Start (S)), 'Terminal Categories' (e.g., 1-10 HisTag (HIS), 1-10 Histidine Binding Site (HIS), 1-10 Histidine complementation site (HIS), 1-10 Histidine complementation site (HIS), 1-10 Open chromatin site (HIS), 1-10 Open chromatin site (HIS), 1-10 Open Vector (HIS), 1-10 Open Chromatin site (HIS), 1-10 Open Chromatin site (HIS)), and 'Unlinked Categories' (e.g., 1-10 Histidine tag for expression constructs (HIS)). The 'Category Rules' section shows 'Child Rules' (e.g., 1-10 Histidine tag for expression constructs (HIS)) and 'Parent Rules' (e.g., 1-10 Histidine tag for expression constructs (HIS)). The 'Category Rules' section is highlighted with a red box.

GenOCAD
CAD Software for Synthetic Biology v2.2.1

STEP 3: DESIGN

Manage Grammar

Training Set 5: Cdk Grammar

Add Import Grammar

Name: Training Set 5: Cdk Grammar

Description: This is a generic grammar for gene expression constructs

Starting Category: Start (S)

Icon Set: select one of icons set

Categories

New Category

Category Detail

Letter: HIS

Description: 1-10 Histidine tag for expression constructs

GenBank Qualifier: misc_binding

Icon: protein-stability-element.png

Category Rules

Child Rules

Code Rule Edit Details

There are no child rules for the selected category.

Parent Rules

Code Rule Edit Details

There are no parent rules for the selected category.

VirginiaTech
Virginia Bioinformatics Institute

Follow us on Facebook, Twitter, YouTube, LinkedIn, and Google+.

Privacy Policy | Terms of Use | Tutorial | History | Help | Downloads | Support

Protein specific sequence

Protein specific sequence

START codon

HIS-TAG

STOP codon

START codon

HIS-TAG

STOP codon

Copyright OpenHelix. No use or reproduction without express written consent

40

- Add a new category to the Training Grammar
- Histidine tags
- Letter: HisTag
- GenBank Qualifier: misc_binding
- Icon: protein-stability-element.png

Copyright OpenHelix. No use or reproduction without express written consent

40

Add Rules to a Grammar

- Cistron with histidine tags need rules: CIS → RBS GEN HIS
- One tag at the end of a cistron (histag-C)

Copyright OpenHelix. No use or reproduction without express written consent

New Rule is Available, Old Rules can be edited

- Cistron-using designs can now have his-tag
- HisTag is now a terminal category because it is used

Copyright OpenHelix. No use or reproduction without express written consent

Rule Editing has Consequences

- Change to rbgn rule affects existing constructs
- View the "Copy of RFP construct"

Copyright OpenHelix. No use or reproduction without express written consent

Validation Status

- Return to the Design list
- Note the change in status
- Examine the status key
- Re-work the design by clicking the steps again until complete

Copyright OpenHelix. No use or reproduction without express written consent

GenoCAD Advanced Agenda

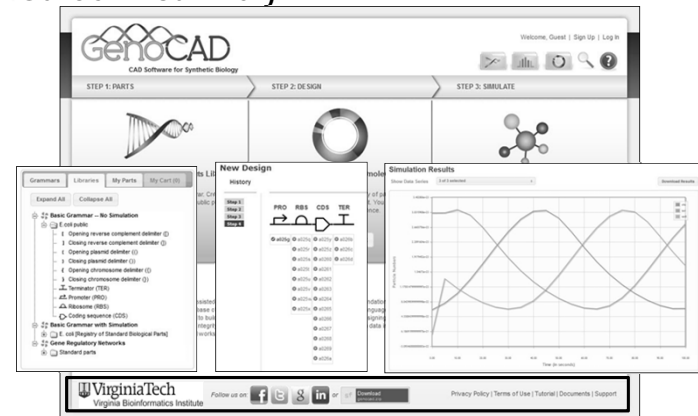
- Introduction and Review
- New Library with Advanced Searching
- Advanced Design
 - Copying and Reworking
 - Export GenBank format → other tools
- Grammar from Scratch
- Modifying (Editing) Grammar
- Summary
- Exercises

GenoCAD: <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent

45

GenoCAD Summary



- GenoCAD is a toolbox for synthetic biology
- Parts, grammars, designs, and simulations

Copyright OpenHelix. No use or reproduction without express written consent

46

GenoCAD Advanced Agenda

- Introduction and Review
- New Library with Advanced Searching
- Advanced Design
 - Copying and Reworking
 - Export GenBank format → other tools
- Grammar from Scratch
- Modifying (Editing) Grammar
- Summary
- Exercises

GenoCAD: <http://www.genocad.org/>

Copyright OpenHelix. No use or reproduction without express written consent

47

 OpenHelix™

Copyright OpenHelix. No use or reproduction without express written consent

48