

Honey and Biotech

The alchemist honey bee: Uncovering the floral resources that create liquid gold - teacher

Identify the plants visited by a colony of bees through DNA analysis of honey.

Background

Plant-pollinator interactions are some of the most important relationships on the planet. At the local scale, the honey bee (*Apis mellifera*) and native pollinators produce critical services to our community flower and vegetable gardens. At the commercial scale, beekeepers travel around the U.S. with thousands of colonies to increase the quality and yield of food crops, including apples, plums, cherries, blueberries, canola, and almonds. Honey bees fly up to two miles away from their colony, which makes it difficult to know what floral resources are used by bees. DNA analysis of bee-collected pollen and honey can reveal the plant preferences of worker bees. This DNA analysis of bee-collected pollen can reveal the presence of rare or endangered plant populations in the local area. Thus, identifying the plant taxa visited by bees is useful in both agriculture and conservation or wildlife biology.

This activity will explore the biotechnology and DNA markers used to distinguish plant taxa through honey and pollen samples. To demonstrate the broad applicability of DNA analysis, the activity will have two scenarios from the perspective of a commercial beekeeping operation and a conservation biologist. Both scenarios include a step-by-step activity using analytical tools in the open-access website DNA Subway.

Scenario 1 - Commercial Beekeeper

Marty McFly, marketing manager at Awesome Bee Honey Farms, won several prestigious awards at the state fair during the honey tasting event. To the dismay of the beekeeper, they lost records on what bee colony produced the winning honey. They have only two broad landscapes where they raise bees: near crop fields and by pasture lands. Luckily some of the honey was left over for laboratory testing and the beekeeper sent out a sample to a diagnostic laboratory for analysis. The laboratory produced a report containing all the DNA sequences from the honey sample. Your job as a DNA analyst (bioinformatician) is to help Awesome Bee Honey Farms identify the plant species, using the given sequences of DNA and basic bioinformatic tools.

Scenario 2 - Wildlife Biologist

Dr. Sue Cameron, conservation biologist, is on the hunt for rare plant species in Ohio. There have been stories circulating in town about a federally-endangered species still persisting in a county where it has not been documented in over 20 years. Unfortunately the landscape is remote and treacherous, making it difficult to survey. Dr. Cameron knew that the rare plant of interest attracts bees and asked Awesome Bee Honey Farms if they have a bee colony near the area. After locating a colony at a nearby site, a honey sample was sent to a diagnostic laboratory for analysis. The laboratory produced a report containing all the DNA sequences from the honey sample. Your job as a DNA analyst (bioinformatician) is to help identify the plant species contained within the honey, using the given sequences of DNA and basic bioinformatic tools.

Materials

computer or tablet with access to internet
DNA sequence files from bee-collected pollen from [GrowNextGen.org](https://grownextgen.org)

Procedure

Download the honey DNA sequences in the Honey Sequences folder to your desktop.
<https://grownextgen.org/go/honey>

Go to: <https://dnasubway.cyverse.org/> then follow the steps below to analyze your sequences.

On the DNA subway page, **enter as a guest**.

Click on **Determine sequence relationships** (blue square).



Honey and Biotech

You are now on the blue line of the DNA Subway. Complete the following steps:

1. Select Project Type - **DNA**.
2. Click **Enter sequences in FASTA format**.
3. Copy and Paste sequences directly into the text box.
4. Give a title under **Name Your Project**.
5. Click **Continue**.

STOP 1 Assemble Sequences

1. Once the sequences are uploaded, click **Sequence Viewer**.
2. The sequences will display in FASTA-format. Below the sequences a scroll bar will appear. Scroll along the entire length of the sequence. Close out of that window by clicking on the **X** in the top right corner.

STOP 2 Add Sequences

1. Click **BLASTN** (Basic Local Alignment Tool – for Nucleotides) to run the algorithm. In the window, click on **BLAST** in the Action column for each sequence. After a few seconds, you will see the sequence hits within the database (Accession # and Details) as well as comparison values (mismatches) between your sequence and the sequences found in the database. Repeat the analysis for each sequence.
2. Click **View Results** in the Action column to answer the questions below.

Answer the following before continuing

1. What are the scientific and common names of the plants from the BLASTN analysis? (Hint: The correct plant ID contains the fewest mismatches with the BLAST results.)

Accession #	Scientific Name	Common Name
(see tables on following page)		

2. What barcoding gene was used in the DNA analysis?
Awesome Honey Farms: tRNA_Leu_(trnL)_gene
3. In which organelle is the barcode located?

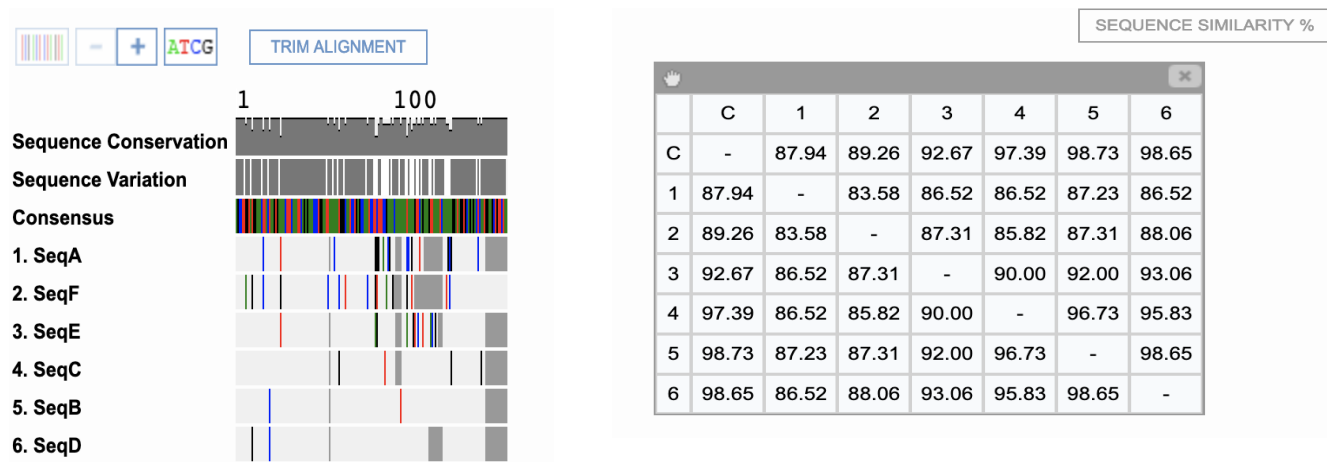
Chloroplast

Honey and Biotech

STOP 3 Analyze Sequences

1. Click **Select Data**.
2. Click **Select All**, make sure it includes the Blast hit you added to the project.
3. Then **Save Selections** in the right box that appears.
4. Click **MUSCLE** (Multiple Sequence Comparison by Log-Expectation) to run the algorithm, then click again to see the Alignment Viewer results.
5. Click **Sequence Similarity** in the upper right of the Alignment Viewer to see the pairwise comparison table. Use this table to answer the following questions.

*The following pairwise comparison is for Awesome Honey Farm sequences.



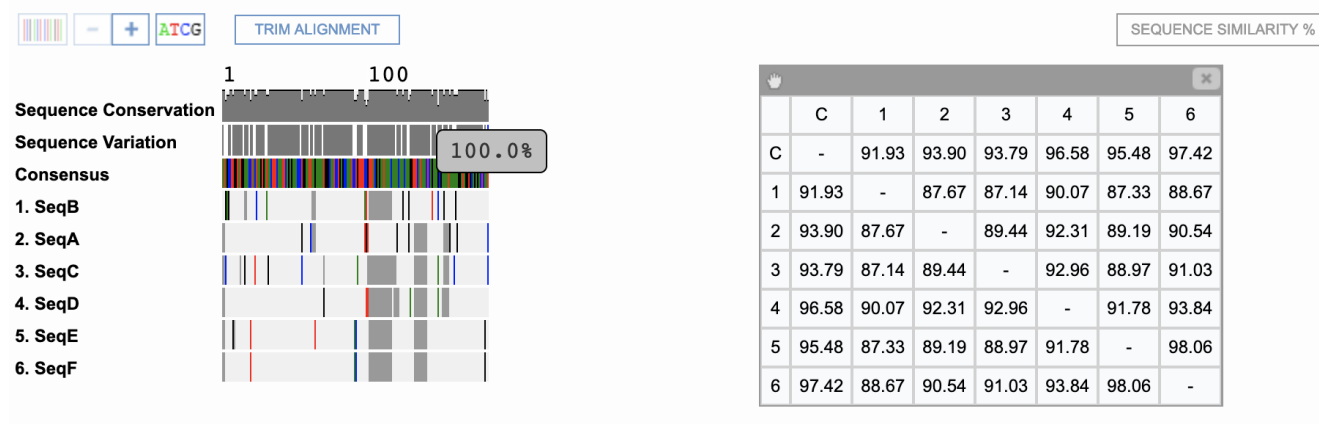
**Note the sequence letters do NOT line up in alphabetical order. Be sure to match the sequence letter with the number in the alignment view to do the comparison.

Which sequences are most similar? They will have the highest % similarity, which is 98.65. This is between Seq D and Seq B (5 and 6 on the table).

Which sequences are most different? They will have the lowest % similarity, which is 83.58. This is between Seq A and Seq F. (1 and 2 on the table).

Honey and Biotech

*The following pairwise comparison is for the conservation biologist sequences.



Which sequences are most similar? They will have the highest % similarity, which is 98.06. This is between Seq E and Seq F (5 and 6 on the table).

Which sequences are most different? They will have the lowest % similarity, which is 87.14. This is between Seq B and Seq C. (1 and 3 on the table).

Answer the following before continuing

1. What two species are the most similar at this gene? What was the % similarity?

Awesome Honey Farms: Alfalfa and Yellow sweet clover

Conservation biologist: Dandelion and Black-eyed Susan

2. What two species contain the most differences at this gene? What was the % similarity?

Awesome Honey Farms: Canola and Buckwheat

Conservation biologist: Spiraea and Milkweed

Reflection and analysis

1. What category of plant type was the main nectar source in the dataset? (Crops/Wildflowers)

Awesome Honey Farms: crops

Conservation biologists: wildflowers

2. How can these results help the person submitting the honey for testing?

Answers will vary, but might include: distance bees are flying to collect nectar, identify threats to their survival (i.e. construction that is removing these plants, change in practices that may impact pollinator feeding patterns, etc).

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Honey and Biotech

Sequence identification

Awesome Honey Farms - Award Winning Honey

Sequence Name	Full Description	Notes
SeqA	DQ131548 _Canola_Brassica_napus_tRNA_Leu_(trnL)_gene, intron; chloroplast	Rapeseed is canola
SeqB	DQ311711 _Alfalfa_Medicago_sativa_tRNA_Leu_(trnL)_gene, intron; chloroplast	Match is #7 in list
SeqC	MW261445 _RedClover_Trifolium_pratense_tRNA_Leu_(trnL)_gene, intron; chloroplast	Match is #10 in list
SeqD	AB546812 _YellowSweetClover_Melilotus_officinalis_tRNA_Leu_(trnL)_gene, intron; chloroplast	Match is #2 in list
SeqE	KF741114 _LeafySpurge_Euphorbia_esula_tRNA_Leu_(trnL)_gene, intron; chloroplast	Match is #3 in list
SeqF	MT572344 _Buckwheat_Fagopyrum_esculentum_tRNA_Leu_(trnL)_gene, intron; chloroplast	Match is #5 in list

Conservation Biology - Rare plant survey

Sequence Name	Full Description
SeqA	MN450068 _RedMaple_Acer_rubrum_tRNA_Leu_(trnL)_gene, intron; chloroplast
SeqB	DQ897599 _Spiraea_virginiana_tRNA_Leu_(trnL)_gene, intron; chloroplast
SeqC	AF214311 _CommonMilkweed_Asclepias_syriaca_tRNA_Leu_(trnL)_gene, intron; chloroplast
SeqD	EU828156 _GattingersFoxglove_Agalinis_gattingeri_tRNA_Leu_(trnL)_gene, intron; chloroplast
SeqE	EF015611 _CommonDandelion_Taraxacum_officinale_tRNA_Leu_(trnL)_gene, intron; chloroplast
SeqF	AY216108 _Black-eyedSusan_Rudbeckia_hirta_tRNA_Leu_(trnL)_gene, intron; chloroplast