

# Webinar DNA barcoding

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12 steps to acquire a DNA barcode

Prepared by:  
iBOL  
International Barcode of Life Consortium

Technical support:  
Centre for Biodiversity Genomics  
University of Guelph



 Centre for  
**Biodiversity Genomics**



Convention on  
Biological Diversity

international  
BARCODE  
OF LIFE



# Acknowledgements

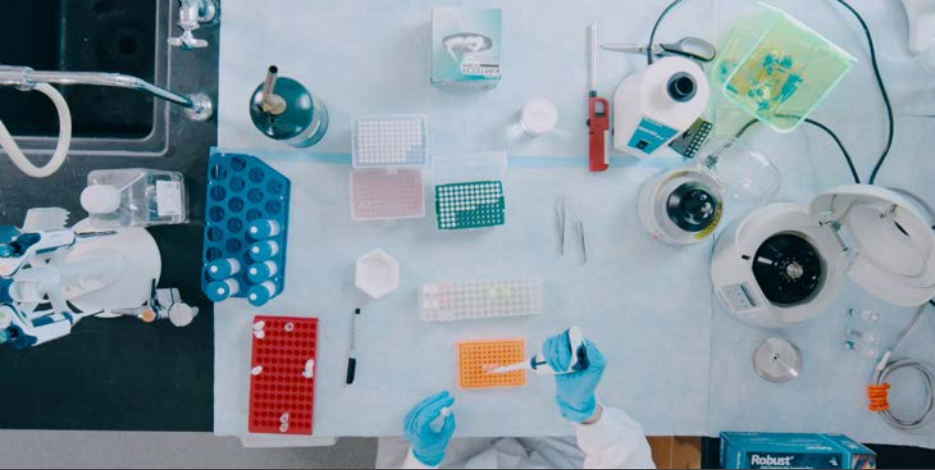
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# Reagents and supplies

- Lysis buffer
- Binding buffer
- Wash buffer
- 96% Ethanol
- ddH<sub>2</sub>O or Elution buffer
- Crushed ice or chilled tube racks
- PCR mastermix
- Agarose powder
- Gel electrophoresis buffer
- Gel loading dye
- Eliminase
- Microcentrifuge tubes (1.5mL, 2.0mL) and sticker labels
- Spin-columns and collection tubes
- PCR tubes (0.5mL)
- Disposable tips for micropipettes





# Laboratory equipment

- Personal protective equipment (lab coat, gloves, safety goggles)
- Sterile tray for specimen
- Burner
- Forceps
- Tube racks
- Micropipettes (10uL, 100uL, 1000uL)
- Vortex mixer
- Microcentrifuge
- Incubator
- PCR thermocycler
- Conical flask
- Microwave
- Gel casting platform
- Power supply
- Gel UV box
- Sequence editing software

# STEP 1

Assemble equipment and reagents.





## STEP 2

Harvest tissue.





## STEP 3

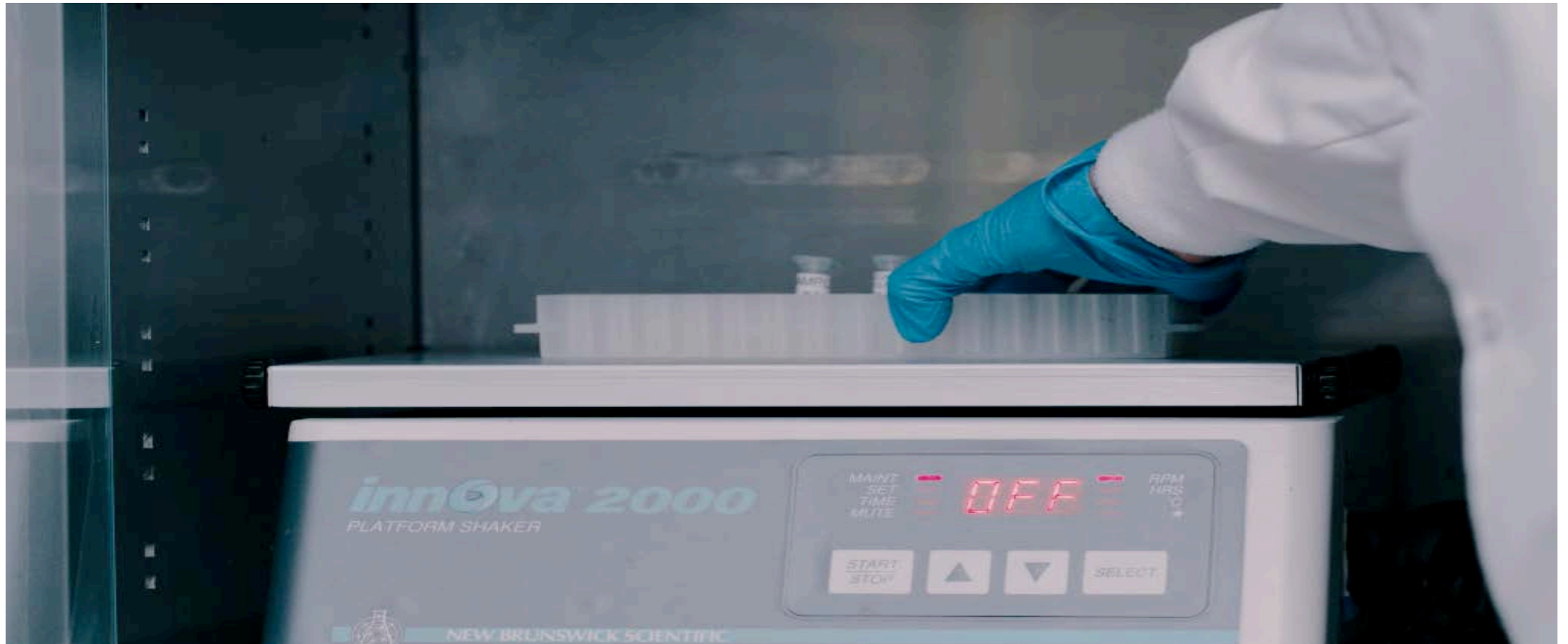
Place tissue in lysis buffer.

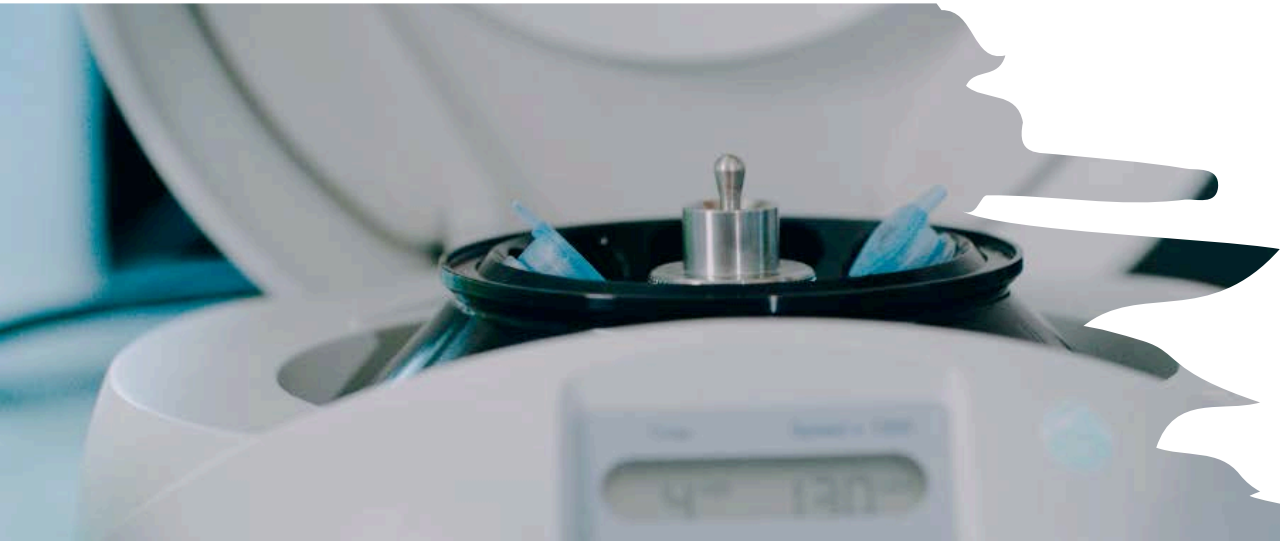
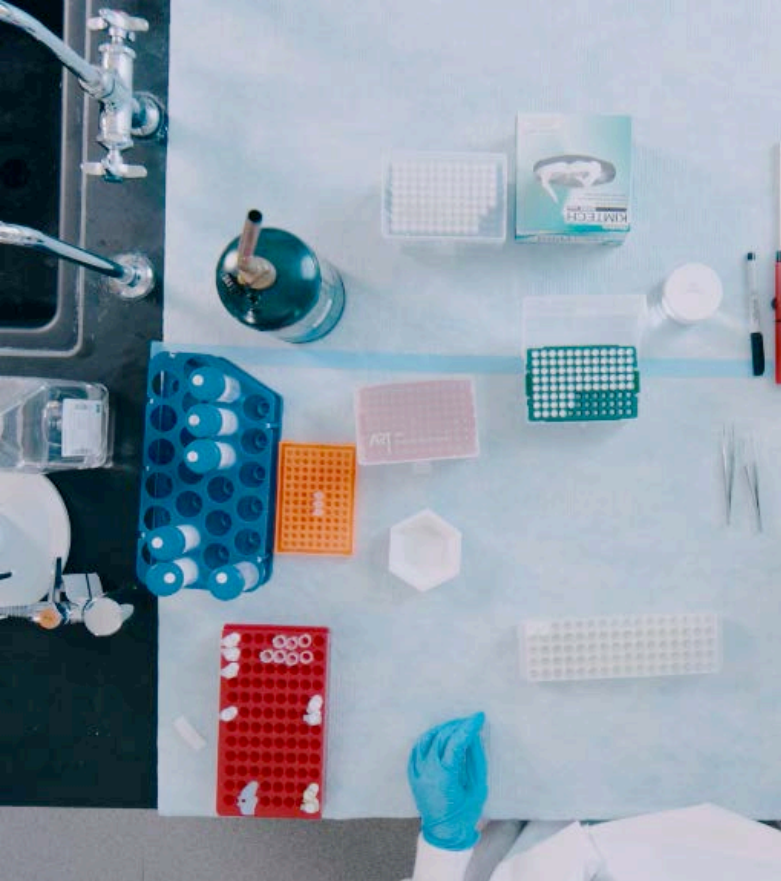
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# EXTRACTION AND PURIFICATION

## STEP 4 Lyse tissue in incubator.





EXTRACTION AND  
PURIFICATION  
**STEP 5** Extract DNA.

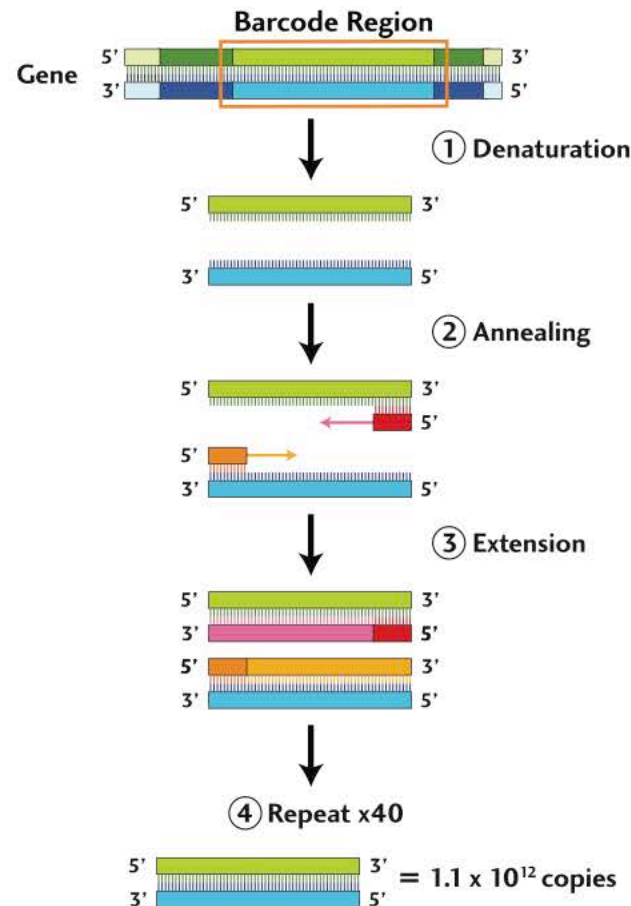
# Polymerase Chain Reaction (PCR)

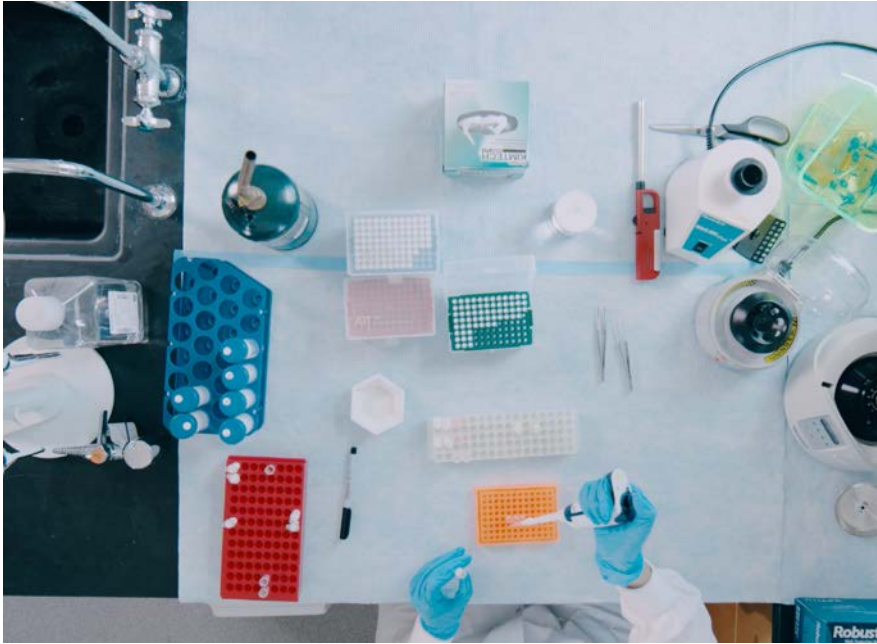
## **STEP 6** Prepare reagents for PCR.





# Set up PCR reaction for barcode region.





PCR  
STEP 7 Set up PCR reaction.

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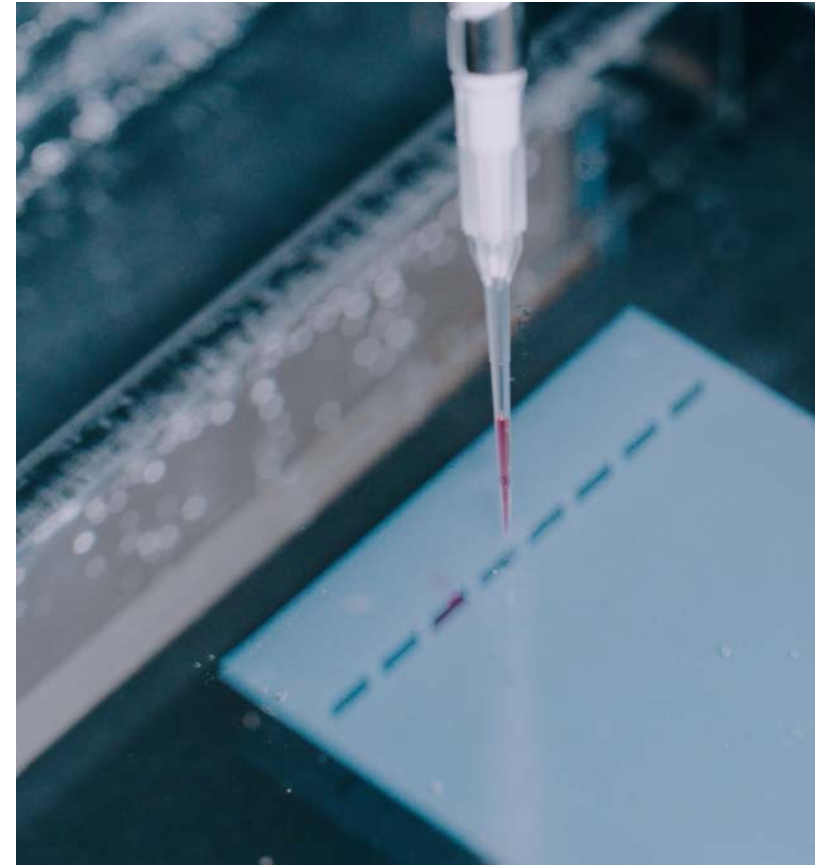
PCR  
**STEP 8** Place tubes in thermocycler.

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Gel electrophoresis  
**STEP 9** Cast a gel.



PCR check  
**STEP 10** Run gel and image.

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## STEP 11

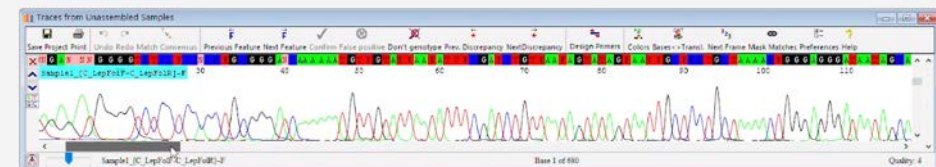
Sequence and  
validate data.



File Edit View Sample Contig Tools View Window Help

Save Project Save Project As Add Samples Add Folder New Folder Undo Redo Reverse Mouse to Trash Clip Ends Make Reference Assemble Assemble in Groups Align to Reference Unassemble Preferences Help


Name	Contents	Length	Quality	Position	Added
Unassembled Samples		1 sample	0	0	08/01/21, 1:15 P
Sample_IC_LapFol-C_LapFolP-F	Trace	680	650	0	08/01/21, 1:15 P
Trash	0 samples	0	0	0	08/01/21, 1:15 P





**BOLD SYSTEMS** DATABASES IDENTIFICATION

Arthropoda / Insecta / Coleoptera / Scarabaeidae / Rutelinae / Anomalini / Popillia / Popillia




Taxonomy  
The Japanese beetle, *Popillia japonica*, is a member of the Scarabaeidae family. It is a common pest of trees and shrubs, feeding on the foliage of many plants.

CC BY-NC-SA CBG Photography Group 2015 Image of *Popillia japonica*

### Statistics

Specimen Records:	446
Specimens with Sequences:	64
Specimens with Barcodes:	63
Subspecies:	0
Subspecies with Barcodes:	0
Public Records:	52
Public Subspecies:	0
Public BINs:	1

### Specimen Depository



- Centre for Biodiversity Genomics [31]
- Mined from GenBank, NCBI [20]
- Canadian National Collection of Insects, Arachnids and Nematodes [8]
- University of Guelph [1]
- Canadian Forest Service, Laurentian Forest Research Institute [1]

**BOLD SYSTEMS** DATABASES IDENTIFICATION TAXONOMY WORKBENCH RESOURCES LOGIN


## BARCODE OF LIFE DATA SYSTEM v4

Advancing biodiversity science through DNA-based species identification.

[EXPLORE THE DATA](#)

DESIGNED TO SUPPORT THE GENERATION & APPLICATION OF DNA BARCODE DATA

BOLD is a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada. It consists of four main modules: a data portal, an educational portal, a registry of BINs (putative species), and a data collection and analysis workbench.




**DATA PORTAL**

A data retrieval interface that allows for searching over 1.7M public records in BOLD using multiple search criteria including, but not limited to, geography, taxonomy, and depository.



**EDUCATION PORTAL**

A custom platform for educators and students to explore barcode data and contribute novel barcodes to the BOLD database.



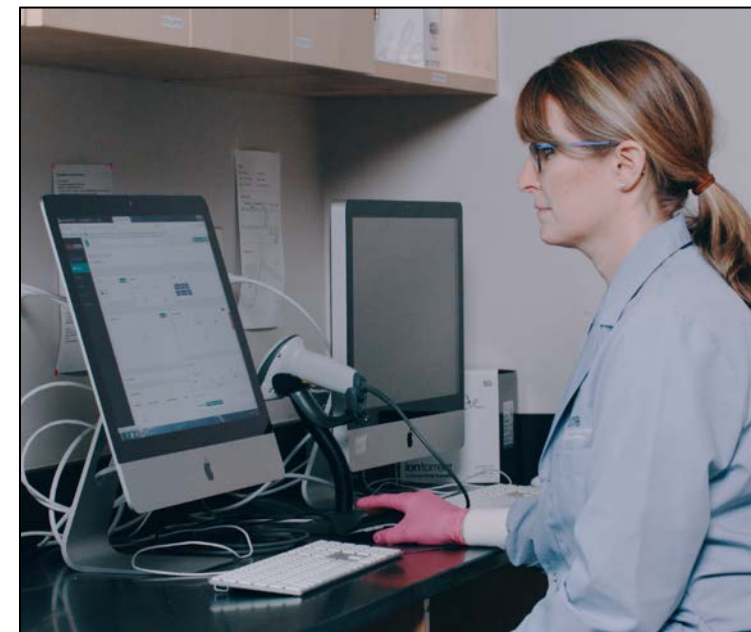
**BIN DATABASE**

A searchable database of Barcode Index Numbers (BINs), sequence clusters that closely approximate species.



**WORKBENCH**

A data collection and analysis environment that supports the assembly and validation of DNA barcodes and other sequences.



# STEP 12

## Analyze data on BOLD.

For more information on the Barcode of Life Data System (BOLD) visit:  
[www.boldsystems.org](http://www.boldsystems.org)



**For more information on DNA  
barcoding as well as technical  
resources and support, please visit:**

- [biodiversitygenomics.net](http://biodiversitygenomics.net)
- [boldsystems.org](http://boldsystems.org)
- [cbd.int](http://cbd.int)
- [ccdb.ca](http://ccdb.ca)
- [ibol.org](http://ibol.org)

