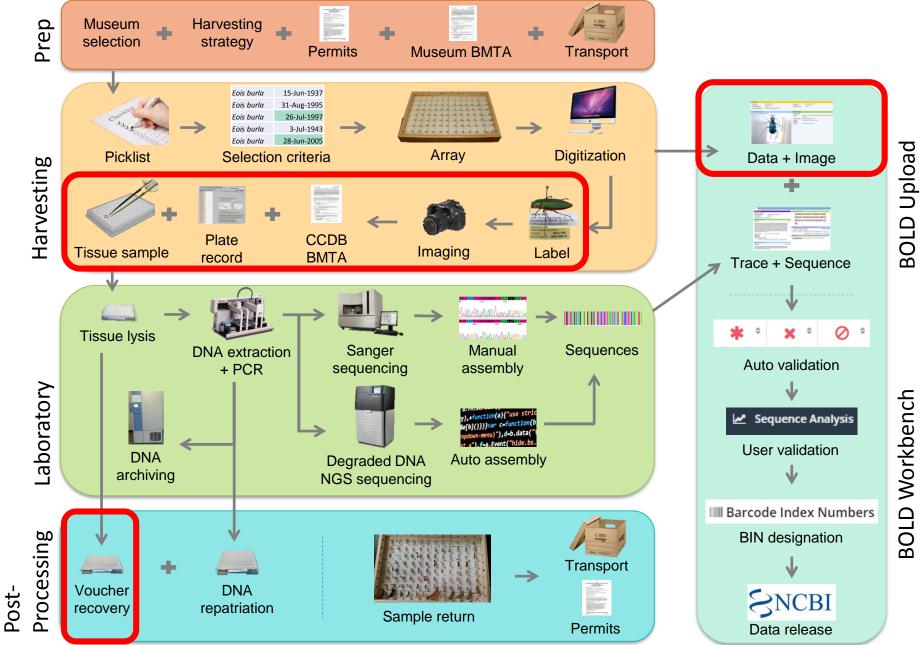


#### 11:45 – 12:30 - Kate Perez and Monica Young Pre-lab Processing

LNAUWB41-17

Centre for Biodiversity Genomics

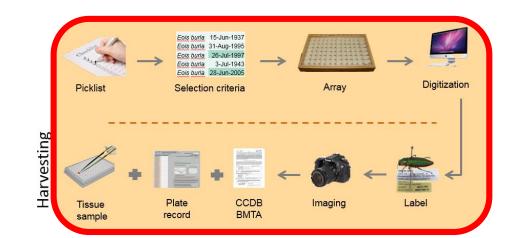
**DNA Barcoding Natural History Collections** 



### Recap

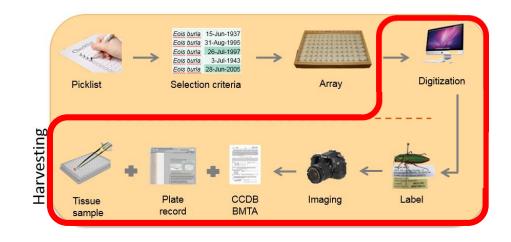
### Sampling on site

- Usually plants and vertebrates
- Completed at the museum



### Borrowing specimens

- Depends on collection, usually for invertebrates
- First 3 steps completed at the museum
- Digitization and pre-lab processing completed at home institution

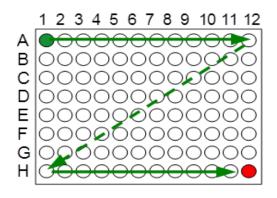


### Before you start...

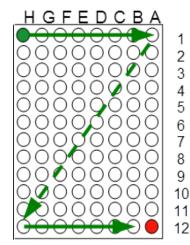
#### **IMPORTANT:** critical to next 3 stages

- Labelling
- Imaging
- Tissue Sampling
- Check orientation of plate and note different controls
  - 12 columns (A01  $\rightarrow$  A12) for animals
  - 8 columns (H01 $\rightarrow$  A01) for plants

#### **12 Column Format**



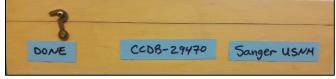
#### 8 Column Format



### Before you start...

Ensure you have labelled all tissue media and all arrays





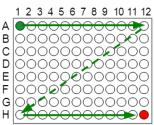
 Ensure you have generated a plate map and use this to verify all stages

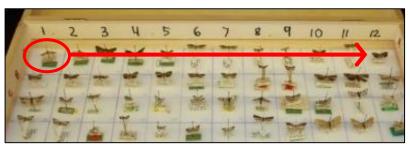
$\begin{array}{c} 1 \ 2 \ 3 \ 4 \ 5 \ 6 \ 7 \ 8 \ 9 \ 10 \ 11 \ 12 \\ A \\ \hline \bullet \\ \bullet \\$		SCROOM BRIDE			<b>Print layout map</b> Ove		пар	CCDB array number: Sample container: rall samples submitted: Map of Sam				
Microplate (animal tissue) SAMPLING ORDER: Begin sampling with position A01 and finish a						sh at H11						
*	01	02	03	04	05	06	07	08	09	10	11	12
Α	USNMENT 00565484	USNMENT 00565485	USNMENT 00565486	USNMENT 00565487	USNMENT 00565488	USNMENT 00565489	USNMENT 00565490	USNMENT 00565491	USNMENT 00565492	USNMENT 00565493	USNMENT 00565494	USNMENT 00565495
В	USNMENT 00565496	USNMENT 00565497	USNMENT 00565498	USNMENT 00565499	USNMENT 00565500	USNMENT 00565501	USNMENT 00565502	USNMENT 00565503	USNMENT 00565504	USNMENT 00565505	USNMENT 00565506	USNMENT 00565507
С	USNMENT 00565508	USNMENT 00565509	USNMENT 00565510	USNMENT 00565511	USNMENT 00565512	USNMENT 00565513	USNMENT 00565514	USNMENT 00565515	USNMENT 00565516	USNMENT 00565517	USNMENT 00565518	USNMENT 00565519
D	USNMENT 00565520	USNMENT 00565521	USNMENT 00565522	USNMENT 00565523	USNMENT 00565524	USNMENT 00565525	USNMENT 00565526	USNMENT 00565527	USNMENT 00565528	USNMENT 00565529	USNMENT 00565530	USNMENT 00565531
Ε	USNMENT 00565532	USNMENT 00565533	USNMENT 00565534	USNMENT 00565535	USNMENT 00565536	USNMENT 00565537	USNMENT 00565538	USNMENT 00565539	USNMENT 00565540	USNMENT 00565541	USNMENT 00565542	USNMENT 00565543
F	USNMENT 00565544	USNMENT 00565545	USNMENT 00565546	USNMENT 00565547	USNMENT 00565548	USNMENT 00565549	USNMENT 00565550	USNMENT 00565551	USNMENT 00565552	USNMENT 00565553	USNMENT 00565554	USNMENT 00565555
G	USNMENT 00565556	USNMENT 00565557	USNMENT 00565558	USNMENT 00565559	USNMENT 00565560	USNMENT 00565561	USNMENT 00565562	USNMENT 00565563	USNMENT 00565564	USNMENT 00565565	USNMENT 00565566	USNMENT 00565567
Н	USNMENT 00565568	USNMENT 00565569	USNMENT 00565570	USNMENT 00565571	USNMENT 00565572	USNMENT 00565573	USNMENT 00565574	USNMENT 00565575	USNMENT 00565576	USNMENT 00565577	USNMENT 00565578	CONTROL

### Before you start...

#### Always follow sampling order

#### **12 Column Format**





### Specimens may not be in array

- Envelopes
- Plants/Fungi
- Vertebrates



\* **NOTE** Plants, Fungi, Vertebrates are usually *labelled*, *imaged* and *tissue sampled* at the **same time** as specimen selection

# **Pre-Lab Processing**

**Specimen Labelling** 

**Specimen Imaging** 

Image Upload

**Tissue Sampling** 

Lab Submission

Voucher Recovery

### Labels to Add When Sampling



Many museums have standards for the format, material, size and colour of voucher labels. Please check prior to labelling.

### **Standard Guidelines**

#### **Materials and format**

- Use acid free paper, ink, and glue
- Durable material e.g. cardstock or fluid resistant
- Colour considerations
- Size and font considerations

### Use forceps to:

- Grasp base of pin and push through label to prevent bent pins
- Remove labels (only if necessary)
- Adjust label spacing and orientation
- Affix glued labels





### **Standard Guidelines: Scannable Labels**

- Link specimen to digital data
- 1D or 2D format
- Consider placement for easy scanning
- Do not obscure or pin through barcode



#### 1D Barcode system



#### 2D Barcode system

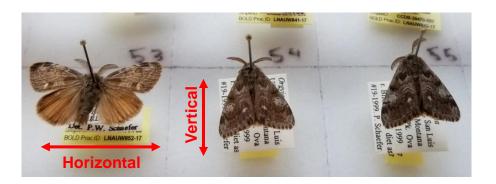




### **Standard Guidelines: Pinned Invertebrates**

### **Orientation and Spacing**

- Align label with specimen preparation and existing labels
- Append new labels at end, label order important
- Ensure labels are spaced evenly
- Ensure scannable labels are accessible





### **Standard Guidelines: Dry Envelopes**

#### **Envelopes**

- Affix label to exterior of envelope using staples or glue (if museum allows) or add to inside
- Use acid free glue
- Option: use sticker labels if acid free





### **Standard Guidelines: Fluid Invertebrates**

- Print on paper intended for fluid immersion
- Caution: printing method important so ink does not rub off
- Samples pulled from parent lots may require parent lot # on label





### **Standard Guidelines: Vertebrates**

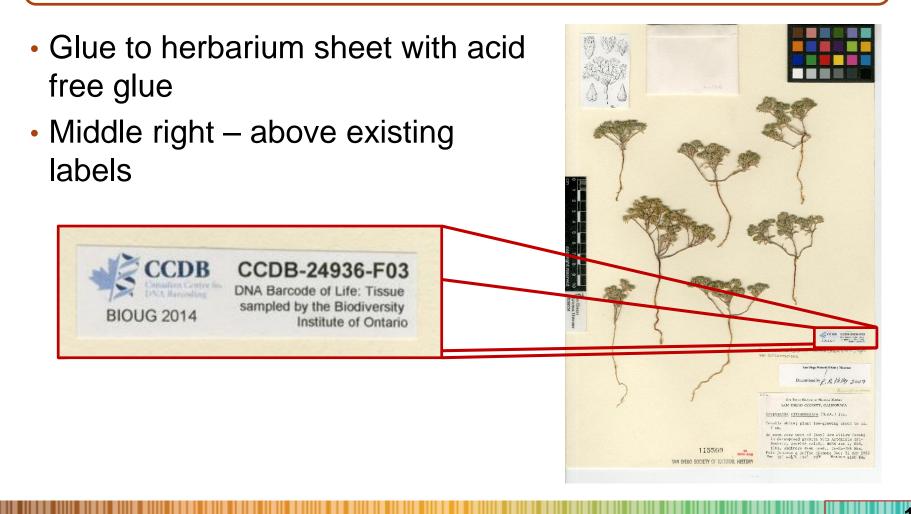
- Generally sampling from tissue archive so you do not affix labels
- More important to add note on digital records that link tissue with skull, skin etc.
- If labelling, then tie to voucher with string that is non-dissolvable and acid free





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### Standard Guidelines: Plants & Fungi



# **Pre-lab Processing**

**Specimen Imaging** 

Image Upload

### **Equipment Overview: DSLR Camera**



### Great for:

- Pinned invertebrates
- Large invertebrates
- Vertebrates

Pros	Cons		
<ul><li>Fast</li><li>Portable</li><li>Inexpensive</li></ul>	<ul> <li>Moderate learning curve</li> <li>Doesn't accommodate very small specimens</li> </ul>		

### **Equipment Overview: Imaging Capable Microscope**



### Great for:

- Small invertebrates
  - Fluid and dry/pinned
- Slides (i.e. genitalia)

Pros	Cons
<ul><li>Reasonably fast</li><li>Built-in software</li></ul>	<ul><li>Relatively expensive</li><li>Less portable</li></ul>

### **Equipment Overview: Scanner**



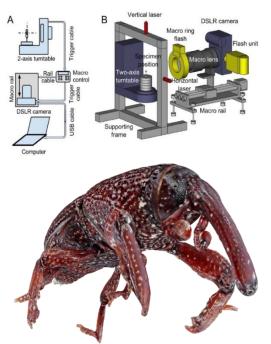
### Great for:

- Plants
- Fungi

Pros	Cons		
<ul> <li>Fast</li> <li>Great for flat</li></ul>	<ul><li>Relatively</li></ul>		
specimens <li>Natural colour</li>	expensive <li>Less portable</li>		

### **Equipment Overview: 3D Imaging**

#### **3D DSLR**



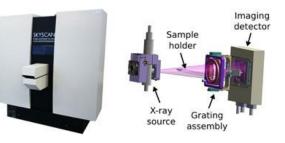
Cedit: Nguyen CV, Lovell DR, Adcock M, La Salle J. Capturing Natural-Colour 3D Models of Insects for Species Discovery and Diagnostics. López-Vaamonde C, ed. *PLoS ONE*. 2014;9(4):e94346. doi:10.1371/journal.pone.0094346

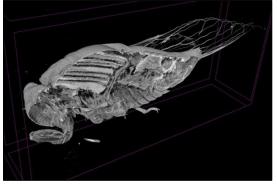




Photo by Eric Erbe; digital colorization by Chris Pooley (USDA, ARS, EMU). This image was released by the Agricultural Research Service, the research agency of the United States Department of Agriculture, with the ID K9077-23

#### **Micro-CT Scanning**





Credit: Chandiramani, S. (2016) Internal anatomy through a cutaway view of the cicada [Digital Image].Retrieved on https://www.microphotonics.com/1376-2/ (Accessed Nov 1, 2017)

### **Equipment Overview: 3D Imaging**

Equipment	Pros	Cons	Great for:
ALL	<ul> <li>Detailed morphology</li> </ul>	<ul> <li>Expensive</li> <li>Not portable</li> <li>Not supported by BOLD</li> </ul>	<ul> <li>Type specimens</li> <li>Species descriptions</li> </ul>
3D DSLR	Natural colour	Time consuming	Pinned specimens
SEM	<ul> <li>Smaller desktop versions available</li> </ul>	<ul> <li>May destroy sample</li> </ul>	<ul> <li>Extremely small specimens</li> </ul>
Micro-CT	<ul> <li>Internal morphology</li> </ul>	<ul><li>Low contrast</li><li>Radiation risk</li></ul>	<ul><li>Histological study</li><li>Variety of taxa</li></ul>

### **Standard Guidelines: Maximum Diagnostic Characters**

### **Example: Diptera**

• Wing venation, bristles, ...

### **Example: Mesostigmata**

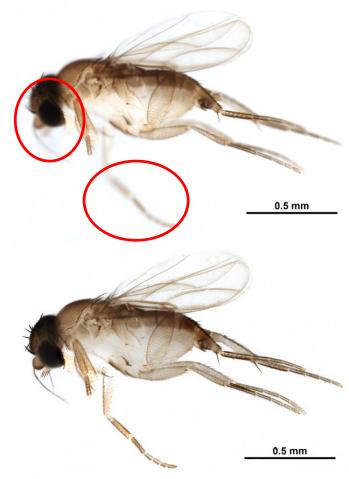
Ventral shield shape & arrangement





### **Standard Guidelines: Z-Stacking**

- Combining a series of images from different focal planes
  - Time consuming but may be necessary to get all of specimen in focus
- Microscopes with automatic z-stacking will have a built in stacking software
- Other software
  - CombineZ by Alan Hadley
  - Helicon Focus
  - Zerene Stacker



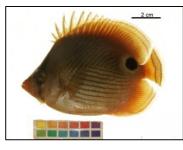
23

### **Standard Guidelines: Specimen Orientation**

#### **BOLD Standards:**



**Dorsal**: Anterior part of specimen face top of image



Lateral: Anterior part of specimen face left of image



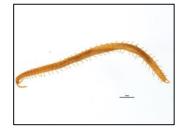
Herbarium: Entire sheet in image (portrait)

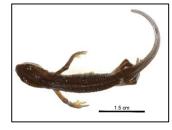
#### **Special Cases:**





Gastropods: Apical and apertural



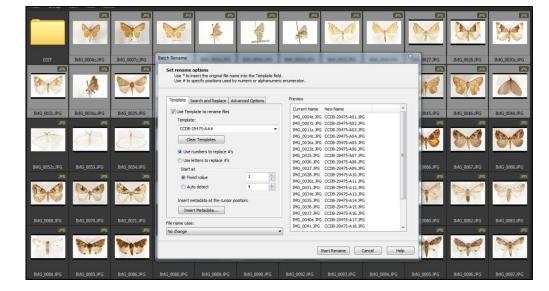


Long Specimens: Dorsal face left of image



### **Standard Guidelines: Image Naming**

- Create folder for each array/plate
- Name image files by Sample ID for easy reference
- Batch renaming
  - Camera software
  - Editing program
  - Free software: Batch



Renamer <a href="https://www.advancedrenamer.com/">https://www.advancedrenamer.com/</a>

\* NOTE Consider museum Requirements

### **Standard Guidelines: Procedure**

#### **Quality assurance**

- One image/specimen or
- Image label before each specimen or
- Include label in image or
- Rename each image after capture
- After completing a plate, rename all images

#### **Background choice**

- White/black
- Leave space between specimen and background





### **Taxon Specific Guidelines: Pinned Invertebrates**

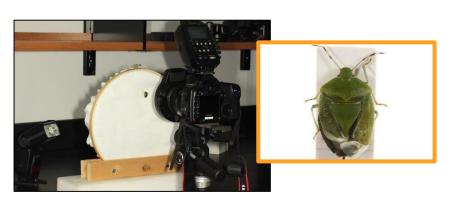
- Image entire array
- Can use for validation after array is disassembled
- Ideal to have array name in image
- Re-name file to array name



### Taxon Specific Guidelines: Pinned Invertebrates (large)

### Equipment

- DSLR camera, macro lens, ring/twin flash and a slave flash
- White fabric and needlepoint ring
- Tripod
- Pin holder



### Procedure

- Pin specimen on fabric or on a piece of foam in front of ring
- **Avoid**: Shadows = flash from behind specimen



### **Taxon Specific Guidelines: Envelopes**

#### Equipment

- DSLR camera, macro lens, ring/twin flash and two slave flashes
- Styrofoam box with glass
- Copy stand
- Ruler

#### Procedure

- Remove specimen from envelope
- Place flat on glass

**N** Avoid: Overexposure on shiny specimens - no direct light on specimen

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### Taxon Specific Guidelines: Fluid Invertebrates (large)

#### Equipment

- DSLR camera, standard/macro lens, master flash and two slave flashes
- Styrofoam box with glass
- Copy stand
- Ruler

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#### **Procedure**

- Place specimen in shallow dish
- Submerge completely in ethanol

**Avoid**: 1) Image distortion from too much fluid – use just enough fluid to cover specimen 2) Colour balance wrong - white balance with camera or when editing

### **Taxon Specific Guidelines: Fluid Vertebrates**

### Equipment

- DSLR camera with a standard lens, master flash and two slave flashes
- Copy stand
- Ruler and colour bar

#### Procedure

- Lay specimen flat on glass or
- Place in shallow dish with ethanol
- Include colour bar if necessary

Avoid: 1) Image distortion from too much fluid = use just enough fluid to cover specimen 2) Colour balance wrong = white balance with camera or when editing

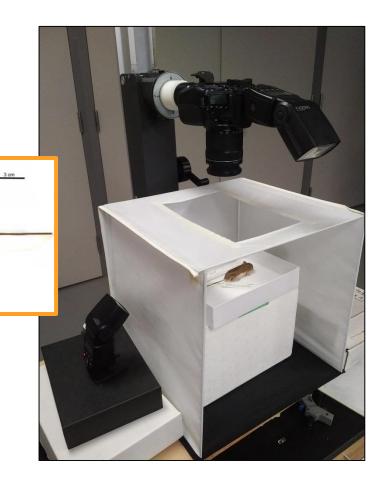
### **Taxon Specific Guidelines: Dry Vertebrates**

### Equipment

- DSLR camera, standard lens, master flash and two slave flashes
- White light box
- Copy stand
- Ruler

#### Procedure

 Place specimen on flat surface inside light box



### Taxon Specific Guidelines: Pinned Invertebrates (small)

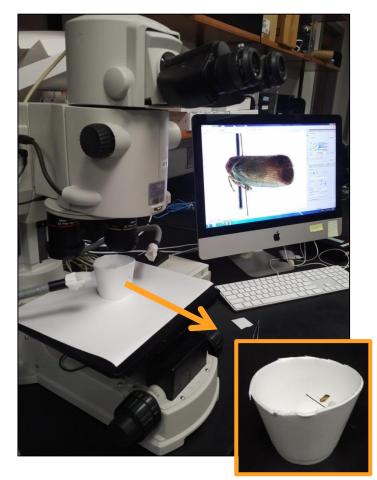
#### Equipment

- Automatic z-stacking microscope
- Styrofoam cup
- Goose neck lights

#### Procedure

- Pin specimen on cup wall (for lateral) or cup bottom (for dorsal)
- Add calibrated scale

Avoid: Overexposure on shiny specimens no direct light on specimen



#### Taxon Specific Guidelines: Fluid Invertebrates (small)







### Equipment

- Imaging microscope
- Goose neck lights
- Dish or slide
- Coverslips



#### Procedure

- Submerge specimen in ethanol
- For z-stacking use a coverslip to keep specimen still

Avoid: Glare from dish = diffuse light with paper caps on goose necks

### **Taxon Specific Guidelines: Herbarium sheets**

### Equipment

- Scanner preferred
- Alternative: DSLR camera, standard lens and external flashes with copy stand

### Procedure

 Scan entire sheet including labels

**Avoid**: Colour balance wrong – white balance before scanning

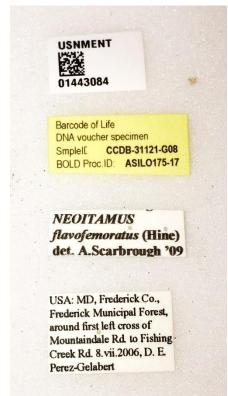


### **Imaging Labels**

- Ensure image is bright and in focus
- May be requirement of museum
- Useful to allow digitization to occur off site without borrowing samples
- Useful for labels that can not be digitized, e.g. illegible or in another language
- Useful as verification of data entry

### OCR

- Program that reads text from images
- May reduce entry error but still requires human verification and parsing
- Can be expensive



#### \* NOTE Label order critical

# **Image Editing: General**

- Keep original images and copy to a new edit folder
- Crop tightly at a 4x3 aspect ratio
  - Or in original aspect ratio
  - Exception: herbarium sheet
- Limit manipulation

#### **Editing Software**

- Free: GIMP, Paint.NET
- Licensed: ACDSee, Photoshop



### Image Editing: Poor Examples

#### Not cropped



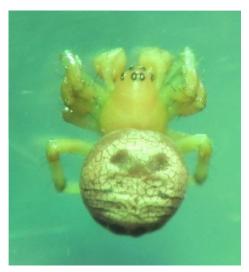
#### Facing wrong direction; dark



#### Incorrect aspect ratio

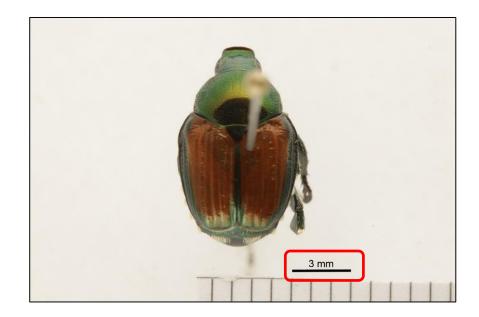


#### Colour balance off



# **Image Editing: Quick Tips**

Create a scale bar



# **Image Editing: Quick Tips**

- Create a scale bar
- Crop image (4:3 ratio)



# **Image Editing: Quick Tips**

- Create a scale bar
- Crop image (4:3 ratio)
- Exposure/levels



# Image Editing: Quick Tips

- Create a scale bar
- Crop image (4:3 ratio)
- Exposure/levels
- Use of repair tool
- Rotate Image
- White Balance



#### Image Submission Template

- Links specimen image(s) with specimen record on BOLD
- Links specimen image(s) with image metadata, e.g.
   Copyright
- Save in folder with images to upload

4	А	В	С	D	E	F	G	Н	l l	J	K	L	M	N	
		Original	View			Measurement				Copyright	Copyright				
l Ir	nage File	Specimen	Metadata	Caption	Measurement	Туре	Specimen ID	Process ID	Copyright Holder	License		Copyright Institution	Copyright Contact	Photographer	
2 C	CDB-29463-A01.JPG	yes	Dorsal				CCDB-29463-A01	LNAUW2448-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Grou
8 C	CDB-29463-A02.JPG	yes	Dorsal				CCDB-29463-A02	LNAUW2449-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Grou
С	CDB-29463-A03.JPG	yes	Dorsal				CCDB-29463-A03	LNAUW2450-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Grou
С	CDB-29463-A04.JPG	yes	Dorsal				CCDB-29463-A04	LNAUW2451-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Grou
С	CDB-29463-A05.JPG	yes	Dorsal				CCDB-29463-A05	LNAUW2452-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Grou
С	CDB-29463-A06.JPG	yes	Dorsal				CCDB-29463-A06	LNAUW2453-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Groi
С	CDB-29463-A07.JPG	yes	Dorsal				CCDB-29463-A07	LNAUW2454-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
С	CDB-29463-A08.JPG	yes	Dorsal				CCDB-29463-A08	LNAUW2455-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
) C	CDB-29463-A09.JPG	yes	Dorsal				CCDB-29463-A09	LNAUW2456-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@qmail.com	CBG Photography	y Gro
C	CDB-29463-A10.JPG	yes	Dorsal				CCDB-29463-A10	LNAUW2457-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
2 C	CDB-29463-A11.JPG	yes	Dorsal				CCDB-29463-A11	LNAUW2458-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
3 C	CDB-29463-A12.JPG	yes	Dorsal				CCDB-29463-A12	LNAUW2459-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@qmail.com	CBG Photography	y Gro
4 C	CDB-29463-B01.JPG	yes	Dorsal				CCDB-29463-B01	LNAUW2460-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
5 C	CDB-29463-B02.JPG	yes	Dorsal				CCDB-29463-B02	LNAUW2461-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
6 C	CDB-29463-B03.JPG	yes	Dorsal				CCDB-29463-B03	LNAUW2462-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
7 C	CDB-29463-B04.JPG	yes	Dorsal				CCDB-29463-B04	LNAUW2463-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
BC	CDB-29463-B05.JPG	yes	Dorsal				CCDB-29463-B05	LNAUW2464-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
) C	CDB-29463-B06.JPG	yes	Dorsal				CCDB-29463-B06	LNAUW2465-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
) C	CDB-29463-B07.JPG	yes	Dorsal				CCDB-29463-B07	LNAUW2466-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
1 C	CDB-29463-B08.JPG	yes	Dorsal				CCDB-29463-B08	LNAUW2467-1	7 CBG Photography Group	CreativeCommons		Centre for Biodiversity Genomic			
2 C	CDB-29463-B09.JPG	yes	Dorsal				CCDB-29463-B09	LNAUW2468-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
3 C	CDB-29463-B10.JPG	yes	Dorsal				CCDB-29463-B10	LNAUW2469-1	7 CBG Photography Group	CreativeCommons		Centre for Biodiversity Genomic			
4 C	CDB-29463-B11.JPG	yes	Dorsal				CCDB-29463-B11	LNAUW2470-1	7 CBG Photography Group	CreativeCommons	2017	Centre for Biodiversity Genomic	s collectionsBIO@gmail.com	CBG Photography	y Gro
5 C	CDB-29463-B12.JPG	ves	Dorsal						7 CBG Photography Group			Centre for Biodiversity Genomic			

 Can be uploaded through batch submissions on BOLD workbench

### Image Submission Template Locations

- On BOLD main console Uploads→Images→Spreadsheet Templates
   BOLD Handbook – Image submission protocol http://v4.boldsystems.org/index.php/Resources
   CCDB website – http://ccdb.ca/resources/
- 4 CCDB submission package received via Email

\* NOTE BOLD will only accept .xls file format
 \* NOTE must be named ImageData.xls (case sensitive)

# **BOLD** SYSTEMS

# Image Submission Template

Image File	Original Specimen	View Metadata	Caption	Measurement	Measurement Type	Sample ID	Process ID
CCDB-29475-A01.jpg	Yes	Dorsal				CCDB-29475-A01	LNAUW1168-17
CCDB-29475-A02.jpg	Yes	Dorsal				CCDB-29475-A02	LNAUW1169-17
CCDB-29475-A03.jpg	Yes	Lateral				CCDB-29475-A03	LNAUW1170-17

#### Image File

- Complete and identical file name of image file
  - Including file extension
  - Case sensitive

\* **NOTE** Image File names that are identical to Sample IDs simplify data entry on the template

# Image Submission Template

Image File	Original Specimen	View Metadata	Caption	Measurement	Measurement Type	Sample ID	Process ID
CCDB-29475-A01.jpg	Yes	Dorsal				CCDB-29475-A01	LNAUW1168-17
CCDB-29475-A02.jpg	Yes	Dorsal				CCDB-29475-A02	LNAUW1169-17
CCDB-29475-A03.jpg	Yes	Lateral				CCDB-29475-A03	LNAUW1170-17

#### **Original Specimen**

Enter Yes if the image shows the actual specimen for this record.
 Otherwise enter No

# Image Submission Template

Image File	Original Specimen	View Metadata	Caption	Measurement	Measurement Type	Sample ID	Process ID
CCDB-29475-A01.jpg	Yes	Dorsal				CCDB-29475-A01	LNAUW1168-17
CCDB-29475-A02.jpg	Yes	Dorsal				CCDB-29475-A02	LNAUW1169-17
CCDB-29475-A03.jpg	Yes	Lateral				CCDB-29475-A03	LNAUW1170-17

#### **View Metadata**

- Controlled Vocabulary: Dorsal, Ventral or Lateral
- Other Standard Orientations on BOLD are:
  - Larva, Eggs, Branch, Flower, Leaf, Stem, Habitat, Collection Site, Fruit, Genitalia, Wing, Pupal Casing, Blind Side, Eyed Side, Apical, Basal, Apertural, Abapertural

### Image Submission Template

Image File	Original Specimen	View Metadata	Caption	Measurement	Measurement Type	Sample ID	Process ID
CCDB-29475-A01.jpg	Yes	Dorsal				CCDB-29475-A01	LNAUW1168-17
CCDB-29475-A02.jpg	Yes	Dorsal				CCDB-29475-A02	LNAUW1169-17
CCDB-29475-A03.jpg	Yes	Lateral				CCDB-29475-A03	LNAUW1170-17

- Caption: Short descriptions are recommended: i.e. part of organism photographed, life stage, sex, etc. (400 char max)
- **Measurement:** Any relevant measurement taken in metric units
- Measurement Type: Item or feature that was measured

#### \* NOTE these fields are not required

# **Image Submission Template**

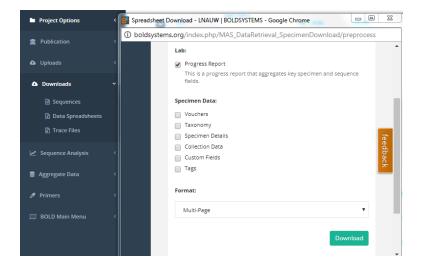
Image File	Original Specimen	View Metadata	Caption	Measurement	Measurement Type	Sample ID	Process ID
CCDB-29475-A01.jpg	Yes	Dorsal				CCDB-29475-A01	LNAUW1168-17
CCDB-29475-A02.jpg	Yes	Dorsal				CCDB-29475-A02	LNAUW1169-17
CCDB-29475-A03.jpg	Yes	Lateral				CCDB-29475-A03	LNAUW1170-17

#### • Sample ID:

- Sample ID for record
  - e.g. CCDB-00000-A01

#### Process ID:

- Generated by BOLD
- Download data spreadsheets to get list



### Image Submission Template

Process ID	License Holder	License	License year	License Institution	License Contact	Photographer
LNAUW1168-17	CBG Photography Group	by-nc-sa	2017	Centre for Biodiversity Genomics	collectionsBIO@gmail. com	CBG Photography Group
LNAUW1169-17	CBG Photography Group	by-nc-sa	2017	Centre for Biodiversity Genomics	collectionsBIO@gmail. com	CBG Photography Group
LNAUW1170-17	CBG Photography Group	by-nc-sa	2017	Centre for Biodiversity Genomics	collectionsBIO@gmail. com	CBG Photography Group

#### License:

Copyright	(C)
<ul> <li>No Rights Reserved</li> </ul>	(nrr)
<ul> <li>CreativeCommons-Attribution</li> </ul>	(by)
<ul> <li>CreativeCommons-Attribution Share-Alike</li> </ul>	(by-sa)
<ul> <li>CreativeCommons-Attribution No Derivatives</li> </ul>	(by-nd)
<ul> <li>CreativeCommons-Attribution Non-Commercial</li> </ul>	(by-nc)
<ul> <li>CreativeCommons-Attribution Non-Commercial Share-Alike</li> </ul>	(by-nc-sa)
<ul> <li>CreativeCommons-Attribution Non-Commercial No Derivatives</li> </ul>	(by-nc-dc)

50

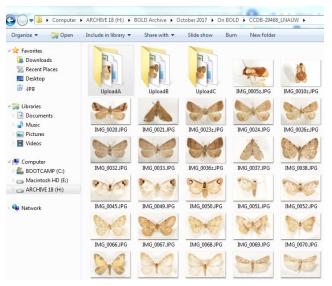
### Image Submission Template

Process ID	License Holder	License	License year	License Institution	License Contact	Photographer
LNAUW1168-17	CBG Photography Group	by-nc-sa	2017	Centre for Biodiversity Genomics	collectionsBIO@gmail. com	CBG Photography Group
LNAUW1169-17	LNAUW1169-17CBG Photography Groupby-nc-saLNAUW1170-17CBG Photography Groupby-nc-sa		2017	Centre for Biodiversity Genomics	collectionsBIO@gmail. com	CBG Photography Group
LNAUW1170-17			2017	Centre for Biodiversity Genomics	collectionsBIO@gmail. com	CBG Photography Group

- License Holder: The primary individual holder of the license
- License Year: The year of license declaration (not the year of submission to BOLD)
- License Institution & Contact: The primary license holder's institution and contact information (email, mailing address, phone number etc.)
- Photographer: The individual or team responsible for photographing and editing the media prior to submission

# **Image Submission Package**

- Ensure all images and "ImageData.xls" spreadsheet are in same folder
- Select folder and compress into zipped folder (WinZip, Winrar)
- Max of 10 images per Sample ID
  - Lateral, dorsal, ventral etc
- Max of 199 MB per zipped folder
  - Split into separate folders if too large
- BOLD only accepts jpgs
  - Consider museums requirements = may need to image in a different format (e.g. tiff) and convert before uploading



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#### **Image Submission to BOLD**

<b>BOLD</b> SYSTEMS	Project & Datase	et Search			Co	de 🔻	Record Search	<b>ଞ</b> ି	<b>.</b> .
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🚯 Main Console		-							
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🛱 🛛 BOLD Main Menu 🛛 <									
	다 Your Datasets: 61			New Dataset		cessed			Тор 20 🔥
	Code	Title		Specimens	Code	Title		Specimens	Accessed
	LATASET- MTBAR12	Revealing the Hyperdiverse Mite Fauna of COI-5P[6290]	Subarctic Canada through DNA Barcoding	6291	DS-IXSCA	Ixodes sca COI-5P[1]	apularis	1	1 day ago ≣
	LATASET- MTBAR12N	Revealing the Hyperdiverse Mite Fauna of 5 2012 COI-5P(6279]	Subarctic Canada through DNA Barcoding Aug 8	6279	TJSD	Ticks of Jo COI-5P[16]	ohn D. Scott Collection	17	2 days ago
	DATASET- MYCOMP12	Comparison of CO1 in Arachnids - full COI-59[1627]		1627	SMINA	Soil Mites COI-5P[83]	s of Israel in Natural and Agricultural Systems	285	7 days ago
	<b>\$</b> DS-161101	2016-11-01 WMON COI-5P[1549]		1603	MYMCA	Terrestria COI-5P[1027]	al Mites of Churchill 2010	1499	8 days ago
	S-CNCGLOR	Gloridonus of CNC COI-5P[80]		80	CHACA	Mites of C COI-5P[608]	Churchill	882	8 days ago
	S-CUNG	Global Cunaxidae COI-5P[309]		309	DS- Water mites of Ontario Dataset WMON2 COL5P(1543]			1597	20+ days ago
	d DS-FALL16	2016-Sent-ttl		1237 *	ELPCG	Fel Lake -	South Frontenac - General Collecting	10000	20+ davs 👻

#### \* **NOTE** Email support@boldsystems.org to delete images

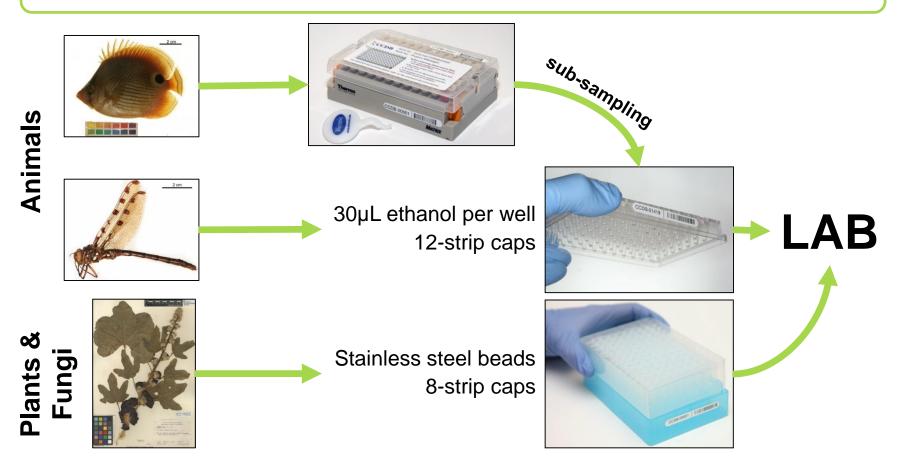
# **Pre-lab Processing**

**Tissue Sampling** 

Lab Submission

Voucher Recovery

#### **Sampling Media**



\* NOTE For DNA extracts and PCR products see CCDB protocols

### Procedure

# Clean sampling station and prepare required items:

- Sterilization tools (e.g. gloves, ethanol)
- Sampling tools (e.g. forceps, scissors, microscope, petri dish)
- Sampling media with label
  - Microplate: Add 30uL of ethanol per well, affix 12-strip caps in proper orientation
  - Tube rack: Add stainless steal bead to each tube; 2<sup>nd</sup> rack (empty) useful for sampling
- Other useful items to have on hand: Kimwipes, gel caps, tube decapper







### Procedure

2 Sterilize tools before starting & after each specimen

#### Ethanol + Flame

(DNA-poor tissue)

- Most invertebrates
- Plants



#### ELIMINase + 3X Water

(DNA-rich tissue)

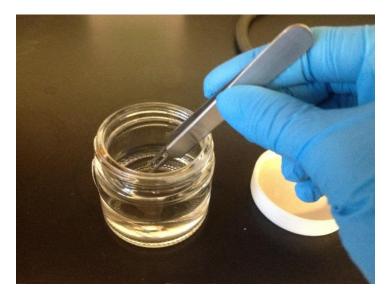
- Vertebrates
- Large marine invertebrates

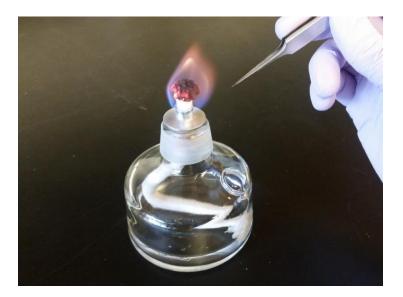


### Procedure

#### 2 Sterilize tools before starting & after each specimen Flame sterilization

 Dip forceps into high concentration ethanol then pass them through flame *quickly* to burn off the ethanol





#### Procedure

#### 2 Sterilize tools before starting & after each specimen Flame sterilization



#### **FIRE RISKS**

- Ethanol fires CANNOT be put out by water
  - Must cover ethanol jar or use fire extinguisher

#### Tie back loose hair and clothing

Look at forceps and ensure flame is completely out before sampling next specimen

Extinguish flame when finished or when you leave the room

NEVER fill ethanol jar more than half way full

NEVER flick forceps when sterilizing

NEVER leave forceps standing in ethanol sterilization jar

#### Procedure

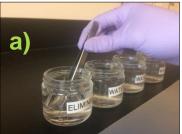
2 Sterilize tools before starting & after each specimen ELIMINase sterilization

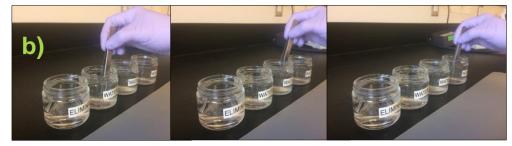


- Label jars to avoid error
- Water levels should be higher than
   ELIMINase levels to ensure thorough rinse

### Procedure

- 2 Sterilize tools before starting & after each specimen ELIMINase sterilization
  - a) Stir forceps in ELIMINase jar for 1 second
  - b) Then rinse in 3 different jars with deionized water
  - c) Wipe with clean Kimwipe before proceeding

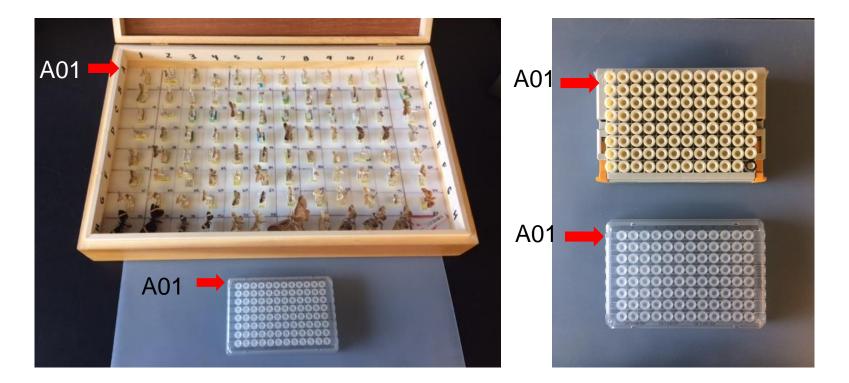






#### Procedure

3 Arrange samples by sampling order. If arrayed, place plate and array in same orientation

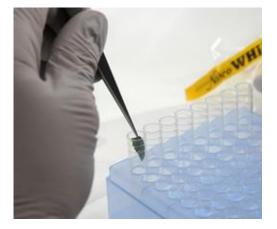


#### **Procedure**

4 Take tissue sample from specimen and place into corresponding well/tube



- Consider museum requirements
- Remove **ONE strip cap at a time** to prevent cross-contamination
- **DO NOT** place foreign objects into wells (e.g. labels)
  - **NEVER** remove and replace samples

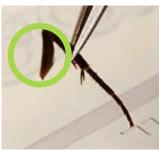


### Procedure

4 Take tissue sample from specimen and place into corresponding well/tube

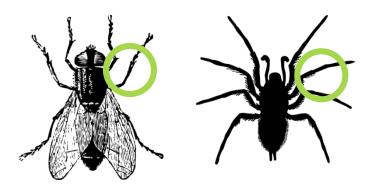
#### Large Arthropods

Tibia or femur **only** (~2-4mm)



#### **Small Arthropods**

Whole leg or antenna (~5-6mm)



Target right side of specimen
 (middle leg → front leg → back leg)

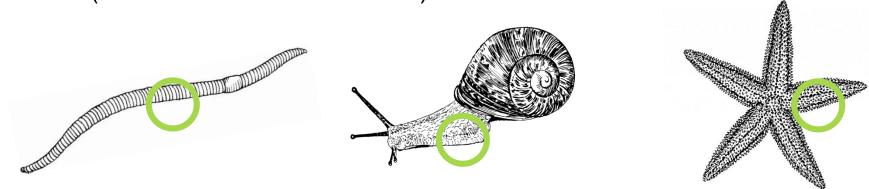
DO NOT take front legs on spiders (may damage pedipalps)

### Procedure

4 Take tissue sample from specimen and place into corresponding well/tube

# Worms, Gastropods, Marine invertebrates & Arthropod Iarvae without legs

 Dense tissue (muscle) from region not critical for identification (~8mm<sup>3</sup> or 2mm in diameter)



#### **Procedure**

Take tissue sample from specimen and place into corresponding well/tube



\* Tissue size critical!!

#### **Plants**

 Coin-sized leafy material, chlorophyllrich (~3-4mm in diameter)



# <u>Fungi</u>

 Stem (~3-4mm in diameter)



# Procedure

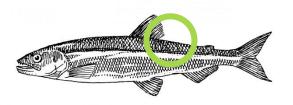
4 Take tissue sample from specimen and place into corresponding well/tube

### **Vertebrates**

- Muscle, e.g. leg or fin region, fin clips (~8mm<sup>3</sup> or 2mm in diameter)
- Skin or body wall (3-4mm in diameter)
- Avoid excessive tissue!

**DO NOT** take tissue from gut, liver, or other internal organs

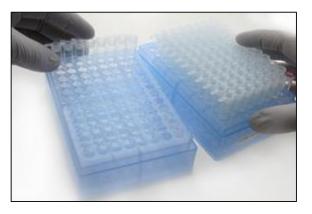






### Procedure

- 5 Sterilize tools between each sample (step 2)!
- 6 Keep track of which specimens have been tissue sampled
  - Confirm that tissue remains in the appropriate well (especially for small tissue)



\* NOTE: Use 2<sup>nd</sup> tube rack



\* **NOTE:** Change specimen position or pinned orientation

### Procedure

Finish sampling entire array and store microplate/tube rack in cool dry place or freezer

- Ensure all cap strips are pressed firmly into the wells/tubes
- Examine microplate from underneath, check for any empty wells!





**O NOT fill control well (H12 or A12)** – negative control

# **Best Practices**

 Always keep tissue sampling area clean to avoid contamination

**DO NOT** sample over microplate/tube rack

#### If you break a specimen:

- Report to museum, they may want to glue back on or put in gel cap
- Keep all specimen pieces together on same pin
- Place above DNA label



# **Best Practices**

- If you placed a tissue sample in the incorrect well:
  - Ideally, start again with a new microplate or tube strip

**NEVER** remove and replace tissue samples!

- If flame and ELIMINase are not permitted:
  - Dip forceps in ethanol and wipe with a clean Kimwipe between samples

# **Special Case: Whole Voucher Lysis**

# **Minute Invertebrates**

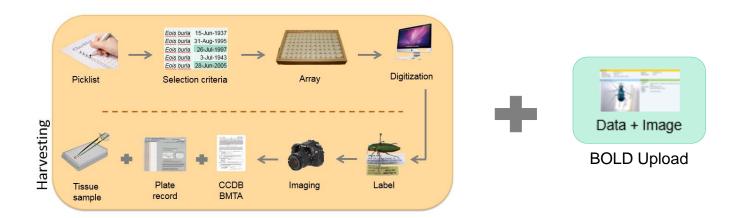
- Place entire specimen into microplate (<3mm)</li>
- Voucher recovery protocol



#### **Destructive Sampling** Discuss with museum FIRST

Can also be used to recover tissue if requested by museum

- Check that ALL prior steps are complete:
  - Data submitted to BOLD
  - Plate map generated
  - Specimens have barcode labels
  - Images taken and uploaded to BOLD
  - 95 tissue samples in microplate/plant tube rack



- Review and complete 3<sup>rd</sup> party agreement with molecular lab
  - Ensure consistency with original donor institution agreement
  - Ownership and storage of DNA extracts
  - Submission protocols

#### CCDB services:

- DNA extracts and PCR products for sequencing (different protocols)
- Voucher/tissue recovery
  - must be indicated BEFORE processing
- DNA repatriation
  - can be requested AFTER processing

Ontario fall under the standard pro 07), and all data submitted to BOLL will comply with the iBOL Data & R v from the iBOL website at <u>http://ww</u> or iBOL Theme Coordinator. A sym	wine, all biological materials shipped to the Biodiversity Institute of visions of the BOL Biological Material Transfer Argenement (v. 2005- ) and generated by the Canadian Centre for DNA Barcoding (CCDB) esource Sharing Policies (v. 2006-7). Full texts can be downloaded w.biolropicet.org or requestedfrom your contactporson at the CCDB opsis of these documents is provided overletal. Prese acknowledge these conditions by filling out and signing the implementing Letter copy form.
IME	PLEMENTING LETTER
agreement between the Provider of Barcoding to abide by the terms and c and the iBOL Data & Resource Sharin filled and signed by the Provider. Th	vide a record of the biological material transfer and to memorialize the biological materials (identified below) and the Canadian Centre for DNA onations of the IBOL Biological Material Transfer Agreement (v. 2009-07) p Rokies (v. 2009-07). The implementing Letter becomes affective when the gardes be securing this implementing Letter because affective when the agree to be bound by these terms for the transfer specified below.
1. Provider	Organization name and address
Name	
Position	
Phone	
E-mail	
University of Gue Phone: +1 (519) 8	e for DNA Barcoding, Biodiversity Institute of Omario biol, 579 Oordon Street, Gueiph, Ontario, Canada N1G 2W1 8242-4 120 ext. 56393 rinla Comments:
University of Gue Phone: +1 (519) 8 3. Description of Biological Mate Type of material sent: whole voucher	ilph, 579 Gordon Street, Guelph, Ontario, Canada N1G 2W1 8242-4120 ext. 56393 srials
University of Gue Phone: +1 (519) 6 3. Description of Biological Mate Type of material sent: whole voucher two sample	ilph, 579 Gordon Street, Guelph, Ontario, Canada N1G 2W1 8242-4120 ext. 56393 srials
University of Gue Phone: +1 (519) 8 3. Description of Biological Mate Type of material sent: whole voucher	ilph, 579 Gordon Street, Guelph, Ontario, Canada N1G 2W1 8242-4120 ext. 56393 srials
University of Gue Phone: +1 (519) 8 3. Description of Biological Mate Type of material sent: whole voucher fissue sample DNA extract	ilph, 579 Gordon Street, Guelph, Ontario, Canada N1G 2W1 8242-4120 ext. 56393 srials
University of Que Phone: +1 (519); 3. Description of Biological Mate Type of material sent: Whole voucher Issue sample DNA extract CR product 4. IBOL Theme / Workgroup: "I hereby certify that I have	Jiph, 579 Gordon Street, Quelph, Ontario, Canada N1G 2W1 82424 120 ext. 56393 rrais Comments: Select /BOL campaign from dropdown list e read and agree to abide by the terms and conditions of al fransfer Agreement (v. 2009/07) and the IBOL Data &
University of Gue Phone: *1 (519) 3. Description of Biological Mater Type of material sent mole voucher Bissue sample ONA extract PCR product 4. IBOL Theme / Workgroup: "I hereby certify that I have the IBOL Biological Materia	Iph 579 Gordon Street, Quelph, Ontario, Canada N1G 2W1 8242-4120 ext. 56393 Trails Comments: Select IBOL campaign from dropdown list e read and agree to able by the terms and conditions of al Transfer Agreement (v. 2009-07) and the IBOL Data & (v. 2009-07)*

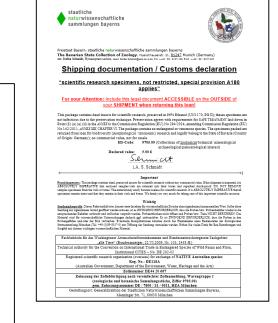
BARCODE 🧐

N CCDB

#### **Transportation**

- Include necessary documentation
  - Customs declaration
  - Loan documents
  - Export permits

	Shipp
	"scientific
Source for Biodiversity Genomics, University of Guelph 50 Store Rd. East, Guelph, Ortario, Canada NG 201 Tel + 15 19 824-4120 ext, 50300 Fax + 15 /8 1624-703	For your A
Shipping documentation / Customs declaration	This package con not infection due Point (8) (a) (b) (i No 142/2011, 202 returned from loa of Origin: Germa
"scientific research specimens; no commercial value"	
This package contains dead insect museum specimens for scientific research, preserved in 95% Elband (UN1170, PG 10) (for Latin species names refer to included ban agreement), Preserved speciments packed are not subject to the initial section list of poducits for veleniary checks at border inspection posts under Art. 3 Council Directive 2007/275EC. Arnex I, EX 9756 000. Presention of specimens agrees with requirements for safe traditionent laid down in Point (8) (a) (a) (b) (b) in the ANNEX to the Commission Regulation (EU) No 2442013. amending Commission Regulation (EU) No 122011. ANNEXXII (LHAPTER VI. I	© Pacetal inspectory: To ABSOLUTELY DAY supplies specimens for specimens random kets
Transfer of scientific specimens	Bendangskontroller Sandrag nor stynnösi entspreckasika: Debit Motsenial wird für mit Probangsfillen unde Statet-samnbase Mite
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Centre for Biodiversity Genomics, Collections unit (BIOUG) 50 Stone Rd. East, Guelph, Ontario, N1G 2W1 Canada	Fachbehörde fi Technical author
	Registered :
To	
Stefan Schmidt	(Aust
Zoologische Staatssammlung München	Zulas
Münchhausenstr. 21 D-81247 München	Gestellun
Germany 49 (0)39-8107 159	Gesteinin
+49 (U)dix-3107 109	
Important	
<u>Path inspectors</u> , The package contains dead preserved insects for scientific research without any communitial value. If the adjournel is neglocal, the <u>Adjournel INTER</u> , <u>NIEERATURE NIEERATURE environmentation</u> their boars and repected abody-arourd CO NOT RELIX/OF Barryanshapediments from the value or board The material any search boarson usakes to relativity exercise the <u>Adjournel INTER</u> , <u>NIEERATURE ADJOURNEL TO ADJOURNEL T</u>	



Mark as "Scientific research specimens, no commercial value"

### **Transportation**

#### Microplates:

- Ensure caps are securely fastened and seal plates in 'ziplock' back
- Mark as "Scientific research specimens, not restricted Special Provision A180 applies"

#### Plant tube racks:

Secure box lid with pieces of lab tape



Consider transporting in a cooling container if necessary

# Post-Extraction: Voucher Recovery

- Recover specimens or tissue (exoskeletal parts) after lysis in the lab
- Taxa with reduced recovery
  - Zooplankton
  - Collembola
  - Mites
  - Soft-bodied flies



 If specimen is destroyed, BOLD "Voucher Status" field is updated to "E-vouchered: DNA/tissue+photo"

> Return to museum after ALL processing is complete (ie. sequencing and BOLD data validation)

# Procedure

- Original microplate and accompanying filter plate obtained from the lab after lysis
- 2 Remove foil slowly from both plates
  - Check for specimens stuck to foil and move them back into corresponding well



#### Procedure

3 Under a microscope, look through each well of a filter plate and transfer specimens to corresponding well in specimen plate

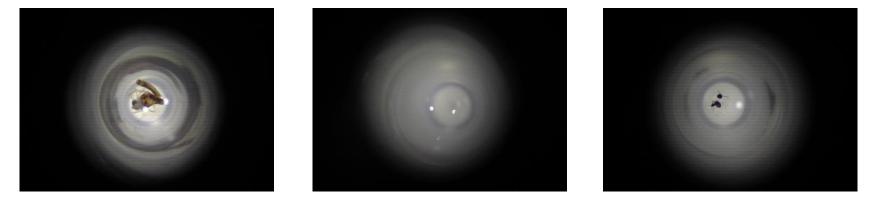


4 After all specimens removed from filter plate, move on to specimen plate.

### Procedure

5 Check all wells under the microscope to confirm that there are specimens in each well

- Specimen considered destroyed if:
  - No specimen in well
  - Specimen not recognizable at order level

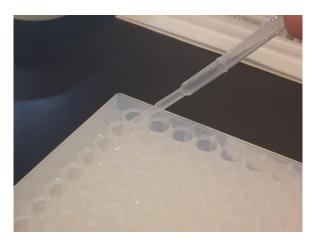


\* **NOTE** damaged specimens and inform museum if required.

#### **Procedure**

#### For potentially translucent organisms (e.g. Mites and Collembola):

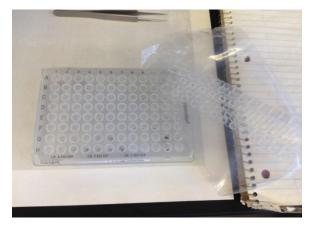
- Use a dark background under voucher plate
- Examine one row at a time and determine missing specimens
- Use pipette and ethanol to wash corresponding well in filter plate
  - Transfer to dish and examine contents on dark background
  - When specimen found, transfer to specimen plate





### Procedure

- 6 Record Sample IDs of destroyed specimens
- 7 If specimen destroyed, change BOLD "Voucher Status" to "E-vouchered: DNA/tissue+photo"
  - Can send as batch submission
- 8 Seal plate using 12-strip caps



Return to museum after ALL processing is complete (ie. sequencing and BOLD data validation)