**Bio-Rad Gel Doc™ XR+ with Image Lab™ Procedure**

1. **PRINCIPLE:**
	1. The Gel Doc™ XR+ with Image Lab™ from Bio-Rad is a high-quality Molecular Imager® that enables quick and easy visualization, documentation, and analysis of nucleic acid and protein gels, blots, and macroarrays. The Gel Doc™ XR+ Imager (instrument) together with Image Lab™ (software) is referred to as the Imaging System.
	2. The Gel Doc™ XR+ Imager is housed by a universal hood, which is equipped with a charged couple device camera (CCD) to capture images in real time.
		1. The universal hood has a light-tight enclosure that contains built-in UV and white light illumination, which allows fluorescent and chemiluminescent images to be captured without a photographic darkroom. This feature can also be used in the extraction of PCR product out of a gel for further processing.
			1. **TO PREVENT DAMAGE TO THE UV TRANSILLUMINATOR, USE EXTREME CARE AND ACCEPTABLE TOOLS, such as a protective screen cover and plastic plungers.**
		2. For easy sample loading, the UV transilluminator is located in the universal hood drawer and can be accessed from the front of the imager. When not imaging, the lights in the darkroom enclosure turn off automatically.
		3. The universal hood has touchpad buttons to perform various functions, but the user does not have to control the lens and light manually. Instead, the user can use Image Lab software to control all functions remotely.
	3. The Image Lab™ software controls the imaging system, acquires, and optimizes images. In addition, the software allows the user to:
		1. Annotate and document images.
		2. Analyze molecular weights when imaging protein and nucleic acid gels.
		3. Determine quantitation and purity of samples.
		4. Export data to other locations (e.g., RICMBLAB\Gel Photos) for further analysis or presentation options.
2. **PROCEDURE FOR OPERATION:**
	1. **Starting the instrument:**
		1. Turn on camera; there is a toggle power button in the back on the left of the camera.
		2. Power on the computer and log in.
		3. Double click “Image Lab™” (the camera software) from the desktop to open the program.
	2. **Shutting down the instrument daily:**
		1. Close out of the Image Lab™ program by clicking the red X on the top right of the screen.
		2. Log out of the computer.
		3. Turn off the camera using the toggle power button in the back of the instrument.
	3. **Image Lab™ Software:**
		1. Image Lab™ is an application used to control the Bio-Rad Gel Doc camera for photographing images. The software has many features that aid in capturing the best possible images along with several tools that can be used to analyze and gather data.
		2. Image Lab™ also allows the user to create and save Protocols, which are steps for photographing and analyzing gels that can be reused to generate reproducible data.
	4. **Protocols for Molecular Genomic Pathology (CMB) Lab:**
		1. **Acrylamide**
			1. Application: GelRed.
			2. Image size: 26.0 cm X 19.4 cm.
			3. Exposure time: 3.000 seconds.
			4. Highlight saturated pixel: Off.
			5. Image color: Gray.
		2. **Agarose Tray A**
			1. Application: GelRed.
			2. Image size: 16.1 cm X 12.0 cm.
			3. Exposure time: 3.000 seconds.
			4. Highlight saturated pixel: Off.
			5. Image color: Gray.
		3. **Agarose Tray B**
			1. Application: GelRed.
			2. Image size: 25.0 cm X 18.7 cm.
			3. Exposure time: 3.000 seconds.
			4. Highlight saturated pixel: Off.
			5. Image color: Gray.
		4. **Agarose Tray E**
			1. Application: GelRed.
			2. Image size: 18.9 cm X 14.1 cm.
			3. Exposure time: 3.000 seconds.
			4. Highlight saturated pixel: Off.
			5. Image color: Gray.
		5. **Faint Bands**
			1. Application: GelRed.
			2. Image size: 26.0 cm X 19.4 cm.
			3. Exposure time: Faint bands.
			4. High saturation pixel: Off
			5. Image color: Gray
	5. **Capturing an image:**
		1. Confirm that the camera and computer are turned on.
		2. Pull out the drawer of the camera, which houses the transilluminator.
		3. Place the gel in the center of the transilluminator and close the drawer.
		4. Double click on the Image Lab™ icon to open the software.
		5. When the Image Lab™ software is opened, a “Start Page” box will appear.
			1. The Start Page box has four sub-sections:

Protocols – the user can select “New” (to create a new protocol) or “Open” (to select desired protocol from the list of CMB Lab protocols).

Recent Protocols – displays a list of the most recent protocols used.

Image Files – the user can select “Open” to access their network drives and documents.

Recent Image Files – displays a list of the most recent images taken.

* + 1. Open desired Protocol. (Found on RICMBLAB$ network drive)
			1. Under “Protocol” sub-section select “Open”.
			2. Navigate to RICMBLAB$ network drive and open “Gel Image Protocols” folder.
			3. Select desired protocol and double click to open.
		2. Click “Position Gel” (yellow button on the bottom left).
			1. A “Filter Position” box will appear; indicating that the filter should be positioned at Filter 1 (standard filter for nucleic acid imaging).

The filter position lever is located at the top of the camera, on the right side.

For standard operation, this lever **MUST** always remain in the “Filter 1” position.

* + 1. Click “OK” to confirm that the filter is positioned correctly.
		2. Adjust the camera zoom with the slider bar below the image until the image is within the gridlines.
		3. Click “Run Protocol” (green button on the left).
		4. An image of the gel will appear and can then be analyzed.
		5. *For oversaturated images with bands that are too intense, thereby reducing faint band visibility, the user can elect to re-capture the image following the standard procedure and selecting “Faint bands” Protocol.*
	1. **Saving a Raw File**:
		1. Immediately after capturing an image, save an unaltered copy (Raw File).
		2. The Raw File allows the Directors to re-analyze and visually optimize an image as needed during review.
		3. To save a Raw File:
			1. Click “File” (toolbar above).
			2. Move cursor down to “Save as…”. Then, click “Save as…”.
			3. Name file by scanning the Soft gel/section label into the file name field.
			4. To save the image, navigate to “This PC”.
			5. Select “Windows (C:)”.
			6. Open the “GelRawFiles” folder and then the appropriate subfolder for the month and year.
			7. Click “save” to save Raw File to applicable folder (month and year).
			8. **Note**: The Raw File must be saved as an Image Lab image (\*.scn).
	2. **Analyzing an image:**
		1. On the left of the screen is the “Analysis Tool Box”, which is a panel of icons used to analyze the image.
		2. The two icons relevant to CMB Lab procedure are:
			1. **Image tools** – allows the user to properly align the image (IF NEEDED) by flipping (horizontal or vertical) and rotating (90° left, 90°right, or custom) the image.

Click “Image Tools” to open icon.

Click “Horizontal” to flip the image horizontally.

Click “Vertical” to flip the image vertically.

Click “90° Left” to rotate the image 90° left.

Click “90° Right” to rotate the image 90° right.

“Custom” is used for manually rotating the image to align straight.

Custom rotating:

* Click “Custom”.
* Then, hold down the left clicker on the mouse and move the red target so that it is in the same orientation as the image (e.g., if the image is slanted left, move the red target so that it is also slanted left).
* When positioned correctly, release the left clicker. Then, right click on the mouse and select “Rotate” to apply the change.

Crop the image by eliminating the background space and overall dimension, which improves storage capability without altering the image quality.

* Click “Crop”.
* Adjust “crop” frame to fit the entire gel image without the background space.
* Right click on the mouse. Then, left click on the mouse and select “crop” to apply the change.
	+ - 1. **Lanes and Bands** – allows the user to add a numerical label (e.g., 1-20) to each lane.

After properly aligning and cropping the image, return to the “Analysis Tool box” by clicking the “back to tool box” arrow (located at the upper left of screen).

Back at the “Analysis Tool Box”, click the “Lanes and Bands” icon (second in the panel).

Click “Lanes” tab.

Under Lanes tab, click “Manual”. Then, enter the number of lanes for which a numerical label should be assigned (number of lanes should equal to gel comb number).

Click “OK”.

Position the cursor at any corner of the “Lane Frame”. Then, using the left clicker on the mouse, drag to resize the “Lane Frame” until it fits the image appropriately (one column per lane).

The “Lane Frame” can be further adjusted by shrinking or expanding the frame from the left or right sides.

* Place cursor on the edge of either side to display “↔”.
* Then, while holding left clicker, slide “↔” left or right to further resize the frame to fit image properly.

When “Lane Frame” is properly aligned, click the “Display Gel Options” icon above. 

Click “Show/Hide All” button at the bottom left corner (this will clear the image of all labels).

Check off “Show Lane Labels” only. Then, click “OK” (to re-label the image with only the lane labels).

* **The color of the numerical lane labels should be white (more visible against the dark background).**
* **To change label color, select:**

**Edit (toolbar above) → Preferences → Colors tab**

**Display items: Lane → Item color → select “White” from pull down window. 🡪 OK**

* 1. **Gel Image Annotation:**
		1. Label the gel in Image Lab
			1. Navigate to the Annotation Tools button.
			2. Select the Test button and curser will appear.
				1. Font: Ariel
				2. Font Size: 22
			3. In the color properties box, change the foreground to white. This will change the text from red to white.
			4. To correctly position the text cursor, left click at the bottom center of the gel.
			5. Scan the Gel Label or Type the Gel Worksheet number for assays that do not have a gel label.
				1. **Note:** If master mixes from two separate clonality assays appear on the same gel, scan, or type the names of **both** worksheets, then add the well positions for the PCR products. An example is included below.



* + - 1. Clonality testing: Please use the correct gene names:
				1. IGH
				2. TRB
				3. TRG
			2. Click anywhere on the gel to complete the labeling process.
				1. At this point, the label can be moved if the position needs to be adjusted. To move it, click on the label and drag it to the desired location on the gel image.
				2. To remove a label from a gel, click on the test so that it is highlighted and press backspace on the keyboard.
	1. **Exporting an image:**
		1. To export the image for uploading into Soft Molecular:
			1. Click “File” (toolbar above).
			2. Move cursor down to “Export”. Then, click “Export for Publication”.
			3. The export settings are as follow:

Publishing Source – “Current View”

Publishing Resolution (dpi) – 300 dpi

Publishing Dimensions (cm) – will auto-populate (Do not change).

* + - 1. Next, click “Export” at the bottom of the export window.
			2. Save image on RICMBLAB$ network drive in folder “Gel Photos”.
				1. **Note:** If master mixes from two separate clonality assays appear on the same gel, export the image twice so each assay will have a copy of the image. Save the images separately with each image named for one of the clonality assays so that both worksheet names appear in the Gel Photos folder separately. Remember to include well positions for the PCR products. An example is included below.



* 1. **To capture additional images:**
		1. Close out the completed image window by clicking the red X in the right corner of the image window (while not in maximized view). Close out the completed image window by clicking the gray X in the right corner of the image window (while in maximized view).
		2. A window will pop up “Would you like to save changes?” Select “No” (the image should already be saved in the Gel Photos folder).
		3. Click the “Start Page” icon (located at the top of the screen) to display the “Start Page” box.
		4. Click on desired protocol from either the Open or Recent Protocols sections. Then, proceed as described above.
1. **QC AND MAINTENANCE:**
	1. **Daily:**
		1. Thoroughly clean the transilluminator with deionized water after each gel has been captured and wipe excess water with lint-free wipes.
		2. Ensure that the unit is completely cleaned and free of buffer residue to prevent salt build up.
		3. Initial the Maintenance Sheet.
		4. Power off camera at the end of each day.
	2. **Weekly:**
		1. Turn the camera off and disconnect the power cord from the outlet.
		2. Using deionized water-soaked paper towels, thoroughly wipe down the interior and exterior of the camera. Follow up with dry lint-free wipes.
		3. Initial the Maintenance Sheet.
	3. **As needed:**
		1. **Replace a bulb when it starts to flicker or does not turn on.**
		2. Replace UV Lamps:
			1. Turn off power and disconnect the power cord from the universal hood.
			2. Remove four screws on corners of transilluminator.
			3. To remove the cover with the UV glass, slide it forward then lift it up.
			4. **Note**: Do not put the UV glass directly on benchtop. Instead, place it on a nonabrasive surface.
			5. **Note**: Use gloves when handling UV lamps.
			6. Rotate each lamp individually until it becomes loose and the pins come to a vertical position.
			7. Remove the lamp.
			8. Install the new lamp by rotating it so the pins are in a horizontal position and the lamp is tight.
			9. Remove the starter by rotating it counterclockwise and then pulling it out.
			10. Replace the old starter with the new starter by rotating it clockwise.
			11. Re-assemble the cover and tighten the four screws.
		3. Replace Epi-illumination Lamp:
			1. Turn off power and disconnect the power cord from the universal hood.
			2. Open the enclosure door.
			3. Locate the epi-white light housing, which is inside of the hood on the right and left walls.
			4. Using a socket wrench, remove the screws on the center of the housing cover.
			5. Pull the cover to remove it.
			6. Remove the lamp by holding the plastic receptacle and pulling the lamp from the receptacle.
			7. Insert the new lamp into the lamp receptacle and push until it is firmly seated.
			8. Reassemble the epi-white light cover.
2. **TROUBLESHOOTING:**
	1. For troubleshooting, refer to Gel DocTM XRS+ and ChemiDocTM XRS+ Imaging Systems with Image LabTM Software Instrument User Guide Appendix B Troubleshooting, page 49. The user guide can be found at the instrument and online:

[**http://www.bio-rad.com/webroot/web/pdf/lsr/literature/10000076955.pdf**](http://www.bio-rad.com/webroot/web/pdf/lsr/literature/10000076955.pdf)

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1. **REFERENCES:**
	1. Gel Doc™XRS+ and ChemiDoc™XRS+ Imaging Systems with Image Lab™ Software Instrument Guide.
2. **REVISIONS:**
	1. 10/10/17: Clarification on exporting an image was added.
	2. 1/15/2020: Updated with steps for Soft Molecular and updated footer to reflect the new lab name.
	3. 10/5/2020: Update made to the instructions on how to save raw files.
	4. 3/6/2022: Update made to the annotation and export instructions.