**DNA, Tissue Automated Extraction Procedure**

1. **PRINCIPLE**:
	1. Isolation of DNA from tissue and body fluid samples is critical for the success of most assays in the Molecular Genomic Pathology Laboratory. The Maxwell RSC DNA FFPE Kit is used in combination with the Maxwell RSC Instrument to provide an easy method for efficient, automated purification of DNA from formalin-fixed, paraffin-embedded FFPE tissue samples.
	2. The Maxwell RSC Instrument is a magnetic particle-handler that allows efficient binding of DNA to the paramagnetic particles in the first well of the prefilled cartridge and moves the samples through the cartridge wells, mixing during processing.
	3. The quality of DNA produced is dependent on the starting material, as well as the method of preparation. DNA of quality satisfactory for PCR can usually be obtained from standard formalin-fixed, paraffin-embedded (FFPE) tissue block samples.
	4. To aid in purification of DNA from tissue, samples are typically digested with the active protease, Proteinase K, in the presence of ionic detergent and the chelating agent EDTA. This protocol rapidly and irreversibly inhibits DNases and results in DNA of sufficient quality for all assays performed in the laboratory. This digestion step also results in the rapid inactivation of conventional infectious agents. This method adapts well to the isolation of DNA from FFPE blocks, following removal of paraffin by treatment with xylene and ethanol.
2. **SAMPLES**:
	1. Sample types: Formalin-fixed, paraffin embedded (FFPE) tissue (slides or Blocks)
3. **REAGENTS**:
	1. Maxwell® RSC FFPE Plus DNA kit: Cat # AS1720. Store at room temperature except for Proteinase K.
		1. Proteinase K: Upon receipt, store at -20°C.
			1. To reconstitute: Resuspend Proteinase K using 500ul of nuclease free distilled water.
			2. Aliquot 100uL of the Proteinase K into 1.5 mL tubes. Store the aliquots at -20°C for up to 1 year. Aliquots cannot be thawed more than 5 times.
				1. **NOTE:** The final aliquot will have about 80uL of Proteinase K instead of 100uL.
	2. Distilled Water (DH20): Reagent grade. Store at room temperature.
4. **PROCEDURE FOR DNA, Tissue Automated Extraction:**
	1. For specimens that exhibit heavy melanin pigmentation, consult Lab Director to determine if the standard protocol or the BSA Method should be utilized. Refer to the PCR Interfering substances BSA Method Procedure.
		1. If BSA is necessary, add an internal note in Soft Molecular.
	2. **Cut Block/Scrape Slides Action (Day 1)**
		1. Log into Soft Molecular.
		2. Open Extractions by using the Extractions tile on the dashboard.
		3. Highlight the Cut Block or Scrape Slides branch in the action tree.
		4. Highlight the Barcode# field. Scan the Soft Molecular specimen label and press **Enter** on the keyboard. Repeat this step for all applicable specimens.
		5. **If a sample is being re-extracted, expand the child level, and change the Volume in the child level tube to 4800. This is necessary to ensure adequate volume in the system for performing any testing.**
		6. Select **Save**.
			1. Two aliquot labels per sample will automatically print upon saving.
				1. If only one 1.5ml tube is being utilized, discard the second aliquot label in the appropriate receptacle.
		7. Select **Back** in the Extractions window.
		8. Exit Soft Molecular application.
		9. Label a 1.5ml tube with an aliquot label for each specimen.
	3. **Record your extraction reagents: Extraction Reagents (Day 1)**
		1. Highlight the Extraction Reagents branch on the action tree.
		2. Highlight the Barcode# field. Scan the aliquot label and press **Enter** on the keyboard. Repeat this step for all applicable specimens.
		3. Enter the final elution volume in the Prod Vol column.
			1. FFPE Block: 70ul
			2. Unstained Slides: 35ul
		4. Select the Spec/Tube Reagents field and in the dropdown, scan the appropriate TQC reagent label to add the Maxwell® RSC FFPE Plus DNA kit lot number to each specimen.
		5. Select **OK** in the Spec/Tube Reagent window.
		6. Mark the **Completed** checkbox and select **Save**.
	4. **Cut Block/Scrape Slides**
		1. Cut FFPE blocks and macrodissect unstained slides according to *FFPE Tissue Preparation for Molecular Testing* Procedure.
		2. Spin tissue to the bottom of the tube in microcentrifuge.
		3. Overlay the tissue with 20ul of Proteinase K and 180ul of Incubation Buffer.
		4. Blanks are prepared by adding reagents to an empty labeled tube and running it through the same process as the patient sample.
		5. Incubate the tubes overnight in a heat block set at 70°C.
	5. **DNA, Tissue Automated Extraction Action (Day 2)**
		1. Log into Soft Molecular.
		2. Open Extractions by using the Extraction tile on the dashboard.
		3. Highlight the Maxwell® DNA Extraction branch on the action tree.
		4. Highlight the Barcode# field. Scan the aliquot label and press **Enter** on the keyboard. Repeat this step for all applicable specimens.
		5. Navigate to the **Print** tab and click the **Print Product Labels** dropdown menu. Select the ‘**…**’ button that appears.
		6. Select **OK** when asked to save before print.
		7. In the Print Product Labels window, verify the correct printer and product label template is selected (PROD LBL V1). Click **Print**. If you desire to label your elution tubes with a product label, print 2 labels, otherwise, manually label the elution tube.
		8. Select **Back** in the Extractions window.
		9. Exit Soft Molecular application.
		10. Label a 1.5ml tube with a product label for each specimen.
	6. **Initialize Maxwell® RSC Instrument**
		1. If the Maxwell® RSC Instrument and the Tablet PC are already powered on, proceed to Step 9 below; otherwise, continue to Step 2.
		2. Start the System by powering on the Maxwell® RSC and the Tablet PC.
		3. The RSC power switch is located next to the power cable connection on the back-left side of the instrument.
		4. Power on the instrument by switching the On/Off power switch to “On”.
		5. Press the power button located on the top of the Tablet PC to power on the Tablet.
		6. Launch the Maxwell® RSC Application Software by touching the software icon on the touchscreen.
		7. A Self-Test is performed automatically when the instrument is powered on, and the software launched.
		8. The Self-Test checks the Maxwell® RSC’s initialization, motion, and heater functions.
		9. The Self-Test can be manually initiated if the System is already on.
		10. From the Home Screen, select the Settings tab and then, select Self-Test to perform the Self-Test.
			1. **NOTE:** A Self-Test must be performed and passed before each use of the instrument.
	7. **Setup Maxwell RSC Instrument:**
		1. From the Home Screen, select **Start**.
		2. Touch the text box at the top of the screen to enter the kit barcode ID.
		3. Using the keypad, use a scanner to scan the 2D barcode (e.g., AS14401041092020-08) on the reagent kit.
			1. **NOTE**: The kit barcode ID is a combination of the catalog ID, lot number, and the expiration date of the kit and must be valid to run a Method.
		4. When the barcode ID is entered correctly, the corresponding extraction Method from the list of preprogrammed Methods will become highlighted.
		5. Confirm that the highlighted Method matches the extraction Kit being used.
		6. Touch the **Proceed** buttonnext to the highlighted Method, to move to the Cartridge Setup screen.
		7. The Cartridge Setup screen displays the layout of the Maxwell RSC Deck Tray with position numbers and the selected run Method.
		8. The Maxwell blank location is rotated on the deck tray each day of testing, in sequential order, so that all locations in the instrument are routinely tested. Use the Maxwell® Maintenance Form to determine the appropriate blank position.
		9. On the maintenance form, document the blank position for the current day.
			1. If there are only a few samples on the Maxwell and they are all spaced apart, there is no requirement to place the blank next to a sample. However, if there is not enough room on the Maxwell to space everything out, then the blank should be placed next to a patient sample.
		10. On the Cartridge Setup screen, the user can select which cartridge positions (based on the number of samples) will be processed by selecting or deselecting cartridge positions.
		11. Select a cartridge position by touching the black rectangle above the number of that position.
		12. A selected position can be deselected/deactivated by touching the black rectangle of that position again.
			1. See the Maxwell® RSC Cartridge Setup Screen in Figure 1 and the Cartridge Setup Screen with Sample ID input in Figure 2:

**Figure 1: Cartridge Setup screen**



**Figure 2: Sample ID input screen**



* + 1. **NOTE:** The instrument will only process cartridge positions that have been selected. Therefore, ensure that no cartridges are loaded in positions that have been excluded from processing.
		2. Next, enter the corresponding Sample ID and Elution Tube ID for all cartridge positions that are to be processed.
		3. To enter the Sample ID and Elution Tube ID for a position, touch the “active” position number (black square) on the Cartridge Setup screen.
		4. Touch the **Sample ID** text box and use the keypad to enter the Sample ID or use a scanner to scan the aliquot label (e.g., MOL-00-0000).
		5. Touch the **Elution Tube** **ID** text box and use the keypad to enter the corresponding Elution Tube position number.
		6. When all Sample IDs and Elution Tube IDs have been entered, the **Proceed** button on the bottom of the screen will become active.
		7. Touch the **Proceed** button to move to the Door screen and then press the OK button to open the Maxwell RSC Instrument door.
		8. Remove the Deck Tray from the Deck for easy loading access. Lift the tray up from the front and pull out.
			1. **NOTE:** If processing fewer than 16 samples, center the cartridges on the Deck Tray as best as possible. If helpful, label the cartridges with the Sample IDs.
		9. Clean the Maxwell® RSC instrument and deck tray before the run using a KimWipe wet with 70% ethanol.
		10. Wrap a Kimwipe wet with 70% ethanol around a magnet and wipe around the plunger bars to pick up any stray magnetic beads.
		11. Place the cartridges in the deck tray according to the previously programmed placement with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down both front and back of the cartridge to snap it into position.
			1. See the Maxwell® RSC Deck Tray with cartridges in Figure 3:

**Figure 3: Placing the Cartridges in the Deck Tray**



* + 1. Carefully peel back the seal, making sure all the plastic comes off. If necessary, use a sterile pipette tip to help remove any remaining plastic.
		2. Verify the cartridges are still firmly snapped into place.
		3. Place one plunger in well #8 (well #8 is the closest well to the Deck Tray position numbers) of each cartridge.
		4. Place elution tubes (provided in kit) into the elution tube position on the deck tray. Label the top of the elution tube with the Molecular Order Number.
		5. Add 50ul of nuclease-free water to the bottom of the elution tube.
		6. Make sure there are no droplets on the side of the tube and there are no air bubbles. Flash spin tubes.
		7. Place the elution tubes back into the deck tray and verify the caps are pointing towards the cartridges.
	1. **Preprocessing of FFPE Samples:**
		1. Remove aliquot tubes from the 70˚C heat block.
		2. Add 400ul Lysis Buffer to each tube.
		3. Briefly vortex tubes and flash spin. Tubes can sit at room temperature overnight if necessary. Do not refrigerate or freeze sample. **Discard gloves**.
		4. Add entire sample (approximately 600ul) to well #1 (in back, furthest from the elution tube) of the cartridge. **Discard gloves**.
		5. With clean gloves, uncap the elution tubes.
		6. Place the deck tray in the instrument.
			1. See Figure 4 for Placing the Deck Tray in the instrument:

**Figure 4: Placing the Deck Tray in the instrument**

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* 1. **Starting a Run:**
		1. When the door to the Maxwell RSC opens, an Extraction Checklist screen is presented. The checklist indicates the steps that need to be performed prior to starting an extraction process.
			1. **NOTE:** Select the **Cancel** button to return to the Cartridge Setup screen.
		2. Once the deck tray in seated in the instrument properly, follow the checklist and ensure that the instrument is setup accordingly.
		3. Touch the **Start** button to begin the extraction run.
			1. **NOTE:** If you wish to abort the run, touch the **Abort** button in the lower right corner of the screen. Any samples being processed will be lost if a run is aborted.
	2. **Completing a Run:**
		1. **NOTE:** It is important to close the Sample Elution Tube caps as soon as the run is finished.
		2. When run is finished, the Protocol Selected screen will change to indicate that the protocol is 100% Completed.
		3. Touch the **Open-Door** button to open the door of the instrument.
		4. Once the Maxwell RSC door is open, verify the plungers are located in well #8.
			1. If the plungers are not located in well #8, please refer to the *Maxwell RSC Instrument Procedure* for troubleshooting steps.
			2. After the door is opened, the UV sanitization window will be presented.
		5. Immediately cap the Sample Elution Tubes to prevent evaporation and/or contamination of the eluates.
		6. Remove the deck tray from the instrument.
		7. Remove sample elution tubes and flash spin to collect eluate at the bottom of the tube.
		8. Place elution tubes in magnetic rack to bring any residual magnetic particles towards the center of the magnetic rack.
		9. Place empty deck tray back into Maxwell® RSC, clean with 70% ethanol and turn on UV light by selecting the sanitize icon on the tablet.
		10. Aspirate each purified sample and place into a clean and appropriately labeled 1.5mL tube.
			1. For any samples that have been split into two tubes, combine the supernatant from each tube into a single clean labeled tube.
		11. If the final eluate looks discolored, consult the Director to determine if BSA should be added to the sample.
	3. **Shutting down the System:**
		1. **NOTE**: Shut down the System as needed (e.g., Technical Support, or prolonged period without use).
		2. Shut down the Maxwell RSC software by pressing the ‘x’ in the upper left corner of the Home screen.
		3. Switch the Maxwell RSC “OFF” using the On/Off power switch in the back of the instrument.
		4. To shut down the Tablet PC:
			1. Swipe inward from the right side of the Home screen.
			2. Select Settings.
			3. Select Power.
			4. Select Shut Down.
	4. **Refer to the NanoDrop 2000 Instrument Procedure to measure the nucleic acid concentration of each sample.**
		1. If a given sample has a DNA concentration of less than 100 ng/ul, proceed with the appropriate clinical assay.
		2. Soft Molecular will automatically calculate dilutions to 50ng/ul.
			1. Please dilute samples that are >100 and <300 to 50 ng/ul.
		3. When the original reading is greater than 300ng/ul, dilute to 100ng/ul and utilize an internal note in Soft Molecular to state original nanodrop reading and how much buffer was added.
			1. Dilute using the following formula:(C1)(V1)=(C2)(V2). Solve for V2, which is the total final volume, so subtract the initial volume in the tube. Recheck the concentration using the Nanodrop 2000 instrument, then proceed with the appropriate clinical assay.

* 1. **DNA/TNA Quantitation Action**
		1. Log into Soft Molecular.
		2. Open Extractions by using the Extraction tile on the dashboard.
		3. Highlight the DNA/TNA Quantitation branch on the action tree.
		4. Select **Tools** tab followed by **Import** to import Nanodrop results.
		5. Choose file location using the ‘**…**’ button next to the Directory field and confirm by clicking **OK**.
		6. Select **INITIAL DNA CONC** from the dropdown in the Template field.
		7. Choose file name using the ‘**…**’ button next to the File Name field and confirm by clicking **Open**.
		8. Select **Import**.
		9. In the Import Finished window select **OK**.
		10. Close the Import from Excel window using the ‘**X**’.
		11. If a dilution is not required, highlight the Barcode# field, scan the product label, and select **Enter** on the keyboard. Repeat this step for all applicable specimens. Select **Save**.
		12. Select **Back** in the Extractions window.
		13. Exit Soft Molecular application.
	2. **First Dilution**
		1. Mark the **Dilute(?)** checkbox for every patient sample that requires a dilution.
		2. Highlight the Barcode# field. Scan the product label and select **Enter** on the keyboard. Repeat this step for all samples pending DNA/TNA Quantitation. Select **Save**.
		3. Highlight the Dilute and Repeat Quantitation branch on the action tree.
		4. Select **Tools** tab followed by **Import** to import Nanodrop results.
		5. Choose file location using the ‘**…**’ button next to the Directory field and confirm by clicking **OK**.
		6. Select **FINAL DNA CONC** from the dropdown in the Template field.
		7. Choose file name using the ‘**…**’ button next to the File Name field and confirm by clicking **Open**.
		8. Select **Import**. If a second dilution is required refer to O. Second Dilution.
		9. In the Import finished window select **OK**.
		10. Close the Import from Excel window using the ‘**X**’.
		11. If a second dilution is not required, highlight the Barcode# field, scan the product label, and select **Enter** on the keyboard. Repeat this step for all applicable specimens. Select **Save**.
		12. Select **Back** in the Extractions window.
		13. Exit Soft Molecular application.
	3. **Second Dilution**
		1. Highlight the correct sample and select the **Internal Note** icon in the Home menu.
		2. Select **Add** and enter ‘Initial DNA Concentration: XYZ ng/uL’ in the text window that appears.
		3. Click **OK** and **Save**.
		4. Mark the **+** sign button in the Initial DNA Conc field to open the multi-run for tube window. Select **New Run** button. Click **OK**.
		5. Enter the value from DNA Concentration field into the Initial DNA Conc field, so that Suggest Add Vol Diluent is calculated.
		6. Select **Tools** tab followed by **Import** to import Nanodrop results.
		7. Choose file location using the ‘**…**’ button next to the Directory field and confirm by clicking **OK**.
		8. Select **FINAL DNA CONC** from the dropdown in the Template field.
		9. Choose file name using the ‘**…**’ button next to the File Name field and confirm by clicking **Open**.
		10. Select **Import**.
		11. Repeat steps 1-10 for additional dilutions as necessary.
		12. Highlight the barcode scanning field. Scan the product label and select **Enter** on the keyboard. Repeat this step for each specimen. Select the **Completed** checkbox and **Save**.
		13. Select **Back** in the Extractions window.
		14. Exit Soft Molecular application.
	4. **Test Aliquot Action: BRAF, IGH-BCL2**
		1. Log into Soft Molecular.
		2. Open Extractions by using the Extraction tile on the dashboard.
		3. Highlight the Test Aliquot branch on the action tree.
		4. Highlight the Barcode# field. Scan the product label and press **Enter** on the keyboard. Repeat this step for all applicable specimens.
		5. Click on the **+** to expand the child level of each patient sample, so the aliquots are displayed.
		6. **If additional aliquots are required:**
			1. Highlight the correct sample.
			2. Select the **Plan Aliquot/Material** button.
			3. Enter the Aliquot value.
				1. **Note**: The Aliquot Value field is alphanumeric, so text (BSA, 1:4 DIL., etc.) can be added to the Aliquot Value column.
			4. Enter the numeric value (no units) in the Volume column.
			5. Attach the test (IGH-BCL2, BRAF).
			6. A window will appear. Select **Yes**.
			7. Select **ATST** in the Protocol field.
				1. **Note**: The Next Action field will automatically populate with Specimen Testing.
			8. Highlight the barcode scanning field. Scan the product label and select **Enter** on the keyboard. Repeat this step for all applicable samples. Select **Save**.
				1. **Note**: When the Test Aliquot action has been completed and saved, the Sample ID will be assigned to the additional aliquots.
		7. Enter the volume with units for each aliquot in the Aliquot Value column.
		8. Enter the numeric value (no units) in the Volume column.
		9. Complete the Test Aliquot action by marking the **Completed** checkbox and select **Save**.
		10. Select **Back** in the Extraction window.
		11. Exit Soft Molecular application.
1. **TROUBLESHOOTING:**
	1. For samples with inhibitors, such as melanin, refer to the PCR Interfering Substances BSA Method Procedure.
	2. If the Maxwell® RSC becomes contaminated:
		1. Use a paper towel wet with 10% bleach to wipe down the inside of the instrument.
		2. Follow the bleach with distilled water, and finish with 70% ethanol.
	3. For additional troubleshooting, see: Genomic DNA Clean and Concentrate Procedure.
	4. Contact information:
		1. Promega 1-800-356-9526
		2. [www.promega.com](http://www.promega.com)
		3. techserv@promega.com
2. **REFERENCES:**
	1. Forsthoefel, K.F., et al., Optimization of DNA Extraction from formalin fixed tissue and its clinical application in Duchenne muscular dystrophy, Amer. J. Clin. Pathol. 98: 98-104, 1995.
	2. Bonin, S. et al. Multicentre validation study of nucleic acids extraction from FFPE tissues. Virchows Arc. 425: 309-317,2010.
	3. Maxwell® RSC FFPE Plus DNA Kit, TM574.
	4. Maxwell® RSC Instrument Operating Manual, AS4500.
3. **REVISIONS:**
	1. 5/11/2022: Update instructions for use with the RSC FFPE Plus DNA kit on the Maxwell RSC Instrument.
	2. 1/16/2024: Clarified Maxwell blank placements and discolored elute resolution.