Forensic Biology Section

Operation of Maxprep Liquid Handler

I. Scope

A. The Maxprep Liquid Handler facilitates the automated processing of DNA extracts. The instrument is an enclosed four-channel pipetting system that performs pre- and post-DNA extraction processes for the Maxwell, as well as setting up assays for DNA quantification and amplification. Using pressure and capacitive liquid sensing technology, the four channels can pipet volumes ranging from 1 µL to 1 mL using pipette tips of 50 µL, 300 µL, and 1000 µL in size. The Maxprep Liquid Handler maintains sample integrity, produces high-quality DNA extracts for downstream analyses, creates PowerQuant standard curves and assays with precision, automatically normalizes samples as it sets up DNA profiling reactions from samples with a wide range of DNA concentrations.

B. The following four automation methods are validated on the Maxprep Liquid Handler: Pre-processing of Maxwell FSC DNA IQ Casework Kit Samples in Tubes (v.1.2.0), Sample Transfer Method (v.1.2.0), PowerQuant System Setup Method (v.1.2.0), DNA Normalization and STR Setup Method (v. 1.2.1).

C. The '**Pre-processing of Maxwell FSC DNA IQ Casework Kit Samples in Tubes**' method automates the preparation and setup of Maxwell FSC cartridges placed in Maxwell deck trays to extract DNA samples using the Maxwell FSC DNA IQ Casework Kit. The Maxprep adds plungers to each cartridge, transfers elution buffer to the elution tubes, adds lysis buffer to the DNA samples, and transfers the DNA samples to the Maxwell cartridges. When the method is finished, the Maxwell deck trays are moved to the Maxwell instrument for extraction.

D. The '**Sample Transfer**' method transfers extracted DNA samples from the Promega 0.5 mL elution tubes to 1.5 mL microcentrifuge tubes, which are better suited for barcoding and long-term storage.

E. The '**PowerQuant System Setup**' method sets up the PowerQuant DNA quantification reactions on 96-well reaction plates. The Maxprep Liquid Handler prepares the master mix, serially dilutes the PowerQuant standards, transfers the master mix to wells in a 96-well plate, and adds the PQ standards and extracted DNA samples (from either 0.5 mL elution tubes or 1.5 mL microcentrifuge tubes).

F. The '**Promega DNA Normalization and STR Setup**' method dilutes ("normalizes") DNA extracts if they are too highly concentrated, prepares the master mix for Fusion and Y23 profiling reactions, and combines the master mix and DNA samples in 96-well plates to be amplified in a Thermal cycler.

G. Sample and run tracking are streamlined through browser-based Portal software which shares the sample names across the various Maxprep Liquid Handler methods, and between the Maxprep Liquid Handler, Maxwell extraction robot, and the QuantStudio 5 quantitation instrument.

II. Instrumentation

A. Daily and/or Weekly Maintenance must be performed before using the instrument for the first time that day and/or week.

B. Do NOT use any bleach or ethanol products to clean the instrument. Only non-bleach wipes or DI water should be used to clean the Maxprep.

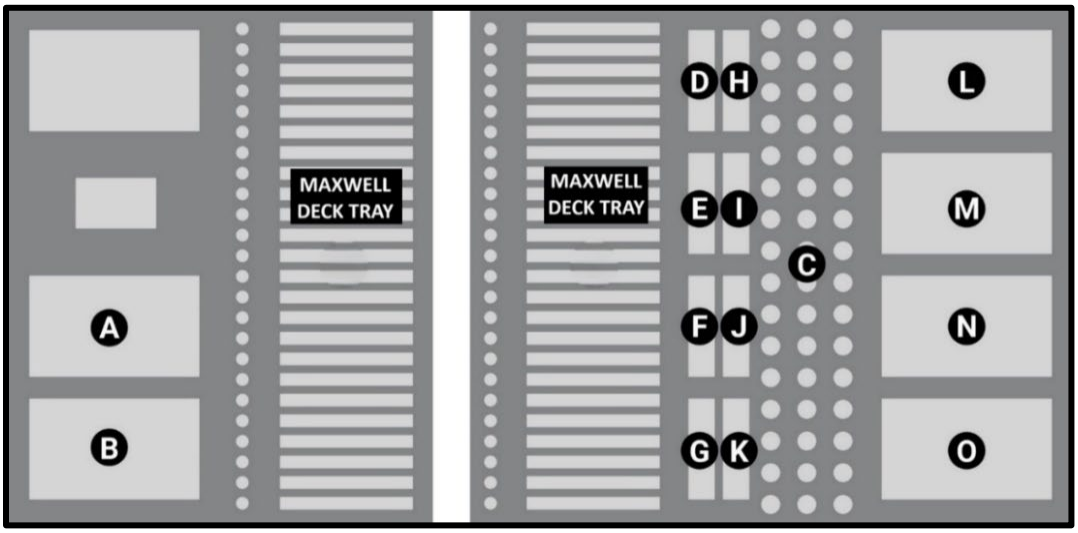
III. Preprocessing of Maxwell FSC DNA IQ Casework Samples in TUBES Method

A. Procedural Notes

1. Final elution tubes must be Promega 1.5ml CW Spin Basket/ClickFit Microtubes. Tubes must be labeled with barcodes with the sample's DNA extract subitem # before loading into the Maxprep sample carriers. The barcode label must be centered vertically under the front lip of the tube.
    a. Case Reagent Blanks may be entered under each case in LIMS for the associated barcode to be printed using the naming convention RB [number/letter], or the tubes can be manually labeled and entered in the software after scanning samples in the Maxprep method setup.
    b. Batch Reagent Blanks (knowns or unknowns extracted as a batch with one blank) can be manually labeled and entered in the software after scanning samples in the Maxprep method setup.
2. Samples MUST be pre-treated using the **Extraction of DNA on Maxwell RSC 48 WITH SPIN BASKETS** protocol, with a final elution volume of 60µl or 100µl.

B. Reagents and Consumables

- Maxwell FSC DNA IQ Casework Kit [Lysis buffer, Elution buffer, FSC Cartridges, Plungers, Elution tubes].
- 1000 µl tip racks, 300 µl tip racks.



Reagents/Consumables	Notes
A. Plungers	Partial or full rack
B. Plungers	Full rack
Maxwell Deck Tray(s)	One opened Maxwell Cartridge and one empty labeled elution tube, per sample
C. Input Samples	1.5 mL Sample Tubes in Sample Carriers
E. Lysis Buffer	In a Maxprep Reagent Reservoir
F. Elution Buffer	In a Maxprep Reagent Reservoir
M. 1000 µl tips	Partial or full rack
N. 1000 µl tips	Full rack
O. 300 µl tips	Partial rack

C. Method

1. Complete a Maxwell Extraction sheet: list the samples and check the box for "Pre-Processing on Maxwell".
2. Follow Sections 7 and 9 of the Extraction of DNA on the Maxwell RSC48 protocol.
3. Place the tubes in a heat block or thermomixer (without agitation) and incubate at **56°C for 30 minutes**.
4. During the incubation period, prepare the Maxwell and Maxprep instruments as follows:
   a. Ensure both Maxwell and Maxprep instruments are clear of any samples, consumables, and reagents from previous runs.
   b. Ensure the Portal Icon  is visible in the Maxwell's menu bar . If not, the Portal needs to be enabled:
   c. From the main screen, **select**: Settings → Admin → Preferences → Portal → Enable. **Click** Test to verify the connection. If there is a problem with the connection, ensure the following matches:
      i. Server name: Maxprep-HP\PromegaPortal
      ii. Database: Portal
      iii. Username: PortalLogin
      iv. Password: PortalLogin
      v. Windows Authentication: Unchecked

d. Open the door of the Maxwell (**click** the Door icon on the top right of the tablet) and remove the required deck tray(s) (front only or front and back).

e. Close the door of the Maxwell (**click** the Door icon again).

f. On the Maxprep Home Screen dashboard, **click** Start.

   i. **Select** the method "Maxwell FSC DNA IQ Tubes" method and then **click** the variant with the desired elution volume, 60µl or 100µl. **Click** Proceed.

   ii. On the next screen, **click** RUN on the bottom left of the software to begin the method. The Maxprep door will lock.

g. **Select** the number of samples with the slider (up to 48) and ensure the appropriate elution volume is selected. **Select** the Maxwell RSC Type of 48. **Click** Next.

h. Place plungers into the plunger holder block and load into the utility carrier(s) at Sites 3 and 4.

   i. A partial rack can be loaded in Site 3, but Site 4 must have a full rack if the number of plungers in Site 3 is not sufficient for the number of samples being processed. Site 4 is optional if Site 3 has enough plungers for the number of samples. If a partial rack is used in Site 3, the rack must be loaded with any empty slots on the left side.

   ii. The plungers are 1 for 1 with the number of samples; you only need to load as many plungers as there are samples.

i. Enter the number of available plungers present in Site 3 and then check the boxes next to the site. Check the box next to Site 4, regardless of whether that site has been used. *Note: The instrument cannot sense the presence of a plunger covering the plunger tool and will perform the entire method without a plunger if the plungers are not available in the expected position.*

j. **Click** Next.

k. Add cartridges and empty elution tubes for the appropriate number of samples to the Maxwell deck tray(s). **Remove cartridge seals** and load the deck tray(s) into the Maxprep.

   i. The front rack must always be loaded in Site 2 with sample 1 towards the back of the instrument.

   ii. Scan the barcodes on the bottom left corner of each tray to transfer the sample information to Portal.

l. **Click** Next.

m. **Click** Next again on the following screen.

5. Load the reagents by removing the reagent carrier.

a. The screen will display the names of the reagents required and where they should be placed on the carrier. There are four sites per Reagent Carrier, which can hold either a Maxprep 3-Position Reagent Tube Holder or a Reagent Reservoir.

b. If present, remove the 3-Position Reagent Tube Holders to make space for the Reagent Reservoirs in Sites 1 and 2 of the first Reagent Carrier.

c. **Click** "Enter Reagent Details" to manually enter the assigned MSP lot number information for each reagent and to see the volume required for each reagent, based on the number of samples entered.

d. Fill the reagent reservoir with enough volume to meet the minimum amount displayed on the screen.

   i. Avoid overfilling, as any remaining reagents cannot be reused.

6. After incubation, centrifuge samples in a benchtop centrifuge (e.g., Eppendorf 5430) for 2 minutes at maximum speed.

a. Confirm all the liquid exited the basket and went into the microfuge tube.

   i. Spin again if any liquid remains.

7. Fully remove as many Sample Carrier(s) as needed for the number of samples in the batch and place them on the bench. Each carrier will hold 16 tubes. Load the barcoded sample tubes into the slots, ensuring the first sample in the batch is loaded into the slot for sample 1. Open each sample lid and **discard all spin baskets**. The tube lids must be even with the raised edge of the carrier, and the barcodes must be visible in the side slot so the scanner can read them.
   a. **Click** SCAN and quickly slide the first carrier into the Maxprep until the carrier is fully in place. **Wait until the gantry moves back slightly and then scan the next carrier, if using, or click Finish on the screen**. If all three sample carriers are being used, the Maxprep will stop scanning on its own after scanning the third carrier.
   b. A pop-up will appear, allowing for the selection of the destination tube type. **Select** 1.5mL Flip Cap tubes for all three racks and **click** Exit.
   c. The number of samples remaining to be scanned will appear on the screen. If the number on the screen is not 0, the instrument did not scan one or more samples. The screen will display the tubes that did and did not scan. Any grey circles indicate a tube that did not scan. To move forward, do one of the following:
      i. **Click** Rescan to repeat the scanning process, being sure to completely remove the carrier from the instrument. Note: If the carrier is not fully removed, an error stating "An item with the same key has already been added" and the run will be aborted.
      ii. **Click** on the grey circle for the missed sample and manually scan the barcode for that tube.
      iii. **Click** the grey circle and manually type in the corresponding reagent blank or sample name. If a sample name is typed, it must match the barcode exactly for downstream processes to access the sample data via Portal. Reagent blank names must always be typed the same way in each method for Portal to properly collate all data for that sample.
8. Ensure the path is clear and **click** Move Arm on the screen, if needed, to access the tip carriers. The tip and plate carriers require two racks of 1000 µl tips and 300 µl tips. The first rack of 1000µl tips and the rack of 300µl tips do not need to be full (partial tip racks should be loaded with any empty spaces to the **left**). The second rack of 1000µl tips must be full.
9. The final screen has a checklist to ensure that all steps have been completed. Visually inspect the setup to ensure everything is in place and flat. Ensure no carriers are out of place and that all components are properly seated in their slots. Check if the tip bucket needs to be emptied.
10. Close the door and **click** Start.
11. Once the process is completed, open the Maxwell door and then the Maxprep door. Remove the Maxwell deck tray(s) from the Maxprep and place the tray(s) into the Maxwell.
12. **Click** START. The Maxwell will prompt you to scan the barcodes of the deck trays that will be used in the procedure (front or front and back). If only using the front tray, leave the back tray field blank.
13. **Click** Continue. Ensure the run data being pulled in matches the date and time of your Maxprep run.
14. **Click** Continue. **Click** on DNA IQ Casework Method and **click** Proceed. Ensure the same data matches the samples in the tray(s) and **click** Proceed. Ensure the setup is acceptable and **click** Start.
    Note: The Maxprep runs for approx. 34 minutes to complete up to 48 samples.
15. While the Maxwell is running, clear any consumables from the Maxprep and remove the empty sample tubes from the sample carriers.
    a. Discard the empty sample tubes.
    b. Empty any excess reagents from the reservoirs into the appropriate hazardous waste collection container and dispose of the reservoirs in the garbage (these should NOT be reused).
    c. Label new 1.5mL tubes with barcodes to transfer extracts into during the Sample Transfer Method.

IV. Sample Transfer Method

A. Procedural Notes

1. "Destination tube matching selection" will ensure the sample will be transferred to the correct destination tube, regardless of the order in which the destination tubes are placed in the sample tube carrier.

B. Method

1. Check the box on the Maxwell Extraction Worksheet to indicate that the Sample Transfer method was utilized.
2. **Click** the Home button on the Maxprep dashboard. **Click** Start.
3. **Click** "Sample Transfer" method and **select** the "Sample Transfer" variant that shows below and **click** Proceed.
4. On the next screen, **click** Run to begin the method. The Maxprep door will lock.
5. Follow the prompts on the screen.
   a. Choose "Input Labware"=**Maxwell RSC 48 Deck Trays,** and "Destination Hardware"=**Samples in tubes**. **Select** the appropriate elution volume (60µl or 100µl). **Click** Next.
   b. Open the door on the Maxwell and remove the deck tray(s). Place deck trays in Maxprep carrier(s). Scan the deck tray(s) to import sample information. If only one deck tray is used, leave the second tray blank. **Click** Exit. *Note: If other runs have been performed between this batch's Maxwell run and this transfer run, you must query the Portal to find your sample data before proceeding.*
   c. Verify the sample info on the Maxprep screen matches the sample info of the samples loaded in the deck tray(s). Check the box next to the tray(s) that were loaded. **Click** Next.
   d. Fully remove the sample carrier(s) from the Maxprep and load with **new, barcoded sample tubes**. The tube lids must be even with the raised edge of the carrier, and the barcodes must be visible in the side slot so the scanner can read them.
      i. **Click** Scan and quickly slide the first carrier into the Maxprep until the carrier is fully in place. **Wait until the gantry moves back slightly and then scan the next carrier, if using, or click Finish on the screen**. If all three sample carriers are being used, the Maxprep will stop scanning on its own after scanning the third carrier.
      ii. A pop-up will appear, allowing for the selection of the destination tube type. **Select** 1.5mL Flip Cap tubes for all three racks and **click** Exit.
      iii. The sample number on the screen should match the number of sample tubes loaded. If the number on the screen does not match the number of samples in the batch, the instrument missed scanning one or more samples. The screen will display the tubes that did and did not scan. Any grey circles indicate a tube that did not scan. To move forward, do one of the following:
         1. **Click** Rescan to repeat the scanning process, being sure to completely remove the carrier from the instrument. Note: If the carrier is not fully removed, an error stating "An item with the same key has already been added" and the run will be aborted.
         2. **Click** on the grey circle for the missed sample and manually scan the barcode for the tube.
         3. **Click** the grey circle and manually type in the corresponding reagent blank or sample name. If a sample name is typed, it must match the barcode exactly for downstream processes to access the sample data via Portal. Reagent blank names must always be typed the same way in each method for Portal to properly collate all data for that sample.
6. Ensure the path is clear and **click** Move Arm to access the pipette tip carrier. Place two racks of 300 µl tips in Sites 2 and 3, and 50 µl in Site 4. The first rack of 300 µl tips in Site 2 and the rack of 50 µl in Site 4 do not need to be full. Partial tip racks should be loaded with empty spaces to the left. The second rack of 300 µl tips in Site 3 must be full. **Click** Next.

7. The final screen will have a checklist to ensure that all steps have been completed. Visually inspect the setup to ensure everything is in place and flat. Make sure no carriers are out of place and that all components are properly seated in their slots. Check if the tip bin needs to be emptied. Close the door and **click** Start.

   Note: The Maxprep runs for approx. 9 minutes to complete 48 samples.

8. Once the method is complete, open the Maxprep door and **ensure all samples have transferred** from the elution tubes in the Maxwell deck tray(s) to the 1.5mL tubes in the sample carriers. If any samples did not transfer, manually transfer the samples to the appropriate final 1.5mL sample tube.

   a. <u>If immediately moving on to PowerQuant:</u>

      i. Leave 1.5mL sample tubes in Sample Carriers.

      ii. Remove the Maxwell deck tray(s) and properly dispose of the waste and consumables as specified in the Maxwell procedure.

      iii. Place the Maxwell deck tray(s) in the Maxwell and allow the **Maxwell** to run a UV Sanitization.

      iv. Remove any other consumables.

      v. Proceed with PowerQuant System Setup Method below

   b. <u>If NOT immediately moving on to PowerQuant:</u>

      i. Remove the Maxwell deck tray(s) and properly dispose of the waste and consumables as specified in the Maxwell procedure.

      ii. Place the Maxwell deck tray(s) in the Maxwell and allow the **Maxwell** to run the UV Sanitization.

      iii. Close and remove the final 1.5mL sample tubes and store appropriately.

      iv. Remove any other consumables and run the UV Sanitization on the **Maxprep**.

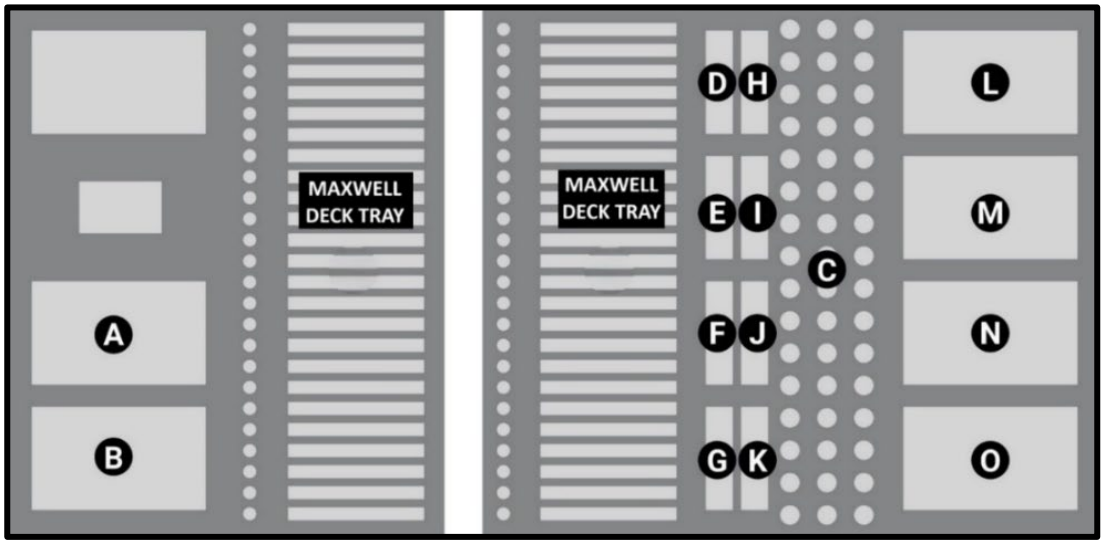
V. <u>PowerQuant System Setup Method</u>

A. Procedural Notes

1. **If a sample has less than 10µl remaining, the sample must be quantified manually.** The Maxprep Liquid Handler requires a minimum volume to ensure precise and proper pipetting, less than 10µl is too small.

B. Reagents and Consumables

- 96-well optical plate; strip tubes.
- PQ Kit [Reaction Mix = Amp. Grade water, 2x Master Mix, 20x Primer/Probe/IPC Mix].
- PQ Male gDNA Standard; PQ Calibrator.
- 300 µl tips; 50 µl tips.



Reagents/Consumables	Notes
B. Amplification Plate in an Amp Plate Base	Optical 96-Well Plate
C. Input Samples	In Maxwell deck trays **OR** 1.5 ml tubes
D. Reaction Mix (empty tube)	In Maxprep 3-Position Reagent Tube Holder
E. Quantification Calibrator	In Maxprep 3-Position Reagent Tube Holder
F. PowerQuant Male gDNA Standard (**Position 1**) and PowerQuant Dilution Buffer (**Position 2**)	In Maxprep 3-Position Reagent Tube Holder
H. PowerQuant 2X Master Mix (Up to 3 tubes)	In Maxprep 3-Position Reagent Tube Holder
I. PowerQuant 20X Primer/Probe/IPC Mix (up to 3 tubes)	In Maxprep 3-Position Reagent Tube Holder
K. Amplification Grade Water (up to 3 tubes)	In Maxprep 3-Position Reagent Tube Holder
L. Strip tubes in Amp Plate Base	In amp plate base in columns 1 and 12
M. 300 µl tips	Partial or full rack
N. 50 µl tips	Partial or full rack
O. 50 µl tips	Full rack

C. Method

1. Complete a Maxprep PowerQuant worksheet listing the samples that will be included in the assay.
2. Retrieve samples to be quantitated from storage. Briefly vortex and centrifuge.
3. Retrieve the appropriate calibrator and quantitation kit reagents. When completely thawed, vortex briefly.
4. On the Maxprep dashboard, **click** START.
5. **Select** the method "PowerQuant System Setup", then **select** the variant named "PowerQuant System Setup" which contains a calibrator. **Click** PROCEED.
6. On the next screen, **click** RUN in the lower left of the screen to begin the method and follow the prompts.
   a. **Select** the input hardware (Samples in tubes). **Select** destination hardware (PCR Plate). **Click** Next.
      i. The final plate layout will be displayed. **Click** Next.
   b. Fully remove as many Sample Carrier(s) as needed for the number of samples in the batch and place them on the bench. Load the barcoded sample tubes into the slots, ensuring the first sample in the batch is loaded in the slot for sample 1. Open each sample lid (a tube opener aids in opening the Click-Fit lids). The tube lids must be flush with the raised edge of the carrier, and the barcodes must be visible in the side slot so the scanner can read them.
      i. **Click** SCAN and quickly slide the first carrier into the Maxprep until the carrier is fully in place. **Wait until the gantry moves back slightly and then scan the next carrier, if using, or click Finish on the screen**. If all three sample carriers are being used, the Maxprep will stop scanning on its own after scanning the third carrier.
      ii. A pop-up will appear, allowing for the selection of the destination tube type. **Select** 1.5mL Flip Cap tubes for all three racks and **click** Exit.
      iii. The sample number on the screen should match the number of sample tubes loaded. If the number on the screen does not match the number of samples in the batch, the instrument missed scanning one or more samples. The screen will display the tubes that did and did not scan. Any grey circles indicate a tube that did not scan. To move forward, do one of the following:
         1. **Click** Rescan to repeat the scanning process, being sure to completely remove the carrier from the instrument. Note: If the carrier is not fully removed, an error stating "An item with the same key has already been added" and the run will be aborted.
         2. **Click** on the grey circle for the missed sample and manually scan the barcode for the tube.

3. **Click** the grey circle and manually type in the corresponding reagent blank or sample name. If a sample name is typed, it must match the barcode exactly for downstream processes to access the sample data via Portal. Reagent blank names must always be typed the same way in each method for Portal to properly collate all data for that sample.

7. Place the 96-well PCR plate on an amplification plate base in carrier 1 at Site 4. Only the brown/grey/black amplification plate bases are suitable for use on the Maxprep (any other plate base would require reprogramming the instrument). **Click** Next.

8. To load reagents, remove the two reagent carriers from the Maxprep and load three Maxprep 3-Position Reagent Tube Holders in Sites 1-3 on the first carrier and Sites 1, 2, and 4 on the second carrier. **Click** "Enter Reagent Details" and follow the prompts to load reagents and enter MSP lot numbers.

a. **In the first carrier:**

i. Site 1: Load one empty 1.5ml tube in the first slot of the 3-position reagent tube holder. Ensure the tube is open with the cap secured in the holder. **Click** Next.

ii. Site 2: Load one tube of the appropriate calibrator in the first slot of the 3-position reagent tube holder. Manually enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

iii. Site 3: Load one tube of PQ Male DNA standard in the first slot and one tube of PQ diluent buffer in the second slot of the 3-position reagent tube holder. Manually enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

9. **Click** "Enter Reagent Details" and follow the prompts to load reagents and enter MSP lot numbers.

a. **In the second carrier:**

i. Site 1: Load as many tubes of **PowerQuant 2X Master Mix** needed to meet the volume to complete the assay, based on the listed required minimum volume, starting with slot 1 of the 3-position reagent tube holder. Manually enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

ii. Site 2: Load as many tubes of **PowerQuant 20X Primer/Probe/IPC Mix** needed to meet the volume to complete the assay, based on the listed required minimum volume, starting with slot 1 of the 3-position reagent tube holder. Manually enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

iii. Site 3: This site is left empty. **Click** Next.

iv. Site 4: Load as many tubes of Amplification grade water needed to meet the volume to complete the assay, based on the listed required minimum volume, starting with slot 1 of the 3-position reagent tube holder. Manually enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

10. Ensure the path is clear and **click** Move Arm to access the pipette tip carrier. One 96-well amplification plate base with one strip cap tube placed in column 1 and one strip cap tube placed in column 12 should be placed on the pipette carrier at Site 1.

11. Place one rack of 300 µl tips in Site 2, and two racks of 50 µl in Sites 3 and 4. The rack of 300 µl tips in Site 2 and the rack of 50 µl tips in Site 3 do not need to be full (partial tip racks should be loaded with empty spaces to the left). The second rack of 50 µl tips in Site 4 must be full. **Click** Next.

12. The final screen will have a checklist to ensure that all steps have been completed. Visually inspect the setup to ensure everything is in place and flat. **Click** Next to start the run. Check if the tip bin needs to be emptied. Note: The Maxprep runs approx. 36 minutes to complete 48 samples .

a. Once the run is complete, remove the PCR plate and plate base and apply the quantitation plate seal.

b. Minimize the Maxprep software and **click** the Chrome icon, which contains the Portal dashboard, or **click** the "Portal Access Web" bookmark in Chrome if it doesn't immediately open.

c. **Click** Runs and find your run in the table. Scroll down to the Export Template field and **select** "PowerQuant Quant Studios" from the drop-down. **Click** Export. Move the downloaded export .xls file to a USB drive and transfer it to H:\Crimelab\DNA\Maxprep Files.

d. Clean the instrument by removing sample tubes for storage, removing all reagents to return to storage, and removing and disposing of any used consumables.

e. Maximize the Maxprep software and run the UV Sanitization.

f. Using a USB drive, transfer the plate record from the networked computer in the post-amplification laboratory to the QS5 computer. Load the plate on the QS5 instrument and import the plate record following the PowerQuant procedure.

g. Once the QS5 run is complete, export the run data as outlined in the PowerQuant procedure. Analyze quantitation data using the Promega PowerQuant Analysis Tool as outlined in the current procedure to produce a quantitation report for the folder.

h. To import quantitation results to the Maxprep, transfer the .xls file exported from the QS5 to the Maxprep using a USB drive. Open the Portal software (Chrome Icon) on the Maxprep computer and **click** Import: Portal Concentration Data. **Click** "PowerQuant" in the table. **Click** Import and navigate to the QS5 .xls file. A chart will appear with the samples found in the file. If everything appears correct, ensure the check box for 'activate on import' is selected for all samples. **Click** Import. You should see a notification that data was successfully imported.

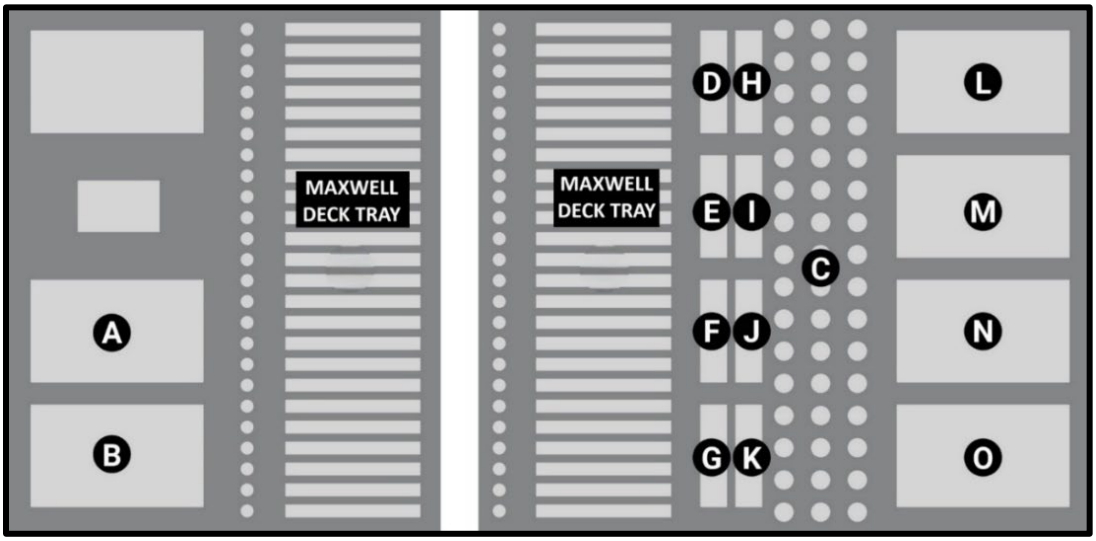
VI. DNA Normalization and STR Setup Method

A. Procedural Notes

1. Sample concentrations from the PowerQuant run need to be loaded into the Portal so the Maxprep has the concentration data for normalization. See section 3.3.4.16 for import instructions.
2. If requantification data is being imported into PowerQuant, **click** "Activate on Import" to override historical concentration data. If multiple concentrations exist for a sample within Portal, **select** the concentration to use within Portal.
3. If a sample concentration is too low to achieve the target of 0.5ng/µl, or if a sample is manually included in the normalization run, the full volume of the neat sample will be added to the reaction.
4. If a **sample concentration is greater than 37.5ng/µl for Fusion or 30ng/µl for Y23**, it needs to be **manually diluted**, and the raw quantitation data manually edited to reflect the diluted concentration **BEFORE** importing the quantitation data into Portal and performing normalization using the Maxprep (or manually amplified).
5. **If a sample has less than 10 µl of volume,** the sample must be **amplified manually**. The Maxprep Liquid Handler requires more than 10 µl in the tube to ensure precise and proper pipetting.

B. Reagents and Consumables

- Deep well plate; 96-well optical plate.
- PQ Kit [Reaction Mix = Amp. Grade water, 2x Master Mix, 20x Primer/Probe/IPC Mix].
- 2800M Male Amp Positive control, TE^{-4}.
- 300 µl tips; 50 µl tips.



Reagents/Consumables	Notes
A. Deep Well Plate	
B. Amplification Plate in Amp Plate Base	Optical 96-Well Plate (can be with barcode)
C. Input Samples	In 1.5 ml tubes
D. Reaction Mix (empty tube)	In Position 1 of Maxprep 3-Position Tube Holder
E. Amplification Controls **(0.25 ng/ul** 2800M DNA**)**	In Position 1 of Maxprep 3-Position Tube Holder
H. PowerQuant 2X Master Mix (up to 3 tubes)	In Maxprep 3-Position Reagent Tube Holder
I. PowerQuant 20X Primer/Probe/IPC Mix (up to 3 tubes)	In Maxprep 3-Position Reagent Tube Holder
K. TE^{-4}	In Reagent Trough
M. 300 µl tips	Partial or full rack
N. 50 µl tips	Partial or full rack
O. 50 µl tips	Full rack

C. Method

1. Complete a Maxprep Fusion or Y23 amplification worksheet listing the samples to be amplified.
2. Retrieve samples to be amplified from storage. Briefly vortex and centrifuge.
3. Retrieve amplification kit reagents and allow them to completely thaw. Briefly vortex.
4. On the Maxprep dashboard, **click** START.
5. **Select** the method "Promega DNA Normalization and STR Setup" method, **select** the relevant variant for the amplification kit, and **click** PROCEED.
6. On the next screen, **click** RUN on the lower left to begin the method.
7. Follow the prompts on the screen:
   a. Manually enter the MSP Lot # for the kit being used. Below the lot #, **select** the kit name (Fusion or Y23).
   b. Next, the input labware must be selected. These will be samples in tubes. Destination labware will always be a PCR plate. If importing quant values from a PowerQuant run, make sure "Import Portal Concentration Data" is checked. **Click** Next once everything is entered correctly.
   c. Fully remove as many Sample Carrier(s) as needed for the number of samples in the batch and place them on the bench. Load the barcoded sample tubes into the slots, ensuring the first sample in the batch is loaded into the slot for sample 1. Open each sample lid (a tube opener aids in opening the Click-Fit lids).

The tube lids must be even with the raised edge of the carrier, and the barcodes must be visible in the side slot so the scanner can read them.

i. **Click** SCAN and quickly slide the first carrier into the Maxprep until the carrier is fully in place. **Wait until the gantry moves back slightly and then scan the next carrier, if using, or click Finish on the screen**. If all three sample carriers are being used, the Maxprep will stop scanning on its own after scanning the third carrier.

ii. A pop-up will appear, allowing for the selection of the destination tube type. **Select** "1.5mL Flip Cap tubes" for all three racks and **click** Exit.

iii. The number of samples scanned at the top of the pop-up and must match the number of sample tubes loaded. If the number on the screen does not match the number of samples in the batch, the instrument missed scanning one or more samples. The screen will display the tubes that did and did not scan. Any grey circles indicate a tube that did not scan. To move forward, do one of the following:

1. **Click** Rescan to repeat the scanning process, being sure to completely remove the carrier from the instrument. Note: If the carrier is not fully removed, an error stating "An item with the same key has already been added" and the run will be aborted.
2. **Click** on the grey circle for the missed sample and manually scan the barcode for the tube.
3. **Click** the grey circle and manually type in the corresponding reagent blank or sample name. If a sample name is typed, it must match the barcode exactly for downstream processes to access the sample data via Portal. Reagent blank names must always be typed the same way in each method for Portal to properly collate all data for that sample. If this is the second time a sample is amplified, manually add **.2** to the end of the sample name on the worksheet. Manually editing the name in the MaxPrep software will prevent the quant value for that sample from being linked to the reamplified sample.

8. Under Assay, **select** "PowerQuant". If all sample names match those imported to Portal, "Sample Number" and "Matching Sample IDs" will be the same number. If the numbers are not the same, a sample name does not match the name in the quant data. Verify the sample name is written the same as when the sample was quantified and that the quant data for that sample has been imported into Portal (section V.12.h above). **Click** Next.

9. The screen only displays samples with concentrations greater than the current "stop at quant" concentration.

a. Any samples with concentrations less than the programmed "stop at quant" concentration, **including Reagent Blanks**, will be automatically moved from the normalization assay to the Excluded tab.

b. The software also has a bug that rounds any concentrations below 0.0006 ng/µl down to 0 ng/µl, therefore excluding the sample from the normalization batch. Any samples with a concentration from 0.0001 – 0.0006 ng/µl will need to be manually included if those samples need to be amplified.

c. There are four options besides the sample list. These show the samples that will be included in the normalization method, those that will be excluded, those that are flagged, and then all samples.

d. Verify all samples that are included on the sample carrier are present on the included tab.

e. **Click** the Excluded tab to ensure there are no samples listed. If samples are listed on the excluded tab and you intend to normalize those for amplification, **click** on each sample and then **click** Include.

f. **Ensure any Reagent Blanks and low concentration samples that should be amplified have been moved from the Excluded Tab to the Included Tab.**

10. Clicking on a sample will display the Sample details to the right (Sample ID, sample concentration , the target mass of the method, whether it is an included or excluded sample, what the quantitation target is, and any flags associated with the sample).

a. There is a known bug in the software that will flag any sample with an Auto/Y flag if the sample has a positive [Auto] concentration and an "Undetermined" [Y] concentration. Consult the PowerQuant Analysis Tool data for the corresponding samples if there is a flag in question.

b. If you would like to use a different target than autosomal, change the target on this screen.

c. If you would like to adjust the target concentration for a sample, **click** "Override Target Mass" and enter the new target concentration in the box that opens.

d. **Click** Next.

11. The final plate layout will be displayed, showing where the controls and the samples will be placed. Click on any samples to display their name to the right.

a. Control 1 is the 2800M Positive Control.

b. Control 2 is the Negative Control.

c. A blank well will be left at the first well of every three columns to accommodate ladders downstream. Ensure all samples are present.

d. **Click** Next.

12. The setup for the utility carrier will be displayed with the appropriate labware needed for this method.

a. On the first carrier, one deep-well dilution plate should be placed at Site 3, and one amplification plate in an amp plate base should be placed at Site 4.

b. A plate detail window will open to scan the barcode on the amp plate. The barcode does NOT need to be scanned. **Click** Close. Once loaded, **click** the box to the left of the site name to insert a check mark.

c. **Click** Next.

13. To load reagents, remove the two reagent carriers from the Maxprep and load three Maxprep 3-Position Reagent Tube Holders in Sites 1-3 on the first carrier and Sites 1-2 on the second carrier. **Click** "Enter Reagent Details" and follow the prompts to load reagents and enter MSP lot #'s.

a. In the first carrier:

i. Site 1: Load one empty 1.5ml tube in the first slot of the 3-position reagent tube holder. Ensure the tube is open with the cap secured in the holder. **Click** Next.

ii. Site 2: Load one tube of the 0.25ng/µl 2800M Male DNA Control in the first slot of the 3-position reagent tube holder. Manually enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

iii. Site 3: This site is left empty. **Click** Next.

iv. Site 4: Load a reagent trough filled with the minimum required volume of TE^{-4} to be used as the diluent and negative control. Manually enter the lot #. **Click** Next.

14. Click "Enter Reagent Details" and follow the prompts to load reagents and enter MSP lot #'s.

a. In the second carrier:

i. Site 1: Load as many tubes of Promega Master Mix to meet the volume needed for the assay, based on the required minimum volume, starting with slot 1 of the 3-position reagent tube holder. Enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

ii. Site 2: Load as many tubes of STR Primer Pair Mix to meet the volume needed for the assay, based on the required minimum volume, starting with slot 1 of the 3-position reagent tube holder. Enter the MSP lot # and ensure the tube is open with the cap secured in the holder. **Click** Next.

iii. Site 3: This site is left empty. **Click** Next.

iv. Site 4: This site is left empty. **Click** Next.

15. Click in the box next to "Samples in Tubes" when loaded. **Click** Next.

16. Ensure the path is clear and **click** Move Arm to access the pipette tip carrier. Place one rack of 300 µl tips in Site 2, and two racks of 50 µl in Sites 3 and 4. The rack of 300 µl tips in Site 2 and the rack of 50 µl tips in Site 3 do not need to be full (partial tip racks should be loaded with the empty spaces to the left). The second rack of 50 µl tips in Site 4 must be full. **Click** Next.
17. The final screen will have a checklist to ensure all the steps have been completed. Visually inspect the setup to ensure everything is in place and flat. **Click** Next to start the run.

    Note: The Maxprep runs for approx. 45 minutes to complete 48 highly concentrated samples.
18. Once the run is complete, remove the PCR plate and apply strip caps or an amplification plate seal to seal the PCR plate. Amplify the samples according to the Fusion or Y23 amplification procedure.
19. Minimize the Maxprep software and **click** the Chrome icon, which contains the Portal dashboard, or **click** the "Portal Access Web" bookmark in Chrome if it doesn't immediately open.
20. **Click** Runs and locate the run in the table below. Scroll down to the Export Template field and **select** 3500 Plate Map from the drop-down. **Click** Export. If a sample was amplified a second time, after importing the 3500 plate map into the 3500, you will need to manually add "**.2**" to the plate record on the 3500.
21. To export the amplification report, **click** Full Report. A file will download containing the concentrations used by the instrument during normalization and the target input mass. Print this file for the folder. This report is the only record of the target concentration that was amplified by the Maxprep and must be printed as a record for the case file.
22. Move both of the downloaded export .xls files to a USB drive and transfer them to the appropriate folder in H:\Crimelab\DNA\Maxprep Files.
23. Once amplification is complete, load the plate in the 3500 Genetic Analyzer per the Fusion or Y23 3500 procedures.
24. Clean the instrument by removing sample tubes for storage, removing all reagents to return to storage, and removing and disposing of any used consumables.
25. Maximize the Maxprep software and run the UV Sanitization.
26. Using a USB drive, transfer the plate record from the networked computer in the post-amplification laboratory to the 3500 computer for import.