Forensic Biology Section

Extraction of DNA on Maxwell RSC48

1. Scope:

1.1. Maxwell DNA Extraction is a semi-automated procedure to extract and purify DNA from nucleated cells using the Casework Extraction Kit and the Maxwell FSC DNA IQ Casework Kit on the Maxwell RSC48 instrument. Samples are manually pre-treated and lysed, and then the Maxwell RSC48 instrument purifies and elutes the DNA.

1.2. The Casework Extraction Kit contains reagents to pre-treat and lyse samples before they are loaded into the Maxwell instrument. An Extraction Master Mix is prepared and manually added to each sample (in tubes or baskets) and incubated at 56℃ for 30 minutes (solid samples can be pre-treated in Spin Baskets or directly in tubes with no basket; liquid samples are pre-treated in tubes with no basket). After incubating in the Extraction Mix, Lysis Buffer is manually added to each sample, vortexed, and then run on the Maxwell instrument.

1.3. The Maxwell FSC DNA IQ Casework Kit has disposable Casework Cartridges that are pre-filled with reagents. To run the pre-treated and lysed samples on the Maxwell instrument, they are manually transferred into well #1 of the cartridges and the Maxwell uses disposable plungers placed in well #8 to automatically transfer the extracts from well to well along the length of the cartridge. Any DNA in the first well will bind to paramagnetic particles and are then transferred by the plungers through the series of prefilled reagents in the cartridge's wells to wash and purify the DNA. Finally, the purified DNA bound to the paramagnetic particles is eluted into 0.5 ml tubes for further testing.

2. Safety:

2.1. Use alcohol to clean up any spills. DO NOT use bleach (bleach may react with Thioglycerol).

3. Specimens:

The Maxwell is suitable for the following biological samples:

- Bloodstain
- Saliva stain
- Tissue
- FTA paper
- Liquid blood
- Epi Swab ("touch DNA")

4. Consumables:

- Casework Extraction Kit [Extraction buffer, Pro-K, 1-Thioglycerol, Nuclease-free water] (Promega)
- Maxwell FSC DNA IQ Casework Kit [Lysis buffer, Elution buffer, FSC cartridges, Plungers, Elution tubes] (Promega)
- 1.5 ml and 0.5 ml Microcentrifuge tubes
- Optional: Casework Spin Baskets and Casework Microfuge Tubes (Promega)

5. Instrumentation:

- Maxwell RSC48 (Promega)
- Thermomixer or heat block [set for 56℃ incubation]
- Centrifuge/Microcentrifuge (benchtop centrifuge required for CW Spin Baskets, e.g. Eppendorf 5430)
- Vortex

6. Procedural Notes:

6.1. Equilibrate reagents to room temperature before use.

6.2. Extraction Master Mix is made before each batch of extractions.

6.3. Casework Spin Baskets must be paired with Casework Microfuge Tubes.

6.4. When pipetting reagents, pipet slowly to avoid foaming.

6.5. Stock Pro-K solution is made by adding 556 µl of Nuclease-free water to one tube of lyophilized Pro-K and mixing gently by inverting. Pro-K solution should be stored frozen (-30℃ to -10℃).

6.6. Eluted DNA extracts can be stored in the refrigerator (1℃ to 8℃) for short term storage.

7. Prepare a master mix of extraction reagents:

7.1. Casework Extraction Buffer, Proteinase K, and 1-Thioglycerol are combined to make an Extraction Master Mix sufficient to pre-treat the total **# of samples + 1** (making an extra 300 µl of master mix):

Extraction Master Mix	Volume Per Sample.
Casework Extraction Buffer	286 µl
Proteinase K (18 mg/ml)	10 µl
1-Thioglycerol	4 µl
Total Reagent Volume	**300 µl**

8. Option 1 - Pre-Treat Samples WITHOUT Spin Baskets:

8.1. Take portions of samples and place in tubes (no spin baskets):

8.1.1. Bloodstains or saliva stains: Cut out stained material and place in a microfuge tube.

Optional: **To test for human hemoglobin (HemaTrace test)**, add 500 µl of TE^{-4} to the microfuge tube and incubate for 15 minutes or more to elute the stain, centrifuge at full speed for 5 minutes to pellet any cells, test 80 µl of the supernatant (see "HemaTrace Human Blood Detection" method), discard **all but 100 µl** of the remainder of the supernatant without disturbing the cell pellet, and *continue with step 8.2 below.*

8.1.2. Cigarette butts (saliva): Cut a ring of paper from the filter end (~0.5 to 1 cm) and place the cutting in a microfuge tube.

8.1.3. Epi swabs: Place the swab head in a microfuge tube.

8.1.4. Solid Samples: Place the sample in a microfuge tube.

8.1.5. Liquid samples: Pipet up to 100 µl sample into a microfuge tube (in a biosafety hood).

8.2. In a biosafety hood, add **300 µl of the Extraction Master Mix** to the microfuge tube, and vortex for five seconds.

8.3. Place the tubes in a heat block or thermomixer (without agitation) and incubate at **56℃ for 30 minutes**.

- Note: While samples are incubating, the Maxwell instrument and cartridges can be prepared for the automated extraction of the pre-treated samples (see below).

8.4. After incubation, briefly spin samples to ensure all the liquid is at the bottom of the microfuge tube.

8.5. In a biosafety hood, add **200 µl of Lysis Buffer** to the samples and vortex for five seconds.

8.6. Briefly spin samples to ensure all the liquid is at the bottom of the microfuge tube.

9. Option 2 - Pre-Treat Samples WITH Spin Baskets:

9.1. Take portions of samples and transfer to CW **Spin Basket** in a CW microfuge tube:

9.1.1. Bloodstains or saliva stains: Place the material in a CW **Spin Basket** in a CW microfuge tube.

NOTE: the HemaTrace test CANNOT be run in the CW Spin Basket. In order to test for human hemoglobin, place the stained material in a standard microfuge tube, add 500 µl of TE^{-4}, incubate for 15 minutes or more to elute the stain, centrifuge at full speed for 5 minutes to pellet any cells, test 80 µl of the supernatant (see "HemaTrace Human Blood Detection" method), discard supernatant without disturbing the substrate or cells, then transfer the substrate and cells to a CW **Spin Basket** and *continue with step 9.2 below.*

9.1.2. Cigarette butts (saliva): Cut a ring of paper from the filter end (~0.5 to 1 cm) and place the cutting in a CW **Spin Basket** in a CW microfuge tube.

9.1.3. Epi swabs: Place the swab head in a CW **Spin Basket** in a CW microfuge tube.

9.1.4. Solid Samples: Place the sample in a CW **Spin Basket** in a CW microfuge tube.

9.2. In a biosafety hood, add **300 µl of the Extraction Master Mix** to the CW Spin Basket and vortex for five seconds.

9.3. Place the tubes in a heat block or thermomixer (without agitation) and incubate at **56℃ for 30 minutes**.

- Note: While samples are incubating, the Maxwell instrument and cartridges can be prepared for the automated extraction of the pre-treated samples (see below).

9.4. After incubation, centrifuge samples in a benchtop centrifuge (e.g. Eppendorf 5430) for **2 minutes at maximum speed**. Confirm all the liquid exited the basket and went into the microfuge tube before removing and discarding the spin baskets. Spin again if any liquid remains.

9.5. In a biosafety hood, add **200 µl of Lysis Buffer** to the samples and vortex for five seconds.

9.6. Briefly spin samples to ensure all the liquid is at the bottom of the microfuge tube.

10. Prepare the Maxwell RSC48 instrument:

10.1. Turn on power to Maxwell instrument (on right side of instrument) and tablet (on top left of tablet).

10.2. Double tap the "**Maxwell RSC48**" icon on the desktop. The instrument will run a 1-minute self-test.

10.3. A prompt to run a 5-minute UV sanitization will appear. Press "**Start**" (if press cancel, the instrument will prompt again when the method is chosen). A 5-minute timer in the corner counts down the time.

10.4. Press the large "**START**" button. A list of methods appears with "DNA IQ Casework" at the top.

10.5. **Scan the barcode** on the side of the Maxwell FSC DNA IQ Casework Kit label to choose the method and automatically enter the Catalog #, Lot #, and Expiration Date of the kit into the Method.

10.6. Press the "**Proceed**" button and the Cartridge Setup screen will appear.

10.7. Choose the "**Front**" or "**Back**" deck tray button.

10.8. Press the cartridge's tall **Rectangle** to select a position, then press that cartridge's **Number** to open the name editor. Type in the Case # and Item # of the sample for the cartridge in that deck tray position.

10.9. After all the samples have been typed in, press the "**Proceed**" button and the Maxwell door will open and present deck trays.

11. Prepare the Casework Cartridges:

11.1. Carry the deck trays to the benchtop and insert one cartridge into the deck trays for every sample being extracted.

11.2. Carry the deck trays to the biosafety hood and place one **0.5 ml Elution tube** (labeled with case # and item #) in the tray next to its corresponding cartridge, then add **approximately 50 µl of Elution Buffer** to each Elution tube and close the caps. (25 to 250 µl of elution buffer can be used if needed).

- Note: 5 to 10 µl of Elution Buffer can evaporate from the Elution tube by the end of the run, so **adding 60 µl of Elution Buffer is appropriate for most forensic samples**, but dilutions of samples with higher yields may need to be diluted to obtain accurate measurements on the Rotor Gene Q.

12. Transfer samples to Casework Cartridges and the Maxwell instrument:

12.1. Optional: turn the deck trays so the cartridges' Well # 1 are positioned toward the front of the hood.

12.2. Peel the foil from one cartridge at a time and carefully pipet each pre-treated/lysed sample into Well # 1 of the corresponding cartridges, slowly pipetting up and down to mix.

- Note: **Pipet slowly** to avoid creating bubbles.

12.3. Place a **plunger** in each cartridge's Well #8 (may be done before or after carrying the deck tray to the instrument).

13. Begin the Automated Extraction on the Maxwell instrument:

13.1. Place the deck tray in the Maxwell instrument and click each deck tray into place (back to front).

13.2. **Open the caps** on all the elution tubes (open caps must be pointing towards the instrument door).

13.3. Ensure that all prompts have been completed on the Extraction Checklist, and then hit the "**Start**" button.

13.4. Once the run has started, the screen shows who began the run, the estimated completion time, and which step the instrument is currently on.

- Note: runs should take 22 minutes, regardless of whether one or two deck trays are used.

14. When the run is complete:

14.1. Press the "**Open Door**" button and **close the cap on each Elution tube**.

14.2. Remove the deck trays from the Maxwell instrument and transfer the Elution tubes containing the Extracts to storage racks.

14.3. A "**Report View**" with the information from the run should be saved to a USB and copied to the "Maxwell Runs" folder on the H-Drive (H:\Crimelab\DNA\MAXWELL Runs).

14.4. Remove and discard the cartridges and plungers from the deck trays, wipe the surfaces inside the instrument and the surfaces of the decks **with alcohol (not bleach)**, and place the deck trays back in the Maxwell instrument.

14.5. The Maxwell instrument will automatically perform a UV cleaning cycle.