Histology Phenotype

High-throughput characterization of the morphological responses of three rat tissues to normoxia, hypoxia and high salt treatments

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MEDICAL COLLEGE OF WISCONSIN

National Heart, Lung, and Blood Institute
PROGRAMS FOR GENOMIC APPLICATIONS

PhysGen

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I. Tissue Acquisition and Preparation

Under anesthesia, the abdominal aorta, heart and one kidney are carefully removed from twelve rats per strain and placed in a labeled specimen cup containing approximately 75 mls of 10% Formaldehyde in phosphate buffer. The rostral end of the aorta is marked with suture to insure that the caudal end will be sectioned. The tissues are allowed to fix overnight at room temperature before they are handled. The following day, the tissues are examined and the heart and kidney are bisected before all three tissues are placed in labeled cassettes prior to automated processing. The rostral-caudal length of the hearts are measured and noted. They are then bisected at their mid-point to insure that sections will be taken from an anatomically consistent level. The kidneys are also bisected at the midsagittal plane to improve fixative penetration. The cassettes are returned to the 10% Formaldehyde solution and are held under house vacuum for a minimum of three days until they are ready to be loaded into the automatic tissue processor.

II. Tissue Processing

The cassettes are placed in the Microm HMP 300 and processed at room temperature, except where noted, according to the following automated protocol:

Step 1. Fixed in 10% Formaldehyde for 1 hour.

Step 2. Fixed in 10% Formaldehyde for 1 hour.

Step 3. Dehydrated in 50% Ethanol for 45 minutes.

Step 4. Dehydrated in 70% Ethanol for 45 minutes.

Step 5. Dehydrated in 95% Ethanol for 45 minutes.

Step 6. Dehydrated in 95% Ethanol for 45 minutes.

Step 7. Dehydrated in 100% Ethanol for 1 hour.

Step 8. Dehydrated in 100% Ethanol for 1 hour.

Step 9. Cleared in Xylene for 1 hour.

Step 10. Cleared in Xylene for 1 hour.

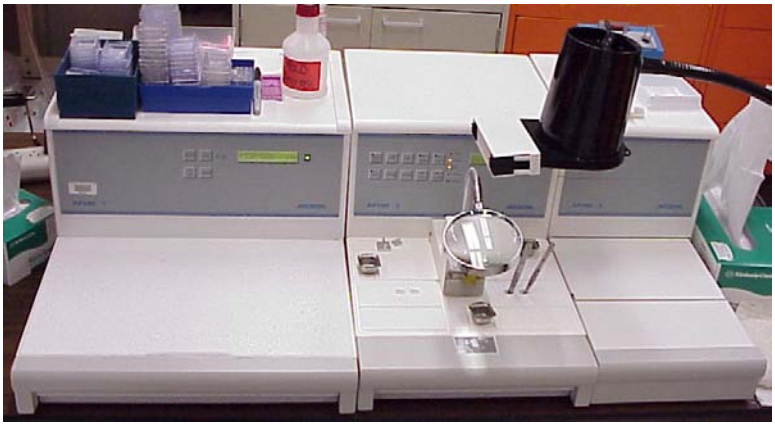
Step 11. Infiltrated with Tissue Prep II paraffin for 1.5 hours at 60°C, under vacuum.

Step 12. Infiltrated with Tissue Prep II paraffin for 2.5 hours at 60°C, under vacuum.

Step 13. Infiltrated with Tissue Prep II paraffin for 4.0 hours at 60°C, under vacuum.

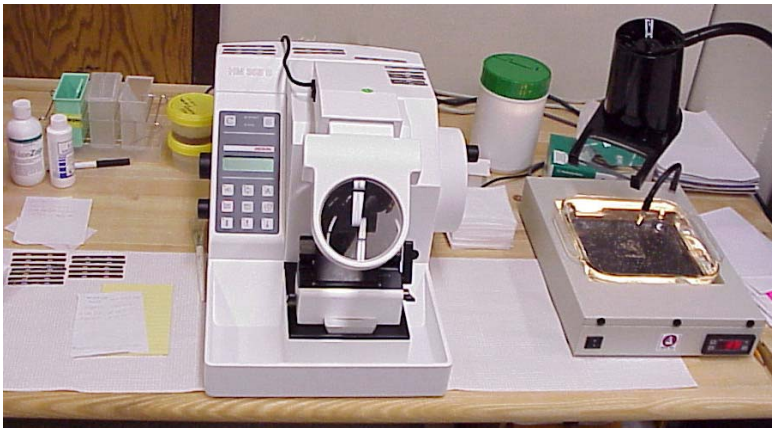
III. Tissue Embedding

After the tissues have been fully infiltrated with paraffin in the processor, they are taken to the Microm AP 280 embedding station. The aortas are trimmed and mounted so that the caudal portion will be cross-sectioned. The rostral half of the heart is mounted for cross sectioning with the caudal half held in reserve in the top half of the cassette. One half of the kidney is mounted so all the layers will be visualized in the midsagittal plane and the other half held in reserve in a separate cassette.



IV. Tissue Sectioning

Three-micrometer thick sections are cut from each tissue on a Microm HM355S microtome. Six slides are produced for each tissue. Tissue sections are mounted on silanized/charged slides with the exception of one uncharged slide dedicated to the Jones Silver Stain. Kidney slides carry two sections, heart slides three, and aorta slides eight sections. One slide is stained with Gomori's One-Step Trichrome for immediate documentation. The rest are held in reserve for future investigation.



V. Tissue Staining

Once all the samples are cut, one slide from each tissue is processed according to this procedure. Note: Steps 1-10 and 21-28 are performed on the Sakura DRS 2000 Automatic Stainer.

Deparaffinization:

1. Xylene, 5 minutes
2. Xylene, 4 minutes
3. Xylene, 3 minutes
4. Xylene, 2 minutes

Hydration:

5. 100% Alcohol, 2 minutes
6. 100% Alcohol, 2 minutes
7. 95% Alcohol, 1 minutes

Rinsing:

8. Distilled Water, 1 minute
9. Distilled Water, 1 minute
10. Distilled Water, 1 minute
11. Remove slides from the Sakura Stainer and place in a Tissue Tek 'white' staining dish containing fresh distilled water.

Pre-treatment - Mordant: Mandatory: Under a Hood

12. Place slides in the pre-warmed 60 °C Bouin's Solution and incubate at 60 °C for 60 minutes. (Optional: Bouin's Solution overnight at room temperature)

13. Remove slides from Bouin's and place in tap water.

Washing:

14. Wash well in running tap water until all the yellow color is removed from the tissue sections.

15. Rinse thoroughly in several changes of Distilled Water. Remove slides

Nuclear Staining:

16. Place slides in the 'Working Weigert's Iron Hematoxylin Solution' for 20 minutes. Agitate slides several times during the 20 minutes. Remove slides.

17. Wash well in running tap water until the water runs clean of excess hematoxylin.

18. Rinse thoroughly in several changes of Distilled Water. Remove slides.

Trichrome Staining:

19. Place slides in the room temperature One-Step Trichrome Stain for 45 minutes. Remove slides.

20. Rinse slides thoroughly in several changes of 0.5% Acetic Acid. Agitate slides to remove excess Trichrome Stain. Total time should be approximately 3 minutes. Do not rinse in Distilled Water.

Dehydration:

21. Transfer slides to the SAKURA DRS2000 Automated Stainer. Use programmed Staining Method '95% start TRICHROME'.

22. 95% Alcohol, 45 seconds

23. 100% Alcohol, 1 minute

24. 100% Alcohol, 2 minutes

Clearing:

25. Xylene, 3 minutes

26. Xylene, 4 minutes

27. Xylene, 5 minutes

28. Xylene, End Station

Coverslipping / Mounting:

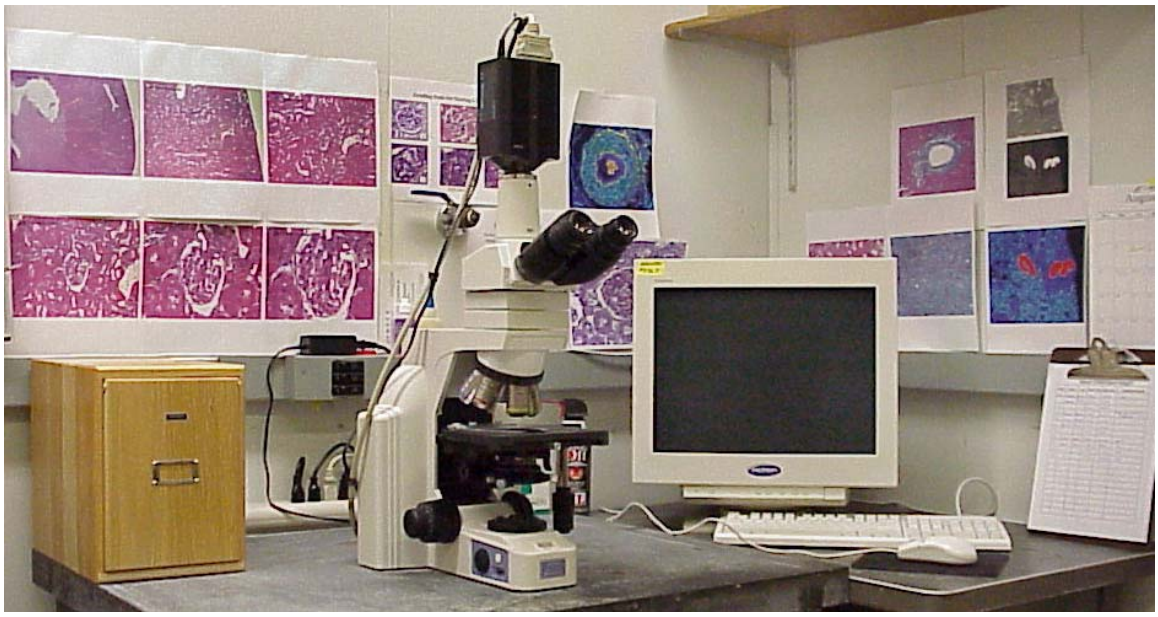
29. Remove the slides from the SAKURA stainer and place the staining rack in a green chemical resistant Tissue-Tek staining dish filled with Clear-Rite 3.

30. From Clear-Rite 3 coverslip using Permount and appropriate sized coverglass.

31. Label slides if necessary and arrange accordingly.

VI. Documentation

Using a Nikon E-400 fitted with a Spot Insight camera, twelve high-resolution color digital micrographs are taken for each of the twelve rats per strain. (Two for the aorta, three for the heart and seven to document the kidney.) The micrographs are taken following a strict protocol that insures that valid comparisons can be made across strains. After all the rats are documented, half of them (a male and female from each treatment) will have their images processed into smaller JPEG files and cataloged for posting on the website. This results in a representative survey of the morphology of three tissues from three treatment protocols as expressed in both genders within a strain.



VII. Solutions

Fixation:

10% Neutral Buffered Formalin
Bouins Solution
Bouins Solution fixed tissue sections MUST be thoroughly washed in running tap water until all the yellow color (the picric acid) is removed from the tissue. Failure to remove the Bouin's Solution can result in inadequate staining. Staining problems have been particularly noticed in Immunohistochemistry staining.

Xylene

Deparaffinization – removal of paraffin from tissue sections
Clearing – removal of alcohol from tissue sections (miscible with permount used for coverslipping)

100% Alcohol, Reagent or Absolute (200 proof)

Hydration – removal of xylene from tissue sections and down a gradual series to distilled water
De-hydration – removal of water from the tissue sections thru a graded alcohols to xylene

95% Alcohol

Alcohol, Reagent	95.0 mls	50.0 mls	190.0 mls
Distilled Water	5.0 mls	950.0 mls	3610.0 mls

Distilled Water

Uncontaminated, no bacterial growth
Must use and rinse thoroughly before hematoxylin

Bouin's

Commercial Solution
Used as a mordant, pretreatment

Weigert's Iron Hematoxylin

Stock Solution A

	100.0 mls	500.0mls	1000.0 mls
Hematoxylin	1.0 gms	5.0 gms	10.0 gms
95% Alcohol	100.0 mls	500.0 mls	1000.0 mls

Stock Solution B

29% Ferric Chloride	4.0 mls	20.0 mls	40.0 mls
Distilled Water	95.0 mls	475.0 mls	950.0 mls
Hydrochloric Acid	1.0 ml	5.0 mls	10.0 mls

Working Weigert's Iron Hematoxylin

FRESHLY PREPARED

USE THE SAME DAY and TOSS

Equal parts of Stock Solution A and Stock Solution B

Stock Solution A	100.0 mls
Stock Solution B	100.0 mls

Trichrome Stain – One-Step 'Gomori's'

STOCK SOLUTION and WORKING SOLUTION

FILTER AFTER USE

SAVE and RE-USE THIS SOLUTION

REFRIGERATE

	500.0 mls	1000.0 mls
Chromotrope 2R	3.0 gms	6.0 gms
Aniline Blue (1.5 gms) (doubled)	3.0 gms	6.0 gms
Acetic Acid	5.0 mls	10.0 mls
Phosphotungstic Acid	4.0 gms	4.0 gms
Distilled Water	500.0 mls	1000.0 mls

0.5% Acetic Acid

Acetic Acid	15.0 mls
Distilled Water	2985.0 mls

Clear-Rite 3

Commercial Solution

Coverslipping / Mounting – is miscible with Permount

Permount

Coverslipping / Mounting Media – a synthetic neutral mounting media.
Does not darken or become acidic with age.
Permount will not affect the stain.

Physiology & BRI Histology Core Laboratory
located: MCW - Department of Physiology

(414) 456-8179
Barbara or Carol

PROJECT #

HISTOLOGY PROJECT REQUEST FORM

Date Received | Received By | Date Completed

PLEASE PRINT THE FOLLOWING INFORMATION

Title of Project

Principal Investigator

Co-Investigator

Account Number to be billed

Affiliation

Project Contact Person

Phone Number

Project Information: Please answer the information below

RADIOACTIVE TISSUE ? **Specify:** _____

Tissue Sections requested check ☐ **Paraffin** ☐ **Frozen** **Type of Tissue** (Animal or Human, Kidney, Heart etc.) _____

Fixation used for tissue specimens ☐ **10% Formalin** ☐ **Bouin's** **Other Fixative Used** _____

Specimen currently / received in _____

Number of UNSTAINED SLIDES per BLOCK ☐ **Section THICKNESS microns** ☐

Tissue SECTIONS per SLIDE ☐ **Number of H & E's per BLOCK** ☐

Specimens are to be

- ☐ **tissue: cut, trimmed**
- ☐ **cassetted**
- ☐ **process & embed**
- ☐ **FS tissue remounted & cut**
- ☐ **paraffin blocks re-cut**
- ☐ **agar orientation**
- ☐ **margins inked**
- ☐ **tissue decalcified**
- ☐ **paraffin block re-embed**
- ☐ **PCR slides**

Special Stains or Procedure Requested

- ☐ **Deparraffinize & Hydrate**
- ☐ **Dehydrate, Clear & Mount**
- ☐ **IHC - Brown Stain etc**
- ☐ **IHC - Fluorescent**
- ☐ **IRON Stain**
- ☐ **Jones Silver Stain**
- ☐ **Counterstain**
- ☐ **Coverslip**
- ☐ **Movat Stain**
- ☐ **PAS Stain**
- ☐ **TRICHROME**
- ☐ **Verhoff's Elastic**

other

Supplies provided to the researcher: amount ???

slides box (½ gross) ☐ **vendor** _____

container with 10% Buffered Formalin ☐

Special Instructions

PGA Histology Images per Strain

144 Images **Post 72**

		Aorta		Heart			Kidney						
Treatment	**Rat ID**	10x	40x	1x	4x	10x	1x	4x	10x	40x	40x	40x	40x
Hypoxia	Female 1												
and	Female 2												
0.4%	Male 3												
Salt	Male 4												
Normoxia	Female 5												
and	Female 6												
4.0%	Male 7												
Salt	Male 8												
Normoxia	Female 9												
and	Female 10												
0.4%	Male 11												
Salt	Male 12												
Normoxia	Female 13												
and	Female 14												
4.0% Salt	Male 15												
+ L-NAME	Male 16												

Notes: