SOUTHERN SEA OTTER TISSUE TRIMMING PROTOCOL

Tissues are grouped by similar density and hardness: Place trimmed tissues from the same or similar groups into the same cassettes to optimize paraffin embedding and sectioning.

Group I: Muscles (Trim longitudinally along long axis of muscle fibers unless noted otherwise; for any lesions, include additional sections of the transition from normal to abnormal tissue)

- 1. Temporal 1 section
- 2. Masseter 1 section (include adjacent zygomatic salivary gland if possible)
- 3. Sternocephalicus (Ventral Neck) 1 section
- 4. Intercostal 1 section
- 5. Diaphragm 1 section
- 6. Tongue 1 transverse section (include dorsal and ventral mucosa)
- 7. Soft Palate/Tonsil 1 transverse section (transection of soft palate, tonsil, and tonsillar crypt)
- 8. Proximal Esophagus 1 cross-section

Group II: Heart (Trim longitudinally from apex to base unless noted otherwise and include epicardial and endocardial surfaces; for any lesions, include additional sections of the transition from normal to abnormal tissue)

- 1. Atrium/Ventricle with A/V Valves (Left) 1 section
- 2. Ventricle with Papillary Muscle (Left) 1 section
- 3. Atrium/Ventricle with A/V valves (**Right**) -1 section
- 4. Ventricle with Papillary Muscle (**Right**) 1 section
- 5. Proximal Interventricular Septum 1 section (just below the atrioventricular border; include left and right ventricular endocardium)
- 6. Apex (Rounded Tip of Left Ventricle) 1 section (distal left ventricular free wall at apex)
- 7. Thoracic Aorta 1 cross-section

Group III: Soft Tissues (Trim as noted below; for any lesions, include additional sections of the transition from normal to abnormal tissue)

- 1. Lung 2 sections (one from each **left** and **right** lobe; include pleural surface and a bronchiole)
- 2. Liver 2 sections (one from each **left** and **right** lateral lobe; include hepatic capsule)
- 3. Gallbladder 1 cross-section (include mucosa and serosa)
- 4. Kidney 2 cross-sections (one from each **left** and **right** kidney; include more than one adjacent renicules with renal capsule, cortex, medulla, and papilla)
- 5. Spleen 1 section (include splenic capsule, cortex, and medulla)
- 6. Pancreas 1 cross-section (duodenal pancreatic limb; include serosa and associated lymph nodes if possible)
- 7. Thymus 1 cross-section (include visceral pleura)
- 8. Omentum 1 cross-section (include serosa)
- 9. Adrenal Gland 2 cross-sections (one from each **left** and **right** adrenal gland; include capsule, cortex, and medulla)
- 10. Thyroid/Parathyroid Gland 2 longitudinal sections (one from each **left** and **right** thyroid gland; place the whole **left** and **right** cranial parathyroid gland into the corresponding cassette)
- 11. Submandibular Salivary Gland 1 cross-section
- 12. Lymph Nodes 1 cross-section from each submandibular, retropharyngeal, axillary, hilar, inguinal, and mesenteric lymph node

Group IV: Gastrointestinal Tract (Trim cross-sections with a sharp blade and handle only along the serosal (outer) surface – do not touch the mucosa; include serosa and mucosa; for any lesions, include additional sections of the transition from normal to abnormal tissue)

- 1. Stomach 2 sections (one from each gastric body and pylorus; trim perpendicular to the rugal folds; include additional sections of any grossly-apparent erosions or ulcers)
- 2. Small Intestine 3 sections (one from each duodenum, jejunum, ileum; include additional sections with any grossly-apparent parasites or abnormal tissue)
- 3. Rectum/Distal Large Intestine 1 section

Group V: Reproductive / Urinary Tract (Trim as noted below; include serosal and mucosal surfaces; for any lesions, include additional sections of the transition from normal to abnormal tissue)

- 1. Ovary 2 longitudinal or cross-sections (one from each **left** and **right** ovary; include any grossly-apparent follicles, corpora lutea, corpora albicantia, or anything that looks like a mass, cyst, or bump)
- 2. Uterus 2 cross-sections (one from each **left** and **right** uterine horn; include additional sections of thickened uterine wall and any abnormal tissue)
- 3. Testicle/Epididymis 4 cross-sections (one from each **left** and **right** testicle/epididymis head and one from each **left** and **right** testicle/epididymis tail)
- 4. Prostate/Distal Ureters/Urethra/Seminal Vesicles 1 transverse section
- 5. Bladder 1 cross-section (include serosa and mucosa)

Group VI: Brain/Pituitary Gland/Ganglion (Trim as described below and as shown in Supplementary Image 1A and 1B; for any lesions, include additional sections of the transition from normal to abnormal tissue)

- 1. Rostral Hippocampus 2 cross-sections (one from each **left** and **right** rostral (anterior) hippocampus; include hippocampus, piriform lobe, thalamus, and hypothalamus; place left and right sections in separate cassettes) (Supplementary Image 1A-2)
 - I. Place brain on cutting board so that the ventral side faces up (Supplementary Image 1A-1)
 - II. Locate the infundibulum and mammillary bodies (Supplementary Image 1A-1)
 - III. Wet brain knife with formalin and, in one continuous motion, make a transverse cut through the entire brain just caudal (posterior) to the mammillary bodies (Supplementary Image 1A-1)
 - IV. With the rostral (anterior) part of the cerebrum, cut a transverse section approximately 4 mm thick
 - V. Cut the interthalamic adhesion to separate left and right hemispheres
 - VI. Collect the sections of cerebrum that include the **left** and **right** rostral hippocampus, piriform lobe, thalamus, and hypothalamus; trim off excess cerebrum as needed to fit sections into cassettes (Supplementary Image 1A-2)
 - VII. Place each section with the side of the first cut facing down into a separate cassette
- 2. Cerebrum Frontal Lobe/Rhinencephalon (**Right**) 1 cross-section (Supplementary Image 1A-3)
 - I. Continue to cut transverse sections of cerebrum approximately 4 mm thick
 - II. Collect a section of cerebrum that includes the **right** frontal lobe and rhinencephalon (Supplementary Image 1A-3)
 - III. Place the section into a cassette

- 3. Cerebrum with Olfactory Bulb 2 cross-sections (one from each **left** and **right** olfactory bulb; place each in a separate cassette) (Supplementary Image 1A-4)
 - I. Continue to cut transverse sections of cerebrum approximately 4 mm thick
 - II. Collect the sections of cerebrum that include the **left** and **right** olfactory bulb (Supplementary Image 1A-4)
 - III. Place each section into a separate cassette
- 4. Caudal Hippocampus 2 cross-sections (one from each **left** and **right** caudal (posterior) hippocampus; include hippocampus, piriform lobe, thalamus, and hypothalamus; place left and right sections in separate cassettes) (Supplementary Image 1A-5)
 - I. With the caudal (posterior) part of the cerebrum and the first cut, cut a transverse section approximately 4 mm thick
 - II. Cut the interthalamic adhesion to separate left and right hemispheres
 - III. Collect the sections of cerebrum that include the **left** and **right** caudal hippocampus, piriform lobe, thalamus, and hypothalamus; trim off excess cerebrum as needed to fit sections into cassettes (Supplementary Image 1A-5)
 - IV. Place each section with the side of the first cut facing down into a separate cassette
- 5. Cerebrum Temporal Lobe (Left) 1 cross-section (Supplementary Image 1A-6)
 - I. Cut another transverse section approximately 4 mm thick
 - II. Collect the section of cerebrum that includes the **left** temporal lobe; trim off excess cerebrum as needed to fit section into cassette (Supplementary Image 1A-6)
 - III. Place the section into a cassette
- 6. Cerebellum with Rostral Medullary Vellum 1 cross-section (include cerebellum, rostral medullary vellum, and pons) (Supplementary Image 1A-7)
 - I. Continue to cut transverse sections through the caudal (posterior) brain approximately 4 mm thick
 - II. Collect a section of cerebellum that includes the rostral medullary vellum and pons (Supplementary Image 1A-7)
 - III. Place the section into a cassette
- 7. Cerebellum with Medulla 1 cross-section (include cerebellum and medulla) (Supplementary Image 1A-8)
 - I. Continue to cut transverse sections through the cerebellum approximately 4 mm thick
 - II. Collect a section of cerebellum that includes the medulla (Supplementary Image 1A-8)
 - III. Place the section into a cassette
- 8. Pituitary Gland 2 sagittal sections (Supplementary Image 1B)
 - I. Bisect the pituitary gland along the midline of the sagittal plane (the line of symmetry between left and right halves) (Supplementary Image 1B)
 - II. Collect both halves of the pituitary gland
 - III. Place both halves with the cut surface facing down in a cassette that contains another section of brain tissue
- 9. Trigeminal Ganglion/Nerve 4 longitudinal sections (two from each **left** and **right** ganglion/nerve)
 - I. Bisect each trigeminal ganglion and nerve longitudinally
 - II. Collect both halves of each ganglion and nerve
 - III. Place all halves with the cut surface facing down in a cassette that contains another section of brain tissue

Group VII: Other

- 1. Eye 2 sections (one from each eye; transect along the mid-sagittal plane and include the lens and optic nerve; place each eye in a separate deep cassette with no other tissues)
 - I. Place each eye on cutting board with the optic nerve facing up
 - II. Locate the superficial blood vessels that run medially and laterally along the surface of the sclera; these blood vessels indicate the left and right halves of each eye
 - III. Locate the medial canthus; this canthus indicates the medial half of each eye
 - IV. Use a sharp blade to bisect each eye perpendicular to the scleral blood vessels and just medial to the entry point of the optic nerve
 - V. Collect the lateral half of each bisected eye that includes the lens and optic nerve
 - VI. Remove a small part of the sclera directly opposite the bisected cut surface so paraffin can penetrate the interior of the eye during embedding
 - VII. Place the bisected (larger) cut surface of each eye facing down into a separate deep cassette with no other additional tissues
- Skin 1 longitudinal section (cut parallel to the direction of hair growth; this sample is usually collected from the ventral abdominal midline during pelt removal; for any lesions, include additional sections of the transition from normal to abnormal tissue; place each skin section in a separate cassette with no other tissues)

Group VIII: Special Requests/Additional Tissues (if collected)

- 1. Spinal Cord Multiple cross-sections (include all areas of spinal cord cervical, thoracic, lumbar, sacral)
 - a. If spinal cord pathology is suspected, separate sections into multiple cassettes and note the location of each section (e.g., cranial/caudal cervical, thoracic, lumbar, sacral, etc.) on the trim sheet
- 2. Any special requests and/or additional tissues Trim as directed in case notes