Washing Hair for Mitochondrial or Nuclear DNA Testing		
Status:Published		Document ID: 1171
DATE EFFECTIVE 09/12/2017	APPROVED BY mtDNA Technial Leader	PAGE 1 OF 3

Washing Hair for Mitochondrial or Nuclear DNA Testing

1 Purpose:

1.1 To prepare hairs for DNA extraction.

2 Demounting

2.1 If the hair is loose, then proceed to the appropriate hair washing procedure. If a "possible root" is observed, the sample should be cut and washed for nuclear DNA testing extraction (see section 4).

2.2 If the hair is mounted:

2.2.1 Process only one mounted slide at a time.

2.2.2 Turn on the heat plate and adjust the heat dial between 100-110^{o}C. Place the slide on a heat plate until the mountant softens and using forceps remove the cover slip. The mountant softens quickly and hairs will scorch if left on the heat plate too long.

2.2.3 The hair will be attached to either the coverslip or the slide. Remove hair and place into a xylene bath for up to 5 minutes or until the mountant completely dissolves. Hairs and slides/coverslip containing hairs can be kept in the xylene bath for longer than 5 minutes if necessary.

2.2.4 Using clean forceps, carefully remove the hair from the xylene bath

2.2.5 It is at the discretion of the analyst to make a picture of the full hair at this time.

2.2.6 Proceed to the appropriate hair washing procedure.

3 Washing the hair for mtDNA testing extraction

3.1 Using forceps and a scalpel cut a 2 cm region of the hair or hair shaft. A picture of the cutting may be taken at this time. If the hair is also to be tested for nuclear DNA, the mitochondrial DNA cutting should be away from the root. Place the unused portion of the hair onto the backing of a post-it note and return to the packaging

3.2 **If "possible tissue" attached to hair is observed, see your supervisor. In some cases the hair will not be washed, proceed to step 3.11 and enter N/A as TergAZyme and Saline lot #.**

3.3 Prepare 5% TergAZyme solution by adding 15ml of Ultra Pure Water water to 0.75g of TergAZyme. Mix well. Record TergAZyme lot #.

3.4 Using clean forceps, place the hair fragment cutting into a 1.5 ml tube with 1 ml of 5% TergAZyme solution. Vortex the tube for 1 minute at high speed, and place into the sonicator for 15 minutes. After sonication, vortex the sample again for 1 minute at high speed.

3.5 Prepare a 50 ml Falcon tube and filter cup. Label the tube and filter cup tab with the sample name. Pre-wet the filter cup membrane with 1 ml of Ultra Pure Water dH_2O.

3.6 Remove the hair from the TergAZyme with clean forceps, and place the hair into the filter cup in the center of the membrane.

3.7 Wash the hair with 1 ml of Ultra Pure Water dH_2O. Allow the liquid to pass through the filter.

3.8 Wash the hair with 1 ml of 0.85% saline. Allow the liquid to pass through the filter. Record Saline Lot #.

3.9 Wash the hair with 1 ml of 100% ethanol. Allow the liquid to pass through the filter.

3.10 Remove the filter cup containing the hair and place on a lint free wipe to let the ethanol evaporate. Once the filter membrane is dry, the hair will be dry as well.

3.11 Transfer the cut hair fragment to the bottom of a clean 1.5 ml tube. Label the tube.

3.12 Store the tube containing the hair fragment in the appropriate “To Be Extracted” cryobox in the pre-amplification laboratory freezer.

3.13 Proceed to Mitochondrial extraction for mitochondrial DNA testing procedure.

4 Washing the hair for nuclear DNA testing extraction

4.1 For nuclear DNA extractions, using forceps and a scalpel cut up to 1.5 cm of the proximal region of the hair, including the root. Place the unused portion of the hair onto the backing of a post-it note and return to the packaging. A picture of the root should be taken at this time.

4.2 If “possible tissue” attached to hair is observed, see your supervisor. In some cases the hair will not be washed, proceed to step 4.8 and enter N/A as Saline lot #.

4.3 Prepare a 50 ml Falcon tube and filter cup set by labeling the tube and filter cup tab with the sample name. Pre-wet the filter cup membrane with 1 ml of 0.85% saline. Document Saline lot #.

4.4 Using clean forceps, place the cut hair into the filter cup in the center of the membrane.

Washing Hair for Mitochondrial or Nuclear DNA Testing		
Status:Published		Document ID: 1171
DATE EFFECTIVE 09/12/2017	APPROVED BY mtDNA Technial Leader	PAGE 3 OF 3

4.5 Wash the hair with 1 ml of 0.85% saline. Allow the liquid to pass through the filter. Repeat that step.

4.6 Wash the hair with 1 ml of 100% ethanol. Allow the liquid to pass through the filter.

4.7 Remove the filter cup containing the hair and place on a lint free wipe to let the ethanol evaporate. Once the filter membrane is dry, the hair will be dry as well.

4.8 Transfer the cut hair fragment to the bottom of a clean 1.5 ml tube. Label the tube.

4.9 Store the tube containing the hair fragment in the appropriate “To Be Extracted” cryobox in the pre-amplification laboratory freezer.

4.10 Within the LIMS system, indicate the cutting was made on the evidence item and schedule the appropriate DNA extraction procedure using the sample creation wizard.

4.11 Proceed to Mitochondrial extraction for nuclear DNA testing procedure