Guidelines for working in the cleanroom facilities of the Henry Wellcome Genetics Labs, University of Cambridge

General information
The following guidelines provide information for the use of cleanroom facilities (also called ‘aDNA labs’ further below in this document) in the Henry Wellcome building that are designed for the work on ancient DNA. The guidelines include information about health and safety aspects specific to the work within the facilities and rules for keeping the labs clean and contamination free. Guidance is provided for best practice for the shared use of equipment and storage space, supply of reagents and waste management. For more general information about health and safety, Human Tissue Act (in case working on human remains younger than 100 years), and other guidance for rules that those working in the labs are expected to comply to and information about sample importation in the Department of Archaeology and Anthropology please refer to the following web pages:

http://www.archanth.cam.ac.uk/safety.html
http://www.safety.admin.cam.ac.uk/subjects/chemicals/coshh
http://www.archanth.cam.ac.uk/safety/humansampleflowchart.pdf

The cleanroom facilities are hosted by the Department of Archaeology and Anthropology (ArchAnth) and are shared for joint use with Department of Zoology (Zool). For any questions about the use of the labs please refer to the following key contacts for further information:

<table>
<thead>
<tr>
<th>Name</th>
<th>Role</th>
<th>Internal number</th>
<th>e-mail</th>
</tr>
</thead>
<tbody>
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<td>9999</td>
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</table>

Location of the facilities: Henry Wellcome Building, Fitzwilliam Street, Cambridge, CB2 1QH

The cleanrooms have a separate entrance on the left side of the building (Tennis Court Rd side). The entrance is card and key access only. For getting your key and card activated for the access, please, contact our Facilities Manager. All new users must confirm that they have read and understood these guidelines, go through Health and Safety and Lab Induction and sign both forms available through the lab website, http://www.arch.cam.ac.uk/research/laboratories/wellcome

The forms should be signed also by the PI and returned to the Facilities Manager. A copy should be kept also with the PI.
Above the general guidance about the work in the lab and inductions each student or researcher working in the lab is expected to receive more specific training relevant for their own research project from the teams of either Archaeology and Anthropology or Zoology departments.

**Basic principles of workflow**

The cleanroom facilities of the Henry Wellcome Genetics Labs are hosted by Department of Archaeology and Anthropology and anyone working in the labs has to comply to the general rules of laboratory use in the premises of the department as outlined in the departmental web page, [http://www.archanth.cam.ac.uk/safety.html](http://www.archanth.cam.ac.uk/safety.html). Also, while working in the facilities you will have to follow the specific rules of our ancient DNA lab in order not to compromise your own work and that of others sharing the lab. The rules to minimize contamination and to keep the labs clean and well maintained are essential for keeping high standards of research of the labs. It is of utmost importance that these guidelines are followed and that any deviation is reported to your PI and Facilities Manager immediately in order to find ways to minimize the effect of the deviation and to find ways to correct it. In case of emergency or accidents make sure the incident is reported to the Facilities Manager and your supervisor. All incidents should be reported including ‘near misses’.

The following guidelines are structured by the following four themes:

1. Guidelines of specific aspects of health and safety in the aDNA labs.
2. Guidelines to minimize contamination risks and secure authenticity of results.
3. Guidelines to keep the labs well stocked with reagents.
4. Guidelines for keeping equipment well maintained and in working order.

All users of the Henry Wellcome cleanrooms must read these guidelines carefully and go through both (i) health and safety and (ii) lab inductions to get access to the labs. In the lab induction form, all new users have to confirm that they have read the guidelines for working in the cleanroom facilities and agree to the rules of the lab use. That form needs to be counter-signed by the PI of the relevant research group who with their signature confirms that the new user has or will be properly supervised and/or trained to carry out their specific project and that the PI has also secured funding to cover the costs related with the project. In case you uncertain about any aspects of the protocol you are following or the facilities in general, please ask for extra guidance either from your supervisor or the Chief Technician.

Clean Lab user meetings will be held at regular intervals and all lab users are expected to participate unless traveling abroad. Please, ask your supervisor about the schedule of these meetings and ask your name to be added to the relevant mailing list.
1. Guidelines of specific aspects of health and safety in the aDNA labs

All basic health and safety rules that apply to other labs in the Department apply also to the labs in the Henry Wellcome Building. For a general guidance on lab health and safety, please, read the departmental Health and Safety manual, http://www.archanth.cam.ac.uk/safety/aahs_dept_safetymanual_oct2015.pdf

The names and telephone extensions of the Departmental First Aiders are displayed on Notice Boards in every Departmental building. The main Security Emergency number can also be contacted to locate a First Aider: Tel. 101. Emergency calls. In the event of a power cut or network problem, the Emergency Phones can be used to reach the University Security. In the event of an emergency which requires the Emergency Services (e.g. fire, serious accident) Dial 9999. There is a telephone that you can use in the main area of the cleanrooms.

Some basic rules

- Eating and drinking are not allowed in any labs.
- Never apply cosmetics in the laboratory.
- Use appropriate care with electrical equipment and sharp objects
- Visitors unauthorized by your PI and supervisor should be kept out of the labs (you cannot take friends or visitors into the labs without relevant coordination)
- Make sure you are familiar with how to use the equipment before using it.
- Make sure you keep the equipment and rooms clean all the time.
- Make sure you are aware of the risks associated with the chemicals you use

Risk assessment forms must be completed by those carrying out lab work to assess and minimize risks that could cause harm to people. For further general information on the procedures see p. 13 in the departmental safety manual. Some items arising under risk assessment may require assessment under different regulations which may include:

- CoSHH regulations (Control of Substances Hazardous to Health) should be followed and you need to familiarize yourself with the CoSHH risk assessment relevant to your work in the lab.

In the aDNA labs there are several chemicals that are hazardous to health. Do not use them without training. Here below is a short guidance on some of these chemicals, what they are and how to deal with them. Some of the chemicals listed are not currently stored in our aDNA labs but since other aDNA labs may use them it is helpful to be aware of these as well. If you are unsure about anything related with these chemicals, ask your supervisor for further guidance.

- Guanidinium thiocyanate is used in some of the silica extraction methods (e.g. Qiagen buffer AL and others). In contact with acids or bleach extremely toxic hydrogen cyanide gas (HCN) may be released that can be fatal. If you spill this reagent DO NOT bring it into contact with acids or bleach. Always wipe up guanidine thiocyanate with dry paper towels first, followed by clean ethanol.

- Guanidinium HCl: Many buffers in the lab contain guanidine salts such as Gu-HCl (e.g. Qiagen PB, ATL and others). If these come into contact with bleach they form chlorine gas that is toxic. If you need to bleach something that has contained these liquids, or wipe up a DNA spill with
such liquids, always first clean with ethanol and/or water. Only after the guanidinium salt is cleaned up first can you then bleach the implement/surface. Remember to clean with ethanol afterwards to avoid bleaching of clothes, more chlorine gas formation, skin irritation etc.

- **Phenol** is highly toxic, it penetrates latex gloves and can be lethal if spilled on the skin. Never use phenol without proper training. Always know what to do about a spill, whether on you or as a leaking tube. NEVER leave a spill unattended. Always use in a vented hood. If possible, centrifuge phenol at a time when few people are in the lab, because vapours are released even in “sealed” tubes.

- **Hydrochloride acid** is highly corrosive, even when diluted. Never use outside a flow hood.

Always add acid to water and not the reverse.

Always store acid bottles on the bottom shelves of the chemical cupboard.

Be cautious when using bleach avoiding any contact of the chemical with your skin. In case of contact with skin, wash immediately at the basin with plenty of water. If symptoms develop obtain immediate medical attention.

In case you get bleach spills into the eye use immediately eyewash at the basin. Speed is essential, IMMEDIATELY irrigate with eyewash solution or clean water, holding the eyelids apart, for at least 10 minutes. Obtain immediate medical attention.

When working with any of the chemicals mentioned above make sure you know exactly where the relevant waste should go.

### Waste management
Some chemical waste is produced in large scale, e.g., TBE, phenol/chloroform and Qiagen products and are therefore collected in 5 L or 20 L plastic containers that are marked with the containers’ contents.

- Never mix buffer wastes in a single bottle but use separate bottles for acids, solvents (e.g. phenol) or Qiagen extraction buffers.
- Use separate bottles for acids, bases, organic solvents and other extraction/purification buffers.
- **Always label** the bottles/containers properly with contents, date and your name so we know what it is.

### WORKING ALONE IN THE LAB
There should be no use of the facilities out of hours unless permission is granted by your supervisor. Users of the lab should also be aware of the risks associated with working alone and take appropriate steps to control them. Make sure someone with the access to the lab (normally your supervisor) knows when you are working alone and let them know when you finish and are out of the lab facilities.
2. Guidelines to minimize contamination risks and to secure authenticity of results.

Some basic rules

- Make sure you follow carefully the details of the routine specified for the entry into the labs.
- Do not bring items unrelated to your work to the labs.
- Do not bring your lab book or laptop into the clean labs.
- All new items and equipment that need to be brought into the labs should be carefully decontaminated first.
- Keep freezers tidy. Only extracts and reagents can be stored permanently.
- Any intermediate stage products of your work can be stored only short term.
- If you have any ideas how to improve these guidelines or to change lab practices in general, please share them with your supervisor.

HOW TO ENTER THE CLEAN LAB IN SIX EASY STEPS

Before entering the clean labs make sure that:

- You have not been to the modern labs that day and that you wear a clean set of clothes, i.e. clothes that have not seen the modern labs since last washing. If you absolutely must go into the clean lab after having been to the modern labs you need to take a shower first and change your clothes. There are showers on the third floor of the Henry Wellcome Building (bring your own towel).

When entering the clean lab you need to:

1. Remove shoes and put blue covers over your socks. If you have long hair, put on a hair net here.
2. Enter the anteroom and put on your first pair of gloves.
3. Put on a facemask and hair net and make sure your hair is inside the net.
4. Put on a (clean) suit and extra pair of sleeves.
5. Put on second pair of gloves and this pair is the only one you can change inside lab.
6. Enter the clean lab, make fresh bleach if necessary, and bleach your gloves.

Once you have entered the clean lab, do not go outside wearing your suit/clogs.

When leaving the clean lab, please do not remove the hair net and ‘fluff’ your hair until you are well outside the lab area.

ANTI-CONTAMINATION GUIDELINES

DO NOT ENTER THE CLEAN LAB AFTER HAVING BEEN IN A MODERN LAB.

- Do not bring anything from the modern lab into the clean lab (this includes lab books, reagents, gel images, pens, computers, iPods, cell phones, etc.)
Always bleach your gloves before you start working.
Always be conscious about what you are touching with your hands – if you touch your face/hair you must change gloves.
Always wipe all work surfaces (and chairs) with bleach followed by water and finally ethanol after use. [the cleaning step with water is necessary because ethanol + bleach = chlorophorm]
Always turn on the UV light in the flow hoods after you finish your lab work.
Only store liquid wastes in the cupboard designated for waste. Bring full waste containers to the chemical storage room when leaving the labs.
Always keep doors closed, i.e. do not sit in the extraction or library preparation room with the door open

DO NOT USE THE THERMOCYCLERS FOR PCRS. They are for non-cyclic incubation only.

CLEANING

To minimize contamination it is absolutely crucial that the clean labs are kept clean. To ensure this, every user is expected to clean their workspace after they are finished in the lab (see everyday cleaning). In addition, each active user will be assigned to a weekly cleaning rotation. The weekly cleaning will be done in teams of two or three and must be done every single week - even if the labs look clean. The weekly cleaning rota is posted on the door to the clean labs as well as inside the labs. Use the checklists as you are cleaning and return them to Chief Technician’s desk. If you can’t clean when it’s your turn you are responsible for finding a replacement. Not cleaning the labs or finding a replacement will have consequences. If you do not assist in these communal duties, other busy people have to step in. If you think you should not be on the rota, let the Chief Technician know.

EVERYDAY CLEANING

Every user must clean their work area after they finish their daily work in the lab. This includes:

- Emptying the table bins and the bins on the floor.
- Washing all surfaces with 5% bleach, water, followed by 70% ethanol.
- Informing your supervisor if any of the consumables are about to run out.
- Cleaning equipment after use (this includes drills, drill bits, centrifuges, etc.)

WEEKLY/FORTNIGHTLY CLEANING (depending on frequency of lab use)

- Wipe all surfaces (freezer/fridges, cupboards, table tops, hoods, doors) with 5% bleach solution, followed by water and 70% ethanol.
- Wipe UV tubes in ceiling and inside LAFs with ethanol soaked towels.
• Sweep and wash the floor with a highly diluted solution of bleach.
• Empty all the bins.
• Move cardboard boxes into the cardboard container in the entrance room.
• Take out waste bags to the entrance room.
• Take any full waste bottles entrance room and inform the Chief Technician that they are ready for pick-up.
• Check and fill stock supplies and inform supervisors before they are running low.
• Use the cleaning form and check off the parts you have completed and leave it in the folder in the storage room.
• Fill out checklist and return it to Chief Technician desk. If any mentioned supplies are low in stock in the storage room/chemical storage room, let your supervisor know ASAP.

GUIDELINES FOR THE WORK IN THE LAB TO AVOID CONTAMINATION

• Always make aliquots. Never pipette a liquid from a communal stock bottle.
• Never over-fill tubes.
• Always wipe up spills immediately. First with 5% bleach followed by water and 70% ethanol unless the spill contains guanidine salts (e.g. PB Buffer), in which case wipe it up with ethanol or water first (for further guidance see instructions above under section 1).
• Do not leave the lids off of pipette tip boxes.
• Always bleach all your benches and centrifuges after use, followed by 70% ethanol.
• Always wipe down the inside of flow hoods. Pay attention to the glass front, not cleaning with ethanol will ruin the glass because bleach etches the glass.
• Always empty waste bags that are full or nearly full.

3. Guidelines to keep the labs well stocked with reagents.

GENERAL LAB ETIQUETTE:

• Do not use anything that has someone else’s name on unless you have explicit permission
• Always report to your supervisor if you THINK things are running low, not when they runs out, but when you can see stock is running low.
• If you need to use a lot of any supply, please ask your supervisor to order it in advance (e.g. Qiagen kits, Amicon filters, scalpels, MinElute columns etc.).
• Storage space in freezers and cabinets is limited, so be considerate and clean out in your drawers regularly.
• Whenever you open a common stock (e.g. a water bottle) always write the date of opening on the bottle.
• When going to the GM reception, look for new packages and bring them to the storage room.
• Do not leave empty boxes in the clean lab area, even if they are not yours.
STOCKS
Make sure that you re-stock the clean lab after use. In other words, do not leave empty or near-empty tips boxes etc. in the labs without moving fresh supplies into the anteroom. Shared storage area for items that can be kept at room temperature outside the cleanrooms is located in the locker on the 1st floor of the HWB. Ask guidance from the supervisor or Chief Technician first on how to use it. There is also cold temporary storage on the ground floor of the HWB.

4. Guidelines for keeping equipment well maintained and in working order.

General guidance: If you think something is broken in the lab, put a note on the machine/item and inform the Manager of the Facilities

Centrifuges
- Always balance centrifuges
- Always use lids on the centrifuges, clean them after use and leave lids open.
- Remove broken tubes/parts of tubes from centrifuges immediately – the pieces can break your tubes or other lab users’ tubes.

Pipettes
- Never pipette a volume outside the range of a pipette (e.g. a P20 pipette does not go above 20 µl). If you use it for 24 µl, you will de-calibrate it and cause mechanical and measuring problems. The appropriate range is written on all pipettes.
- Inaccurate or not working pipettes must be left in the designated box just inside the storage room before shipped for repair and calibration.

Thermocyclers are only used for incubation at certain temperature in aDNA labs

NEVER USE THERMOCYCLERS FOR PCRS

PCR and amplification of libraries should be performed in separate lab facilities designed for modern DNA, e.g. those in the premises of Sir David Attenborough Building, Department of Zoology.
**CLEAN-LAB BOOKING**

Always book lab time using the online calendar (link to be shared with you by your supervisor) and specify what you will be doing (extractions, building libraries etc.)

- Only schedule the amount of time you actually need.
- Finish your work and clean the bench within your allotted time.
- If you have to change a booking or cannot use the time you have booked in the lab, make sure to change it and to inform people who might be affected (i.e. those who have booked time before or after you).

For any other questions, including stocking of the reagents, lab-ware, primers etc. please talk to your supervisor.
Henry Wellcome Genetics Labs Cleanrooms induction form

Name of the new user/guest: ___________________________ e-mail: ______________________

I confirm that I have read the Guidelines for working in the cleanroom facilities of the Henry Wellcome Genetics Labs, and agree to comply with them in order to conduct my work in the labs according to these guidelines. I am familiar with the risk assessment forms related to the protocols I will be using in the lab and I understand that health and safety is my responsibility. I understand that my supervisor and the Facilities Manager are available for advice and can prohibit access to the labs in case of any activity that not complying to these guidelines or good laboratory practice.

Signature: ___________________________

Date: ___________________________

Lab and Health and Safety induction completed by: ___________________________ Date: __________

As the PI supporting the work of the new user/guest in the cleanrooms I can confirm that there are sufficient funds to cover the costs related to the project they are undertaking.

PI name: ___________________________ Signature: ___________________________

Supervisor/host:

I, the undersigned, hereby acknowledge that I have introduced my guest to our laboratory practices and protocols to be used in the HW Genetics Labs Cleanrooms and that I will provide adequate level of supervision and I am responsible for her/his behaviour in the labs for the duration of her/his stay.

Name: ___________________________

Date: ___________________________

Signature: ___________________________