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| **Title of resource** |
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| GN_14: DNA Barcoding introduction to good laboratory practice guide |
| **Authored by** |
| When using this protocol, the following should be referenced:<br><br>Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3. |
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| **Description** |
| Basic introduction to good laboratory practice, including notes on personal protective equipment, laboratory equipment, reagents, samples and training. Protocol from the Gnatwork Bangladesh workshop, September 3-6th 2018. |
| **Intended use** |
| Scientific research use and training purposes. |
| **Restrictions on use** |
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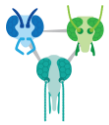
| **Resource history** |
| Updated version of: Harrup, L.E. (2014). The Pirbright Institute *Culicoides* DNA Barcoding Protocols, Version 2, available at www.ibvnet.com |



# GN_14: DNA Barcoding introduction to good laboratory practice guide

Citation: Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3

## A. Introduction

During DNA Barcoding studies cross-contamination between samples in a batch and between sample-batches can arise from several different sources including the repeated isolation of nucleic acids (DNA and RNA templates) and the handling of amplified PCR products (amplicons) in an area. With DNA templates typically more prone to contamination than RNA templates as they are more stable, hence contaminants are more persistent in the environment. Consistently observing a network of protocols focused on maintaining laboratory areas in a contamination-free condition is essential for the operation of a successful molecular biology laboratory and for the completion of a successful DNA Barcoding study. When arranged in a linear fashion the steps involved in a DNA barcoding studies can be separated into three major groups, the pre-PCR activities (sample preparation and PCR preparation), the post-PCR activities (PCR execution and agarose gel electrophoresis) and sequencing. The essential parts of a contamination control program should include space and time separation of pre-PCR, post-PCR and sequencing activities, use of physical aids to reduce and/or eliminate contamination of work areas, the use of aliquoted reagents and the incorporation of negative controls. Further specific suggestions for controls to reduce the risk of cross-contamination occurring are given below.

## B. Personnel Protective Equipment

Personnel protective equipment (PPE) should include 'Howie'-style laboratory-coats and single-use disposable nitrile (or equivalent) laboratory gloves, these items protect you from contaminating your samples and protect yourself from your samples or reagents being handled which may contain hazardous reagents. 'Howie'-style laboratory coats are part of an international recognised codified standard of protective wear. The coat style is named after J.W Howie who chaired the UK department of Health and Social Security committee whose 1978 report codified standards of clinical laboratory practice. The 'Howie'-style laboratory coat has buttons on the left flank, full length sleeves, elasticated wrists and a mandarin collar. It is designed to minimize pathogen/hazardous material contact with street clothes. Laboratory coats should be washed regularly, and not shared between pre-PCR, post-PCR and sequencing work areas and/or laboratories. Disposable plastic oversleeves worn over the laboratory coat sleeves can also provide additional protection from contaminating laboratory coat sleeves.

## C. Laboratory Equipment

Dedicated laboratory equipment, in particular pipettes, should be used for the pre-PCR, post-PCR and sequencing procedures and not shared between these areas or between laboratories. Pipettes should always be used with sterile disposable pipette tips with hydrophobic filters to prevent the carryover of aerosols created during pipetting and transfer of contaminants between reactions.

PCR reactions should be setup in a dedicated PCR laminar flow cabinet (or equivalent). Ideally two cabinets should be available such that mastermix preparation and template addition activities can be physically separated. Working areas including cabinets used for mastermix preparation and/or template addition and equipment in particular pipettes in PCR laboratories should be wiped with a DNA degradation solution e.g. DNAZap™ (ThermoFisher Scientific) or 10% hypochlorite (bleach) solution, before and after use. Where available, ultraviolet (UV) sterilisation of PCR laminar flow cabinets and equipment before and after use can further reduce the risk of cross-contamination. Ultraviolet radiation is effective for reducing contamination of PCR process for amplicons >300 bp. Manufacturer's instructions regarding the use of UV decontamination of laminar flow hoods should be followed to prevent exposure of staff and students to UV radiation.

Steam autoclaving of items at 121˚C for 20 minutes at 15 psi is generally sufficient to destroy DNA contamination and DNases from most items (RNases cannot be removed by autoclaving), and can be used to provide an additional level of routine protection from cross-contamination or in response to a spillage contaminating an item. However, times, temperatures and pressures of autoclave cycles can vary depending on the type and settings of the autoclave used. Ensure items are suitable and will not be damaged by autoclaving at the temperature setting of the autoclave to be used.

### D. Reagents and Samples

Sterile, nuclease (RNase and DNase) free water should be used for all pre-PCR, post-PCR and sequencing reactions. Reagents including PCR mastermixes, primers, nuclease free water should be aliquoted in appropriate volumes for the assay and to minimise the number of freeze-thaw cycles. Primers are particularly sensitive to contamination and aliquots of primers should never be open when tubes containing DNA extractions, PCR products etc. are open in the work area. New batches of reagents e.g. primers, Taq DNA polymerase etc. need to be assessed for performance against well-characterized positive control material.

### E. Samples

The steps of the PCR protocol should be streamlined to minimise manipulation of samples during DNA extraction and PCR setup, reducing the number of occurrences during which cross-contamination could occur. A negative or 'no template' amplification control, e.g. nuclease free water, should always be included in each reaction batch to check for potential contamination of the reagent mastermix and equipment used to prepare the PCR reactions. In addition a positive amplification control should be used for every batch of PCR reactions set-up. The positive control should normally be a DNA extract that amplifies consistently to a moderate level, use of a strong positive is an unnecessary risk due to high amplicon copy number it would contain.
The maintenance of an identifiable chain between voucher specimen, DNA extraction, PCR product and COI sequence is essential for accurate DNA Barcoding studies. All samples should be double labelled with their unique identifying code using either solvent-resistant cryo-labels or solvent-resistant cryopen. Accurate records of work undertaken should be made by user in laboratory notebooks on a daily basis and on all forms required for sample management. The Barcode of Life Data System (BOLD) hosts an extensive online workbench of data management and analysis pipelines for DNA Barcode data http://v4.boldsystems.org/. Including pipelines for free analysis and the hosting of specimen collection data together with matched morphological and molecular identification data, and pipelines for submission of data to GenBank and provision of DOI numbers for datasets facilitating the public open access release of datasets. Further information on guidelines and minimum quality standards for DNA Barcode data can be found at www.boldsystems.org/docs/dwg_data_standards-final.pdf. Further information on the International Barcode of Life Initiatives see the International Barcode of Life Project (iBOL) www.ibol.org.

### F. Training

Maintaining a clean and contamination-free laboratory is the responsibility of all users. All staff and students using the pre-PCR, post-PCR and sequencing areas should read, understand and receive appropriate training in all relevant protocols and local rules for using the laboratory areas and be aware of the issue raised above and the specific preventative measures and understand how they play an important role in the prevention of contamination.