**Dr. Monroy’s Lab Student/Employee Training Checklist**

All training documents are located at <http://www2.nau.edu/~fpm/>

**Student/Employee Name (& initials):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Supervisor Name (& initials):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**PURPOSE: To provide standardized training for all laboratory workers at Dr. F. Monroy’s Lab at Northern Arizona University.**

1. *Student/Employee and supervisors are both responsible for implementing this training checklist.*
2. *Students/Employee are* ***not allowed to do lab work*** *without approved training.*
3. *Retraining is recommended if a student has not used an instrument or work area for several months.*

**INSTRUCTIONS: Student/**Employees sign their initials on every line item. Supervisors sign their initials in boxes and DATE each main topic (or page).

**REQUIRED BASICS 1:** This training is required before lab personnel can enter the lab without supervision.

1. *Immediate supervisors are expected to conduct the majority of BASICS 1 training for a new student/employee.*
2. *Complete this training in the first week of employment.*

**REQUIRED BASICS 2:** This training is required before lab personnel can work in the lab **with direct supervision**.

1. *Immediate supervisors are expected to conduct the majority of BASICS 2 training for a new employee.*
2. *Complete this training by the end of the first month of employment.*

AUTOCLAVE (advance training required)

\_\_Keep door closed when not in use.

\_\_Media can be run anytime. (Waste only at end of workday)

\_\_If you remove another person’s media with agar, store it at 55°C. Inform the person where you placed the media. , store it at 55°C. Inform the person where you placed the media. If you don’t know who ran it, email the entire F. Monroy lab group.

\_\_Waste should only be run at the end of the workday. Upon successful sterilization (check autoclave tape!), place waste bags in large Biohazard bins. When Biohazard bins are full, drag them to the hallway storage location, and replace with an empty bin.

FREEZERS/REFRIDGERATORS

\_\_Close AND CHECK that freezer doors remain shut. Do not simply walk away from freezers.

\_\_Some freezers (-20C) need to be defrosted 2-3x per year.

\_\_Biohazard storage.

\_\_ Special conditions and labeling are required to store ethanol and isopropanol in freezers. Acetone & other flammables MAY NOT be stored in lab freezers.

\_\_Know the different styles of freezer racks and their location.

\_\_Organize samples using freezer boxes. Do not leave tubes unsecured in flip racks with no tops – this almost guarantees a spill. Clear tops for flip rack are available, but must be taped securely.

\_\_Label boxes & racks with CONTENTS/ PROJECT/INITIALS/ DATE, etc.

\_\_Use foil lids on 96-well and 384-well plates. DO NOT store plates loosely. They need extra protection, such as in a white fiberboard box or a stainless steel rack designed specifically for rack storage.

\_\_Use plastic freezer boxes in refrigerators to prevent mold growth

\_\_Temporary storage of DNA: store at +4°C for up to 30 days to avoid multiple freeze/thaw cycles).

\_\_Long-term storage of DNA: store at -20°C. Only save original DNA stocks (dilutions can usually be thrown out after data collection).

\_\_**Please purge items that are no longer needed!**

GENETICS and GENOMICS

\_\_DNA, RNA, and PROTEIN require special handling.

\_\_Samples are precious.

\_\_Prevent contamination across samples.

\_\_Protecting samples (gloves, aerosol-resistant tips).

\_\_Defined work stations for certain protocols.

GOOD LAB PRACTICES

\_\_See Sambrook & Russell Manual (3 volumes) for a wide variety of laboratory reagents & protocols. Return these manuals to their Computer Room shelf.

\_\_Form good work habits (precision, consistency, repeatability, do not attempt shortcuts).

\_\_Give yourself plenty of time for lab work. Focus. Most tasks require 2-4 hr blocks of time.

\_\_Use legible hand writing.

\_\_Include enough details on each tube or plate that another F. Monroy lab employee/student could discern contents and project if they found your samples lying around.

\_\_Organize your samples.

\_\_USE CONTROLS (what are appropriate repeatable CTLS for DNA, RNA, and Protein?)

\_\_Cleanup after you are done! Plan 5-10 minutes.

NOTEBOOKS

\_\_Keep up to date

\_\_Use ink

\_\_Write out exact procedures until they are routine.

\_\_Refer to pages that have a detailed version of common procedures.

\_\_More information is better.

\_\_Never obliterate mistakes, strike through with a single line.

\_\_Data generation, quality, & standards.

\_\_Signature & date at bottom of page: generally not required, but up to supervisor and PI. Required for work on forensics and patents.

PIPETTES & TIPS (**external checkout required**)

\_\_Rainin and Gilson pipettes, single-channel models

\_\_Pipette sets: pipettes are stored together in color-coded sets. DO NOT mix and match. Return pipettes to their home base when finished.

\_\_Know how to properly adjust the thumbwheel to change volume. Many models have a thumbwheel lock.

\_\_Check that ejector arm is at a proper position; push it back into position if it becomes loose. Double-check that is does not interfere with tip seal.

\_\_Pipettes are most accurate in their mid-range settings. Upper & lower limits are less accurate. DO NOT set a pipette outside of its limits!

\_\_Multichannel (12 vs 24)

\_\_Electronic

\_\_Single-use pipette tips (p10, p100, p200, p1000, aerosol-resistant, TRD vs LTS)

\_\_**DO NOT use excessive force to seat pipette tips. It leads to scarring, bending, and breakage of pipette barrels. Know how to properly twist-on tips if needed for a better seal.**

\_\_Match pipette tip boxes with appropriate tips. Reuse boxes, autoclave to sterilize. Recycle when they can no longer be used.

\_\_Proficiency training (check dye dilution series on NanoDrop).

\_\_Serological pipettes (1mL, 5mL, 10mL, 25mL, 50mL)

\_\_Proper use of GeneMate battery-powered serological pipette pumps. DO NOT pipet liquid into the nose cone. There is a filter in the nose cone and when it gets wet, the pipette will leak. If this happens change the filter, or get help from the Primary/Secondary person for pipettes.

REAGENTS

\_\_Everything needs a Hazard Diamond label! This includes any container, of any size, that has a reagent in it. Exceptions are DNA stocks in a nonhazardous solution (water or TE). Only certain abbreviations are allowed to assist fire & emergency personnel.

\_\_Certain reagents (ethanol, isopropanol, Qiagen reagents, and nonhazardous reagents) may be stored on benchtop racks, so long as the rack has a Hazard Diamond. If stored in this way, individual containers do not need a Hazard Diamond. However, only the individual reagent listed on the rack label may be stored in a rack.

\_\_Use ice or StrataCoolers (-20°C vs. +4°C) for working with temperature-sensitive reagents on the benchtop.

\_\_Know locations of common lab stocks. Find recipes in the Sambrook & Russell manual.

\_\_Handling: DO NOT pipette reagents directly from stock bottles. Make disposable working stocks into smaller tubes first.

\_\_Storage of personal lab stocks in lab drawers is okay, but all items need a Hazard Diamond label

**ADVANCED TRAINING:** This training **is required** before students/employees are allowed to perform a procedure or use an instrument without supervision.

1. *ALL employees and supervisors are encouraged to seek assistance from experienced lab members for advanced training.*
2. *This list is flexible. Trainings are only required for instruments and work areas that an individual employee will use.*
3. *Note that certain items are in the category“****external checkout required”****. Training & certification must be provided by the supervisor for that instrument or work area.*

AGAROSE GEL ELECTROPHORESIS

\_\_Safety glasses required

\_\_Electrophoresis theory. Know appropriate agarose density for different applications (0.7% for genomic DNA, 1.5-2.0% for PCR, etc.).

\_\_UV Hazards. GelDoc UV light tray and benchtop UV light.

\_\_Hazards associated with DNA intercalating dyes. Use of SybrSAFE.

\_\_Buffers: 50xTAE vs. 10xSB

\_\_DI water only: DO NOT need E-Pure water!

\_\_LABEL everything directly on gel rig: INITIALS, DATE buffer was made, & NUMBER of uses (buffer is good for about 3 uses or 3 days)

\_\_Learn how to properly use power supplies. Appropriate settings for an agarose gel depend on gel size and % agarose.

\_\_TURN OFF power source and unplug leads BEFORE removing gel.

\_\_GelDoc camera use & saving photos.

\_\_Cleanup after taking photos: remove your gel from the UV light, cover the tank you used, straighten tank area. Double-check that the power source is off.

\_\_Gel disposal in blue bin containing non-RCRA waste.

\_\_E-gel: protocol to remove target amplicon from gel and purify.

\_\_Specialized agarose types: NuSieve, Metaphor, Low Melting Point agarose.

AUTOCLAVE (**brief specialized training required**)

\_\_Learn how to properly set up a waste run and a liquid media run.

\_\_Typical settings: Waste =\_\_\_\_\_\_\_min. @ \_\_\_\_\_\_C @ \_\_\_\_\_\_\_psi

Liq Media=\_\_\_\_\_\_\_min. @ \_\_\_\_\_\_C @ \_\_\_\_\_\_\_psi

\_\_Keep door closed when not in use.

\_\_Media can be run anytime. (Waste only at end of workday)

\_\_If you remove another person’s media with agar, store it at 55°C. Inform the person where you placed the media. , store it at 55°C. Inform the person where you placed the media. If you don’t know who ran it, email the entire MGGen lab group.

\_\_Waste should only be run at the end of the workday. Upon successful sterilization (check autoclave tape!), place waste bags in large Biohazard bins. When Biohazard bins are full, drag them to the hallway storage location, and replace with an empty bin.

BALANCES

\_\_Adjust balancing bubble to its center point before EVERY use. (Turn OFF during this step)

\_\_Clean after EVERY use with brush.

\_\_Clean balance and benchtop after EVERY use.

\_\_Care & maintenance of analytical balances. Treat them with care.

\_\_Proper technique for moving/sliding.

BSL2 pathogens work area (**specialized training & external checkout required**)

\_\_See BSL2 supervisor for specialized training

CENTRIFUGES

\_\_Rotor types (fixed angle vs swinging bucket).

\_\_Learn appropriate settings for refrigerated protocols. Return to RT after EVERY use. Prolonged chilling is hard on these machines.

\_\_Always balance rotors. Use balance tubes/plates if needed.

\_\_Always use rcf (relative centrifugal force) to set speed (rcf=g force). Rpm is not a setting that translates across different rotors or protocols.

\_\_Learn care & maintenance. (Keep central spindle clean. Dust out back panel & motor fan every 6 months.)

\_\_ Use hinge lubricant ONLY for swinging buckets. Do NOT use lubricant on central spindle!!! Heat turns it into an adhesive!

\_\_ If samples leak out of tubes during a spin, immediately wipe up any liquid in the rotor with a paper towel or Kimwipe. After any Qiagen extraction, rinse thoroughly with tap water and dry rotor upside down on paper towels.

Cell Culture ROOM

\_\_NO exposed bacteria/contamination

\_\_Proper entry & exit: ENTER only through hallway door. Don disposable lab coat & gloves. To EXIT, remove disposable lab coat, and dispose of gloves. Wash/rinse hands thoroughly.

\_\_Clean before and after use. Tear a large Kimwipe in half. Apply a moderate amount of 70% ethanol to Kimwipe and clean the hood surfaces you will use. Be sure to apply UV light before use and after.

\_\_Organize fiberboard boxes in freezer & fridges. Purge items no longer needed.

\_\_ Maintain materials and clean up protocols

\_\_Turn off all microscopes and equipment used before exiting

\_\_ Check Lab assignment schedule for Cell Culture room.

DNA

\_\_Proper handling techniques.

\_\_Appropriate temperatures for handling & storage.

\_\_Dilutions: what is generally appropriate for prokaryotic genomes (1ng/uL) vs eukaryotic genomes (20-50 ng/uL).

\_\_96-well DNA array plate and 96-well maps.

\_\_Diversity panel (samples from diverse geographic sources).

\_\_CONTROLS:

\_\_NTC (No Template Control, same as negative control. Water is used instead of template).

\_\_RBC (Reagent Blank Control)

\_\_POS CTL (Known Positive Control)

\_\_Cross-species Control

\_\_Synthetic POS (PCR-generated POS CTL)

ELECTROPORATOR

\_\_Cloning theory, electro competent cells, vector insert ratio.

\_\_Proper settings & usage

\_\_Cleaning & maintenance

ENZYMES

\_\_Labile enzymes (T4 Ligase, restriction enzymes, etc.).

\_\_Proper handling & storage of enzymes. Avoid freeze-thaw cycles. Use on ice or +4°C StrataCooler.

\_\_Expiration dates (vendor recommendations vs. actual expiration).

\_\_DNA Polymerases: antibody *Taq* (Plat *Taq*) vs. normal *Taq*.

FUME HOODS (***external checkout required***)

\_\_Understand proper PPE

\_\_Minimize clutter to maintain air flow.

\_\_Hazardous chemicals in use.

\_\_Storage in cabinets under hoods.

\_\_Mixed waste bottles for temporary storage.

\_\_Clean up after yourself. For multi-day procedures, write a reminder to yourself to clean up any solid or liquid waste.

HAZARDOUS WASTE DISPOSAL (***external checkout required***)

\_\_Schedule waste pickups with NAU-EHS using online reservation (<http://nau.edu/research/compliance/environmental-health-and-safety/>). Contact person is Matt Freyer.

\_\_Waste must be placed in a leak-proof container, such as a bottle with a tight-fitting lid for liquids, or double-bagged Ziplocs for solid waste.

\_\_Fill out waste tag and affix. Waste tags are stored by West hood.

\_\_Hazardous waste categories (flammables, oxidizers, corrosives, toxins).

LIQUID N2

\_\_Cryosafety and handling. Wear cryogloves for protection.

\_\_Appropriate use of pressurized 200L tank, mid-sized floor dewars on wheels, and small 5L dewars.

\_\_Do not ride with the elevator when exchanging the 200L tank, in case it vents a large amount of N2 gas. Send the tank on the elevator and use the stairs.

\_\_Communicate with other users before placing an order. Email to get an estimate of projected usage.

\_\_Praxair ships to NAU on Wednesdays. Order by Tuesday afternoon.

MICROBIOLOGY BENCH

\_\_Safety glasses required

\_\_Aseptic technique

\_\_Media (proper preparation, dispensing, and storage)

\_\_Incubators

\_\_Shaker-incubator

\_\_Reserving workspace and communication

\_\_Cleaning

MICROSCOPES

\_\_Appropriate handling

\_\_Storage

\_\_Cleaning

NANODROP

\_\_Spectrophotometer basics and DNA quantification theory. Different methods include NanoDrop, Picogreen, Agarose, and Agilent bioanalyzer.

\_\_Software

\_\_ **Separate access via EnGGen (see supervisor)**

\_\_Cleaning

\_\_Buffers storage & replacement

\_\_Accuracy, repeated reads

\_\_Results

\_\_Logoff computer

\_\_Cleaning work station

PCR

\_\_Theory

\_\_Applications

\_\_Quantitative PCR (qPCR)

\_\_Reagents

\_\_Cost

\_\_Temporary storage on top shelf of 4°C.

\_\_Long-term storage in -20°C freezers.

PCR HOODS

\_\_Scheduling & communication

\_\_Clean before and after use. Tear a large Kimwipe in half. Apply a moderate amount of DNA Away to Kimwipe and clean the inside surfaces of the PCR hood. Follow with 70% ethanol using the other ½ Kimwipe.

\_\_Learn when to change gloves.

\_\_Requirement for clean pipettes.

\_\_Setting up master mixes.

\_\_96 vs. 384 well plates.

\_\_plates: skirted ($3) vs. unskirted ($2).

\_\_Adhesive lids: Type B, green, foil. Learn how to properly affix Type B lids onto PCR plates to prevent evaporation! Also learn proper technique for affixing foil lids to avoid evaporation during frozen storage.

\_\_Straighten up after work. Clean with DNA Away again, followed by 70% ethanol.

\_\_Reorder supplies. Write down reminders.

PCR MACHINES

\_\_Proper care & use.

\_\_Entering/editing programs

\_\_Special features: ramp times, gradient PCR, and gradient calculator

\_\_Cycling conditions.

\_\_Chill step (max of 24hrs @16C).

\_\_Reserve with a piece of tape stating date, time, and initials

POST-PCR BENCH

\_\_Preparation (70% ethanol only)

\_\_Handling

\_\_Dilution

\_\_Allowable pipettes

\_\_Ethanol precipitation

pH METER

\_\_Safety glasses required

\_\_Theory

\_\_Probe care

\_\_Calibrate before use

\_\_Storage in KCl buffer

\_\_Clean after EVERY use

PRIMERS

\_\_Software

\_\_Designing a good primer

\_\_Labels & modifications

\_\_Cost

\_\_Correct ordering format

\_\_Resuspend in Clean environment

\_\_100uM stock vs. working stock

QIAGEN KITS

\_\_Theory (silica binding matrix, pH & salt dependent, DNA solubility)

\_\_Handling (DO NOT pipette reagents from stock bottles)

\_\_Vacuum manifolds

\_\_Waste disposal into ethanol/Qiagen waste

\_\_Use older reagents first

\_\_Label reagent bottles with DATE/INITIALS

\_\_Order new kits before they run out!

\_\_Cleaning

SHARPS SAFETY

\_\_**NAU Needle stick Safety & Prevention**

\_\_Minimize use of needles & razor blades; use safer alternatives when possible

\_\_Storage in original containers or hard-sided box

\_\_Proper handling – DO NOT re-cap needles! DO NOT re-use Sharps

\_\_Discard needles, scalpels, and razor blades in red plastic Sharps containers

\_\_

SPECTROPHOTOMETERS

\_\_Theory

\_\_Software

\_\_Proper handling

\_\_Calibration

\_\_Cleaning

Flow Cytometer (***external checkout required***)

\_\_Training process:

\_\_Hands-on training with authorized user

\_\_External checkout by authorized user

\_\_Reserve using with sign/email

\_\_Theory of machine: Lasers, fluorescent dyes

\_\_Software and run parameters for variable samples

\_\_Know how to set up and activate the machine.

\_\_Check that a clean SIP/work station is available BEFORE you start to prepare samples for loading.

\_\_Know how to properly save data

\_\_Know how to check for errors.

\_\_Sample hazard. Dispose plates/samples in proper waste bin

\_\_After any run: run rinse/cleaning cycle tank to clean.

OTHER

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