Lab Reproducibility

Holly K. Dressman, PhD
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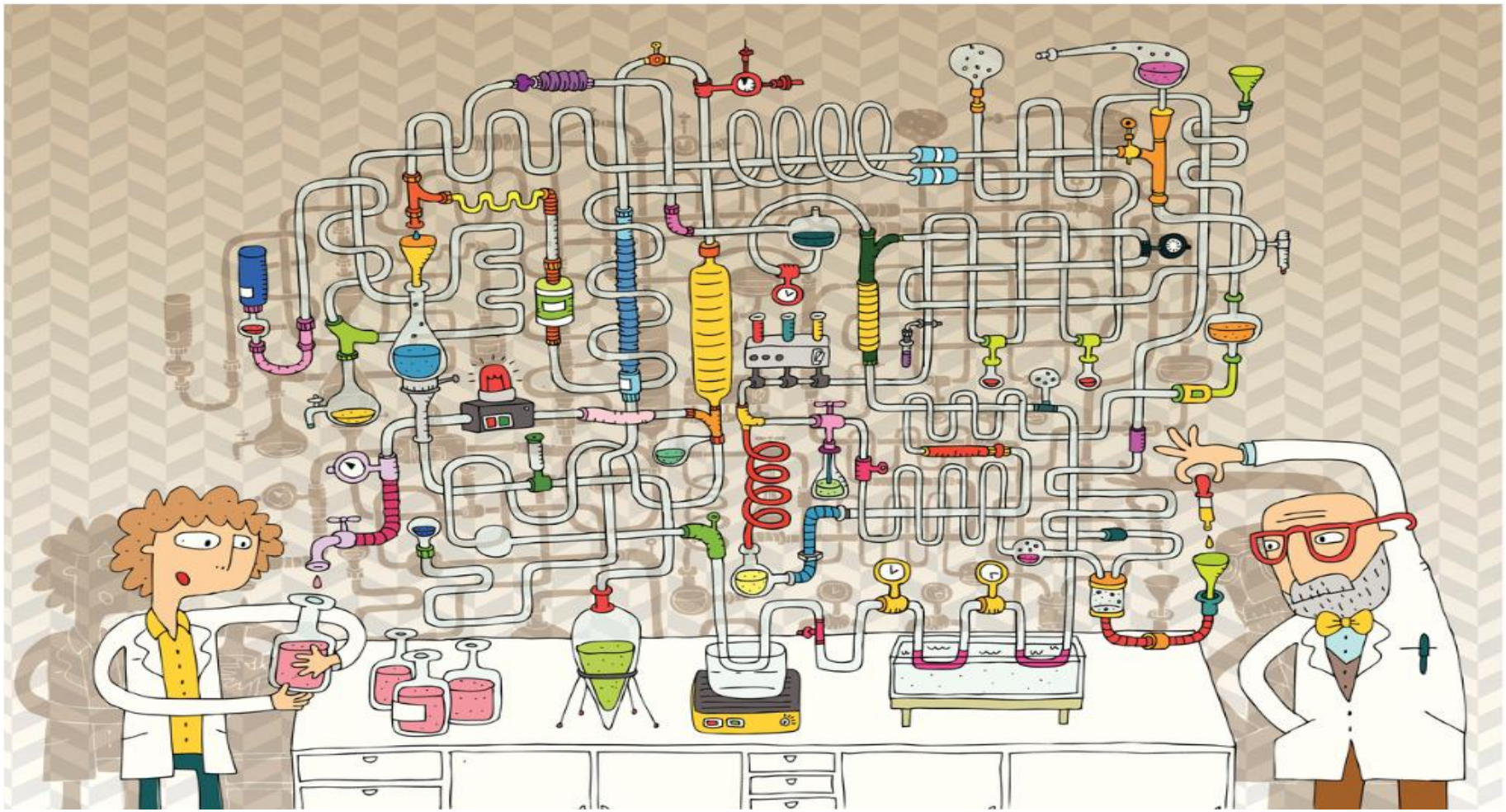
"Availability of the data underlying a published study is probably the most significant way in which journals can, now, ensure reproducibility of the published literature." PLOS ONE blog

Being able to review the data does indeed allow one to see if a researcher's analysis and conclusions drawn are accurate for that dataset. HOWEVER, it does little to validate the quality and accuracy of the dataset itself.



Sackler Colloquium on Improving the Reproducibility of Scientific Research
13 papers presented in PNAS March 2018 (11) edition

Issues with data and analyses: Errors, underlying themes, and potential solutions

Andrew W. Brown[a,1], Kathryn A. Kaiser[a,2], and David B. Allison[a,3,4]

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Training replicable predictors in multiple studies

Prasad Patil[a,b] and Giovanni Parmigiani[a,b,1]

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Scientific progress despite irreproducibility: A seeming paradox

Richard M. Shiffrin[a,1], Katy Börner[b], and Stephen M. Stigler[c]

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An empirical analysis of journal policy effectiveness for computational reproducibility

Victoria Stodden[a,1], Jennifer Seiler[b], and Zhaokun Ma[b]

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Standards for design and measurement would make clinical research reproducible and usable

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The American Journal of Pathology, Vol. 185, No. 1, January 2015

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

EDITORIAL

Science Isn't Science If It Isn't Reproducible

Kevin A. Roth* and Audra E. Cox†

From the Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama (Editor-in-Chief); and the American Society for Investigative Pathology,† Bethesda, Maryland (Managing Editor)*

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Home » Research & Training » Rigor and Reproducibility

RIGOR AND REPRODUCIBILITY

Rigor and Reproducibility

- Reporting Guidelines
- Application Instructions
- Training
- Funding Opportunities
- Meetings and Workshops
- Announcements
- Publications
- Resources

Principles and Guidelines for Reporting Preclinical Research

NIH held a joint workshop in June 2014 with the Nature Publishing Group and Science on the issue of reproducibility and rigor of research findings, with journal editors representing over 30 basic/preclinical science journals in which NIH-funded investigators have most often published. The workshop focused on identifying the common opportunities in the scientific publishing arena to enhance rigor and further support research that is reproducible, robust, and transparent.

The journal editors came to consensus on a set of principles to facilitate these goals, which a considerable number of journals have agreed to endorse. These principles are shown below.

Open all | Close all

- Rigorous statistical analysis
- Transparency in reporting
- Data and material sharing
- Consideration of refutations
- Consider establishing best practice guidelines for:
- Endorsements — Principles and Guidelines for Reporting Preclinical Research
- Adapted Guidelines

This page last reviewed on December 12, 2017

Related Links

Rigor and Reproducibility FAQs

Study Design

- Best practices are enforced
- NIH and lending foundations require courses at all levels that provide principles and guidelines on scientific reproducibility

Biological Reagents and Reference Materials

- Vendors offer only validated reagents and broad utilization by PIs
- Research funder policies require documented use of validated and non-contaminated reagents
- Procedures to document reagent validation and lack of contamination required by publishers.
- Incentivize the continued development of tools for reagent validation
- Define standard operating procedures for biological materials handling throughout the materials lifecycle.

PLOS | BIOLOGY

PLoS Biol. 2015 Jun 9;13(6):e1002165

PERSPECTIVE

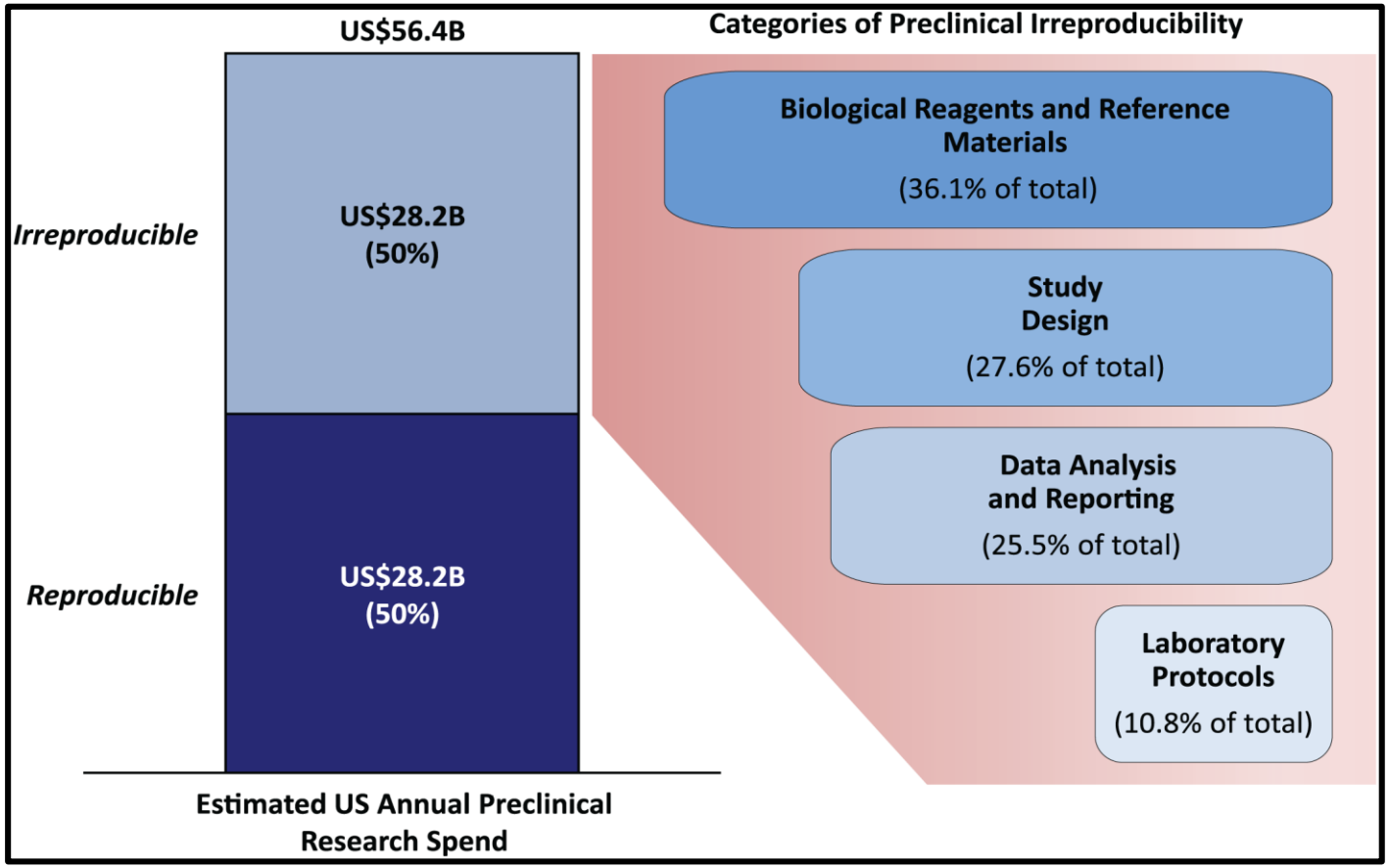
The Economics of Reproducibility in Preclinical Research

Leonard P. Freedman[1]*, Iain M. Cockburn[2], Timothy S. Simcoe[2,3]

1 Global Biological Standards Institute, Washington, D.C., United States of America, 2 Boston University School of Management, Boston, Massachusetts, United States of America, 3 Council of Economic Advisers, Washington, D.C., United States of America

* lfreedman@gbsi.org

"replication and cumulative knowledge production are cornerstones of the scientific process"



NATURE | NEWS FEATURE

1,500 scientists lift the lid on reproducibility

Survey sheds light on the 'crisis' rocking research.

Monya Baker

25 May 2016 Corrected: 28 July 2016

- The survey e-mailed to *Nature* readers and advertised on affiliated websites and social-media outlets for assessing research reproducibility.
- About 80% of respondents thought that funders and publishers should do more to improve reproducibility, suggesting that people would welcome required guidelines.

Factors that contribute to irreproducible research

- Selective reporting
- Pressure to publish
- Low statistical power or poor analysis
- Not replicated enough in original lab
- Insufficient oversight/mentoring
- Methods and analysis code unavailable
- Poor experimental design
- Raw data not available from original lab
- Fraud
- Insufficient peer review
- Problems with reproduction efforts
- Variability of standard reagents

Factors that could boost reproducibility

- Better understanding of statistics
- Better mentoring/supervision
- More robust experimental design
- Better teaching
- More within-lab validation
- Incentives for better practice
- Incentives for formal reproduction
- More external-lab validation
- More time for mentoring
- Journals enforcing standards
- More time checking notebooks

There are at least six things in this picture that a quality-assurance manager would try to improve. Can you spot them?

QUALITY TIME

IT MAY NOT BE SEXY, BUT QUALITY ASSURANCE IS BECOMING A CRUCIAL PART OF LAB LIFE.

BY MONYA BAKER

Rebecca Davies remembers a time when quality assurance terrified her. In 2007, she had been asked to lead accreditation efforts at the University of Minnesota's Veterinary Diagnostic Laboratory in Saint Paul. The lab needed to ensure that the tens of thousands of tests it conducts to monitor disease in pets, poultry, livestock and wildlife were watertight. "It was a huge task. I felt sick to my stomach," recalls Davies, an endocrinologist at the university's College of Veterinary Medicine.

She nevertheless accepted the challenge, and soon found herself hooked on finding — and fixing — problems in the research process. She and her team tracked recurring tissue-contamination issues to how containers were being filled and stored; they traced an assay's erratic performance to whether technicians let an enzyme warm to room temperature; and they established systems to eliminate spotty data collection, malfunctioning equipment and neglected controls. Her efforts were crucial to keeping the diagnostic lab in business, but they also forced her to realize how much researchers' work could improve. "That is the beauty of quality assurance," Davies says. "That is what we were missing out on as scientists."

456 | NATURE | VOL 529 | 28 JANUARY 2016

Nature 529:456-458, 2016

Quality Assurance (QA) systems

Strengthens research and improves reproducibility

- QA is ad hoc on most academic research
- Scribbling data on paper towels
- Repeating experiments without running controls
- Lab notebooks are not rigorously monitored, need auditing
- Hassle of upfront adopting of QA system
- NIH does not require it at this time for research projects

Common issues in research laboratories

DISORGANIZED SAMPLE STORAGE
Clear labelling and proper organization are important for incubators and freezers. Everyone in the lab should be able to identify a sample, where it came from, who did what to it, how old it is and how it should be stored.

INADEQUATE DATA LOGGING
Data should be logged in a lab notebook, not scribbled onto memo paper or other detritus and carelessly transcribed. Notebooks should be bound or digital; loose paper can too easily be lost or removed.

VARIABLE EXPERIMENTS
Protocols should be followed to the letter or deviations documented. If reagents need to be kept on ice while in use, each lab member must comply.

UNSECURED DATA ANALYSIS
Each lab member should have their own password for accessing and working with data, to make it clear who works on what, when. Some popular spreadsheet programs can be locked down so that manipulating data, even accidentally, is difficult.

MISSED MAINTENANCE
Instruments should be calibrated and maintained according to a regular, documented schedule.

OLD AND UNDATED REAGENTS
These can affect experimental results. Scientists should specify criteria for age and storage of all important reagents.

Standard Operating Procedures (SOPs) provide documentation to trace provenance

QA systems in industry

- Good Laboratory Practice (GLP)
- Good Clinical Practice (GCP)
- Good Manufacturing Practice (GMP)

Hospital and Industry QA system

- Clinical Laboratory Improvement Admendments (CLIA)

Nature 529:456-458, 2016

What is GLP?

Good Laboratory Practices

GLP requires a Quality Assurance System

- Quality System
  A structured and documented management system for ensuring quality.
- Quality Assurance
  All the planned and systematic actions that are established to ensure that the experiment is performed and data generated, documented and reported in compliance with applicable regulatory requirements.
- Quality Control
  The operational techniques and day-to-day activities undertaken within the QA system to verify that the requirements for quality of the activities have been fulfilled.

Why GLP guidelines in a research lab?

Quality = Conformance to requirements. Characteristics of a product that bear on its ability to satisfy stated or implied needs. (*i.e.,* works the way you said it would, was performed the way you said you would)

- Check box on SOP/runsheet, initial and date
- Create paper trail of connected records and logs

Integrity = State of being whole, undiminished, unadulterated. (*i.e.,* no changes in electronic records, no degradation in sample, failure in equipment or contamination in reagents)

- Record time in/out of temperature storage
- Verify reagents are good and equipment is working
- Track reagents and equipment used

Verification

- Verify accuracy of sample analysis
- Verify that the system performs according to analysts expectations
- Proof that analytical procedure does what it proposes to do
- Proof suitability of the instrument for intended use

When do you need GLP?

Drug Development Process and Medical Device Development Process

- Good Laboratory Practice (GLP) – human and other species samples
- Good Clinical Practice (GCP) – human subjects
- Good Manufacturing Practice (GMP) – purity of drug product
- Quality System Regulation (QSR) – device product meets requirements

Processes in Quality System

Quality System
• Roles and responsibilities • Organization Chart • Map GLP needs to specific SOPs • Quality Assurance, management meetings, audits

Standard Operating Procedures (SOPs) = Describe routine procedures

Document Control	Operations	Equipment	Methods	Reagents	Personnel & Training	Informatics
• Approve and change SOPs • Numbering system • Restrict access to paper originals • Record retention • *Protocols & Final Reports*	• Facilities with separate space • Safety • Power Outage • Emergency	• Installation • Calibration • Maintenance • Cleaning • Operation • Repair	• Specimen receipt and storage • Method 1 • Method 2	• Reagent receipt and storage • Restricted access • Quarantine and certification	• Document education and experience • Method and forms to document lab training • Method to prove competency	• LIMS and sample tracking • Restricted access to electronic raw data and logs • Change control • Analysis pipelines • Backup and retrieval from archives

Equipment Categories

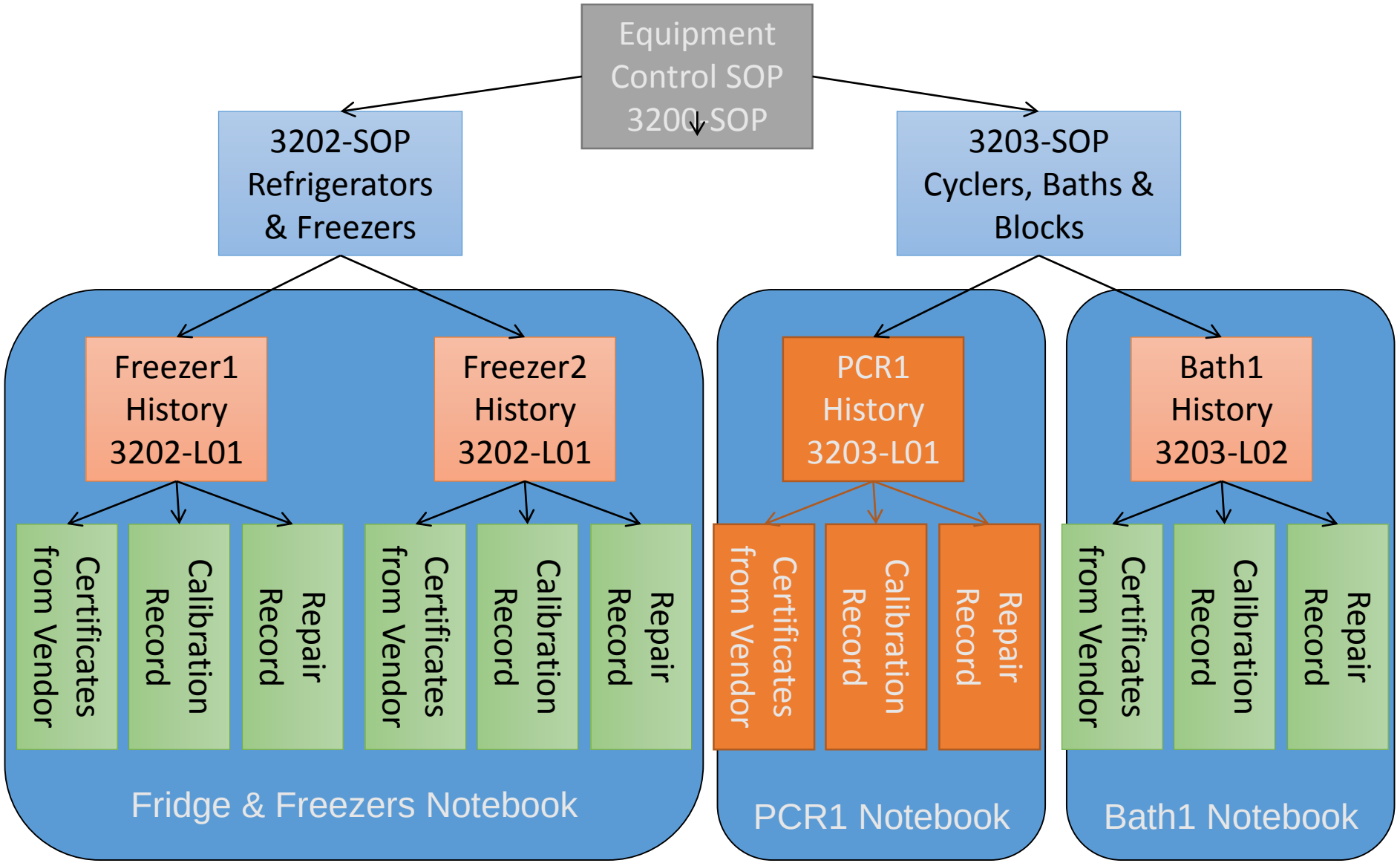
Group A Exempt	Group B Standard	Group C Complex
No measurements	Measurements	Measurements
Low risk to results	Controls physical parameters (temp)	Includes computerized analysis
No Equip SOP	Group Equip SOP	Individual Equip SOP
	Qualification, Calibration & Maintenance	Qualification, Calibration & Maintenance
Speed-vac Vortex microfuge	Balance Thermal cycler	Sequencer, Bioanalyzer, Promega GlowMax, Nanostring nCounter

Equipment Control SOP is a process which:

1) enables detailed tracking of all equipment used in a test system; and
2) documentation of routine and non-routine activities that assure the performance of each piece of equipment prior to its use

- Equipment used in the generation of data shall be of **appropriate design and adequate capacity** to function according to the protocol.
- Equipment shall be adequately **inspected, cleaned, and maintained.** Equipment used for the generation, measurement, or assessment of data shall be adequately **tested, calibrated and/or standardized**.
- The **written SOPs** shall set forth in sufficient detail the **methods, materials, and schedules** to be used and **designate the person** responsible for the performance of each operation.
- **Written records** shall be maintained…These records, containing the date of the operation, shall describe whether the maintenance operations were **routine** and followed the written SOPs. Written records shall be kept of **non-routine repairs.** Such records shall document the nature of the defect, how and when the defect was discovered, and any remedial action taken in response to the defect.

Many SOPs, Records & Notebooks



Chip Priming Station History

Equipment ID_____

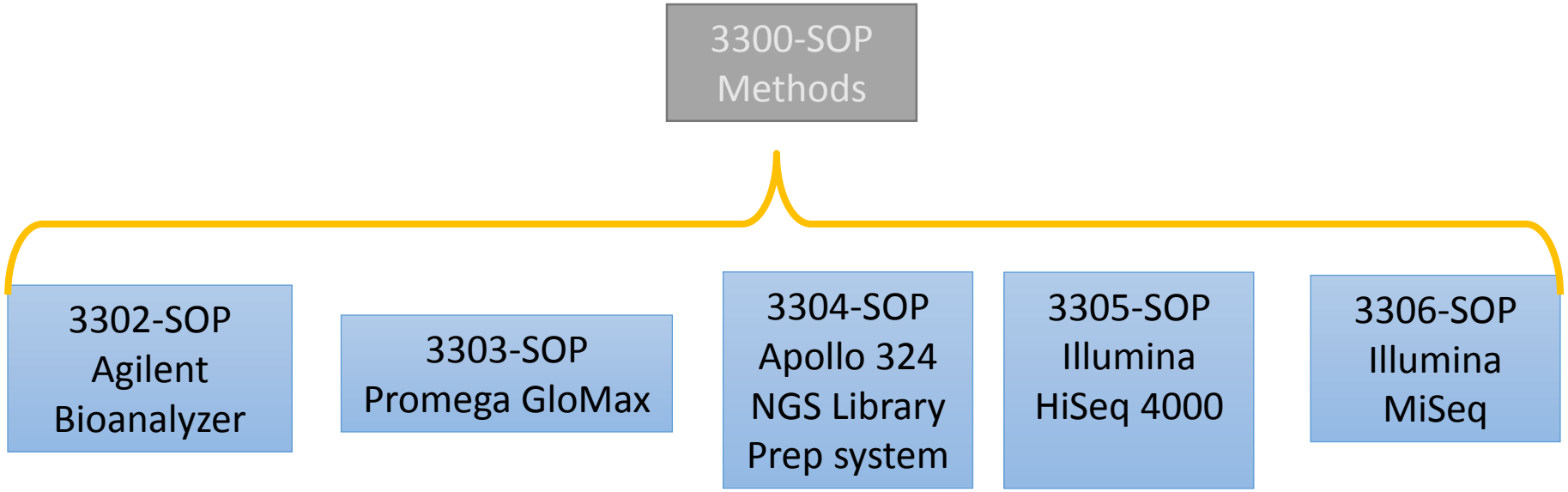
Date	Initials	Replace Syringe	Replace Gasket	Replace Adapter	Test Seal	Other Procedures or comments

Agilent 2100 Bioanalyzer History

Equipment ID_____

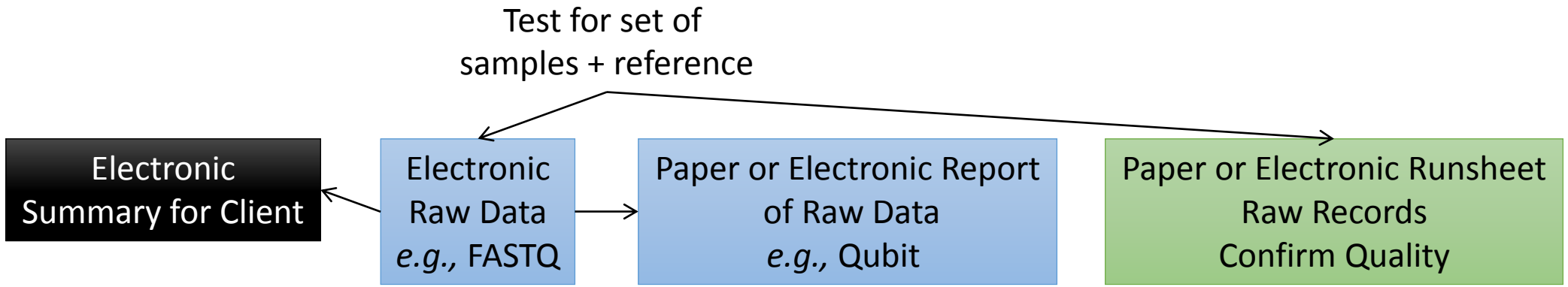
Date	Initials	Clean Lens	Clean Pin Set	Run Diagnostics	Other Procedures or Comments

Method SOPs


3300-SOP
Methods
3302-SOP
Agilent
Bioanalyzer
3303-SOP
Promega GloMax
3304-SOP
Apollo 324
NGS Library
Prep system
3305-SOP
Illumina
HiSeq 4000
3306-SOP
Illumina
MiSeq

Methods SOPs and Runsheet

- List master mix and sample volumes
- Track reagents and equipment used
- Check boxes to confirm steps performed
- Include QC review and reference sample
- Record observations and deviations



Qiagen MagAttract PowerSoil DNA Extraction (96-Well)

WORK INSTRUCTION 05-22-2017	WI-005-B Revision: 3.2	Page 1 of 6

1.0 PURPOSE

1.1 The purpose of this document is to provide instructions for extracting DNA from microbiome samples, in a high-throughput 96-well format.

2.0 SCOPE

2.1 All persons who perform molecular work in the Sequencing and Genomic Technologies Lab. All personnel will have the required Duke IRB training.

3.0 ASSOCIATED DOCUMENTS

3.1 WI-005-T01 Microbiome-DNA-sampleProcessingTracking-template to document all sample procedures and store in project ID folder on server or/and Duke dropbox.
3.2 WI-S01 DNA Quantification – Qubit
3.3 WI-S01-T01 GloMax_QubitConcTemplate

4.0 REQUIRED REAGENTS, EQUIPMENT, AND CONSUMABLES

4.1 Qiagen MagAttract PowerSoil DNA EP Kit (384) (27100-4-EP)
4.2 Multi-channel Pipettor(s) (volumes required 10 µl – 1000 µl)
4.3 WeLLevator™ Pipette Guide (MoBio, 11970)
4.4 Life Technologies DynaMag 96 side plate Magnet (12331D)
4.5 Plate Shaker (MoBio, 11996; Retsch MM400)
4.6 Plate Shaker Adapter Plates (MO BIO, 11990; Retsch MM400)
4.7 Barnstead/Lab-Line Titer plate shaker
4.8 Benchmark Benchmixer/vortexer
4.9 Centrifuge with speed of 4000g and Plate Adapters
4.10 Weigh boats
4.11 Kimwipes
4.12 Liquid transfer troughs (sterile reservoirs)
4.13 Needle nose tweezers and/or disposable tweezers or Q-tips
4.14 Pipette tips (filtered for pipettors and non filter for tweezers)
4.15 Regular 96-well sample plate
4.16 GloMax Microplate Reader

5.0 SAFETY REQUIREMENTS

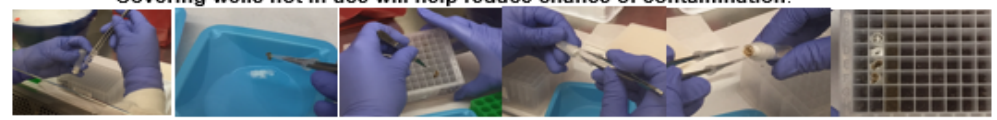
5.1 Handle any potentially infectious materials at the appropriate biosafety levels.
5.2 Gloves are required, safety glasses and lab coat highly recommended.

6.0 INSTRUCTIONS

6.1 Procedure follows. Print WI-005-T01 and check steps and fill in as appropriate during the procedure.
6.2 Adapted from notes from MoBio Tech Support & the PowerMag Soil DNA Isolation Kit intructions (Version: 09182014) and .
6.3 ⓘ indicates time saving tip. **NOTE: If using repeater – tip must not have contact with samples to prevent cross-contamination.**
6.4 **NOTE:** This protocol has been modified to fit centrifuges with only 3000xg capabilities, if your centrifuge can reach 4500xg, refer to original MoBio protocol for times.

- **Qiagen MagAttractMoBio PowerSoil DNA Isolation Kit (27100-4-EP) – Kit Lot #**_____
- Ice Bucket with Ice
- Water Bath at 70C (Turn on ***prior*** to starting ☐)
- 50 ml conicals
- 250 ml sterile media bottle

1. ☐ Use sample tracking Excel template file generate Sample Matrix (Sample Barcode/ID, Tube #, etc.).
2. ☐ Retrieve samples and place on ice..
3. ☐ For solid sample, add **400 µl** of the RNase A Solution (stored at RT) to **75 ml** of the PowerBead Solution for every 96 well plate you plan to process. (or can use 50ml tube, 37.5ml + 200ul RNase A, x2 tubes)
4. ☐ For 200ul liquid sample, add **400 µl** of the RNase A Solution (stored at RT) to **55 ml** of the PowerBead Solution for every 96 well plate you plan to process. . (or can use 50ml tube, 27.5ml + 200ul RNase A, x2 tubes)
5. ☐ Carefully peel off the **Square Well Mat** that covers the PowerBead DNA Plate and set aside.
6. ☐ For solid samples, add **750 µl** of PowerBead Solution / RNase A Solution to each well of the PowerBead Plate.
7. ☐ For liquid samples, add **550 µl** of PowerBead Solution / RNase A Solution to each well of the PowerBead DNA Plate.
8. ☐ Samples may be swabs, watery, or solid stool. For watery stool, vortex, then take **200 µl**. For solid samples**, add 0.1-0.2 grams** (this may vary due to PCR inhibitors in fecal samples). May use pipet tips or Q-tips to transfer from clients collection tube to plate, **BE CAREFUL!! Covering wells not in use will help reduce chance of contamination**.



9. ☐ Add **60ul** of **Solution SL** to each well. Seal plate tightly with the mat. Briefly using plate shaker, shake the plate (10-20 seconds at speed 6-8).
Note: *Check the **Solution SL** (**lysis solution** before using. If the SL solution has precipitated, heat the solution at 60℃ until the precipitate has dissolved. Mix gently. (**Note**: **SL solution** contains SDS. If it gets cold, it will precipitate. Heating at 60℃ will dissolve the SDS. SL solution can be used while it is still warm)*

10. ☐ **Weigh plate** prior to incubation, write down weight (g).

and place the **Collection Plate 2ml** on plate shaker, **setting 10**. Mix for **5 minutes**.

35. ☐ ☐ (C) **Remove** the **Collection Plate 2ml** from the plate shaker and place the DWP on the WeLLevator™ equipped with the PowerMag Magnetic Separator.
36. ☐ ☐ (D) Keep the **Collection Plate 2ml** in place for **5 minutes** to allow for complete collection of the **ClearMag® Zorb Reagent** (beads) on the bottom of each well.
37. ☐ ☐ (E) Keeping the **Collection Plate 2ml** on the magnet, **remove** the **Collection Plate 2ml** disposable plate seal. Load your multichannel pipettor ith tips and aspirate **500 µl** of liquid from each sample well. (You may want to set your pipettor to 555 µL in case there is residual fluid volume from previous steps.)
38. ☐ **Repeat Steps (A) through (E).**
39. ☐ **Remove** the **Collection Plate 2ml** from the plate magnet and add **250 µl** of **ClearMag® Wash Solution** to each well containing sample. Mix the sample by repeated pipetting (5 – 10 times) to completely resuspend the **ClearMag® Zorb Reagent** (beads) and transfer the complete volume of beads to a **96 well microplate**.
40. ☐ Place the **96 well microplate** on the **magnet plate** and keep the **96 well microplate** in place for **3 minutes** to allow for complete concentration of the ClearMag® Zorb Reagent (beads) on the bottom of each well.
    Note: *You do not need to use the WeLLevator for this step, just place the* ***magnet plate*** *on the benchtop.*
41. ☐ Keeping the **96 well microplate** on the magnet, **aspirate** all of the liquid from each sample well. Note were the magent is pulling the beads and aspirate fluid away from the beads, do not touch the beads with pipet tip.
42. ☐ When the **ClearMag® Zorb Reagent (beads)** are visibly **dry (2-3 mins), remove** the **96 well microplate** from the magnet, resuspending the beads in each well with **100 µl** of **ClearMag® Elution Buffer (EB).** Seal the **96 well microplate** with a disposable plate seal and place it on the plate shaker. Mix (**speed 8-10) for 5 minutes** to ensure that all bead surfaces come in contact with the water. (for low yield samples, alternatively can be eluted with less volume, e.g. 50ul instead of 100ul)
43. ☐ Place the **96 well microplate** on the PowerMag Magnetic Separator (or equivalent) and keep the **96 well microplate** in place for **3 minutes** to allow for complete concentration of the **ClearMag® Zorb Reagent** (beads) on the bottom of each well.
    Note: *You do not need to use the WeLLevator for this step, just place the magnetic plate on the benchtop.*
44. ☐ Keeping the **96 well microplate** on the magnet, remove the Sealing Tape and aspirate the complete volume of eluted nucleic acids, taking care to avoid the concentrated pellet of **ClearMag® Zorb Reagent (beads). Transfer** the eluent to a fresh 96 well sample plate.
45. ☐ For human or primate stool samples, create a 10x dilution plate for PCR use for later steps. 36ul of EB + 4 ul of samples. Label plate with ProjectID_submissionID_DNA_10xDilu_initial_date.
46. The eluted DNA is ready for use. You can measure on the QuBit DNA HS or BR assay to determine yield. Use the "WI-020 Amplicon Quantification – Qubit" for instructions.
47. ☐ If you will not use the eluents right away, seal the plate with a **Elution Sealing Mat** and store the samples at -20°C until needed. Label the plate with submission ID, PI and date.

7. 0 Revision History

Summary of Changes	Date	Version
N/A – 1st version	02/01/2016	1
Adapted from LAD lab, modified for other tissue types by SGT lab	09/08/6106	2
1. Corrected error 55ml bead solution 2. Changed to 250 µl of ClearMag® Wash to accommodate 300ul well vol. 3. Corrected plate type for final sample storage to regular conical bottom 96 well sample plate. 4. A full 96 well plate will not float, so changed use a weight to weigh the plate down during 70C incubation to optional.	10/05/2016	3
5. Added numbered steps. Added vol options for solid and liquid samples. Solid sample range 0.1-0.2grams	11/4/2016	3.1
Qiagen acquired MoBio, changing protocol to reflect Qiagen kit protocol	4/27/2017	3.2
Changed protocol name & some formatting	5/22/2017	3.2
Add a 10x dilution step at the end for human and primate stools	3/28/2018	3.2

illumina

TruSeq Stranded mRNA

Consumables and Equipment List

For Research Use Only. Not for use in diagnostic procedures.

Consumables

	Consumable	Supplier
☐	1.5 ml RNase/DNase-free nonsticky tubes	Thermo Fisher Scientific, part # AM12450
☐	10 µl barrier pipette tips	General lab supplier
☐	10 µl multichannel pipettes	General lab supplier
☐	10 µl single channel pipettes	General lab supplier
☐	1000 µl barrier pipette tips	General lab supplier
☐	1000 µl multichannel pipettes	General lab supplier
☐	1000 µl single channel pipettes	General lab supplier
☐	200 µl barrier pipette tips	General lab supplier
☐	200 µl multichannel pipettes	General lab supplier
☐	200 µl single channel pipettes	General lab supplier
☐	96-well storage plates, round well, 0.8 ml ('midi' plate)	Thermo Fisher Scientific, part # AB-0859
☐	Agencourt AMPure XP 60 ml kit	Beckman Coulter Genomics, part # A63881
☐	Agilent DNA 1000 Kit	Agilent Technologies, part # 5067-1504
☐	Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
☐	Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
☐	Nuclease-free ultrapure water	General lab supplier
☐	RNaseZap (to decontaminate surfaces)	General lab supplier
☐	RNase/DNase-free 8-tube strips and caps	General lab supplier
☐	RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
☐	SuperScript II Reverse Transcriptase (1 per 48 reactions)	Thermo Fisher Scientific, part # 18064-014
☐	Tris-HCl 10 mM, pH8.5	General lab supplier
☐	Tween 20	Sigma, part # P7949
☐	**[Optional - to aliquot reagents]** 96-well 2 ml deep well plates	Thomson Instrument Company, part # 951652
☐	**[Optional - to determine input RNA integrity]** Certified low range ultra agarose	Bio-Rad, part # 161-3107
☐	**[Optional - positive control]** Human UHR total RNA	Agilent Technologies, part # 740000
☐	**[Optional - for starting material quality assessment]** One of the following: • Standard Sensitivity RNA Analysis Kit (20nt Lower Marker) • Agilent RNA 6000 Nano Kit	 • Advanced Analytical Technologies, part # DNF-489 • Agilent Technologies, part # 5067-1511

Consumables for HS Workflow

	Consumable	Supplier
☐	96-well Hard-Shell 0.3 ml PCR plate	Bio-Rad, part # HSP-9601
☐	Microseal 'A' film	Bio-Rad, part # MSA-5001

Consumables for LS Workflow

	Consumable	Supplier
☐	96-well 0.3 ml PCR plates	General lab supplier

Equipment

	Equipment	Supplier/Description
☐	Basic lab equipment: safety glasses, lab coats, powder-free protective gloves, stopwatch or timer, ice bucket	
☐	96-well thermal cycler (with programmable heated lid)	General lab supplier
☐	One of the following: • Fragment Analyzer Automated CE System • 2100 Bioanalyzer Desktop System	 • Advanced Analytical Technologies, part # FSv2-CE2 or FSv2-CE10 • Agilent Technologies, part # G2940CA
☐	Magnetic stand-96	Thermo Fisher Scientific, part # AM10027
☐	Microplate centrifuge	General lab supplier
☐	Vortexer	General lab supplier

Equipment for HS Workflow

	Consumable	Supplier
☐	High-Speed Microplate Shaker	VWR, catalog # • 13500-890 (110 V/120 V) or • 14216-214 (230 V)
☐	Midi plate insert for heating system Note: Two inserts are recommended to support successive heating procedures.	Illumina, catalog # BD-60-601
☐	Stroboscope	General lab supplier
☐	One of the following: Note: Two systems are recommended to support successive heating procedures. • SciGene TruTemp Heating System • Hybex Microsample Incubator	 • Illumina, catalog # SC-60-503 (115 V) or SC-60-504 (220 V) • SciGene, catalog # 1057-30-0 (115 V) or 1057-30-2 (230 V)

Product Contents

	Library Prep Component	Catalog #
☐	TruSeq Stranded mRNA Library Prep (48 Samples)	20020594
☐	TruSeq Stranded mRNA Library Prep (96 Samples)	20020595

	Index Adapter Component	Catalog #
☐	IDT for Illumina-TruSeq RNA UD Indexes (24 indexes, 96 samples)	20020591
☐	IDT for Illumina-TruSeq RNA UD Indexes (96 indexes, 96 samples)	20022371
☐	TruSeq RNA Combinatorial Dual Indexes (96 indexes, 96 samples)	20019792
☐	TruSeq RNA Single Indexes (12 indexes, 24 samples) Set A	20020492
☐	TruSeq RNA Single Indexes (12 indexes, 24 samples) Set B	20020493

Signatures

FAS/FSE	Customer
	

Illumina Stranded mRNA sample prep protocol

Table of Contents

Illumina Stranded mRNA sample prep protocol

Item	Quantity	Storage	Supplied By
96-well 0.3 ml PCR Plate	1	15°C to 30°C	User
Microseal 'B' Adhesive Seals	3	15°C to 30°C	User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	6	15°C to 30°C	User
RNase/DNase-free Eight-Tube Strips and Caps (if using multichannel pipettes)	6	15°C to 30°C	User

Preparation

- Remove the following from -15°C to -25°C storage and thaw them at room temperature:
  - Bead Binding Buffer
  - Bead Washing Buffer
  - Elution Buffer
  - Fragment, Prime, Finish Mix
  - Resuspension Buffer

NOTE
The Resuspension Buffer can be stored at 2°C to 8°C after the initial thaw.

NOTE
After use in this procedure, store the Bead Binding Buffer, Bead Washing Buffer, and Elution Buffer at 2°C to 8°C for subsequent experiments.

- Remove the RNA Purification Beads tube from 2°C to 8°C storage and let stand to bring to room temperature.
- Pre-program the thermal cycler with the following programs:
  - Choose the pre-heat lid option and set to 100°C
  - 65°C for 5 minutes, 4°C hold—save as **mRNA Denaturation**
  - 80°C for 2 minutes, 25°C hold—save as **mRNA Elution 1**
  - 94°C for 8 minutes, 4°C hold—save as **Elution 2 - Frag - Prime**

NOTE
For inserts larger than 120–200 bp with a median size of 150 bp, see Appendix B Alternate Fragmentation Protocols.

- Set the centrifuge to 15°C to 25°C, if refrigerated.
- Apply an RBP barcode label to a new 96-well 0.3 ml PCR plate.

Make RBP

1. Dilute the total RNA with nuclease-free ultra pure water to a final volume of 50 µl in the new 96-well 0.3 ml PCR plate labeled with the RBP barcode.
2. Vortex the room temperature RNA Purification Beads tube vigorously to resuspend the oligo-dT beads.
3. Add 50 µl of RNA Purification Beads to each well of the RBP plate to bind the polyA RNA to the oligo dT magnetic beads. Gently pipette the entire volume up and down 6 times to mix thoroughly.
4. Seal the RBP plate with a Microseal 'B' adhesive seal.

Incubate 1 RBP

1. Place the sealed RBP plate on the pre-programmed thermal cycler. Close the lid and select **mRNA Denaturation** (65°C for 5 minutes, 4°C hold) to denature the RNA and facilitate binding of the polyA RNA to the beads.
2. Remove the RBP plate from the thermal cycler when it reaches 4°C.
3. Place the RBP plate on the bench and incubate at room temperature for 5 minutes to allow the RNA to bind to the beads.

Wash RBP

1. Remove the adhesive seal from the RBP plate.

Reagent Control

Reagents

- Reagent receipt and storage
- Restricted access
- Quarantine and certification

- **Document receipt, condition, storage**
- **Reagent Receipt Log**
- **Create expiration dates**
- **Track which reagents were used when**
  - **Runsheet – record for samples in a study**
  - **Reagent Use – record of equipment or kit use**
  - **Reagent Prep – record of reagent preparation**

Reagent Control & Receipt

Number: 3501-SOP **Date Effective:** 2012Jan04

Version: 2011Dec21 **Date Archived:**

Initial Approval	Signatures	Date
Document Owner		
Laboratory Director		
Quality Assurance Unit		

Table of Contents

1.0 Purpose

This procedure describes the reagent control and receipt process followed in the Duke Microarray Facility.

2.0 Scope

This procedure applies to all reagents used in GLP studies performed in adherence with the Quality System described in 3100-SOP.

3.0 Roles & Responsibilities

Role	*Responsibilities*
Personnel	Perform procedures as described in this SOP. Record reagent deliveries in the Reagent Receipt Log and verify that reagents are approved for use. Document solution preparation and any deviations in 'Forms for Reagent Preparation' and/or Runsheets associated with specific SOPs.
Laboratory Director	Confirm personnel are trained in this SOP before performing work. Approve reagents to be used without manufacturer documentation. Generally supervise the performance of this SOP.
Quality Assurance Unit	Maintain and review the Reagent Receipt Log. Periodically audit the performance of this SOP.

4.0 Definitions & Abbreviations

Common definitions, acronyms and abbreviations are included in the Glossary 3100-W01.

4.1 Reagent

A reagent is an item that is used in a test system for sample analysis. Many reagents contribute to a chemical reaction. Reagents include, but are not limited to: enzymes, chemicals, water, microarrays, columns for nucleic acid isolation and prepackaged kits.

4.2 Solution

A solution is a liquid reagent that is prepared in the Duke Microarray Facility, typically by dissolving solutes in a solvent. The final solution as well as the input solutes and solvent are under Reagent Control. Solutions include, but are not limited to: master mixes and ethanol dilutions.

4.3 Consumable

A consumable is an item that is intended to be used once and then discarded. Consumables may be used in a test system, but are not under Reagent Control. Consumables include, but are not limited to: disposable gloves, plastic tubes, filters, 96-well plates, pipet tips and paper towels.

5.0 Support Documents & References

Number	Document Title
3501-F01	Form for Reagent Receipt
3501-W01	List of Exempt Reagents

6.0 Reagent Control

6.1 Overview

- Reagent Control is a process which: 1) enables detailed tracking of all reagents used in a test system; and 2) assures the performance of each reagent prior to its use.
- The Duke Microarray Facility shall use reagents of sufficient grade, purity and/or activity for the intended use.
- Deteriorated or expired reagents and solutions shall not be used.
- While the Duke Microarray Facility may contain a variety of reagents and solutions, items approved for use in GLP studies will be identified by the presence of a 'GLP Approved' label.

6.2 Reagent ID Numbers

- Each container of a GLP Approved reagent or solution will be assigned a unique Reagent ID to track its use in test systems.
- Reagents packaged as multiple boxes/bags in manufacturer kits will be assigned a single Reagent ID.
- Each Reagent ID will contain a 6-digit date followed by a hyphen and a suffix.
- The date will be the Date Received written in the following format: MMDDYY.
- e suffix includes a 3- to 4-digit abbreviation for the reagent category, assigned as follows:

Reagent Category	Abbreviation
Ambion Message Amp Premier Kit	MAP
Ambion WT Expression Kit	WTX
Affymetrix Poly A Controls	PAC
Affymetrix HWS Kit	HWS
Affymetrix Hyb Controls	HybC
Agilent RNA 6000 Nano kit	NANO
Agilent RNA 6000 Pico Kit	PICO
Universal Human Reference	HRef
Water, RNase-free	H2O
Water, Deionized at Duke University	DIW
Ethanol	ETH
Isopropanol	ISO
RNase Zap	ZAP
Nanodrop Calibration Kit	NDC
Nanodrop Reconditioning Kit	NDR
Miscellaneous	MISC

- If more than one reagent or solution in the same category is received or prepared on the same day, a 2-digit number will be added at the end of the suffix in the Reagent ID to distinguish a specific container (*e.g.*, 090101-XXX03 identifies the third container on September 1, 2011).

6.3 Reagent Labels

- All reagents and solutions in the laboratory areas shall be labeled to indicate identity, concentration, storage requirements, and expiration date.
- The Date Received for reagents or Date Prepared for solutions will be recorded on the label.
- When reagents and solutions are first used in a test system, a Date Opened will be recorded on the label.
- Labels may be applied by the reagent manufacturer, personnel in the Duke Microarray Facility or a combination of both.
- Label information may be typed, hand written or a combination of both.
- 'GLP Approved' labels will be affixed to reagents and solutions that are approved for use.
- 'Quarantine' labels will be affixed to suspect reagents and solutions that may not be used.
- When applicable, a label may be affixed to a supplemental container (*e.g.*, plastic bag, kit box) that contains a group of related reagents or solutions.
- Information on labels affixed to a supplemental container shall apply to all items within that container.

6.4 Expiration Dates

- Expiration dates for each reagent will be assigned by the manufacturer, whenever possible.
- Employees will assign expiration dates to solutions and reagents without manufacturer dates.
- The following rules will be used to assign expiration dates, when necessary.

Reagent Type	Unopened	Opened
Dry Reagent	4 years after Date Received	2 years after Date Opened
Liquid Reagent	3 years after Date Received	2 years after Date Opened
Solution	not applicable	1 year after Date Prepared

- Expiration dates may be modified as a reagent is used. For example, the length of time until an expiration date may be shortened after an item is opened.
- The expiration date of a prepackaged kit shall apply to all reagents within the kit, even if the individual contents of the kit have labels with unique expiration dates.
- Any reagent or solution that is past its expiration date will be discarded.

6.5 Certificate of Analysis

- The specifications of each reagent will be documented in a Certificate of Analysis (COA) or equivalent (*e.g.*, Declaration of Conformity) before use.
- For most reagents, a COA or equivalent is available from the manufacturer.
- As described in Section 8.1, COAs will be labeled with the same Reagent ID as the reagent/kit and will be kept in the Reagent Receipt Notebook.
- Reagents without a COA or equivalent may be used in a test system if either:
  - The Laboratory Director approves the reagent use and lists the item in the List of Exempt Reagents 3501-W01.
  - An Employee verifies the performance of the reagent by using it in a test system with reference samples.

6.6 Quarantine

- Suspect reagents shall be placed in a separate location and labeled as 'Quarantine' to prevent possible use.

- Reagents may be removed from Quarantine and used in a test system if either:
  - The Laboratory Director approves the reagent use, after suitable investigation.
  - An Employee verifies the performance of the reagent by using it in a test system with reference samples.

6.7 Reagent Receipt Record & Notebook

- A running record of all reagents received shall be maintained as described in Section 8.1 using the Form for Reagent Receipt 3501-F01.
- Employees will print and add a page number to new pages in the Reagent Receipt Records, as needed.
- The QAU will routinely review the Reagent Receipt Records to verify that all fields were correctly completed. Upon approval, the QAU will sign and date the bottom of each page.
- The Reagent Receipt Records, COAs and other reagent documentation shall be stored in the Reagent Notebook, as described in 3100-SOP Quality System.

7.0 Solution Preparation

- Some test systems require the preparation of common solutions (*e.g.*, wash buffers) that will be stored and used on multiple days of testing.
- Detailed instructions for these reagent preparations are provided in the associated SOPs.
- As previously described, solutions shall be labeled to indicate identity, concentration, storage requirements, and expiration date.
- Instructions for solution storage and assigning expiration dates are included in the associated SOPs.
- When appropriate, solutions shall be labeled with a Date Prepared (*e.g.*, "prepared MMDDYY"), rather than a Date Received or a Date Opened.
- Solutions will be approved for use if they were prepared from GLP Approved reagents.
- The preparation of common solutions will be documented in 'Forms for Reagent Preparation' associated with specific SOPs.
- The preparation of common master mixes within an approved kit will be documented in the 'Runsheet' associated with the specific SOP.

8.0 Procedures

8.1 Receiving Reagents

1. Employee opens package and inspects contents for damage, potential contamination (*e.g.*, broken seals) or compromised condition (*e.g.*, warm ice packs or evaporation of dry ice).
2. Employee confirms contents have appropriate labels, and match the Packing Slip(s).
3. Employee records Reagent ID (as described in section 6.2), receipt condition and other information in Reagent Receipt Record.
   Note: Suspect reagents are labeled and placed in Quarantine.
4. Employee enters Reagent ID and Date Received on 'GLP Approved' label and affixes it to each container, as described in Section 6.3 above.
5. Employee writes the Reagent ID on the COA for each reagent and places it in Reagent Notebook.
   Note: Reagents without COAs are labeled and placed in Quarantine, unless the item is included on the List of Exempt Reagents.
6. Employee records the reagent approval method in the Reagent Receipt Log.

7. Employee places each reagent in the appropriate location given its storage requirements.
8. Employee initials and dates the Packing Slip(s) and places it XXXXX.

9.0 Version History

Version	*Modifications*
YYYYMmmDD	Initial Release

	Signature	Date of Annual Review
Document Owner		
Document Owner		
Document Owner		

Reagent Preparation & Use Record for RNA Nano 6000 Kit

Date Received		Duke ID for kit	
Agilent Kit Lot		Date & Time Opened	
Expiration Date		Date & Time Discarded	

DNA Ladder Preparation

Initials_____
Date_____

	Time Removed	Time Returned
Ladder stock tube		n/a
Ladder aliquots	n/a	

☒ Heat Ladder stock tube 70 degrees C Equipment ID:_____

☒ Quick cool on ice

☒ Transfer 7 uL aliquots to 0.5 mL tubes Tubes Labeled:_____

A) Spin Filter

☒ Filter 550 uL Gel Vial#1 Initials & Date_____

B) Spin Filter

☒ Filter 550 uL Gel Vial#1 Initials & Date_____

C) Spin Filter

☒ Filter 550 uL Gel Vial#2 Initials & Date_____

D) Spin Filter

☒ Filter 550 uL Gel Vial#2 Initials & Date_____

Preparation of RNA 6000 Pico Ladder

1. Thaw the RNA 6000 Pico Ladder on ice.

   Pico Ladder Lot No.: _____

2. Transfer the entire ladder (10 µL) to a 0.2 mL thin wall PCR tube.

3. Heat the ladder at 70°C for 2 minutes.

   Equipment ID: _____

4. Place the ladder on ice for 2 minutes.

5. Add 90 µL of nuclease-free water to the ladder.

   Nuclease-free water Lot No.: _____

6. Aliquot 7 µL of ladder into 0.5 mL safe-lock tubes and label tubes as "RNA Pico Ladder", include the lot number and expiration date (i.e. Pico Ladder Lot No. followed by the date.).

   RNA Pico Ladder Lot No.: _____

   Expiration Date: _____

7. Store ladder at -80°C.

   Storage Location: _____

Personnel Qualifications

Personnel & Training

- Document education and experience
- Method and forms to document lab training
- Method to prove competency

- All personnel in the lab shall have the necessary education, training, and experience to perform his/her assigned functions.
- The QAU shall maintain documentation of these qualifications for each individual in the **Personnel Notebook.**
    1. Curriculum Vitae
    2. Copy of a Diploma or Transcripts
    3. Documentation of Training
    4. Job Description
- Documents shall be retained for a period of at least 5 years following the date on which the results of the GLP study are submitted to the FDA.

Training Personnel Documentation

Subject of Training	Manner of Training	Documentation	Maintenance
Quality System	Self Training	Record of Self Training	Annual review
Laboratory Methods	Methods Training with Competency Review	Record of Methods Training	Competency Review at least once per 6 months
Safety Topics	On-line Course	Certificate	Varies, typically annual update is required
Compliance Topics	On-line Course	Certificate	Varies, typically annual update is required
Miscellaneous	Group Meeting	Record of Meeting Attendance	None

Quality System Self Training

Trainee: _____ **Date of Hire:** _____

Position: _____ **Supervisor:** _____

My initials and date below indicate that I have read the SOP as well as any support documentation. I understand and will follow the procedures presented. If I have questions, I will contact my supervisor.

Document	*Version*	*Original Training*	*Annual Review*	*Annual Review*
3100-SOP Quality System				
3101-SOP Document Control				
3102-SOP Study-Specific Documents				
3103-SOP Quality Control Plan				
3104-SOP Quality Assurance Plan				
3105-SOP Personnel Training				
3500-SOP Workplace Safety				
3501-SOP Reagent Control				
3200-SOP Equipment Control				
3400-SOP Informatics				

Methods Training & Competency Review

SOP Title: _____

Number: _____ **Trainee Name:** _____

Version: _____ **Instructor Name:** _____

A. **Reading** = Trainee has read the SOP and support documentation.
Trainee (initial/date)_____

B. **Observation** = Trainee observed Instructor perform the method SOP and fulfill documentation.
Trainee (initial/date)_____Instructor (initial/date)_____
Samples:_____
File Name for Results: _____

C. **Supervised Work** = Instructor observed Trainee perform the method SOP and fulfill documentation.
Trainee (initial/date)_____Instructor (initial/date)_____
Reference Sample:_____Lot No: _____
File Name for Results:_____

D. **Competency Review** = Trainee completed the method and documentation without supervision.
Trainee (initial/date)_____Instructor (initial/date)_____
Reference Sample:_____Lot No: _____
File Name for Results:_____

Quality Control Criteria

D. **Competency Review** = Trainee completed the method and documentation without supervision.

Trainee (initial/date)_____Instructor (initial/date)_____

Reference Sample:_____Lot No: _____

File Name for Results:_____

QC Criterion	*Expected Results*	*Trainee Results*	*Pass or Fail*

Approved by Supervisor_____Reviewed by QAU_____

MICROBIOME

Best practices for analysing microbiomes

Rob Knight[1,4,6,12*], Alison Vrbanac[2,12], Bryn C. Taylor[2,12], Alexander Aksenov[3], Chris Callewaert[4,5], Justine Debelius[4], Antonio Gonzalez[4], Tomasz Kosciolek[4], Laura-Isobel McCall[3], Daniel McDonald[4], Alexey V. Melnik[3], James T. Morton[4,6], Jose Navas[6], Robert A. Quinn[3], Jon G. Sanders[4], Austin D. Swafford[1], Luke R. Thompson[7,8], Anupriya Tripathi[9], Zhenjiang Z. Xu[4], Jesse R. Zaneveld[10], Qiyun Zhu[4], J. Gregory Caporaso[11] and Pieter C. Dorrestein[1,3,4]

Nat Rev Microbiol. 2018 Jul;16(7):410-422.

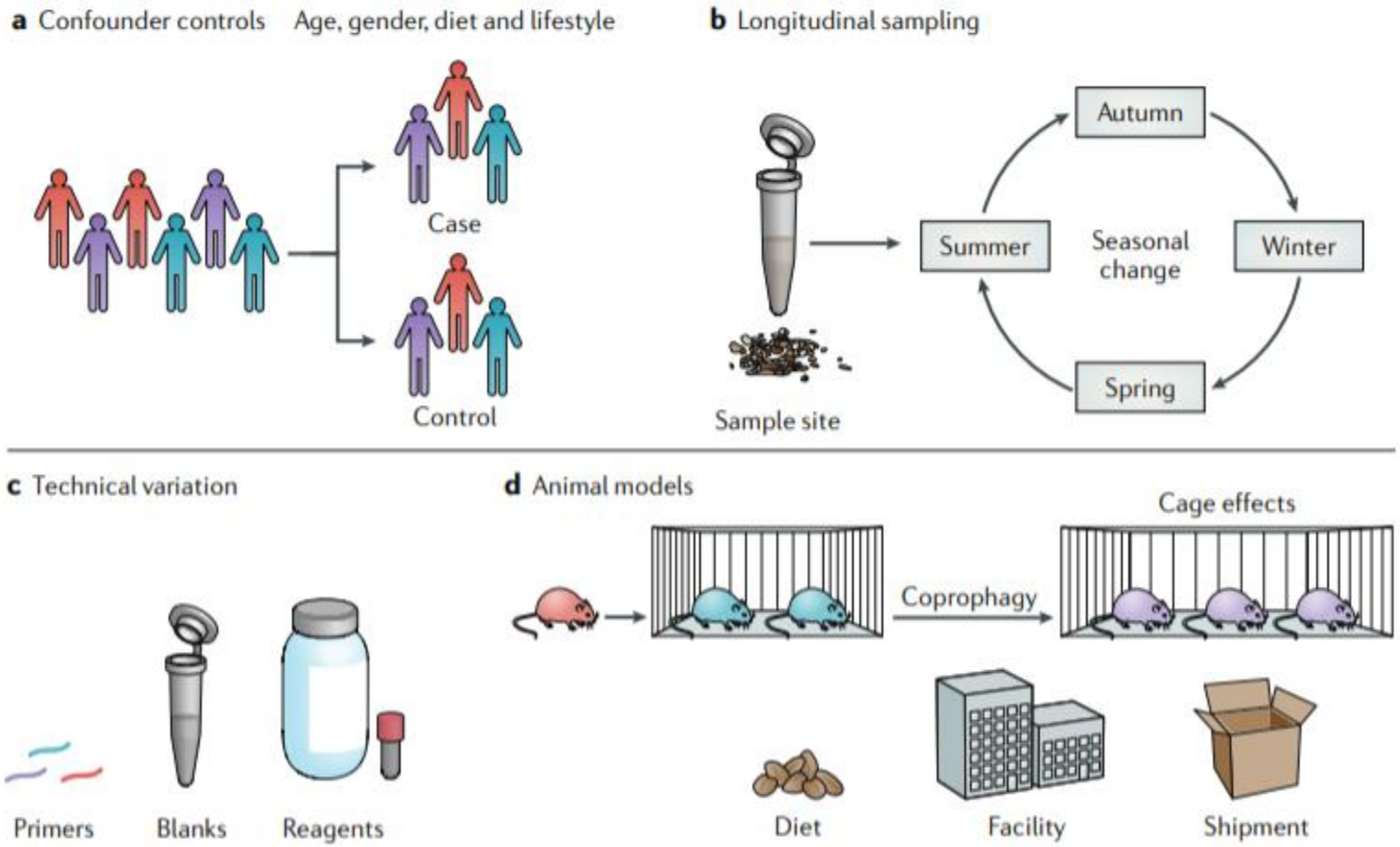


Fig. 1 | **Experimental design considerations for microbiome experiments.** Conducting a robust microbiome experiment warrants careful attention to numerous factors. **a** | Stratification by potential confounders (for example, age, gender, diet, lifestyle factors and medications) can help resolve differences in microbiota between groups of interest that might otherwise be masked by a confounder effect[5]. **b** | Longitudinal studies are especially powerful as they both control for confounding factors and allow for the assessment of community stability[7]. **c** | For all studies, standardizing technical factors and sample processing are essential to control for variation introduced by kit reagents, primers, sample storage and other factors. The collection and curation of metadata about all aspects of each sample, from clinical variables to sample processing, are crucial for data interpretation; without metadata, it is difficult to draw meaningful conclusions from sequencing data. **d** | Similar considerations apply to animal studies, though the additional impact of coprophagy must be addressed in experimental design.

Example of Metadata collected from Laboratory experiment

#SampleID	BarcodeSequence	LinkerPrimerSequence	PrimerID	group	kit	Notes	Cage	tissue	DOB	additional notes	Age	gender	platform	libProtocol	seqOrder	Project
A1	CGTACCAGATCC	CAAGCAGAAGACGGCATACGAGAT	806rcbc864	Prox-WT	Qigen DNeasy PowerSoil	3609	319F	Prox	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
A2	TTAAGCGCCTGA	CAAGCAGAAGACGGCATACGAGAT	806rcbc876	Mid-WT	Qigen DNeasy PowerSoil	3609	319F	Mid	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
A3	GTTATGACGGAT	CAAGCAGAAGACGGCATACGAGAT	806rcbc888	Ile-WT	Qigen DNeasy PowerSoil	3609	319F	Ile	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
A4	CCAATGATAAGC	CAAGCAGAAGACGGCATACGAGAT	806rcbc900	Liver-WT	Qigen DNeasy PowerSoil	3609	319F	Liver	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
A5	TCAATGACCGCA	CAAGCAGAAGACGGCATACGAGAT	806rcbc912	Heart-WT	Qigen DNeasy PowerSoil	3609	319F	Heart	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
B1	CTCTCATATGCT	CAAGCAGAAGACGGCATACGAGAT	806rcbc924	Prox-WT	Qigen DNeasy PowerSoil	3574	317F	Prox	3/18/2018	none	9.3	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
B2	CATGTTGGAACA	CAAGCAGAAGACGGCATACGAGAT	806rcbc936	Mid-WT	Qigen DNeasy PowerSoil	3574	317F	Mid	3/18/2018	none	9.3	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
B3	CGAGATAGTTTG	CAAGCAGAAGACGGCATACGAGAT	806rcbc948	Ile-WT	Qigen DNeasy PowerSoil	3574	317F	Ile	3/18/2018	none	9.3	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
B4	ATGTTTAGACGG	CAAGCAGAAGACGGCATACGAGAT	806rcbc865	Liver-WT	Qigen DNeasy PowerSoil	3574	317F	Liver	3/18/2018	none	9.3	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
B5	TGCGGGATTCAT	CAAGCAGAAGACGGCATACGAGAT	806rcbc877	Heart-WT	Qigen DNeasy PowerSoil	3574	317F	Heart	3/18/2018	none	9.3	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
C1	AGCCTCATGATG	CAAGCAGAAGACGGCATACGAGAT	806rcbc889	Prox-WT	Qigen DNeasy PowerSoil	3586	319F	Prox	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
C2	TTAAACCGCGCC	CAAGCAGAAGACGGCATACGAGAT	806rcbc901	Mid-WT	Qigen DNeasy PowerSoil	3586	319F	Mid	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
C3	CTATCGGAAGAT	CAAGCAGAAGACGGCATACGAGAT	806rcbc913	Ile-WT	Qigen DNeasy PowerSoil	3586	319F	Ile	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
C4	CCAGTATCGCGT	CAAGCAGAAGACGGCATACGAGAT	806rcbc925	Liver-WT	Qigen DNeasy PowerSoil	3586	319F	Liver	3/16/2018	touched skin	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
C5	ATGGGACCTTCA	CAAGCAGAAGACGGCATACGAGAT	806rcbc937	Heart-WT	Qigen DNeasy PowerSoil	3586	319F	Heart	3/16/2018	none	9.6	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
D1	CGCGTCAAACTA	CAAGCAGAAGACGGCATACGAGAT	806rcbc949	Prox-WT	Qigen DNeasy PowerSoil	3592	319F	Prox	3/19/2018	none	9.1	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065
D2	ACATGTCACGTG	CAAGCAGAAGACGGCATACGAGAT	806rcbc866	Mid-WT	Qigen DNeasy PowerSoil	3592	319F	Mid	3/19/2018	none	9.1	f	MiSeqv2_250PE	EMP16s_515_806	4984	5065

Electronic Lab Notebooks (ELNs)

1. SCINOTE

4.7 - Excellent

PROS:

- Very easy to use and quick to set up
- Unique experimental workflow
- Inventory management
- Free account with unlimited project users
- Automatically generates reports & manuscript drafts

CONS:

- No option for drawing molecules

2. BENCHLING

4.3 - Excellent

PROS:

- Very user-friendly and quick to set up
- Useful DNA tools (CRISPR guide and primer design)
- Templates for sequence mapping and sharing
- Free account with 10 GB of storage space

CONS:

- The free account is tied to a single user
- Report structure is not flexible

3. RSPACE

4.0 – Very good

PROS:

- Possible archive management, built-in metrics, and analytics
- Can connect to the eCAT sample tracking system
- Supports chemical structures
- Free to use
- Many integrations

CONS:

- Not open source
- GUI could use improvements

4. DOCOLLAB

3.7 – Very Good

PROS:

- Easy to use with useful tips
- Free account
- Clean GUI

CONS:

- Local installation not possible
- Not open source

5. LABFOLDER

3.3 – Very good

PROS:

- Sketching
- Free account for smaller teams and free mobile app
- Integration with Mendeley

CONS:

- Not very intuitive
- Unflattering structured design
- The free version is limited to 3 team members

6. LABARCHIVES

3.3 – Very good

PROS:

- Pubmed references entry editor
- Interface with GraphPad Prism

CONS:

- Graphical user interface needs improvements
- Quite complicated, additional training necessary
- Not a lot of storage space in the free version
- Not open source

7. MBOOK

3.0 - Good

PROS:

- Supports all operating systems and browsers
- Suitable for different fields of science

CONS:

- No free account, only free trial
- Niche interface, not very intuitive
- Not open source

8. LABGURU

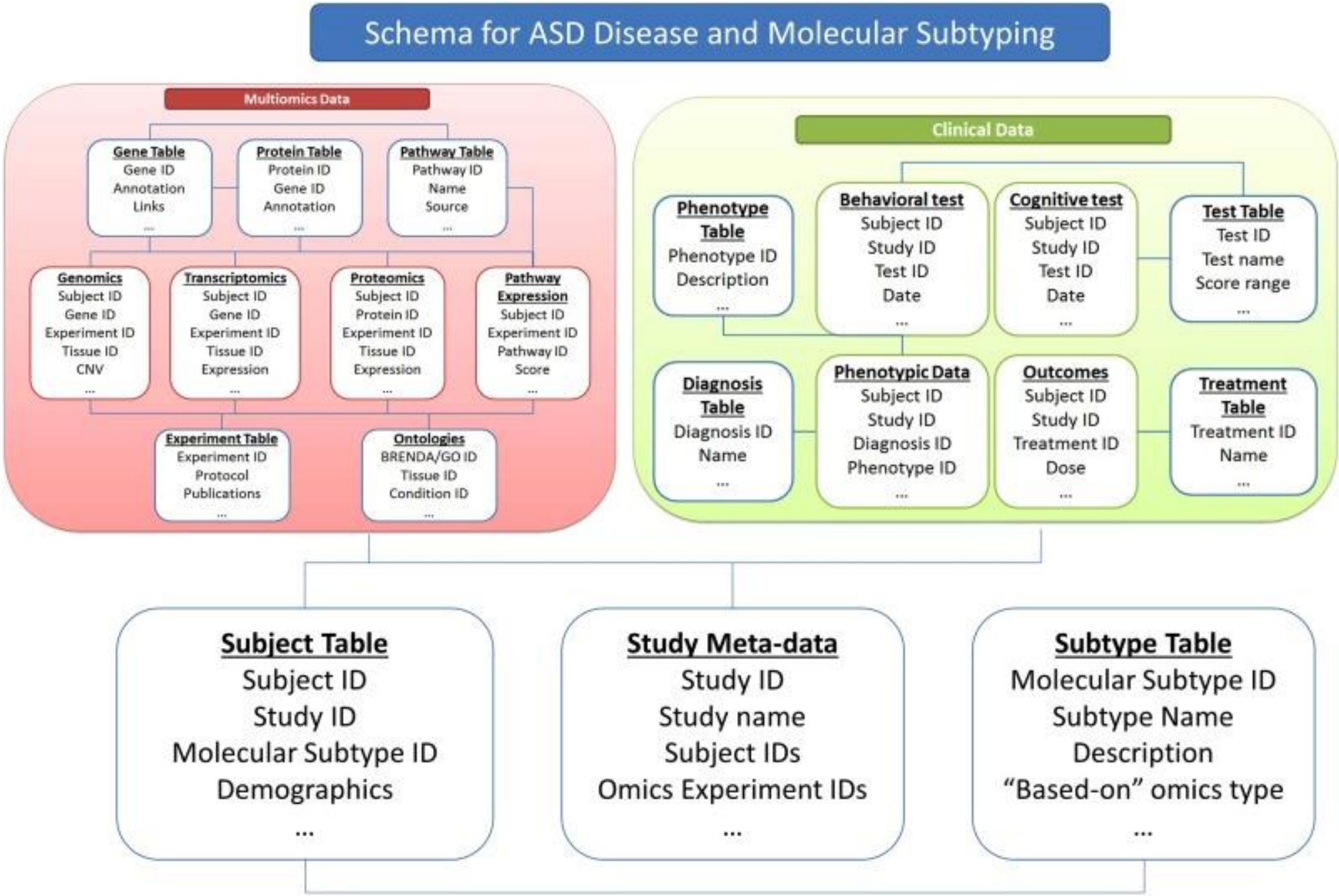
2.7 - Good

PROS:

- Advanced tagging system for easy search
- Track recording from batch number to concentration

CONS:

- Not very intuitive
- Project view too complex
- No free plan available
- Expensive monthly subscription



Autism Spectrum Disorder

OMICS. 2015 Apr 1; 19(4): 197–208.