EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Nucleic Acid Extraction Manual/Column Based

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented: 1 March 2010

Updated:	Ashima Amin **Name**	12 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:
061520: Review, Protocols split out into appendixes. Renumbered SOPs. Previously EIGC.003.

Changes to previous procedures:
041116: Review

Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.

Principles:

RNA isolation is the process of purifying RNA molecules from cells by lysing the cells open and precipitating RNA. All samples are registered into our Laboratory Information Management System (LIMS) and evaluated for quality by the EIGC staff members. All isolated RNA is quantitated by spectrophotometric methods (either NanoDrop N-100 Spectrophotometer by Thermo Scientific or Tecan Infinite M200Pro), to determine 260/280 and 260/230 ratios and is evaluated for quality using the BioAnalyzer.

a. See ***EIGC.005_Quantitation with NanoDrop*** for a detailed protocol on how to perform QC using the Nanodrop N-100.

b. See ***EIGC.004_Quantitation with Fluorescence*** for a detailed protocol on how to perform QC using the Tecan Infinite M200Pro.

c. See ***EIGC.006_QC with the BioAnalyzer*** for a detailed protocol on how to perform QC using the BioAnalyzer.

DNA isolation is the process by which DNA is separated from proteins, membranes, and other cellular material contained in the cell from which it is isolated. All samples are registered into our Laboratory Information Management System (LIMS) and evaluated for quality by the EIGC staff members. All isolated dsDNA is quantitated by spectrophotometric methods using the Tecan Infinite M200Pro to determine 260/280 and 260/230 ratios and is evaluated for quality using the BioAnalyzer. All quality of isolated dsDNA is analyzed using agarose gels.

a. See ***EIGC.004_Quantitation with Fluorescence*** for a detailed protocol on how to perform QC using the Tecan Infinite M200Pro.

b. See ***EIGC.006_QC with the BioAnalyzer*** for a detailed protocol on how to perform QC using the BioAnalyzer.

c. See ***EIGC.008_General Agarose Gel Electrophoresis*** for a detailed protocol on how to perform QC using agarose gels.

Specimen Types:

Whole blood, buffy coat, PMBC, plasma and serum, tissue, FFPE, cells, saliva

Safety: All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook).

Reagents and Consumables:

RNA Kits

Nucleic Acid	Sample Source	Kit
RNA	Tissue (< 5 mg)	miRNeasy Micro Kit (Qiagen, Cat# 217084)
RNA	Tissue (5-50 mg)	miRNeasy Mini Kit (Qiagen, Cat# 217004)
RNA	Cells (< 1 million)	miRNeasy Micro Kit (Qiagen, Cat# 217084)
RNA	Cells (1-10 million)	miRNeasy Mini Kit (Qiagen, Cat# 217004)
RNA	Whole blood- Frozen, 1.5 mL	miRNeasy Mini Kit (Qiagen, Cat# 217004)
RNA	Whole Blood - Tempus Tubes	Tempus RNA Isolation Reagent Kit (Cat# 4380204)
RNA	PBMC	miRNeasy Mini Kit (Qiagen, Cat# 217004)
RNA	Buffy Coat	miRNeasy Mini Kit (Qiagen, Cat# 217004)
RNA	Serum/Plasma	miRNeasy Serum/Plasma Kit (Qiagen, Cat# 217184)
RNA	FFPE	EZNA FFPE RNA Kit (Omega, Cat# R6954)
RNA	Poly-A - Standard - mRNA RNAseq	Use either RNeasy Mini Kit (Qiagen, Cat# 74104), RNeasy Micro Kit (Qiagen, Cat# 74004), or miRNeasy Kit
RNA	***for total RNA (miRNA and mRNA)***	***You must use miRNeasy Kits.***

DNA Kits

Nucleic Acid	Sample Source	Kit
DNA	Tissue	QIAamp DNA Mini Kit (Qiagen, Cat# 51304/51306)
DNA	Cells	QIAamp DNA Mini Kit (Qiagen, Cat# 51304/51306)
DNA	Whole Blood - Frozen - 1.5mL	QIAamp DNA Mini Kit (Qiagen, Cat# 51304/51306)
DNA	Whole blood - Tempus Tubes	Omega SQ Blood DNA Kit II (Omega, Cat# D0714-50)
DNA	PBMC	QIAamp Blood Mini Kit (Qiagen, Cat# 51104/51106)
DNA	Buffy Coat, Plasma, Serum	QIAamp Blood Mini Kit (Qiagen, Cat# 51104/51106)
DNA	FFPE	EZNA FFPE DNA Kit (Omega, Cat# D3399)
DNA	Microbiome for 16S	DNeasy PowerSoil Kit (Qiagen, Cat# 12888)
DNA	Whole Blood - KingFisher	Mag-Bind SQ Blood DNA Isolation Kit (Omega, Cat# M6213-02)
DNA	Saliva - KingFisher	Mag-Bind SQ Saliva DNA Isolation Kit (Omega, Cat# M0312-EUW)

RNA Isolation:

1. **Whole Blood or Buffy Coat:** Total RNA is extracted from 200 µl blood or buffy coat using Trizol LS / chloroform protocol followed by the miRNeasy Mini Kit.

    a. See **EIGC.002_Appendix_A** for a detailed protocol on how to process blood or buffy coat.

2. **Plasma and Serum**: RNA is extracted from 200 µl of plasma or serum using the miRNeasy Serum/Plasma Kit.

    a. See **EIGC.002_Appendix_F** for a detailed protocol on how to process plasma or serum.

3. **PBMC:** RNA is extracted from 5 million PBMC cells using the miRNeasy Mini Kit.

    a. See **EIGC.002_Appendix_B** for a detailed protocol on how to process PBMC cells.

4. **FFPE Tissue:** Total RNA is extracted from FFPE curls or slides from 5-10 paraffin sample sections, 5-10 µm thick, using the E.Z.N.A. FFPE RNA kit, Heat Extraction Method.

    a. See **EIGC.002_Appendix_G** for a detailed protocol on how to process FFPE.

5. **Tissue:** Total RNA is extracted from up to 50 mg of tissue. If the provided tissue is up to 5 mg of tissue, RNA is extracted using the miRNeasy Micro Kit. If the starting material is between 5 mg and 50 mg of tissue, total RNA is extracted using the miRNeasy Mini Kit.

    a. See **EIGC.002_Appendix_E** for a detailed protocol on how to process tissue using the miRNeasy Micro Kit.
    b. See **EIGC.002_Appendix_C** for a detailed protocol on how to process tissue using the miRNeasy Mini Kit.

6. **Cultured Cells and Cell Pellets:** Total RNA is extracted from up to 10 million cells. If the provided material is up to 1 million cells, RNA is extracted using the miRNeasy Micro Kit. If the starting material is between 1 million and 10 million cells, total RNA is extracted using the miRNeasy Mini Kit.

    a. See **EIGC.002_Appendix_D** for a detailed protocol on how to process cells using the miRNeasy Micro Kit.
    b. See **EIGC.002_Appendix_B** for a detailed protocol on how to process cells using the miRNeasy Mini Kit.

***Please proceed to appropriate RNA QC steps. QC protocols are listed above.**

DNA Isolation:

1. **Whole Blood:** Genomic DNA is extracted from 200 µl blood or 2-5 mL of blood. If less than 200 µl of blood is provided, extraction occurs using the QIAamp DNA Mini Kit. If 2-5 mL of blood is provided, DNA is extracted using the SQ DNA II Blood kit or the Mag-Bind SQ Blood DNA Kit in conjunction with the KingFisher Flex Magnetic Particle Processor.

    a. See **EIGC.002_Appendix_L** for a detailed protocol on how to process blood using the QIAamp DNA Mini Kit.
    b. See **EIGC.002_Appendix_O** for a detailed protocol on how to process blood using the SQ DNA II Blood Kit.
    c. See **EIGC.001_Nucleic Acid Extraction KingFisher Flex** for a detailed protocol on how to process blood using the Mag-Bind SQ Blood Kit with King Fisher.

2. **Buffy Coat:** Genomic DNA is extracted from 200 µl of buffy coat using the QIAamp Blood Mini Kit.
    a. See **EIGC.002_Appendix_L** for a detailed protocol on how to process buffy coat using the QIAamp Blood Mini Kit.

3. **Plasma and Serum:** Genomic DNA is extracted from 200 µl of plasma or serum using the QIAamp Blood Mini Kit.

    a. See **EIGC.002_Appendix_L** for a detailed protocol on how to process plasma or serum using the QIAamp Blood Mini Kit.

4. **PBMC**: Genomic DNA is extracted from 5 million PMBC cells using the QIAamp Blood Mini Kit.
    a. See **EIGC.002_Appendix_L** for a detailed protocol on how to process PBMC cells using the QIAamp Blood Mini Kit.
    b. See **EIGC.001_Nucleic Acid Extraction KingFisher Flex** for a detailed protocol on how to process PBMC using the Mag-Bind Blood & Tissue DNA HDQ 96 Isolation Kit with the King Fisher.

5. **FFPE Tissue**: Genomic DNA is extracted from 8-10 paraffin sample sections, 5-10 µm thick, using the E.Z.N.A. FFPE DNA Kit.

    a. See **EIGC.002_Appendix_P** for a detailed protocol on how to process FFPE using the E.Z.N.A. FFPE DNA Kit.
    b. See **EIGC.001_Nucleic Acid Extraction KingFisher Flex** for a detailed protocol on how to process FFPE using the Mag-Bind FFPE DNA kit with the King Fisher Flex.

6. **Tissue:** Genomic DNA is extracted from 25 mg of tissue using the QIAamp Mini Kit.

a. See **EIGC.002_Appendix_N** for a detailed protocol on how to process tissue using the QIAamp DNA Mini Kit.

7. **Cultured Cells or Cell Pellets**: Genomic DNA is extracted from 5 million cells using the QIAamp DNA Mini Kit.

a. See **EIGC.002_Appendix_M** for a detailed protocol on how to process cells using the QIAamp DNA Mini Kit.

8. **Saliva**: Genomic DNA is extracted from saliva collected in Oragene (DNA Genotek) collection tubes using the Mag-Bind Saliva DNA Isolation kit in conjunction with the King Fisher Flex Magnetic Particle Processor.

a. See **EIGC.001_Nucleic Acid Extraction KingFisher Flex** for a detailed protocol on how to process saliva using the Omega Mag-Bind Saliva DNA Isolation Kit.

9. **Soil:** Genomic DNA is extracted from fecal, gut material, vaginal swabs, rectal swabs, or buccal swabs using the Qiagen DNeasy PowerSoil Kit.

a. See **EIGC.011_16S Microbome** for a detailed protocol on how to process 16s samples using the DNeasy PowerSoil Kit.

***Please proceed to appropriate DNA QC steps. QC protocols are listed above.**