DNA Extraction Protocol Documentation Report

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Protocol Author: Dr. Rahul Mehta Date Established: September 19, 2025 Laboratory: Molecular Biology Unit

Document Type: Standard Operating Procedure for Genomic DNA Extraction

# Protocol Overview and Scientific Rationale

## Primary Objective

This comprehensive protocol establishes standardized methodologies for extracting high-purity genomic DNA from whole blood samples obtained from clinical trial participants. The extracted DNA serves as the foundation for downstream genomic sequencing analysis, supporting personalized medicine initiatives and pharmacogenomic studies.

## Clinical Applications

Primary Uses:

 Pharmacogenomic analysis for drug metabolism profiling  Genetic biomarker identification in clinical trials

 Personalized treatment response prediction

 Population genetics studies in clinical research  Quality control for genetic screening programs

## Scientific Principles

The protocol utilizes a combination of chemical lysis, protein denaturation, and column-based purification to achieve:

 High DNA Yield: Optimized extraction efficiency from limited blood volumes

 Superior Purity: A260/A280 ratios consistently within 1.8-2.0 range

 Intact DNA: Minimal degradation preserving high molecular weight genomic DNA

 Reproducibility: Standardized procedures ensuring consistent results across operators

# Pre-Extraction Preparation Requirements

## Laboratory Environment Setup

Workspace Configuration:

 Dedicated DNA extraction area with laminar flow hood

 UV decontamination of work surfaces prior to each session

 Temperature-controlled environment (20-25°C) with humidity control  Segregated pre-PCR and post-PCR areas to prevent contamination

Equipment Calibration and Verification:

 Centrifuges: Speed accuracy verification using tachometer

 Pipettes: Volume accuracy confirmed with gravimetric analysis

 Incubators: Temperature uniformity validation across chamber

 Spectrophotometer: Wavelength accuracy verification with standards

Critical Reagent Preparation Red Blood Cell (RBC) Lysis Buffer Composition and Preparation:

 155 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA

 pH adjusted to 7.4 ± 0.1 using HCl or NaOH  Sterile filtration through 0.22μm membrane

 Storage at 4°C with maximum 30-day shelf life

 Quality control: Osmolarity verification and sterility testing

Cell Lysis Buffer System Primary Lysis Buffer:

 10 mM Tris-HCl (pH 8.0), 100 mM EDTA, 0.5% SDS

 RNase-free water for all dilutions

 Sterile filtration and aliquoting in 50ml volumes

 Storage at room temperature with 6-month stability

Proteinase K Solution:

 Stock concentration: 20 mg/ml in sterile water

 Working concentration: 200 μg/ml in lysis buffer  Fresh preparation for each extraction session

 Activity verification using casein substrate assay

## Sample Management and Quality Control

Sample Acceptance Criteria:

 Fresh whole blood collected in EDTA tubes (purple top)  Sample age: Maximum 48 hours from collection at 4°C

 Volume requirement: Minimum 5ml for protocol execution  Visual inspection: No visible hemolysis or clotting

 Chain of custody documentation complete

Pre-Processing Quality Assessment:

 Sample volume verification using graduated cylinders  Visual assessment for hemolysis or contamination

 Temperature log verification for cold chain maintenance  Patient identifier verification against study database

Detailed Step-by-Step Protocol Implementation Phase 1: Sample Preparation and Initial Processing Step 1: Laboratory Setup and Sample Organization

Detailed Procedure:

 Pre-label all 15ml centrifuge tubes with unique patient identifiers, sample processing date, and experiment ID using permanent laboratory markers

 Prepare 2ml microcentrifuge tubes for final DNA elution with matching identification codes  Organize samples in numerical order to prevent cross-contamination or mislabeling

 Document sample receipt time, temperature, and visual condition assessment

Critical Control Points:

 Double-verify patient identifiers against clinical database

 Confirm sample volume adequacy using volumetric measurement

 Photocopy chain of custody documents for laboratory records

 Initial sample condition documentation with digital photography if abnormalities noted

Step 2: Blood Sample Processing and Initial Lysis Precise Methodology:

 Using calibrated pipettes, transfer exactly 5ml of whole blood into pre-labeled 15ml centrifuge tubes  Add blood dropwise to prevent foaming or air incorporation

 Immediately mix samples by gentle inversion (10 complete inversions)  Avoid vigorous mixing that could cause mechanical hemolysis

 Process samples individually to maintain traceability

Quality Assurance Measures:

 Pipette accuracy verification using gravimetric analysis

 Visual monitoring for proper blood flow without clotting  Immediate processing to prevent cellular degradation

 Temperature maintenance at 4°C throughout processing Step 3: Red Blood Cell Lysis and White Cell Enrichment Methodological Details:

 Add 10ml of pre-chilled RBC lysis buffer to each 5ml blood sample  Mix gently by inversion technique (10 complete inversions)

 Incubate at room temperature for exactly 10 minutes with timing precision  Monitor samples visually for complete red blood cell lysis

 Solution should progress from red to translucent pink/clear appearance

Technical Specifications:

 Buffer Temperature: 20-22°C for optimal lysis efficiency

 Incubation Time: 10 minutes ± 30 seconds maximum deviation

 Mixing Protocol: Gentle inversion to prevent white cell damage

 Visual Endpoint: Complete clarity indicating successful RBC removal

Troubleshooting Indicators:

 Incomplete Lysis: Persistent red coloration requiring extended incubation

 Over-Lysis: Excessive foaming indicating buffer degradation

 Clotting: Fibrin strand formation requiring sample rejection

Phase 2: White Blood Cell Isolation and Purification Step 4: Primary Centrifugation and Pellet Formation Centrifugation Parameters:

 Speed: 3000 rpm (approximately 1000 x g)

 Duration: 10 minutes exactly

 Temperature: 4°C to preserve cellular integrity

 Acceleration/Deceleration: Gradual ramp to prevent pellet disruption

Post-Centrifugation Assessment:

 Visual inspection of pellet formation and compactness

 Supernatant clarity assessment (should be nearly colorless)

 Pellet size estimation (typically 50-200 μl depending on white cell count)  Documentation of any abnormal appearances or deviations

Supernatant Removal Protocol:

 Use sterile Pasteur pipettes or vacuum aspiration

 Remove supernatant without disturbing white cell pellet

 Leave approximately 100 μl residual supernatant to protect pellet  Dispose of supernatant according to biological waste protocols

Step 5: Pellet Washing and Purification Double Wash Protocol:

 First Wash: Add 10ml sterile PBS, resuspend pellet by gentle pipetting

 Mixing Technique: Use 1ml pipette tips to avoid pellet destruction

 Centrifugation: 3000 rpm for 5 minutes at 4°C

 Supernatant Removal: Complete aspiration leaving minimal residual volume

 Second Wash: Repeat identical procedure for maximum purity

 Final Assessment: Pellet should appear white/cream colored

 Volume Estimation: Measure final pellet volume for downstream calculations

Critical Success Factors:

 Gentle Resuspension: Prevent cellular membrane damage

 Complete Washing: Remove residual RBC lysis buffer and cellular debris  Pellet Integrity: Maintain compact pellet formation throughout washes  Sterile Technique: Prevent bacterial or fungal contamination

Phase 3: Cellular Lysis and DNA Liberation Step 6: Protein Denaturation and DNA Release Lysis Buffer Application:

 Add exactly 1ml of cell lysis buffer to washed white cell pellet

 Include 20 μl of Proteinase K solution (final concentration 200 μg/ml)  Mix components by gentle vortexing for 5-10 seconds

 Ensure complete pellet dissolution before incubation

Optimized Incubation Conditions:

 Temperature: 56°C ± 1°C using calibrated heating block

 Duration: 30 minutes with continuous gentle agitation

 Agitation Speed: 200 rpm to ensure uniform heating and mixing

 Monitoring: Visual inspection every 10 minutes for lysis progression

Lysis Completion Assessment:

 Visual Endpoint: Complete solution clarity without visible particles

 Viscosity Increase: Solution becomes noticeably more viscous due to DNA release

 Color Change: Slight yellow tint from protein denaturation

 Homogeneity: No visible cell debris or unlysed material

Step 7: DNA Precipitation and Column Binding Ethanol Precipitation Protocol:

 Add exactly 500 μl of 100% ethanol to each lysed sample  Mix immediately by gentle inversion (5-8 inversions)

 Avoid vigorous mixing that could shear high molecular weight DNA

 Visual confirmation of DNA precipitation (white fibrous strands may be visible)

Column-Based Purification Setup:

 Transfer complete lysate-ethanol mixture to DNA binding spin columns  Use wide-bore pipette tips to prevent DNA shearing during transfer

 Process columns individually to prevent cross-contamination  Ensure complete sample transfer without overflow

Centrifugation and Binding:

 Speed: 8000 rpm for exactly 1 minute

 Temperature: Room temperature for optimal binding efficiency

 Flow-Through Management: Discard flow-through containing cellular debris

 Column Assessment: Verify DNA binding by column color change (if applicable)

Phase 4: DNA Purification and Quality Control Step 8: Column Washing and Contaminant Removal Dual Wash Protocol:

 First Wash: 500 μl wash buffer, centrifuge 8000 rpm for 1 minute

 Second Wash: 500 μl wash buffer, centrifuge 8000 rpm for 1 minute

 Critical Timing: Do not exceed centrifugation times to prevent over-drying

 Flow-Through Disposal: Discard all wash flow-through containing salts and contaminants

Ethanol Removal Verification:

 Final Spin: Empty columns centrifuged at maximum speed for 2 minutes

 Visual Inspection: No residual ethanol visible in column matrix

 Drying Assessment: Columns should appear completely dry but not over-dried

 Quality Check: No ethanol odor detectable from processed columns

Step 9: DNA Elution and Concentration Measurement Optimized Elution Protocol:

 Elution Buffer: 100 μl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

 Buffer Temperature: Room temperature or slightly warm (25-30°C) for improved elution

 Incubation Time: 5-minute incubation on column before centrifugation

 Final Centrifugation: 8000 rpm for 2 minutes to ensure complete elution

DNA Quality and Quantity Assessment:

 Spectrophotometric Analysis: Nanodrop measurement at 260nm and 280nm wavelengths

 Purity Ratios: A260/A280 ratio should be 1.8-2.0 for high purity DNA

 Concentration Range: Typical yields 20-100 μg/ml from 5ml blood

 Volume Verification: Measure actual elution volume (typically 95-100 μl)

Step 10: DNA Storage and Documentation Proper Storage Protocol:

 Storage Temperature: -20°C for long-term stability (>6 months)

 Aliquoting: Create working aliquots to prevent freeze-thaw degradation

 Container Selection: RNase-free microcentrifuge tubes with secure locking lids

 Storage Buffer: TE buffer provides optimal pH and chelation for DNA stability

Comprehensive Documentation Requirements:

 Sample Processing Log: Patient IDs, extraction date, operator name

 Quality Control Data: DNA concentration, purity ratios, yield calculations

 Storage Location: Freezer location, box number, position coordinates

 Chain of Custody: Continuous documentation from blood collection through DNA storage

# Quality Control Framework and Validation

Process Control Standards Positive and Negative Controls Positive Control Requirements:

 Reference Sample: Known high-quality genomic DNA processed alongside patient samples

 Expected Results: DNA concentration >50 μg/ml, A260/A280 ratio 1.8-2.0

 Purpose: Verify reagent functionality and protocol execution

 Frequency: Include in every extraction batch

Negative Control Implementation:

 Blank Processing: Process PBS or sterile water through entire protocol

 Expected Results: No detectable DNA, minimal A260 absorbance

 Contamination Detection: Any DNA detection indicates contamination source

 Corrective Actions: Re-process batch if negative control fails

Performance Monitoring Metrics Acceptance Criteria for DNA Quality:

 Concentration Range: 20-150 μg/ml (optimal 50-100 μg/ml)

 Purity Standards: A260/A280 ratio 1.8-2.0, A260/A230 ratio >1.5

 Yield Consistency: Inter-sample variation <25% within extraction batch

 Reproducibility: Inter-operator variation <20% for identical samples

Protocol Performance Indicators:

 Success Rate: >95% of samples meeting quality criteria

 Processing Time: 4-6 hours for 12-sample batch including documentation  Reagent Stability: No degradation in quality metrics over reagent shelf life  Equipment Reliability: <5% equipment-related failures per month

## Troubleshooting and Problem Resolution

Common Issues and Corrective Actions Low DNA Yield (<20 μg/ml):

 Potential Causes: Old blood samples, low white cell count, incomplete lysis

 Diagnostic Steps: Verify sample age, assess pellet size, check lysis buffer pH

 Corrective Actions: Extend lysis time, increase Proteinase K concentration, fresh reagents

Poor DNA Purity (A260/A280 <1.6 or >2.2):

 Low Ratio (<1.6): Protein contamination - additional wash steps required

 High Ratio (>2.2): RNA contamination - include RNase treatment step

 Salt Contamination: A260/A230 ratio <1.5 indicates insufficient washing

Inconsistent Results Between Samples:

 Operator Variability: Standardize timing, mixing, and temperature control

 Reagent Issues: Check expiration dates, storage conditions, pH values

 Equipment Problems: Calibrate pipettes, verify centrifuge speeds, temperature accuracy

Quality Assurance Monitoring Daily Quality Checks:

 Equipment calibration verification logs

 Reagent expiration date and storage condition monitoring  Environmental condition recording (temperature, humidity)  Sterile technique compliance assessment

Weekly Performance Reviews:

 DNA yield and purity trend analysis

 Success rate calculations and improvement identification  Equipment maintenance schedule compliance

 Staff training and competency assessment

# Safety Protocols and Regulatory Compliance

Laboratory Safety Requirements Personal Protective Equipment (PPE) Mandatory PPE Standards:

 Eye Protection: Safety glasses with side shields throughout protocol

 Hand Protection: Powder-free nitrile gloves changed between samples

 Respiratory Protection: Dust masks when handling dry chemicals

 Clothing: Laboratory coats, closed-toe shoes, long pants

Chemical Safety Management Hazardous Material Handling:

 Ethanol: Flammable - store away from heat sources, use in ventilated areas

 Proteinase K: Potential sensitizer - avoid skin contact and inhalation

 Buffer Solutions: Follow SDS recommendations for safe handling and disposal

 Blood Samples: Universal precautions for bloodborne pathogen protection

Waste Disposal Protocols:

 Biological Waste: Autoclave all blood-contaminated materials before disposal

 Chemical Waste: Segregate organic solvents, acids, and bases for proper disposal

 Sharps Disposal: Immediate disposal of needles, blades in puncture-resistant containers

 Documentation: Maintain waste disposal logs for regulatory compliance

## Regulatory Compliance Framework

Good Laboratory Practice (GLP) Adherence Standard Operating Procedure (SOP) Requirements:

 Version Control: Current protocol version identification and distribution

 Training Documentation: Staff training records and competency assessments

 Change Control: Formal approval process for protocol modifications

 Audit Trail: Complete documentation of all protocol deviations and corrective actions

Data Integrity Standards Documentation Requirements:

 Real-Time Recording: All measurements and observations recorded immediately

 Permanent Records: Use permanent ink, no erasures allowed

 Error Corrections: Single line through errors with initials and date

 Backup Systems: Electronic data backup with secure access controls

Traceability Standards:

 Sample Chain of Custody: Continuous documentation from collection to analysis

 Equipment Records: Maintenance, calibration, and usage logs

 Reagent Tracking: Lot numbers, expiration dates, storage conditions

 Personnel Records: Training, authorization, and protocol execution logs

# Technology Integration and Future Developments

## Automation Opportunities

Potential Automation Applications:

 Liquid Handling: Automated pipetting systems for improved precision and throughput

 Sample Processing: Robotic systems for consistent mixing and incubation

 Quality Control: Automated spectrophotometry with result interpretation

 Data Management: Electronic laboratory notebooks with real-time data capture

## Protocol Optimization Strategies

Continuous Improvement Initiatives:

 Method Validation: Comparative studies with alternative extraction methods  Cost Optimization: Reagent usage minimization without quality compromise  Throughput Enhancement: Batch size optimization and parallel processing

 Quality Enhancement: Advanced purification techniques for specialized applications

# Conclusion and Implementation Guidelines

## Protocol Strengths and Advantages

Key Benefits:

1. High-Quality DNA: Consistent production of sequencing-grade genomic DNA
2. Reproducibility: Standardized procedures ensuring consistent results across operators
3. Scalability: Batch processing capability for clinical trial requirements
4. Cost-Effectiveness: Optimized reagent usage with minimal waste generation
5. Regulatory Compliance: Full GLP adherence with comprehensive documentation

## Implementation Success Factors

Critical Requirements:

 Staff Training: Comprehensive training program with hands-on experience

 Equipment Maintenance: Regular calibration and preventive maintenance schedules

 Quality Control: Robust QC program with clear acceptance criteria

 Documentation: Meticulous record-keeping for traceability and compliance

 Continuous Monitoring: Regular performance assessment and improvement initiatives

## Performance Expectations

Anticipated Outcomes:

 DNA Yield: 50-100 μg high-quality genomic DNA per 5ml blood sample

 Processing Time: 4-6 hours for 12-sample batch including QC analysis

 Success Rate: >95% samples meeting quality specifications

 Reproducibility: <15% coefficient of variation between operators

 Regulatory Readiness: Full compliance with GLP and clinical trial standards

This comprehensive DNA extraction protocol provides the analytical foundation necessary for genomic analysis in clinical research settings. Strict adherence to specified parameters ensures data integrity, regulatory compliance, and optimal scientific outcomes while maintaining the highest standards of laboratory safety and quality assurance.

Protocol Status: Validated and Implementation-Ready

Effective Date: September 20, 2025

Next Review Date: March 19, 2026

Authorized by: Dr. Rahul Mehta, Laboratory Director

Quality Assurance Approval: Laboratory Quality Manager