Standard Operating Procedure

Title: 2,3-BISPHOSPHOGLYCERATE MUTASE Full Gene Sequencing Analysis
Effective Date: [Date]
Reviewed by: [Name/Position]
Approved by: [Name/Position]

1. PURPOSE

The purpose of this SOP is to provide a detailed method for performing full gene sequencing analysis of the 2,3-bisphosphoglycerate mutase (BPGM) gene. This protocol includes the steps for sample preparation, DNA extraction, PCR amplification, sequencing, data analysis, and reporting of results.

2. RESPONSIBILITY

Designated laboratory personnel are responsible for carrying out this procedure. It is the responsibility of all staff performing the analysis to ensure the protocol is followed and documented accurately. Any deviations or issues should be promptly reported to a supervisor.

3. SPECIMEN

Acceptable Specimens

- EDTA blood: 2-5 mL
- Fresh or frozen tissue: ≤100 mg
- Cultured cells: ≥10^6 viable cells

Unacceptable Specimens

- Specimens with hemolysis
- Contaminated specimens
- Insufficient quantity of sample
- Samples not stored or transported properly

4. MATERIALS AND REAGENTS

- EDTA blood collection tubes
- DNA extraction kit (e.g., Qiagen DNA Mini Kit)

- PCR reagents (e.g., Taq polymerase, primers specific for BPGM gene)
- Sequencing kit (e.g., Illumina MiSeq Reagent Kit)
- Agarose gel electrophoresis equipment
- Pipettes and tips (aerosol-resistant)
- Nuclease-free water
- 70% Ethanol
- Sequencing analysis software

5. EQUIPMENT

- Thermocycler
- Nanodrop spectrophotometer
- Agarose gel electrophoresis apparatus
- DNA sequencer (e.g., Illumina MiSeq)
- Computer with sequencing analysis software

6. PROCEDURE

Step 1: Sample Preparation

1.1 **Blood Samples:**

- Collect 2-5 mL of peripheral blood in EDTA tubes.

1.2 **Tissue Samples:**

- Ensure tissue is fresh or properly frozen, not exceeding 100 mg.

1.3 **Cell Cultures:**

- Confirm ≥10^6 viable cells.

Step 2: DNA Extraction

2.1 **Using the DNA Extraction Kit:**

- Follow the manufacturer's protocol for the extraction of DNA from the specified sample type.
- Quantify and check purity of the extracted DNA using a Nanodrop spectrophotometer. Acceptable A260/A280 ratio is between 1.8 – 2.0.

Step 3: PCR Amplification of BPGM Gene

3.1 **Primer Design:**

- Use primer sets that flank the exons and exon-intron boundaries of the BPGM gene.

3.2 **PCR Reaction Setup:**

- Prepare the PCR mix as follows:
  - DNA template: 50 ng
  - Forward primer: 10 pmol
  - Reverse primer: 10 pmol
  - Taq polymerase: as per manufacturer instructions
  - dNTPs: 200 µM each
  - Buffer: supplied with Taq enzyme
  - Nuclease-free water: to 50 µL total volume

3.3 **PCR Cycling Conditions:**

- Initial denaturation: 95°C for 3 minutes
- 35 cycles of:
  - Denaturation: 95°C for 30 seconds
  - Annealing: [primer-specific temperature] for 30 seconds
  - Extension: 72°C for 1 minute
- Final extension: 72°C for 10 minutes

Step 4: Gel Electrophoresis

4.1 **Prepare Agarose Gel:**

- Prepare a 1.5% agarose gel with DNA-safe dye.

4.2 **Load PCR Product:**

- Mix 5 µL of PCR product with 1 µL loading buffer and load into the gel.

4.3 **Run Gel Electrophoresis:**

- Run the gel at 100V for 45 minutes. Visualize the bands using a UV transilluminator.

Step 5: Sequencing

5.1 **PCR Product Purification:**

- Purify the PCR products using an appropriate purification method (e.g., ExoSAP-IT).

5.2 **Library Preparation:**

- Prepare the sequencing library using the Illumina MiSeq Reagent Kit following the manufacturer's instructions.

5.3 **Sequencing Reaction:**

- Load the prepared library into the DNA sequencer and start the sequencing run.

Step 6: Data Analysis

6.1 **Sequence Alignment:**

- Align the raw sequence data to the reference BPGM gene sequence using bioinformatics software.

6.2 **Variant Calling:**

- Identify variants using variant calling software and annotate using an appropriate database (e.g., ClinVar, dbSNP).

6.3 **Data Interpretation:**

- Interpret variants in the context of known functional consequences and clinical significance.

Step 7: Reporting Results

7.1 **Report Preparation:**

- Compile a report including patient information, sequencing methodology, findings, interpretation, and recommendations.

7.2 **Review and Verification:**

- Review the report for accuracy and completeness. Obtain verification from a senior scientist or supervisor.

7.3 **Results Transmission:**

- Send the finalized report to the ordering physician or healthcare provider. Ensure all documentation complies with regulatory standards.

7. QUALITY CONTROL

- Perform positive and negative controls with each sequencing run to ensure the accuracy and validity of results.
- Ensure reagents and equipment are within their usage life and maintain calibration of instruments regularly.

8. REFERENCES

- Manufacturer's instructions for DNA extraction kit, thermocycler, DNA sequencer, and reagents.
- Current literature and database for BPGM gene analysis.

This SOP provides a comprehensive guide for the full gene sequencing of the 2,3-bisphosphoglycerate mutase gene, ensuring

consistency, accuracy, and compliance with regulatory standards throughout the analytical phase.