# Ecological Study Protocol: Human Microbiome and Gene Expression Regulation in Type 2 Diabetes Mellitus (T2DM)

## 1. Background & Rationale

Type 2 Diabetes Mellitus (T2DM) is a growing public health challenge in India, with increasing prevalence in both urban and rural populations. Emerging evidence indicates that alterations in gut microbiome composition and dysregulated host gene expression may contribute to insulin resistance, chronic inflammation, and metabolic dysfunction. This ecological study aims to investigate regional and population-level patterns in microbiome diversity, functional gene expression, and their association with T2DM prevalence.

## 2. Objectives

Primary Objective: To evaluate the association between gut microbiome composition, functional diversity, and host gene expression profiles with T2DM prevalence in diverse ecological settings across India.

Secondary Objectives:

1. 1. To assess regional variations in microbiome profiles and identify potential protective or risk-associated microbial taxa.
2. 2. To correlate dietary patterns, lifestyle factors, and environmental exposures with microbiome and gene expression variations.
3. 3. To identify potential microbial and genetic biomarkers predictive of T2DM risk.

## 3. Study Design

Design: Multi-site ecological study.  
Sites: Selected urban, peri-urban, and rural clusters across different Indian states.  
Population: Adults aged 30–65 years, stratified by T2DM status (diagnosed vs. non-diabetic).  
Sample Size: Minimum 500 participants per ecological region.  
Sampling Frame: Random cluster sampling within each ecological region.

## 4. Methodology

4.1 Data Collection:

* • Demographics, medical history, anthropometric measurements.
* • Dietary recall (24h + food frequency questionnaire).
* • Stool samples for microbiome analysis (16S rRNA + metagenomics).
* • Blood samples for fasting glucose, HbA1c, and RNA extraction for transcriptomics.

4.2 Laboratory Analysis:

* • DNA extraction using standardized kits (Qiagen or equivalent).
* • Microbiome profiling: 16S rRNA sequencing (Illumina MiSeq).
* • Shotgun metagenomics for functional profiling.
* • Host gene expression: RNA-seq using Illumina NovaSeq platform.

## 5. Data Analysis Plan

* • Alpha & beta diversity metrics for microbiome.
* • Differential abundance analysis (DESeq2, LEfSe).
* • Correlation between microbiome features and gene expression modules.
* • Ecological regression models adjusting for confounders.

## 6. Ethics & Intellectual Property Considerations

This study will be conducted following ICMR ethical guidelines and will seek IRB/IEC approval at each participating site. Novel microbiome or genetic biomarkers identified may be patented under Indian patent law, with institutional ownership.

## 7. Timeline

• Months 1–3: Site selection, ethics approvals, SOP finalization.

• Months 4–9: Participant recruitment & sample collection.

• Months 10–15: Laboratory analyses.

• Months 16–18: Data analysis & dissemination.