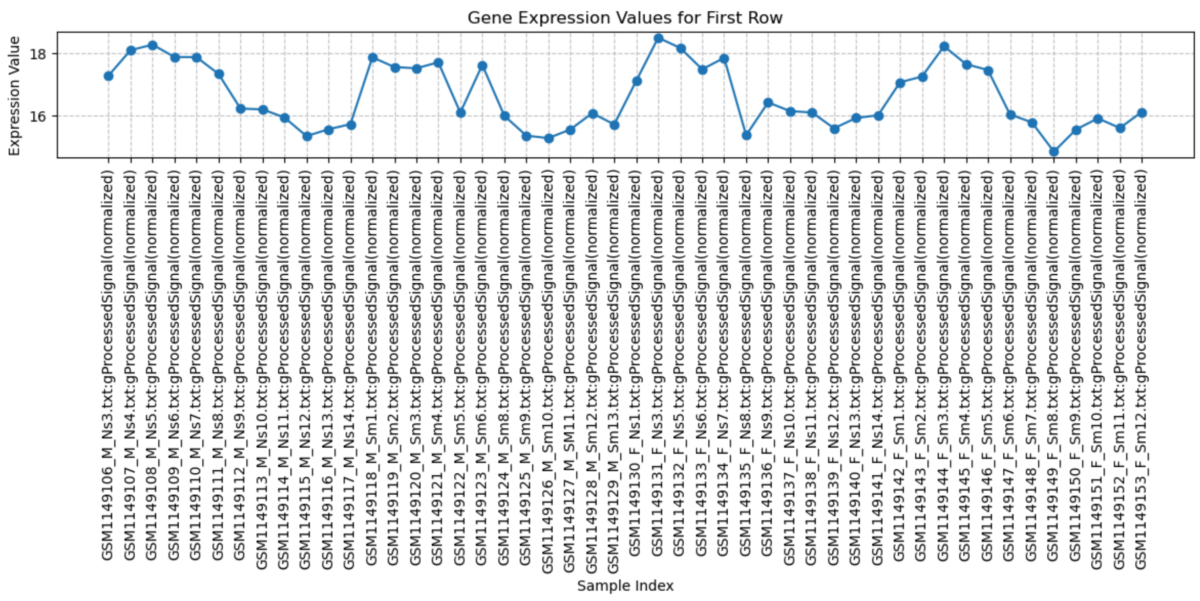
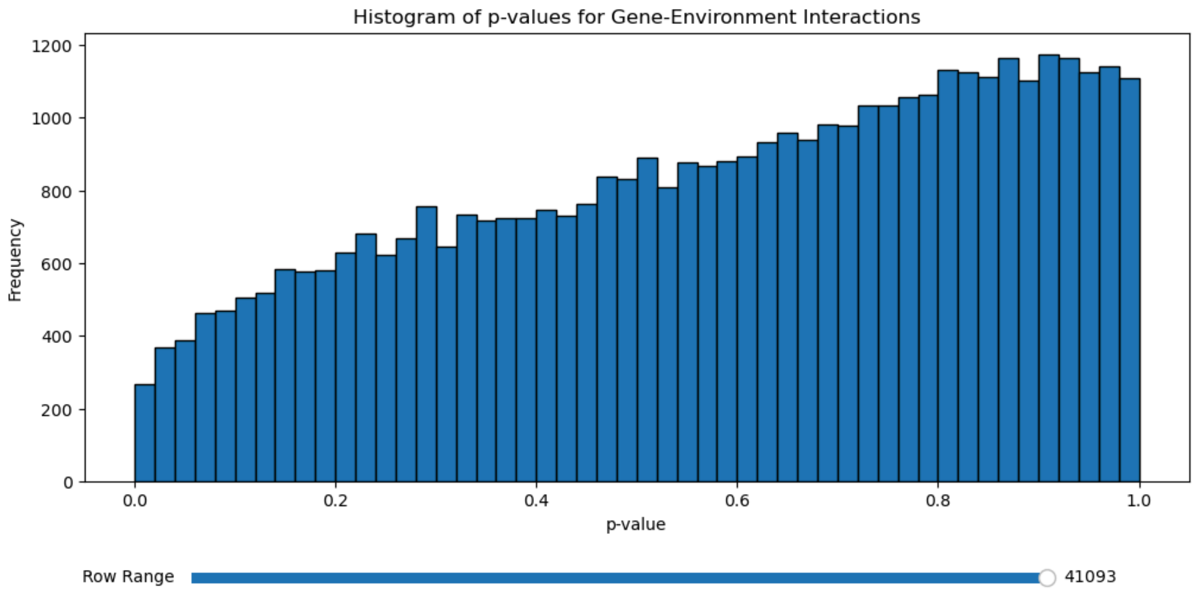
Report

- **Visualization of data values for the first row:**



- **Histogram of p values**



Based on the histogram of p-values for gene-environment interactions, we can interpret the results as follows:

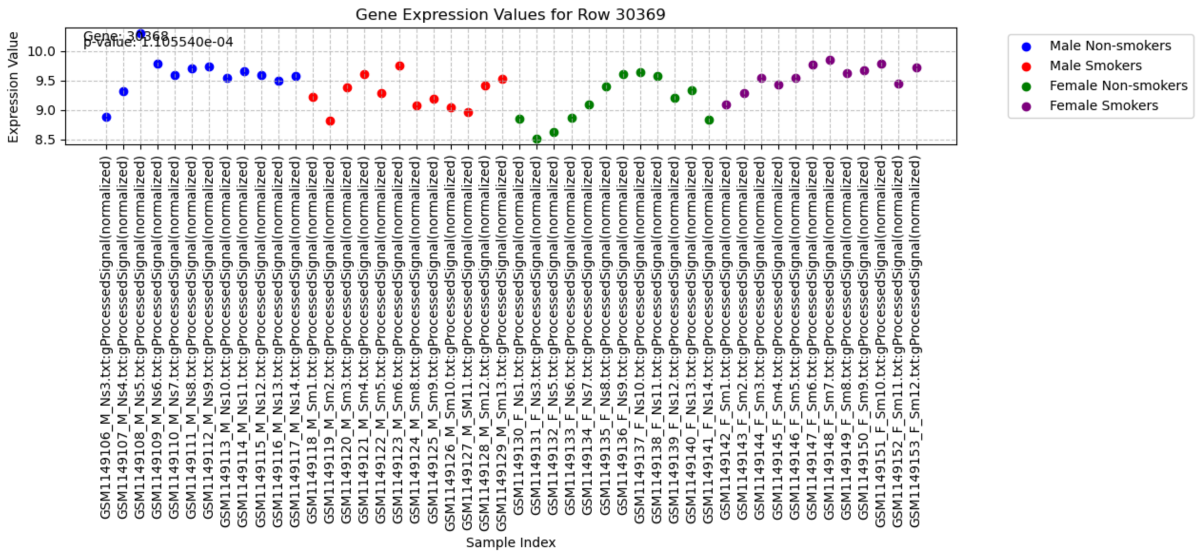
1. Distribution shape: The histogram shows a relatively uniform distribution of p-values across the range from 0 to 1, with a slight increase in frequency towards higher p-values.

2. Interpretation:
- P-values close to 0 indicate strong evidence against the null hypothesis (i.e., there is likely a significant gene-environment interaction).

- P-values close to 1 indicate weak evidence against the null hypothesis (i.e., there is likely no significant gene-environment interaction).

3.Findings: There is a slight increase in frequency for higher p-values, suggesting that for many genes, there may not be strong evidence of gene-environment interactions. However, there are still a considerable number of genes with low p-values, indicating potential significant interactions for some genes.

- **Gene expression value for a row with a low p-value**



Data preparation

Data Description

Data is generated from white blood cells from 48 individuals. Key details:

- A single file with 48 columns of data, plus some auxiliary columns
- Auxiliary columns: Probe name, Gene Symbol, Entrez Gene Id (ignore the rest)
- A single gene (identified by a Gene Symbol or Entrez Gene Id) could have multiple probes
- Total of 41,094 probes

Data Columns:

1. 12 Male Non-smokers (106-117)
2. 12 Male Smokers (118-129)
3. 12 Female Non-Smokers (130-141)
4. 12 Female Smokers (142-153)

Important notes:

- Values are logs to the base 2 of the original value
- Some 0 values are present due to thresholding low values before taking the log

```
import pandas as pd
import numpy as np


# Load the data
data = pd.read_csv('Raw Data_GeneSpring.txt', sep='\t')  # Load data using tab as separator


# Display the first few rows and basic information about the dataset
print(data.head())
print(data.info())
```

```
        ProbeName  GSM1149106_M_Ns3.txt:gProcessedSignal(normalized)  \
0  GE_BrightCorner                                          17.288560
1       DarkCorner                                           2.172766
2      A_24_P66027                                          11.954556
3      A_32_P77178                                           6.224496
4     A_23_P212522                                           9.328137

   GSM1149107_M_Ns4.txt:gProcessedSignal(normalized)  \
0                                          18.103434
1                                           0.075925
2                                          11.681619
3                                           6.963845
4                                           9.164498

   GSM1149108_M_Ns5.txt:gProcessedSignal(normalized)  \
0                                          18.280110
1                                           0.232402
2                                          11.831579
3                                           7.096990
4                                           8.934509

   GSM1149109_M_Ns6.txt:gProcessedSignal(normalized)  \
0                                          17.883734
1                                           0.052573
2                                          12.262896
3                                           7.319873
4                                           9.166321

   GSM1149110_M_Ns7.txt:gProcessedSignal(normalized)  \
0                                          17.879555
1                                           0.483300
2                                          11.233925
3                                           6.611215
4                                           9.175541

   GSM1149111_M_Ns8.txt:gProcessedSignal(normalized)  \
0                                          17.340961
1                                           0.240482
2                                          11.722222
3                                           6.760409
4                                           8.810351

   GSM1149112_M_Ns9.txt:gProcessedSignal(normalized)  \
0                                          16.226210
```

```
1                                    0.327676
2                                   11.582652
3                                    7.810502
4                                   10.665818

   GSM1149113_M_Ns10.txt:gProcessedSignal(normalized)  \
0                                   16.203045
1                                    0.041980
2                                   13.039122
3                                    8.377619
4                                   10.681737

   GSM1149114_M_Ns11.txt:gProcessedSignal(normalized)  ...  \
```

```
# Separate auxiliary columns and gene expression data
auxiliary_columns = ['ProbeName', 'GeneSymbol', 'EntrezGeneID', 'Go']
gene_expression_columns = [col for col in data.columns if col not in auxiliary_columns]


# Create separate dataframes for auxiliary information and gene expression data
auxiliary_data = data[auxiliary_columns]
gene_expression_data = data[gene_expression_columns]


# Convert gene expression data to numeric type, replacing any non-numeric values with NaN
gene_expression_data = gene_expression_data.apply(pd.to_numeric, errors='coerce')


# Display basic statistics of the gene expression data
print(gene_expression_data.describe())
```

```
       GSM1149106_M_Ns3.txt:gProcessedSignal(normalized)  \
count                                      41093.000000
mean                                           6.128693
std                                            4.114148
min                                            0.000000
25%                                            2.932579
50%                                            6.374927
75%                                            9.182739
max                                           18.298971

       GSM1149107_M_Ns4.txt:gProcessedSignal(normalized)  \
count                                      41093.000000
mean                                           6.350905
std                                            4.154255
min                                            0.000000
25%                                            3.195622
50%                                            6.636967
75%                                            9.374315
max                                           18.557070

       GSM1149108_M_Ns5.txt:gProcessedSignal(normalized)  \
count                                      41093.000000
mean                                           6.293946
std                                            4.206224
min                                            0.000000
25%                                            3.023633
50%                                            6.596164
75%                                            9.382986
max                                           18.698050

       GSM1149109_M_Ns6.txt:gProcessedSignal(normalized)  \
count                                      41093.000000
mean                                           6.531112
std                                            4.031424
min                                            0.000000
25%                                            3.623112
50%                                            6.660977
75%                                            9.434568
max                                           18.612185

       GSM1149110_M_Ns7.txt:gProcessedSignal(normalized)  \
count                                      41093.000000
mean                                           6.335960
std                                            4.083656
min                                            0.000000
25%                                            3.301992
50%                                            6.543399
75%                                            9.283957
max                                           18.552126

       GSM1149111_M_Ns8.txt:gProcessedSignal(normalized)  \
count                                      41093.000000
mean                                           6.227665
std                                            4.124976
min                                            0.000000
25%                                            3.113181
50%                                            6.465138
75%                                            9.219945
```

```
# Check for missing values
print("Missing values in gene expression data:")
print(gene_expression_data.isnull().sum().sum())
```

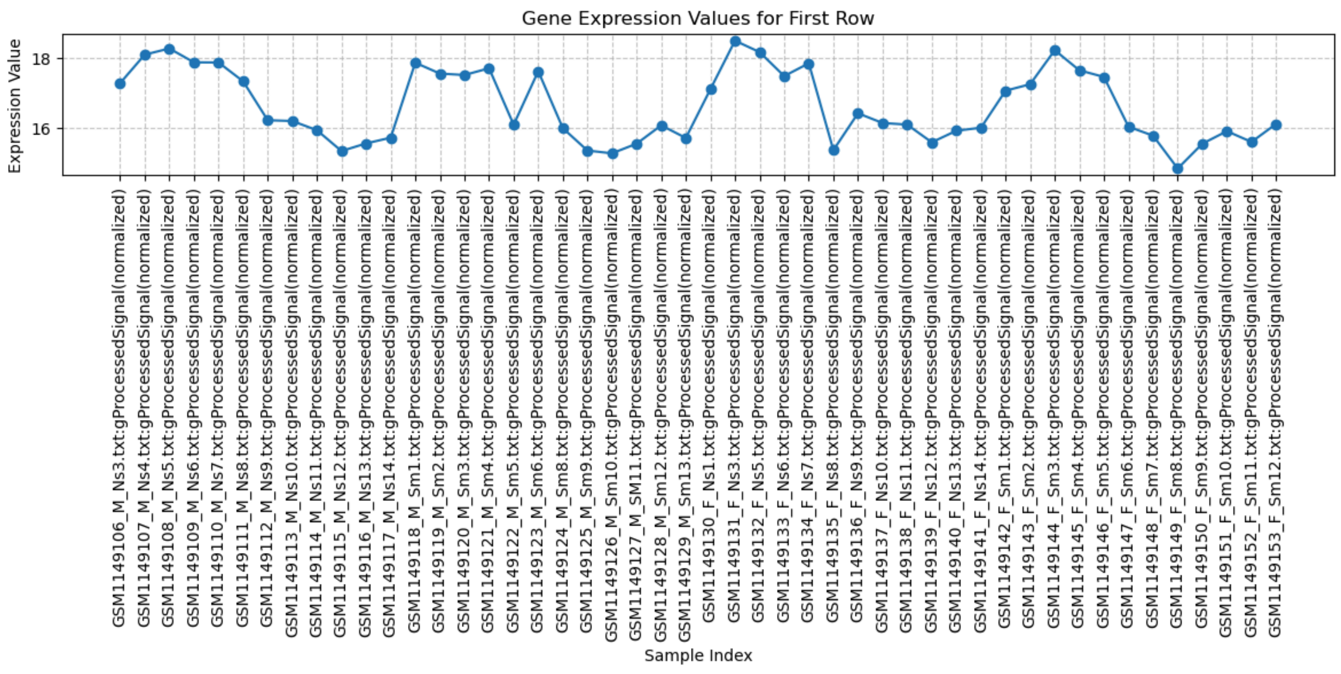
```
Missing values in gene expression data:
0
```

```
# Group data by gender and smoking status
male_non_smokers = gene_expression_data.iloc[:, 0:12]
male_smokers = gene_expression_data.iloc[:, 12:24]
female_non_smokers = gene_expression_data.iloc[:, 24:36]
female_smokers = gene_expression_data.iloc[:, 36:48]
```

```
# Display the shape of each group
print("Shape of each group:")
print(f"Male Non-smokers: {male_non_smokers.shape}")
print(f"Male Smokers: {male_smokers.shape}")
print(f"Female Non-smokers: {female_non_smokers.shape}")
print(f"Female Smokers: {female_smokers.shape}")
```

```
Shape of each group:
Male Non-smokers: (41093, 12)
Male Smokers: (41093, 12)
Female Non-smokers: (41093, 12)
Female Smokers: (41093, 12)
```

```
import matplotlib.pyplot as plt  # Import matplotlib for plotting
import nbformat  # Import nbformat to check its version

# Get the first row of gene expression data
first_row = gene_expression_data.iloc[0]

# Create a line plot of the first row values
plt.figure(figsize=(12, 6))  # Set figure size
plt.plot(range(len(first_row)), first_row.values, marker='o')  # Plot values with markers

# Customize the plot
plt.title('Gene Expression Values for First Row')  # Add title
plt.xlabel('Sample Index')  # Label x-axis
plt.ylabel('Expression Value')  # Label y-axis
plt.grid(True, linestyle='--', alpha=0.7)  # Add grid lines

# Rotate x-axis labels for better readability
plt.xticks(range(len(first_row)), first_row.index, rotation=90)

# Adjust layout to prevent cutting off labels
plt.tight_layout()

# Show the plot
plt.show()
```

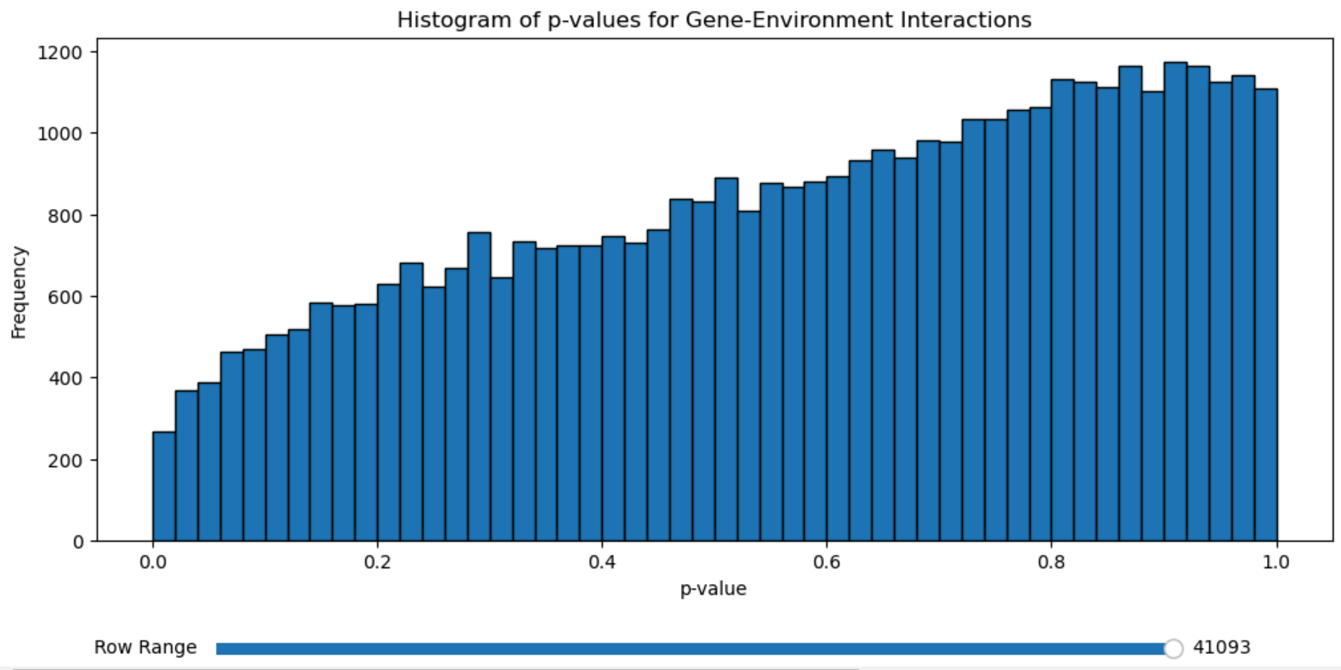


```
def custom_two_way_anova(data, factor1, factor2):
    n = len(data)  # Total number of observations
    grand_mean = np.mean(data)  # Overall mean

    # Calculate SST (Total Sum of Squares)
    sst = np.sum((data - grand_mean)**2)  # Calculate total sum of squares

    # Calculate SS for Factor 1
    ss1 = sum(len(group) * (np.mean(group) - grand_mean)**2 for group in [data[factor1 == level] for level in np.unique(factor1)])  # S

    # Calculate SS for Factor 2
    ss2 = sum(len(group) * (np.mean(group) - grand_mean)**2 for group in [data[factor2 == level] for level in np.unique(factor2)])  # S

    # Calculate SS for Interaction
    ss_interaction = sum(len(group) * (np.mean(group) - grand_mean)**2 for group in [data[(factor1 == f1) & (factor2 == f2)] for f1 in

    # Calculate SSE (Error Sum of Squares)
    sse = sst - ss1 - ss2 - ss_interaction  # Error sum of squares

    # Degrees of freedom
    df1 = len(np.unique(factor1)) - 1  # Degrees of freedom for factor 1
    df2 = len(np.unique(factor2)) - 1  # Degrees of freedom for factor 2
    df_interaction = df1 * df2  # Degrees of freedom for interaction
    dfe = n - (len(np.unique(factor1)) * len(np.unique(factor2)))  # Degrees of freedom for error

    # Mean Squares
    ms1 = ss1 / df1  # Mean square for factor 1
    ms2 = ss2 / df2  # Mean square for factor 2
    ms_interaction = ss_interaction / df_interaction  # Mean square for interaction
    mse = sse / dfe  # Mean square error

    # F-values
    f1 = ms1 / mse  # F-value for factor 1
    f2 = ms2 / mse  # F-value for factor 2
    f_interaction = ms_interaction / mse  # F-value for interaction

    # P-values
    p1 = 1 - stats.f.cdf(f1, df1, dfe)  # P-value for factor 1
    p2 = 1 - stats.f.cdf(f2, df2, dfe)  # P-value for factor 2
    p_interaction = 1 - stats.f.cdf(f_interaction, df_interaction, dfe)  # P-value for interaction

    return (f1, p1), (f2, p2), (f_interaction, p_interaction)  # Return F-values and p-values for both factors and interaction


def perform_two_way_anova(row_index):
    row_data = data.iloc[row_index]  # Get the row data for the specified index

    # Prepare data for ANOVA
    male_non_smoker = row_data[male_non_smokers.columns].values  # Extract male non-smoker values
    male_smoker = row_data[male_smokers.columns].values  # Extract male smoker values
    female_non_smoker = row_data[female_non_smokers.columns].values  # Extract female non-smoker values
    female_smoker = row_data[female_smokers.columns].values  # Extract female smoker values

    # Combine all data into a single array
    all_data = np.concatenate([male_non_smoker, male_smoker, female_non_smoker, female_smoker])  # Combine all data

    # Create factor arrays
    gender = np.repeat(['Male', 'Female'], len(all_data) // 2)  # Create gender factor array
    smoking = np.tile(np.repeat(['Non-smoker', 'Smoker'], len(male_non_smoker)), 2)  # Create smoking factor array

    # Perform two-way ANOVA using custom function
    _, _, (_, p_interaction) = custom_two_way_anova(all_data, gender, smoking)  # Perform custom two-way ANOVA

    return p_interaction  # Return p-value for interaction (different response to smoke in men vs women)


# Create an array to store p-values for each row
p_values = np.zeros(data.shape[0])  # Initialize array with zeros for each row

# Iterate through each row and calculate p-value
for i in range(data.shape[0]):
    p_values[i] = perform_two_way_anova(i)  # Calculate and store p-value for each row
    print(f"Processing row {i+1}/{data.shape[0]}, p-value: {p_values[i]:.4f}")  # Print progress and p-value for each row

# Print the shape of the p_values array to confirm
print(f"Shape of p_values array: {p_values.shape}")  # Display the shape of the resulting array
```

```
Processing row 1/41093, p-value: 0.9761
Processing row 2/41093, p-value: 0.5642
Processing row 3/41093, p-value: 0.4624
Processing row 4/41093, p-value: 0.6588
Processing row 5/41093, p-value: 0.8536
Processing row 6/41093, p-value: 0.5111
Processing row 7/41093, p-value: 0.7951
Processing row 8/41093, p-value: 0.5469
Processing row 9/41093, p-value: 0.8972
Processing row 10/41093, p-value: 0.9469
Processing row 11/41093, p-value: 0.5371
Processing row 12/41093, p-value: 0.6049
Processing row 13/41093, p-value: 0.6592
Processing row 14/41093, p-value: 0.3179
Processing row 15/41093, p-value: 0.5989
Processing row 16/41093, p-value: 0.8493
Processing row 17/41093, p-value: 0.3753
Processing row 18/41093, p-value: 0.8546
Processing row 19/41093, p-value: 0.2126
Processing row 20/41093, p-value: 0.3606
Processing row 21/41093, p-value: 0.7641
Processing row 22/41093, p-value: 0.5702
Processing row 23/41093, p-value: 0.6062
Processing row 24/41093, p-value: 0.7621
Processing row 25/41093, p-value: 0.7680
Processing row 26/41093, p-value: 0.7760
Processing row 27/41093, p-value: 0.8274
Processing row 28/41093, p-value: 0.2674
Processing row 29/41093, p-value: 0.9726
Processing row 30/41093, p-value: 0.7795
Processing row 31/41093, p-value: 0.1684
Processing row 32/41093, p-value: 0.2675
Processing row 33/41093, p-value: 0.9584
Processing row 34/41093, p-value: 0.7100
Processing row 35/41093, p-value: 0.2198
Processing row 36/41093, p-value: 0.7664
Processing row 37/41093, p-value: 0.9002
Processing row 38/41093, p-value: 0.5480
Processing row 39/41093, p-value: 0.7606
Processing row 40/41093, p-value: 0.8798
Processing row 41/41093, p-value: 0.1550
Processing row 42/41093, p-value: 0.6512
Processing row 43/41093, p-value: 0.2734
Processing row 44/41093, p-value: 0.0233
Processing row 45/41093, p-value: 0.4668
Processing row 46/41093, p-value: 0.1403
Processing row 47/41093, p-value: 0.3644
Processing row 48/41093, p-value: 0.7771
Processing row 49/41093, p-value: 0.3745
Processing row 50/41093, p-value: 0.5188
Processing row 51/41093, p-value: 0.3238
Processing row 52/41093, p-value: 0.4046
Processing row 53/41093, p-value: 0.3527
Processing row 54/41093, p-value: 0.6807
Processing row 55/41093, p-value: 0.7222
Processing row 56/41093, p-value: 0.3963
Processing row 57/41093, p-value: 0.8501
Processing row 58/41093, p-value: 0.6599
```

Note that the row 1 in data corresponds to the entry at index 0 in the p_values array.

```
import matplotlib.pyplot as plt
from matplotlib.widgets import Slider

# Create the main figure and axis
fig, ax = plt.subplots(figsize=(12, 6))  # Create a figure with a larger size for better visibility
plt.subplots_adjust(bottom=0.25)  # Adjust the bottom margin to make room for the slider

# Plot the initial histogram
n, bins, patches = ax.hist(p_values, bins=50, edgecolor='black')  # Create histogram with 50 bins and black edges

# Set labels and title
ax.set_xlabel('p-value')  # Label for x-axis
ax.set_ylabel('Frequency')  # Label for y-axis
ax.set_title('Histogram of p-values for Gene-Environment Interactions')  # Title for the plot

# Create the slider axis
slider_ax = plt.axes([0.2, 0.1, 0.6, 0.03])  # Position of the slider [left, bottom, width, height]
slider = Slider(slider_ax, 'Row Range', 1, data.shape[0], valinit=data.shape[0], valstep=1)  # Create slider object

# Function to update the plot when the slider is moved
def update(val):
    num_rows = int(slider.val)  # Get the current value of the slider
    ax.clear()  # Clear the current plot
    ax.hist(p_values[:num_rows], bins=50, edgecolor='black')  # Plot new histogram with updated data
    ax.set_xlabel('p-value')  # Reset x-axis label
    ax.set_ylabel('Frequency')  # Reset y-axis label
    ax.set_title(f'Histogram of p-values for Gene-Environment Interactions (Rows 1-{num_rows})')  # Update title with row range
    fig.canvas.draw_idle()  # Redraw the figure

# Connect the update function to the slider
slider.on_changed(update)  # Call update function when slider value changes

plt.show()  # Display the plot
```



Based on the histogram of p-values for gene-environment interactions, we can interpret the results as follows:

1. Distribution shape: The histogram shows a relatively uniform distribution of p-values across the range from 0 to 1, with a slight increase in frequency towards higher p-values.
2. Interpretation:
    - P-values close to 0 indicate strong evidence against the null hypothesis (i.e., there is likely a significant gene-environment interaction).
    - P-values close to 1 indicate weak evidence against the null hypothesis (i.e., there is likely no significant gene-environment interaction).

3.Findings: There is a slight increase in frequency for higher p-values, suggesting that for many genes, there may not be strong evidence of gene-environment interactions. However, there are still a considerable number of genes with low p-values, indicating potential significant interactions for some genes.

```
# Find rows with p-values < 0.05
significant_rows = data[p_values < 0.05]  # Filter data for rows with p-values less than 0.05

# Print the number of significant rows
print(f"Number of rows with p-values < 0.05: {len(significant_rows)}")  # Display count of significant rows

# Display the first few significant rows
print("\nFirst few significant rows:")
print(significant_rows.head())  # Show the first 5 rows of significant data

# Calculate the percentage of significant rows
percentage_significant = (len(significant_rows) / len(data)) * 100  # Calculate percentage of significant rows
print(f"\nPercentage of rows with p-values < 0.05: {percentage_significant:.2f}%")  # Display percentage with 2 decimal places

# Optional: Save significant rows to a CSV file
significant_rows.to_csv('significant_gene_environment_interactions.csv', index=False)  # Save significant rows to CSV
print("\nSignificant rows have been saved to 'significant_gene_environment_interactions.csv'")  # Confirm save operation
```

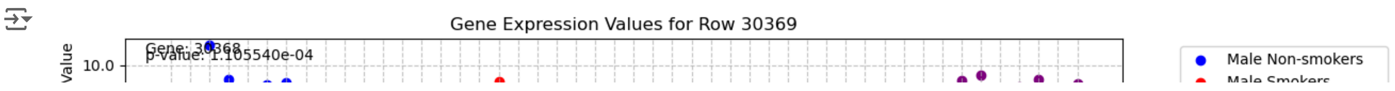
```
Number of rows with p-values < 0.05: 811

First few significant rows:
        ProbeName  GSM1149106_M_Ns3.txt:gProcessedSignal(normalized)  \
43   A_23_P111020                                           6.274315   
62   A_23_P151294                                           4.025540   
88   A_24_P926770                                           7.474671   
123  A_23_P145024                                          11.561870   
127  A_24_P910381                                           6.558103   

     GSM1149107_M_Ns4.txt:gProcessedSignal(normalized)  \
43                                            6.972955   
62                                            6.789796   
88                                            7.623500   
123                                          11.799589   
127                                           6.041059   

     GSM1149108_M_Ns5.txt:gProcessedSignal(normalized)  \
43                                            7.077835   
62                                            4.723725   
88                                            7.720977   
123                                          11.227537   
127                                           4.144054   

     GSM1149109_M_Ns6.txt:gProcessedSignal(normalized)  \
43                                            7.144897   
62                                            6.423915   
88                                            8.082088   
123                                          10.797735   
127                                           5.286856   

     GSM1149110_M_Ns7.txt:gProcessedSignal(normalized)  \
43                                            6.687301   
62                                            4.090033   
88                                            8.051618   
123                                          11.376524   
127                                           5.351962   

     GSM1149111_M_Ns8.txt:gProcessedSignal(normalized)  \
43                                            6.699010   
62                                            7.397929   
88                                            7.837849   
123                                          11.506655   
127                                           4.442767   

     GSM1149112_M_Ns9.txt:gProcessedSignal(normalized)  \
43                                            7.144043   
62                                            6.831710   
88                                            7.911806   
123                                          12.245893   
127                                           6.438065   

     GSM1149113_M_Ns10.txt:gProcessedSignal(normalized)  \
43                                            6.988871   
62                                            5.415668   
88                                            8.179203   
123                                          11.970752   
127                                           7.659210   
```

```
# Find the index of the minimum non-NaN p-value
min_p_value_index = np.nanargmin(p_values)  # Get the index of the lowest non-NaN p-value in the numpy array

# Get the minimum non-NaN p-value
min_p_value = np.nanmin(p_values)  # Get the minimum non-NaN p-value
```

```python
# Print the row number (index + 1) and its p-value
print(f"Row number with minimum p-value: {min_p_value_index + 1}")  # Print row number (index + 1 because indexing starts at 0)
print(f"Minimum p-value: {min_p_value:.6e}")  # Print minimum p-value in scientific notation
```

```
Row number with minimum p-value: 30369
Minimum p-value: 1.105540e-04
```

```python
# Get the row 30369 of gene expression data
row_30369 = gene_expression_data.iloc[30368]  # Subtract 1 because indexing starts at 0

# Create a scatter plot of the row 30369 values
plt.figure(figsize=(12, 6))  # Set figure size

# Define groups and their corresponding colors
groups = ['Male Non-smokers', 'Male Smokers', 'Female Non-smokers', 'Female Smokers']
colors = ['blue', 'red', 'green', 'purple']

# Plot each group separately
for i, (group, color) in enumerate(zip(groups, colors)):
    start = i * 12
    end = start + 12
    plt.scatter(range(start, end), row_30369.values[start:end], color=color, label=group)  # Plot values with colored markers

# Customize the plot
plt.title('Gene Expression Values for Row 30369')  # Add title
plt.xlabel('Sample Index')  # Label x-axis
plt.ylabel('Expression Value')  # Label y-axis
plt.grid(True, linestyle='--', alpha=0.7)  # Add grid lines

# Move legend outside the plot area
plt.legend(bbox_to_anchor=(1.05, 1), loc='upper left')  # Move legend outside and to the right

# Rotate x-axis labels for better readability
plt.xticks(range(len(row_30369)), row_30369.index, rotation=90)

# Add gene name or identifier as text annotation
gene_name = gene_expression_data.index[30368]  # Get gene name or identifier
plt.text(0.02, 0.98, f"Gene: {gene_name}", transform=plt.gca().transAxes, verticalalignment='top', fontsize=10)

# Add p-value as text annotation
p_value = p_values[30368]  # Get p-value for this gene
plt.text(0.02, 0.93, f"p-value: {p_value:.6e}", transform=plt.gca().transAxes, verticalalignment='top', fontsize=10)

# Adjust layout to prevent cutting off labels and legend
plt.tight_layout()
plt.subplots_adjust(right=0.85)  # Make room for legend on the right

# Show the plot
plt.show()

# Print additional information about the gene
print(f"Gene: {gene_name}")
print(f"p-value: {p_value:.6e}")
print(f"Mean expression value: {row_30369.mean():.4f}")
print(f"Standard deviation: {row_30369.std():.4f}")
print(f"Minimum expression value: {row_30369.min():.4f}")
print(f"Maximum expression value: {row_30369.max():.4f}")
```


Gene Expression Values for Row 30369
Gene: 30368
p-value: 1.105540e-04
Value
10.0
Male Non-smokers
Male Smokers