QTL Analysis Pipeline - Complete Pipeline & Output Documentation

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Pipeline Overview

What the Pipeline Does

The QTL Analysis Pipeline performs comprehensive genetic association analysis to identify variants that influence molecular traits:

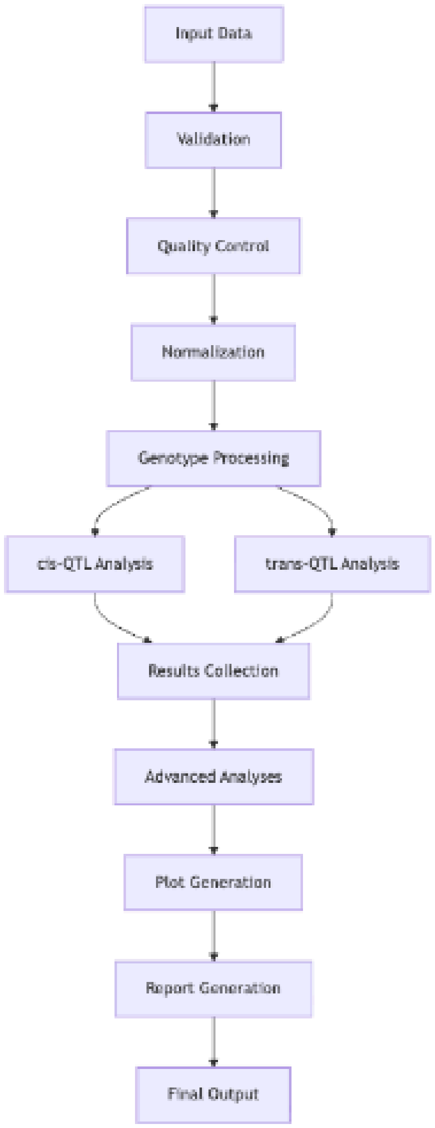
- cis-QTL Analysis: Tests variants within 1Mb of each gene
- trans-QTL Analysis: Tests variants across the entire genome
- Multi-omics Integration: Simultaneous analysis of expression, protein, and splicing
- Quality Control: Comprehensive data quality assessment
- Advanced Analyses: Interaction testing, fine-mapping, and visualization

Pipeline Architecture

```
text
```

```
Input Data → Validation → QC → Normalization → QTL Mapping → Results → Reports
     ↓           ↓         ↓         ↓            ↓           ↓        ↓
   VCF/BCF    Format    Sample    Data       Statistical  Summary  Interactive
   Phenotypes Checks    QC        Transformation  Tests    Files    Reports
   Covariates          Variant QC              Association
   Annotations         PCA                    Testing
```

Complete Workflow



High-Level Pipeline Flowchart

Runtime Expectations

Analysis Type	Sample Size	Variant Count	Expected Runtime	Memory Usage
cis-eQTL	100 samples	1M variants	1-2 hours	8-16GB
cis-eQTL	500 samples	5M variants	4-8 hours	16-32GB
trans-eQTL	500 samples	5M variants	24-48 hours	32-64GB
Multi-omics	1000 samples	10M variants	2-3 days	64-128GB

Step-by-Step Pipeline Process

Step 1: Input Validation

Purpose: Ensure all input files are correct and compatible

Process:

- Checks file existence and permissions
- Validates file formats (VCF, BED, TSV)
- Verifies sample concordance across files
- Checks chromosome naming consistency
- Validates configuration parameters

Key Outputs:

- Validation report in logs

- Sample concordance summary
- Format compatibility check

Step 2: Quality Control

Purpose: Identify and filter low-quality data

Process:

- Sample-level QC:
    - Missingness rate calculation
    - Heterozygosity analysis
    - Sex check validation
    - Relatedness detection
- Variant-level QC:
    - Missingness rate
    - Hardy-Weinberg Equilibrium
    - Minor Allele Frequency
    - Call rate thresholds
- Phenotype QC:
    - Missing value analysis
    - Outlier detection
    - Distribution assessment

Key Outputs:

- QC summary reports
- Filtered genotype data
- Sample and variant exclusion lists

Step 3: Data Normalization

Purpose: Transform data to meet statistical assumptions

Process:

- eQTL Normalization:
    - VST (Variance Stabilizing Transformation)
    - Log2 transformation

    - Quantile normalization
    - TPM normalization
- pQTL Normalization:
    - Log2 transformation with pseudocount
    - Z-score standardization
    - Quantile normalization
- sQTL Normalization:
    - Log2 transformation for PSI values
    - Arcsinh transformation
    - Z-score standardization

Key Outputs:

- Normalized phenotype files
- Normalization comparison plots
- Transformation parameters

Step 4: Genotype Processing

Purpose: Prepare genotype data for efficient analysis

Process:

- Format conversion (VCF → PLINK/BCF)
- Chromosome normalization
- Variant filtering (MAF, missingness, HWE)
- Multi-allelic site handling
- Sample matching across datasets

Key Outputs:

- Processed genotype files
- Filtering statistics
- Format-converted data

Step 5: QTL Mapping

Purpose: Perform statistical association testing

Process:

- cis-QTL Analysis:
    - Linear regression for variant-gene pairs
    - Permutation testing for FDR calculation
    - Window-based testing (default: 1Mb)
- trans-QTL Analysis:
    - Genome-wide association testing
    - Multiple testing correction
    - Conditional analysis

Statistical Model:

```
text

Phenotype ~ Genotype + Covariates + ε
```

Where:

- Phenotype: Normalized molecular trait
- Genotype: Genetic variant dosage
- Covariates: Technical and biological confounders
- ε: Error term

Key Outputs:

- Association statistics
- Nominal p-values
- FDR-corrected results
- Effect size estimates

Step 6: Advanced Analyses

Purpose: Provide deeper biological insights

Process:

- Interaction Analysis: Test for context-specific effects
- Fine-mapping: Identify causal variants
- Conditional Analysis: Independent signal detection
- Pathway Enrichment: Biological context interpretation

Key Outputs:

- Interaction results
- Credible sets
- Conditional association statistics
- Pathway analysis results

Step 7: Visualization and Reporting

Purpose: Generate interpretable results and summaries

Process:

- Manhattan plots for genome-wide results
- QQ plots for inflation assessment
- Volcano plots for effect size visualization
- Locus zoom plots for regional association
- Interactive HTML reports

Key Outputs:

- Static plots (PNG/PDF)
- Interactive plots (HTML)
- Comprehensive reports
- Summary statistics

Output Structure

Complete Directory Tree

```
text

results/
├── QTL_results/                   # Main QTL analysis results
│   ├── eqtl/                     # Expression QTL results
│   │   ├── cis/                  # cis-eQTL results
│   │   │   ├── nominals.txt     # All association results
│   │   │   ├── significant.txt  # FDR-significant results
│   │   │   ├── permutations/    # Permutation results
│   │   │   └── summary.txt      # Analysis summary
│   │   └── trans/               # trans-eQTL results
│   │       ├── nominals.txt
│   │       ├── significant.txt
│   │       └── summary.txt
│   ├── pqtl/                     # Protein QTL results
│   └── sqtl/                     # Splicing QTL results
├── GWAS_results/                  # GWAS results (if enabled)
│   ├── gwas_combined_results.txt
│   ├── individual_phenotypes/    # Per-phenotype results
│   └── qc_report.txt
├── QC_reports/                    # Quality control outputs
│   ├── genotype_qc/
│   │   ├── sample_missingness.png
│   │   ├── maf_distribution.png
│   │   ├── heterozygosity.png
│   │   └── hwe_violations.txt
│   ├── phenotype_qc/
│   │   ├── expression_qc.png
│   │   ├── protein_qc.png
│   │   └── splicing_qc.png
│   ├── sample_concordance/
│   │   ├── overlap_summary.txt
│   │   └── concordance_plot.png
│   └── comprehensive_qc_report.html
├── plots/                         # All generated visualizations
│   ├── manhattan/                # Manhattan plots
│   │   ├── eqtl_cis_manhattan.png
│   │   ├── eqtl_trans_manhattan.png
│   │   └── gwas_manhattan.png
│   ├── qq/                       # QQ plots
│   ├── volcano/                  # Volcano plots
│   ├── distribution/             # Distribution plots
│   ├── locuszoom/                # Locus zoom plots
│   ├── interactive/              # Interactive plots (HTML)
│   └── summary/                  # Summary plots
├── reports/                      # Comprehensive reports
│   ├── analysis_report.html      # Main HTML report
│   ├── pipeline_summary.txt      # Text summary
│   ├── results_metadata.json     # Results metadata
│   └── methods_section.txt       # Methods for publications
├── interaction_results/          # Interaction analysis
```

```
│   ├── age_interaction/
│   ├── sex_interaction/
│   └── summary.txt
├── fine_mapping_results/         # Fine-mapping outputs
│   ├── credible_sets/
│   ├── susie_results/
│   └── finemap_results/
├── normalization_comparison/     # Normalization assessment
│   ├── eqtl/
│   │   ├── distribution_comparison.png
│   │   ├── normalization_report.html
│   │   └── statistical_summary.txt
│   ├── pqtl/
│   └── sqtl/
├── genotype_processing/          # Processed genotype data
│   ├── filtered_genotypes.vcf.gz
│   ├── plink_format/
│   └── processing_log.txt
├── temp/                         # Temporary files (cleaned up)
└── logs/                         # Pipeline execution logs
    ├── pipeline_YYYYMMDD_HHMMSS.log
    ├── validation.log
    ├── qc.log
    └── analysis.log
```

File Formats and Interpretation

Main QTL Results Files

1. Nominal Association Results (`nominals.txt`)

Format: Tab-separated values

```text
phenotype_id  variant_id  chromosome  position  p_value  beta  se  maf
gene1         chr1_1000   1           1000      2.5e-08  0.32  0.05 0.15
gene1         chr1_2000   1           2000      1.2e-06  0.25  0.06 0.12
```

Columns:

- `phenotype_id`: Gene/protein/splicing event ID
- `variant_id`: Genetic variant identifier
- `chromosome`, `position`: Genomic coordinates
- `p_value`: Association p-value
- `beta`: Effect size (change in phenotype per additional effect allele)
- `se`: Standard error of effect size
- `maf`: Minor allele frequency

2. Significant Results (`significant.txt`)

Format: Tab-separated values with FDR information

```text
phenotype_id  variant_id  p_value  beta  p_fdr  q_value
gene1         chr1_1000   2.5e-08  0.32  0.001  0.001
gene2         chr2_5000   3.2e-07  0.28  0.015  0.015
```

Additional Columns:

- `p_fdr`: False Discovery Rate adjusted p-value
- `q_value`: Storey's q-value (similar to FDR)

3. Permutation Results (`permutations/`)

Directory containing:

- `permutation_pass_1.txt`: Results from first permutation round
- `permutation_stats.txt`: Summary of permutation distribution
- `empirical_pvalues.txt`: Empirical p-values from permutations

Quality Control Files

1. Sample QC Report

```text
Sample ID    Missing Rate  Heterozygosity  Status
```

```
sample1      0.02          0.32            PASS
sample2      0.15          0.45            FAIL_MISSING
sample3      0.01          0.29            PASS
```

2. Variant QC Report

```
text

Variant ID   Chromosome  Position  MAF  Missing Rate  HWE_P  Status
rs12345      1           1000      0.12 0.01          0.85   PASS
rs67890      1           2000      0.005 0.08         1e-08  FAIL_HWE
```

3. Sample Concordance Report

```
text

Dataset      Total Samples  Overlap Samples  Overlap Percentage
Genotypes    500           -                -
Expression   480           475              95.0%
Covariates   490           485              97.0%
```

Results Interpretation

Key Metrics to Evaluate

1. Genomic Control Lambda (λ)

What it is: Measure of test statistic inflation
Interpretation:

- λ = 1.0: Perfectly calibrated (ideal)
- 1.0 < λ < 1.05: Slight inflation (acceptable)
- λ > 1.05: Significant inflation (potential confounding)
- λ < 1.0: Deflation (rare, may indicate issues)

2. Number of Significant Associations

Expected ranges:

- cis-eQTLs: 10-80% of genes typically have cis-eQTLs
- trans-eQTLs: 1-10% of genes typically have trans-eQTLs
- pQTLs: 5-50% of proteins typically have pQTLs
- sQTLs: 5-30% of splicing events typically have sQTLs

3. Effect Size Distribution

Typical ranges:

- cis-eQTLs: |beta| = 0.1-1.0 (moderate to large effects)
- trans-eQTLs: |beta| = 0.05-0.3 (small to moderate effects)
- pQTLs: |beta| = 0.1-0.8 (moderate effects)
- sQTLs: |beta| = 0.2-1.5 (moderate to large effects)

How to Read Output Files

Example cis-eQTL Result Interpretation

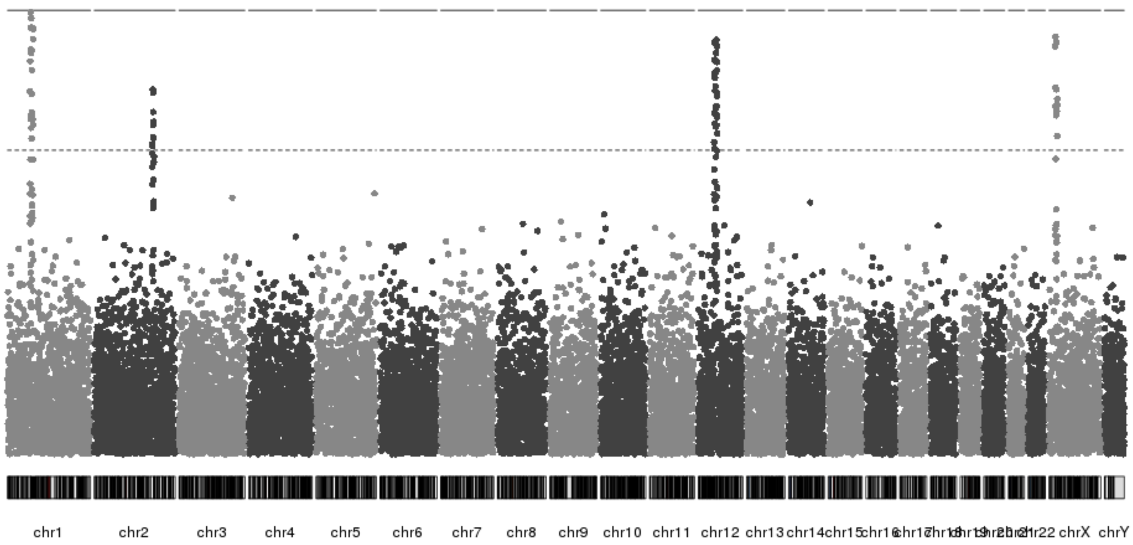
bash

```
# From significant.txt:
# gene1  chr1_1000_A_T  2.5e-08  0.32  0.001

Interpretation:
- Gene 'gene1' has a significant cis-eQTL at variant chr1:1000
- Association p-value: 2.5e-08 (highly significant)
- Effect size (beta): 0.32 → Each effect allele increases expression by 0.32 units

- FDR-adjusted p-value: 0.001 → 0.1% false discovery rate
```

Example Manhattan Plot Interpretation



What to look for:

- Peaks above red line: Genome-wide significant hits (p < 5e-8)
- Peaks above orange line: Suggestive hits (p < 1e-5)
- Chromosome patterns: Should be relatively uniform
- Inflation: Points above diagonal in QQ plot indicate inflation

Quality Control Outputs

Sample QC Assessment

1. Sample Missingness Plot

File: `QC_reports/genotype_qc/sample_missingness.png`
What to check:

- Most samples should have <5% missingness
- Remove samples with >10% missingness
- Look for bimodal distribution indicating batch effects

2. MAF Distribution Plot

File: `QC_reports/genotype_qc/maf_distribution.png`
What to check:

- Should show exponential decay (many rare variants, few common)
- Check MAF threshold is appropriate (vertical line)
- Ensure no unusual peaks or gaps

3. Heterozygosity Plot

File: `QC_reports/genotype_qc/heterozygosity.png`
What to check:

- Most samples should cluster around population mean
- Outliers may indicate sample contamination or issues
- Different clusters may indicate population stratification

Phenotype QC Assessment

1. Expression Distribution

File: `QC_reports/phenotype_qc/expression_qc.png`
What to check:

- Distribution should be smooth without extreme outliers
- Missingness pattern should be random, not systematic
- Batch effects visible as blocks in missingness heatmap

2. Sample Concordance

File: `QC_reports/sample_concordance/concordance_plot.png`
What to check:

- Overlap should be >80% between genotypes and phenotypes
- Low overlap indicates sample ID mismatches
- Investigate samples present in one dataset but not others

Advanced Analysis Outputs

Interaction Analysis Results

1. Interaction Summary

```text
phenotype_id  variant_id  p_nominal  p_interaction  beta_interaction
gene1         chr1_1000   2.5e-08    0.01          0.15
```

Interpretation:

- `p_interaction`: Significance of interaction term
- `beta_interaction`: Effect size of interaction
- Example: The genetic effect on gene1 differs by the interaction covariate

2. Stratified Results

Files: `interaction_results/age_stratified/`

- Contains results split by interaction covariate levels
- Useful for understanding direction of interaction effects

Fine-mapping Results

1. Credible Sets

```text
phenotype_id  variant_id  posterior_probability  credible_set
gene1         chr1_1000   0.45                  1
gene1         chr1_2000   0.35                  1
gene1         chr1_3000   0.15                  1
```

Interpretation:

- `posterior_probability`: Probability variant is causal
- `credible_set`: Set of variants containing causal variant with 95% probability
- Variants in same credible set should be in high LD

2. Fine-mapping Summary

- Number of credible sets per locus
- Size of credible sets (smaller = better resolution)
- Posterior probabilities of top variants

Troubleshooting Outputs

Common Issues and Diagnostic Files

1. Memory Issues

Check: `logs/pipeline_*.log` for memory warnings
Solutions:

- Increase `memory_gb` in configuration
- Enable `process_by_chromosome: true`
- Use `force_plink: true` for large datasets

2. No Significant Results

Diagnostic files to check:

- `QC_reports/comprehensive_qc_report.html` - Data quality issues
- `normalization_comparison/` - Normalization effectiveness
- `plots/qq/` - Test statistic inflation

Common causes:

- Insufficient sample size
- Poor data quality
- Inappropriate normalization
- Overly strict multiple testing correction

3. Long Runtime

Check: `logs/analysis.log` for bottleneck steps
Optimization strategies:

- Increase `num_threads`
- Use `qtl_mode: "cis"` instead of "both"
- Reduce `num_permutations`
- Use BCF instead of VCF format

Log File Interpretation

Example Log Entry Analysis

```text
2024-01-15 10:30:15 - QTLPipeline - INFO - 🔍 Running eQTL cis analysis...
2024-01-15 10:35:22 - QTLPipeline - INFO - ✅ eQTL cis: 1250 significant associations
2024-01-15 10:35:23 - QTLPipeline - WARNING - ⚠️ High genomic inflation detected: λ = 1.12
2024-01-15 10:35:24 - QTLPipeline - ERROR - ❌ trans-eQTL analysis failed: Memory allocation failed
```

Interpretation:

- cis-eQTL analysis completed successfully with 1250 hits
- Genomic inflation suggests potential confounding
- trans-eQTL failed due to memory limits

Best Practices for Results Analysis

1. Start with QC Assessment

- Always examine QC reports before interpreting results
- Check sample and variant filtering thresholds
- Verify normalization effectiveness

2. Validate Key Findings

- Check top hits in external databases (GTEx, eQTL Catalogue)
- Verify effect directions make biological sense
- Consider replication in independent datasets

3. Use Multiple Visualization Types

- Manhattan plots for genome-wide overview
- QQ plots for inflation assessment
- Locus zoom for regional context
- Volcano plots for effect size distribution

4. Consider Biological Context

- Annotate significant hits with known genes
- Check for enrichment in functional categories
- Consider tissue/cell type specificity

5. Document Analysis Decisions

- Keep configuration files for reproducibility
- Document filtering thresholds and normalization methods
- Record any manual curation steps

Example Results Workflow

bash

```
# 1. Check pipeline completed successfully
cat results/pipeline_summary.txt

# 2. Examine QC reports
open results/reports/analysis_report.html

# 3. Check significant hit counts
wc -l results/QTL_results/eqtl/cis/significant.txt

# 4. Generate custom visualizations for top hits
# (Using the provided plotting utilities)

# 5. Export results for downstream analysis
cp results/QTL_results/eqtl/cis/significant.txt my_analysis/top_qtls.txt
```