

## Step5: Functional Association Analysis

This module integrates differential or pattern-specific features to elucidate biological mechanisms through three complementary approaches:

- Pathway Overlay Analysis:** Count the number of pathways co-annotated by differentially expressed genes/metabolites, and those annotated solely by either genes or metabolites.
  - Pathway Annotation Analysis:** Visualize pathways with the highest number of annotated differential pattern-specific features.
  - Pathway Centric Feature Integration:** Integrate pathway topology with spatial distributions of key molecules.
- By analyzing pathways co-annotated by spatially significant features, we uncover critical biological mechanisms underlying tissue phenotypes. The platform supports two feature input types:
- Differential features:** Top 300 DEGs/DATs (by adjusted p-value) from differential analysis
  - Pattern-specific features:** Top 300 features per spatial module (by correlation score)

Default KEGG annotation is provided. For custom pathways, select 'upload annotation data'.

## Analysis Parameters

Select data for functional association analysis:

Differential features

Select up/down-regulated differential features for analysis

Up-regulated and down-regulated features

Annotation source

Built-in KEGG database

Note: Pathway mapping scales with feature count. Please avoid duplicate submissions.

Launch Functional Analysis

## Annotation Summary

Export results

| number_of_annotated_pathways | Count_max | Count_min |
|------------------------------|-----------|-----------|
| 212                          | 14.00     | 1.00      |

## Pathway Overlay Analysis

Venn diagram showing shared and module-specific KEGG pathway annotations.

## Pathway Annotation Overlay

Intersection of pathways annotated by input features:

- Left circle: Gene-annotated pathways
- Right circle: Metabolite-annotated pathways
- Overlap: Co-annotated pathways



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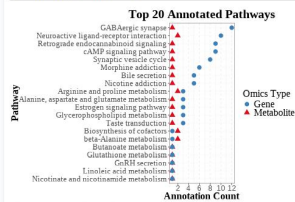
## Pathway Annotation Analysis

Dot plot visualizing pathways with the most mapped features (Top 20 by Count).

## Pathway Annotation Count

Filter by pathway type:

Gene-metabolite co-annotated pathways



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Export data

## Pathway-Centric Feature Integration

Co-annotation network mapping of genes and metabolites within biological pathways. Select pathways below to explore.

## Pathway Topology Mapping

Annotation type:

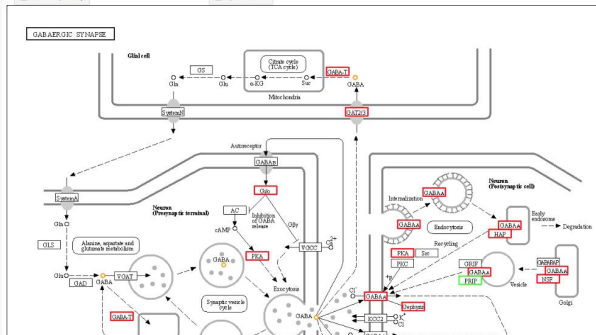
Co-annotated pathways

Download pathway

Export features

Select pathway:

GABAergic synapse



## Pathway Feature Table: Metabolites

Key metabolites in selected pathway:

| metabolite              | KEGG ID | Status | p_val_adj | log2_Fold_Change |
|-------------------------|---------|--------|-----------|------------------|
| gamma-aminobutyric acid | C00314  | Up     | 0.00      | 0.50             |

## Pathway Feature Table: Gene

Key genes in selected pathway:

| gene    | KEGG ID | Status | p_val_adj | log2_Fold_Change |
|---------|---------|--------|-----------|------------------|
| Slc6a11 | K05044  | Up     | 0.00      | 1.29             |
| Hsp1    | K04647  | Up     | 0.00      | 1.52             |
| Gng3    | K04640  | Up     | 0.00      | 0.87             |
| Gabra1  | K05175  | Up     | 0.00      | 1.01             |
| Hnf     | K06027  | Up     | 0.00      | 0.87             |
| Gphn    | K15376  | Up     | 0.00      | 0.43             |
| Gabra2  | K05188  | Up     | 0.00      | 0.99             |
| Pfkfb3  | K04345  | Up     | 0.00      | 0.72             |
| Abat    | K13824  | Up     | 0.00      | 0.65             |

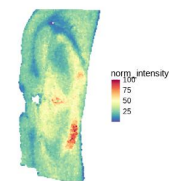
## Spatial Activity Visualization

Examine spatial distributions of key pathway components:

## Metabolite Spatial Distribution

Select pathway metabolite:

gamma-aminobutyric acid



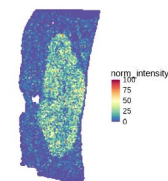
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## Gene Spatial Distribution

Select pathway gene:

Slc6a11



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