

Use “LinkedOmics” platform for analyzing cancer-associated multi-dimensional data

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1. Open browser “Chrome/Safari” (Chrome is preferable)
2. Go to <http://linkedomics.org/>
3. Read introduction “About”, “OMICS Data type” and go through user manual “Manual and Tutorial”
4. REGISTER to sign-in or click on ENTER AS GUEST
5. Query page has two panels (a) navigation and (b) query/result panel. Click NEW ANALYSIS button in navigation panel
6. Perform association analysis using LinkFinder
 - a. Select the cancer cohort: TCGA Bladder Cancer, BLCA
 - b. Select search dataset containing the attribute of interest: MUTATION Data type (gene level).
 - c. *Optional*, Select population with particular characteristics: ER positive patients [do not select in this example]
 - d. Select attribute/gene of interest: RB1 gene
 - e. Select target dataset with which possible pair-wise association analyses will be calculated: HiSeq RNA Data Type (gene level)
 - f. Select statistical method: T-test
 - g. Click SUBMIT QUERY
7. View Results
 - a. Table: Association result
 - i. Select view
 - ii. Gene/protein attribute
 - iii. Signal strength: log₂FC (T-Test)
 - iv. P-value
 - v. FDR(BH)
 - vi. Event_SD (Mutation events in search dataset, MUTATION)
 - vii. Event_TD (non-zero events in target dataset, RNAseq)
 - b. Volcano Plot: association signal strength vs. p-value
 - c. Download table result as .text and plot as .png
8. Click View CDKN2A [Mutation in RB1 vs. CDKN2A expression Plot]
9. Repeat analysis with mutation in “RB1” gene in TCGA Breast cancer (BRCA) cohort
10. Click on data cart “ANALYZED RESULTS” in left panel to view all query results
11. Perform pathway enrichment analysis for association output (from LinkFinder) using LinkInterpreter
 - a. Click on view radio button “RB1 MUTATION vs RNAseq” query output
 - b. Click on the “LinkInterpreter” tab, which is next to the tab “LinkFinder”
 - c. Select enrichment method: ORA (over-representation analysis)

- d. Select Functional Database: KEGG PATHWAY
 - e. Select Rank Criteria: FDR
 - f. Select Sign (or direction): Positively correlated
 - g. Significance Level: 0.001
 - h. Click SUBMIT
12. View Results
- a. Click on Enrichment Results to “View Enrichment Results”
 - b. Click on top enriched pathway: [hsa04110 Cell cycle - Homo sapiens \(human\)](#)
 - c. Click on SUMMARY to download the results
 - d. Click on View Filtered Data on upper pane
 - e. View Filtered table based in input selection criteria
 - f. Click Ideograviewer to explore chromosomal view [<1000 genes]
13. Repeat the analysis with “GO analysis [biological process]” and different significant criteria
14. Repeat the analysis with “GSEA (gene set enrichment analysis)”
15. To perform comparison between the multi-omics association across gene-level or cancer cohort using LinkCompare
16. Click on Click on data cart “ANALYZED RESULTS” in left panel to view all query results
17. Select checkbox next to LinkFinder query between RB1 mutation and RNA expression in the bladder cancer cohort (BLCA) in “Association Result panel”
18. Select checkbox next to LinkFinder query between RB1 mutation and RNA expression in the breast cancer cohort (BRCA)
19. Select COMPARE
20. View Results
- a. Table: Comparison result
 - i. Attribute (gene/protein/site)
 - ii. ID presenting Query result [ID- signal strength, p-value, FDR]
 - iii. Meta-analysis result: meta statistic (sumz_stat), meta P-value (sumz_P), and meta FDR (sumz_FDR)
 - b. Visualization
 - i. Scatter Plot
 - ii. Venn Plot
 - iii. Heatmap (for more than 2 query comparison)
 - c. Download table result as .text and plot as .png
21. Perform pathway enrichment analysis for comparison output (from LinkCompare) using LinkInterpreter (repeat step 10 & 11)
22. To submit **user data** into LinkedOmics follow the instructions in the manual “Data Sharing and Annotation”
23. For any queries reach out at linkedomics.zhanglab@gmail.com

Please Note:

- Only the same level data type results are comparable (“gene level”, “site level”, “analyte level”, “focal level”, “miRNA level,” and “phosphosite level”)

Additional Case Studies

1. Explore impact of Copy number variation (SCNA) in ERBB2 on phosphorylation in Breast cancer (BRCA)
 - a. Perform Association analysis (LinkFinder)
 - i. Cancer Cohort: Breast Cancer (BRCA)
 - ii. Search Dataset: Copy Number Variation (SCNA, gene level GISTIC)
 - iii. Search Attribute: ERBB2
 - iv. Target Dataset: Phosphoproteomics (phosphosite level)
 - v. Statistical method: Pearson/ Spearman correlation
 - vi. SUBMIT
 - b. Perform ORA Enrichment analysis for Reactome pathway (LinkInterpreter)
 - i. Go to LinkInterpreter
 - ii. Select Reactome pathway
 - iii. Rank criteria: p-value
 - iv. Select direction: Positively correlated
 - v. Significance level: 0.05
 - vi. SUBMIT
2. Explore association between Pathological Stage [Clinical Data type] and protein expression [gene Level] in Colorectal cancer (COADREAD).
 - a. Perform Association analysis
 - i. Cancer Cohort: Colorectal Cancer (COADREAD)
 - ii. Search Dataset: Clinical
 - iii. Search Attribute: pathologic_stage
 - iv. Target Dataset: Proteome (gene level)
 - v. Statistical method: JT test
 - vi. SUBMIT
 - b. Perform GSEA enrichment analysis for KEGG pathway
3. Explore Scatter plot showing the relation between miRNA has-mir-200c and *ZEB1* gene expression in Breast Cancer
 - a. Perform Association analysis
 - i. Cancer Cohort: Breast Cancer (BRCA)
 - ii. Search Dataset: miRNAseq
 - iii. Search Attribute: has-mir-200c
 - iv. Target Dataset: RNAseq (HiSeq)
 - v. Statistical method: Pearson correlation
 - vi. SUBMIT
 - b. Perform ORA enrichment analysis for Gene ontology biological process.
4. Identify survival signatures for epithelial cancer types (BLCA, BRCA, HNSC, CESC)
 - a. Perform Association analysis

- i. Cancer Cohort: Breast Cancer (BLCA)
- ii. Search Dataset: Clinical
- iii. Search Attribute: Overall_survival
- iv. Target Dataset: RNAseq (HiSeq)
- v. Statistical method: COXPH analysis
- vi. SUBMIT

- vii. Click “New Analysis” on left panel

- viii. Repeat (4a, i-vi) steps for BRCA
- ix. Similarly, Repeat (4a, i-vi) steps for HNSC
- x. Similarly, Repeat (4a, i-vi) steps for CESC
- xi. Click “Analysis results” on left panel
- b. Select CHECKBOX for respective queries (4 here, BLCA,BRCA,HNSC,CESC). Click button “Compare” (LinkCompare).
 - i. View Table and Plots
- c. Perform GSEA enrichment analysis for KEGG pathway