Testing Sets of Genomic Features aka Pathways

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Background

Where we start...

The typical result of an experiment:

A list of deferentially expressed features.

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Accession Number	Description	Fold change AB/DC	p-value
IPI00791534	Solute carrier family 4, anion exchanger member 1	49.91	0.033
IPI00022418	Isoform 1 of fibronectin	33.71	0.006
IPI00020101	Histone H2B type 1-C/E/F/G/I	5.47	0.013
IPI00026314	Isoform 1 of gelsolin	5.33	0.048
IPI00418431	ASPN protein	5.21	0.009
IPI00021405	Isoform A of prelamin-A/C	5.19	0.009
IPI00009802	Isoform V0 of versican core protein	5.07	0.003
IPI00418169	Isoform 2 of annexin A2	4.98	0.0003
IPI00453473	Histone H4	4.46	0.024
IPI00022200	Isoform 1 of collagen alpha-3(VI) chain	1.25	0.036
IPI00410241	Periostin isoform thy6	- 1.67	0.047
IPI00218585	Isoform 2 of Periostin	- 1.71	0.034
IPI00006114	Pigment epithelium-derived factor	- 1.71	0.044
IPI00007960	Isoform 1 of periostin	- 1.83	0.012

(Salmon et al., Journal of Proteomics:2013)

Where we start...

The typical result of an experiment:

A list of deferentially expressed features.

Biological theory is about groups of features that are ...

–Involved in a biological process

–Involved in a series of chemical reaction

$\rightarrow$ Biological theory is about sets of features.

Advantages

- Easier to interpret in terms of biology
- Has more statistical power

  Ref: [Subramanian et al., 2005] & [Khatri et al., 2012]

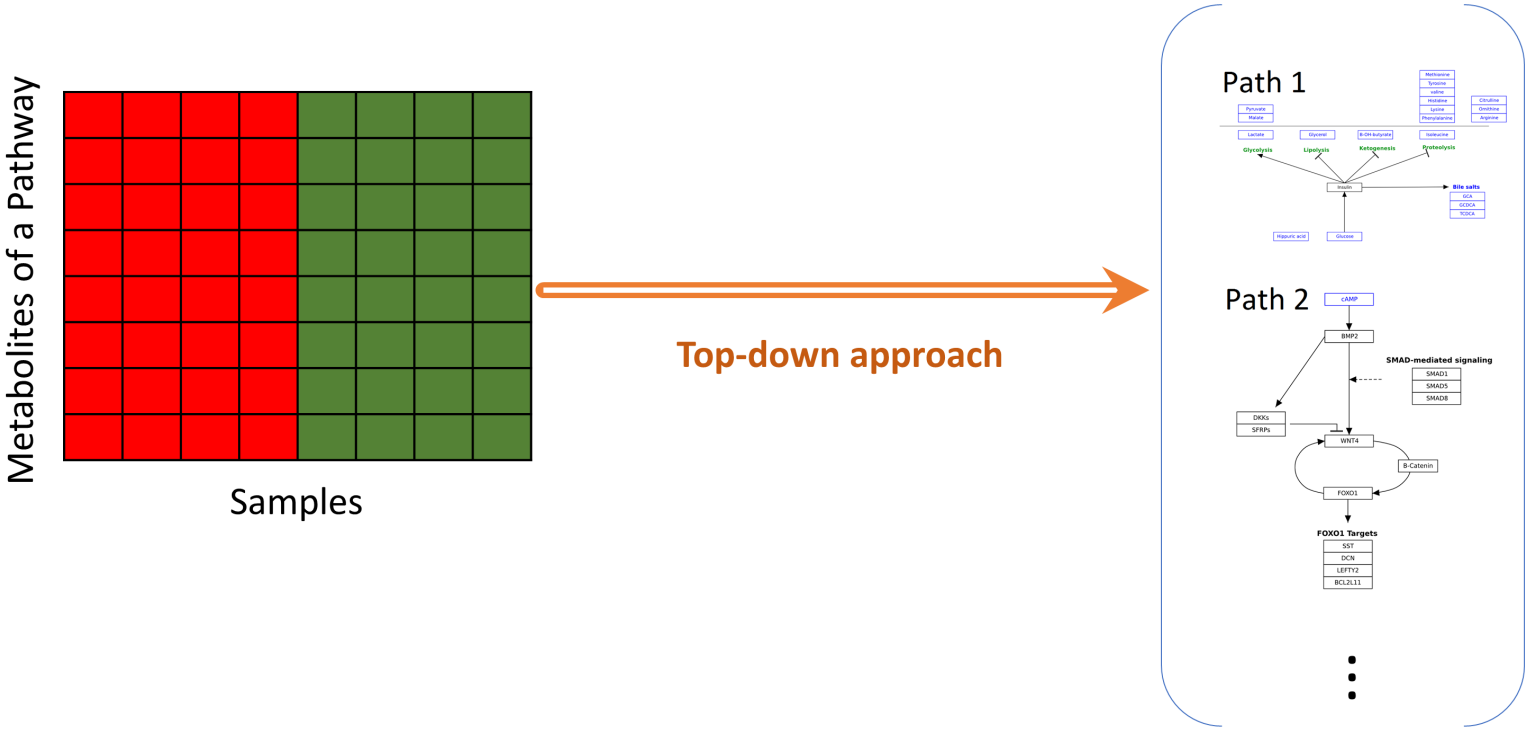
Existing Methods

Testing Pathways : Approach

Two approaches

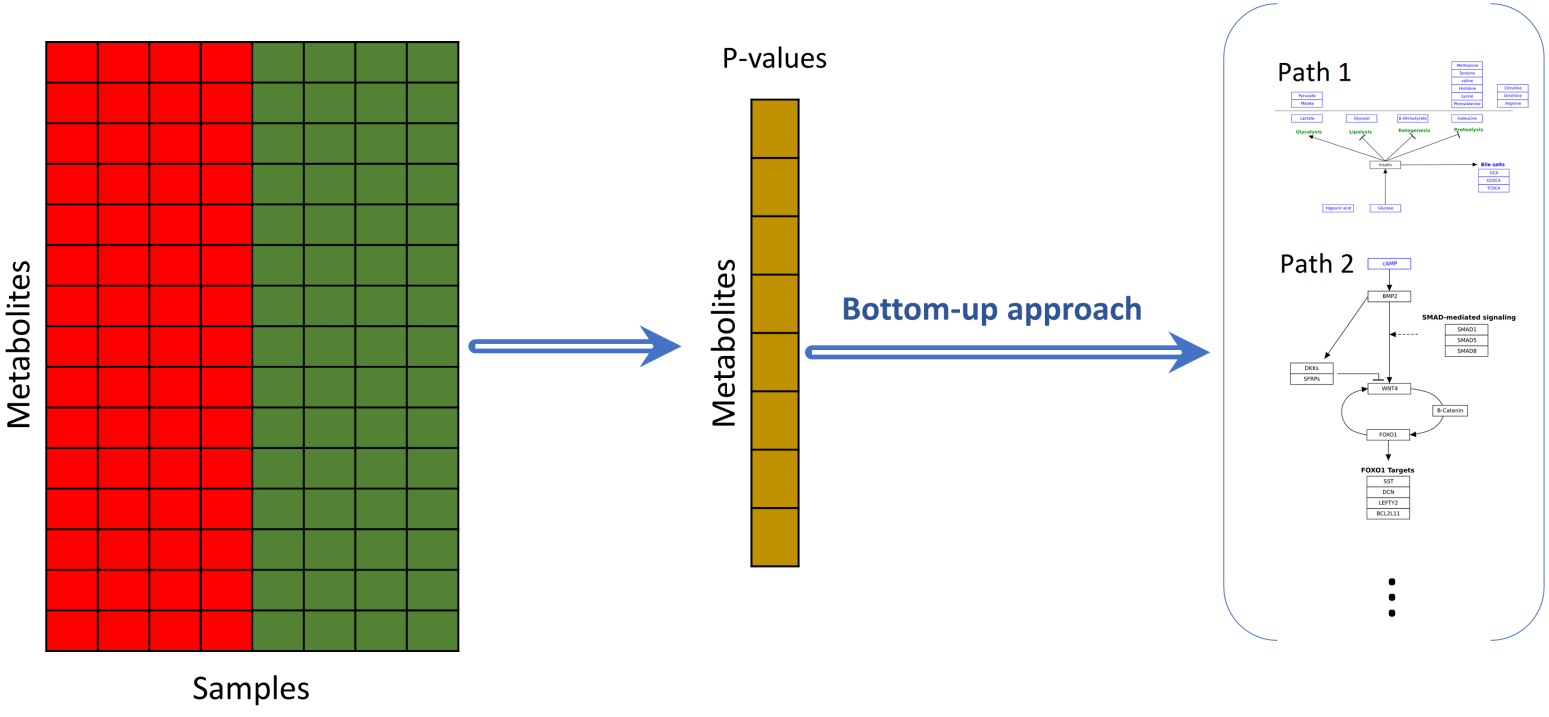
Two approaches

- Top-down: Model the metabolites jointly at the pathway level e.g. Globaltest



Two approaches

- Bottom-up: Test the metabolites separately and combine the p-values e.g. Fisher's exact test



SEA follows a Bottomn-up approach.

Notation

- m features
- 2^m possible feature-sets identified by S

There are many sources to define S.

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– self-contained null hypothesis : Are there *any* active features in the set?

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"Same results if there are few active features."

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Are there any active features in the selected set?

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Global test : example

- Association with response y
- Measured features $=$ a set of covariates $(x_1, \ldots, x_p)$
- Clinical covariates: $(z_1, \ldots, z_m)$
- Linear predictor: $\alpha + \sum_{j=1}^{m} z_i \gamma_j + \sum_{i=1}^{p} x_i \beta_i$
- Null hypothesis:

$$H_0 : \beta_1 = \ldots = \beta_p = 0$$

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Fisher's exact test: example
20000 features; 100 feature-sets; 200 Sig. features

Could arise due to chance: $p = 0.26$

	Sig. features	*non-Sig. features*	*total*
in feature-set	2	98	100
not in feature-set	198	19702	19900
total	200	19800	20000

Could not arise due to chance: $p = 0.0005$

	Sig. features	*non-Sig. features*	*total*
in feature-set	6	94	100
not in feature-set	194	21706	19900
total	200	19800	20000

Self-contained or Competitive?

Self-contained

- lower specificity

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Competitive

- Seems biologically more relevant
- Not over-powered
- Permutes the features

Where we end up

Current methods

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- All methods depend on the choice of database and feature-set

- All methods need multiple comparisons corrections with multiple feature-sets

Simultaneous Enrichment Analysis (SEA)

Unified null hypothesis

$$H_0^U(S, c) : \pi(S) \leq c$$

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Self-contained

$$H_0^{self}(S) : \pi(S) = 0$$

Competitive

$$H_0^{Comp}(S) : \pi(S) \leq \pi(\mathbb{S})$$

Hommel (1988)

The set S of hypotheses is rejected by Simes if and only if:

$$\min_i \frac{|S|}{i} p_{(i:S)} < \alpha$$

Assumption:

Certain form of dependence among features

Same assumption required for using BH

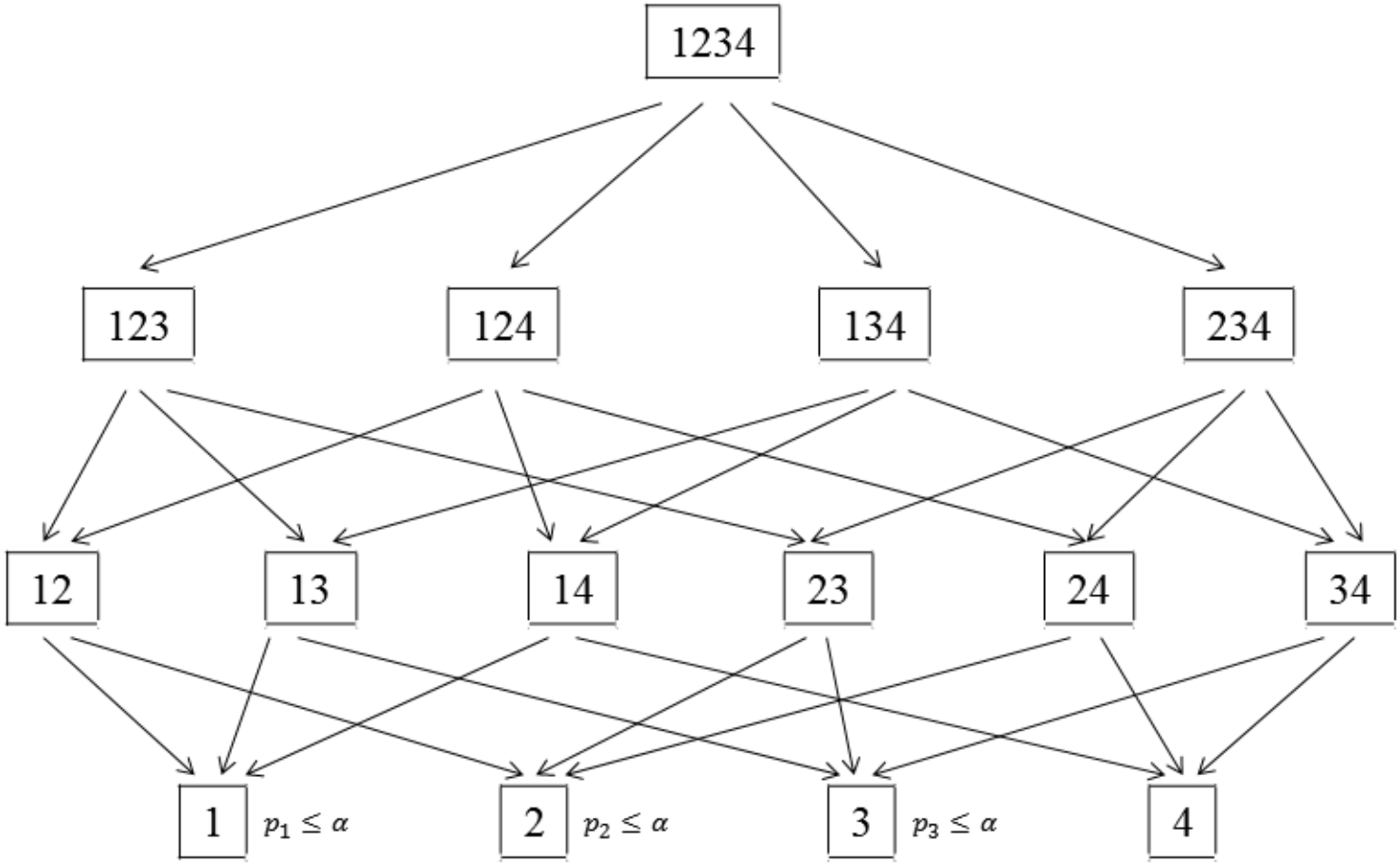


Figure 1: Closed Testig Example: $\alpha/2 < p1 \leq p2 \leq p3 \leq 2\alpha/3$, and $p4 > \alpha$

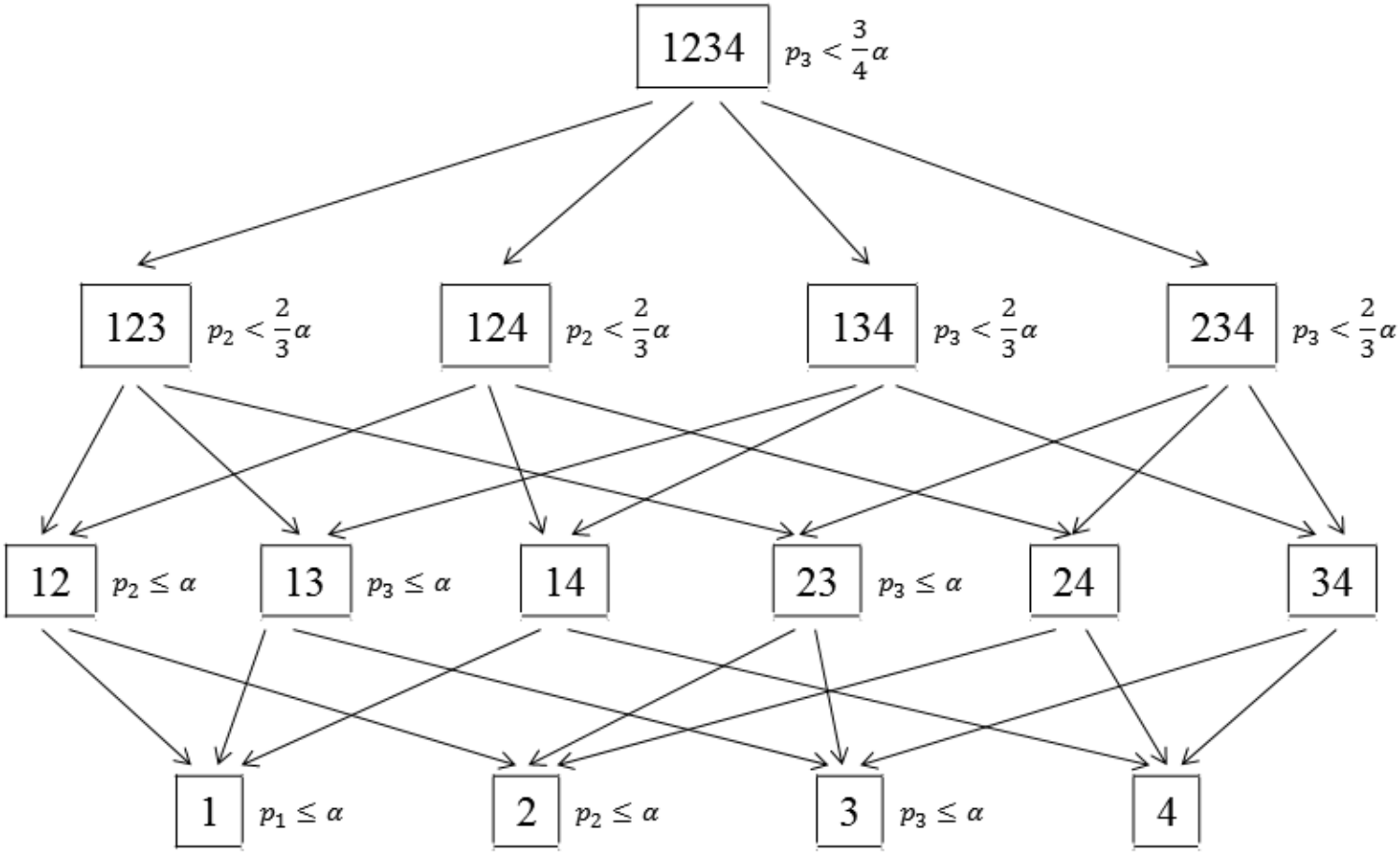


Figure 2: Closed Testig Example: $\alpha/2 < p1 \leq p2 \leq p3 \leq 2\alpha/3$, and $p4 > \alpha$

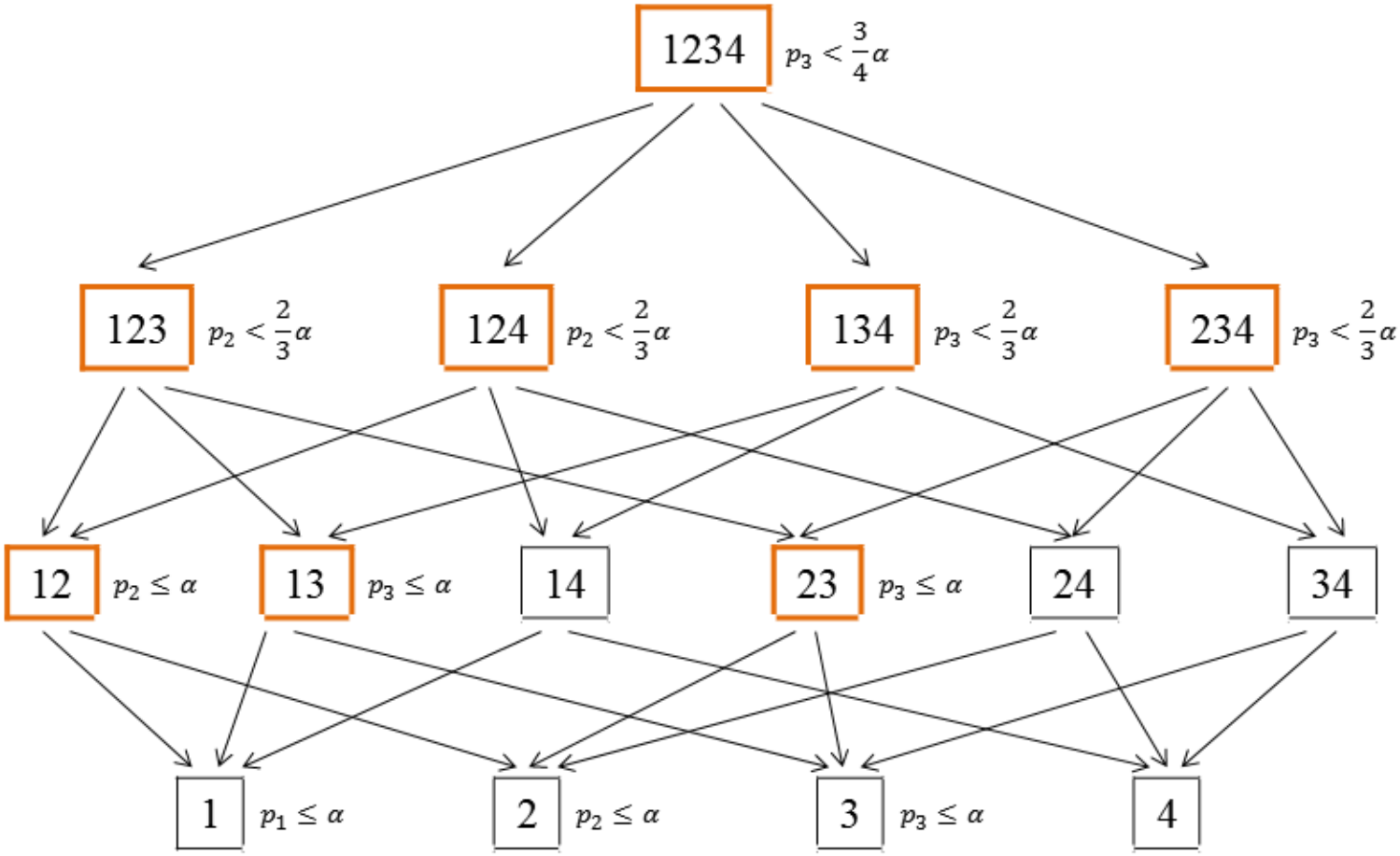


Figure 3: Closed Testig Example: $\alpha/2 < p1 \leq p2 \leq p3 \leq 2\alpha/3$, and $p4 > \alpha$

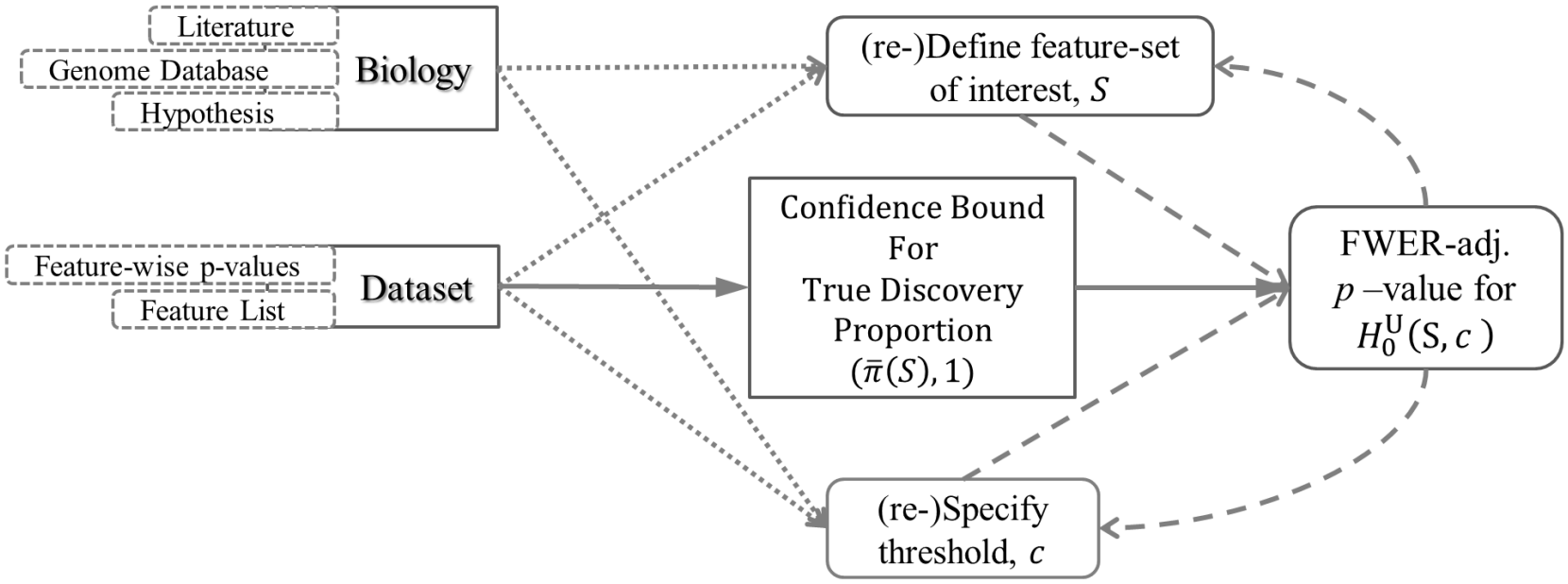
Goeman et al. (2016)

$$P\left(\begin{array}{c}\hat{\pi}(S_1) \leq \pi(S_1)\\ \hat{\pi}(S_2) \leq \pi(S_2)\\ \cdot\\ \cdot\\ \cdot\\ \hat{\pi}(S_{2^m}) \leq \pi(S_{2^m})\end{array}\right) \geq 1-\alpha$$

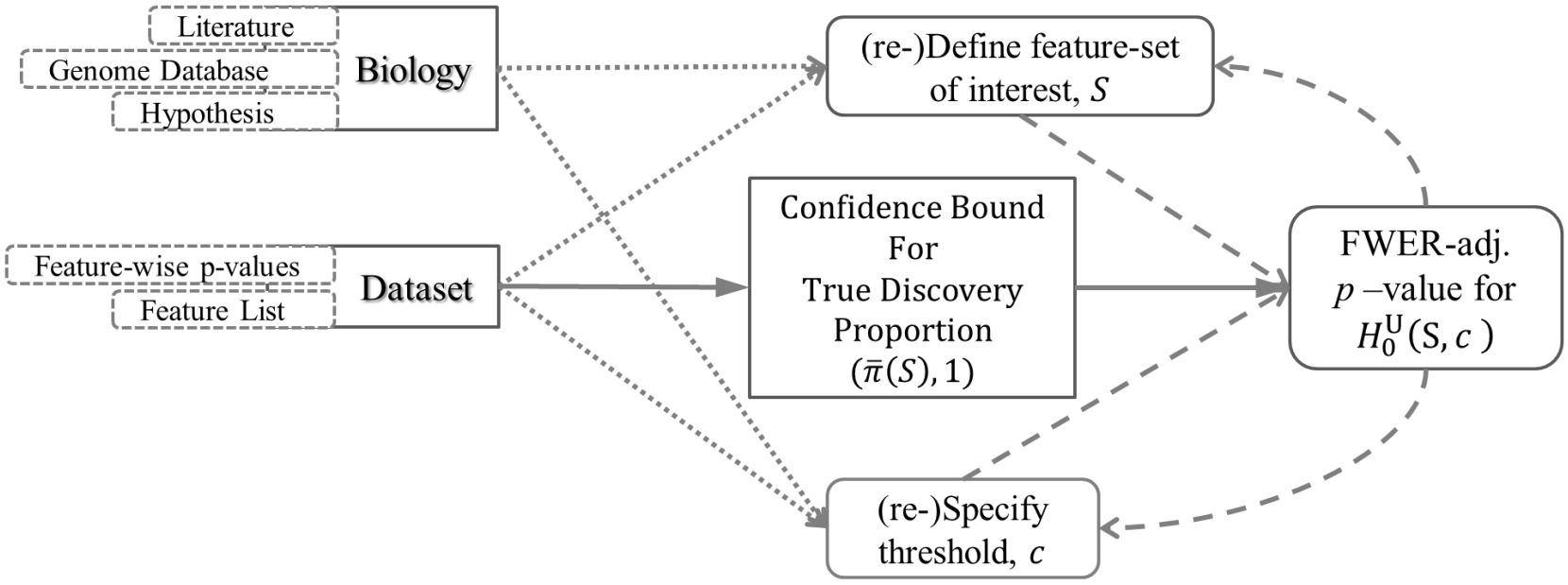
- Overall FWER control
- Simultaneity over all S:

  Choice of S after analysis

  S can be revised as many times as desired


Literature
Genome Database
Hypothesis
Biology
Feature-wise p-values
Feature List
Dataset
(re-)Define feature-set of interest, S
Confidence Bound For True Discovery Proportion $(\bar{\pi}(S), 1)$
(re-)Specify threshold, c
FWER-adj. p –value for $H_0^U(S, c)$

SEA pipeline



- Get raw p-values per feature
- Choose S and c
- Build a confidence interval for $\bar{\pi}(S)$
- Check if the CI includes c
- Calculate the adjusted p-value
- Modify S and/or c as needed

Discussion: Properties of SEA

- Valid competitive testing
- Combined feature-wise and feature-set testing
- Flexibility $\times$ 3
- Short computation time
- Power comparable to classical feature-set testing

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- An exploratory tool

That is all!

Any questions?