

Hurdle model

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What is Hurdle model?

Hurdle model is a statistical test for differential analysis that utilizes a two-part model, a discrete (logistic) part for modeling zero vs. non-zero counts and a continuous (log-normal) part for modeling the distribution of non-zero counts. In RNA-Seq data, this can be thought of as the discrete part modeling whether or not the gene is expressed and the continuous part modeling how much it is expressed if it is expressed. *Hurdle model* is well suited to data sets where features have very many zero values, such as single cell RNA-Seq data.

On default settings, *Hurdle model* is equivalent to MAST, a published differential analysis tool designed for single cell RNA-Seq data that uses a hurdle model [1].

Running Hurdle model

We recommend normalizing your data prior to running *Hurdle model*, but it can be invoked on any counts data node.

- Click the counts data node
- Click the **Differential analysis** section in the toolbox
- Click **Hurdle model**
- Select the factors and interactions to include in the statistical test (Figure 1)

Numeric and categorical attributes can be added as factors. To add attributes as factors, check the attribute check boxes and click **Add interactions**. To add interactions between attributes, click the attribute check boxes and click **Add interaction**.

Select factors for analysis

☒ P53
☒ Treatment
☐ Group

Add factors Add interaction

Selected factors

Factor	Delete
P53	X
Treatment	X

Cross tabulation

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Figure 4. Adding factors to the statistical test

- Click **Next**
- Define comparisons between factor or interaction levels (Figure 2)

Adding comparisons in *Hurdle model* uses the same interface as [ANOVA/LIMMA-trend/LIMMA-voom](#). Start by choosing a factor or interaction from the *Factor* or drop-down list. The levels of the factor or interaction will appear in the left-hand panel. Select levels in the panel on the left and click the > arrow buttons to add them to the top or bottom panels on the right. The control level(s) should be added to the bottom box and the experimental level(s) should be added to the top box. Click **Add comparison** to add the comparison to the *Comparisons* table. Only comparisons in the *Comparisons* table will be included in the statistical test.

Define comparisons

Factor
P53*Treatment

p53+*Control
p53+*H-RasV12
p53-*Control
p53-*H-RasV12

Vs

p53-*Control

Add comparison
Reset comparison

Comparisons

Comparison	Delete
p53+*H-RasV12 vs. p53+*Control	✕

Advanced options

Option set
-- Default --
Configure

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Finish

Figure 5. Adding comparisons

- Click **Finish** to run the statistical test

Hurdle model produces a *Feature list* task node. The results table and options are the same as the *GSA* task report except the last two columns (Figure 3). The percentage of cells where the feature is detected (value is above the background threshold) in different groups (Pct(group1), Pct(group2)) are calculated and included in the *Hurdle model* report.

Gene list

Results: 21565		Optional columns											
Filter		View			Gene ID			Ensembl ID			Gene name		
MS vs Ctrl		P-value			FDR step up			Ratio			Fold change		
		LSMean(MS)			LSMean(Ctrl)			Pct(MS)			Pct(Ctrl)		
<input type="checkbox"/> Gene ID		1	A1BG	A1BG	A1BG	0	0	0.806	-1.241	1.331	1.652	0.043	0.083
<input type="checkbox"/> Ensembl ID		2	NAA25	NAA25	NAA25	0	0	0.683	-1.464	1.843	2.699	0.097	0.165
<input type="checkbox"/> Gene name		3	NAA30	NAA30	NAA30	0	0	0.828	-1.208	1.351	1.632	0.049	0.085
<input type="checkbox"/> P-value		4	NAA35	NAA35	NAA35	0	0	0.775	-1.290	1.506	1.943	0.065	0.112
<input type="checkbox"/> FDR step up		5	NAA38	NAA38	NAA38	0	0	0.622	-1.607	2.073	3.332	0.112	0.199
<input type="checkbox"/> Ratio		6	NAA50	NAA50	NAA50	0	0	0.867	-1.154	1.596	1.841	0.073	0.105
<input type="checkbox"/> Fold change		7	NAA60	NAA60	NAA60	0	0	0.977	-1.024	1.320	1.352	0.044	0.052
<input type="checkbox"/> LSMean		8	NAAA	NAAA	NAAA	0	0	0.963	-1.038	1.145	1.189	0.022	0.031
<input type="checkbox"/> Low expressed		9	NAALAD2	NAALAD2	NAALAD2	0	0	0.872	-1.146	1.383	1.586	0.054	0.081
<input type="checkbox"/> Pct(MS)		10	NAALADL2	NAALADL2	NAALADL2	0	0	0.491	-2.038	3.986	8.125	0.203	0.322
<input type="checkbox"/> Pct(Ctrl)		11	NAA20	NAA20	NAA20	0	0	0.752	-1.330	1.493	1.984	0.064	0.117
<input type="checkbox"/> Save filter		12	NAB1	NAB1	NAB1	0	0	0.893	-1.120	1.431	1.603	0.057	0.080
<input type="checkbox"/> Clear filter		13	NACAD	NACAD	NACAD	0	0	0.811	-1.233	1.983	2.444	0.104	0.146
<input type="checkbox"/> Saved filters		14	NACC2	NACC2	NACC2	0	0	0.824	-1.214	1.671	2.028	0.077	0.114
<input type="checkbox"/> (No saved filters available)		15	NADSYN1	NADSYN1	NADSYN1	0	0	0.887	-1.127	1.172	1.321	0.025	0.047
<input type="checkbox"/> Generate filtered node		16	NAE1	NAE1	NAE1	0	0	0.844	-1.184	1.529	1.811	0.067	0.102
<input type="checkbox"/> Save as managed list		17	NAF1	NAF1	NAF1	0	0	0.897	-1.115	1.288	1.436	0.041	0.063
		18	NAGK	NAGK	NAGK	0	0	0.905	-1.105	1.297	1.433	0.041	0.061
		19	NAGLU	NAGLU	NAGLU	0	0	0.904	-1.106	1.095	1.212	0.014	0.033
		20	NALCN	NALCN	NALCN	0	0	0.352	-2.845	7.060	20.085	0.292	0.463
		21	NAMPT	NAMPT	NAMPT	0	0	1.148	1.148	1.805	1.573	0.086	0.075
		22	NACA	NACA	NACA	0	0	0.503	-1.987	4.814	9.566	0.232	0.356
		23	NANS	NANS	NANS	0	0	0.897	-1.115	1.096	1.222	0.014	0.036
		24	NAA16	NAA16	NAA16	0	0	0.725	-1.380	1.736	2.395	0.087	0.146
		25	N4BP2L2	N4BP2L2	N4BP2L2	0	0	0.353	-2.830	4.425	12.524	0.227	0.398

Figure 6. Hurdle model task report

Hurdle model advanced options

Low value filter

Low-value filter allows you to specify criteria to exclude features that do not meet requirements for the calculation. If there is filter feature task performed in the upstream analysis, the default of this filter is set to **None**, otherwise, the default is **Lowest average coverage** is set to 1.

Lowest average coverage: the computation will exclude a feature if its geometric mean across all samples is below than the specified value

Lowest maximum coverage: the computation will exclude a feature if its maximum across all samples is below the specified value

Minimum coverage: the computation will exclude a feature if its sum across all samples is below than the specified value

None: include all features in the computation

Multiple test correction

Multiple test correction can be performed on the p-values of each comparison, with **FDR step-up** being the default. If you check the *Storey q-value*, an extra column with q-values will be added to the report.

Use only reliable estimation results

There are situations when a model estimation procedure does not fail outright, but still encounters some difficulties. In this case, it can even generate p-value and fold change on the comparisons, but they are not reliable, i.e. they can be misleading. Therefore, the default of *Use only reliable estimation results* is set **Yes**.

Data has been transformed with log base

Shows the current scale of the input data for this task

Background expression level

Set the threshold for a feature to be considered expressed for the two-part hurdle model. If the feature value is greater than the specified value, it is considered expressed. If the upstream data node contains log-transformed values, be sure to specify the value on the same log scale. Default value is **0**.

Shrinkage of error term variance

Applies shrinkage to the error variance in the continuous (log-normal) part of the hurdle model. The error term variance will be shrunk towards a common value and a shrinkage plot will be produced on the task report page if enable. Default is **Enabled**.

Shrinkage of regression coefficients

Applies shrinkage to the regression coefficients in the discrete (logistic) part of the hurdle model. The initial versions of MAST contained a bug that was fixed in its R source in March 2020. However, for the sake of reproducibility the fix was released only on a topic branch in MAST Github [2] and the default version of MAST remained as is. To install the fixed version of MAST in R, run the following R script.

```
# Uninstall the default version of MAST, if it's installed.
remove.packages("MAST")
# Install devtools, if it's not installed yet.
library("devtools")
install_github("https://github.com/RGLab/MAST/tree/fix/bayesglm")
library(MAST)
```

In Flow, the user can switch between the fixed and default version by selecting **Fixed version** or **Default version**, respectively. To disable the shrinkage altogether, choose **Disabled**.

References

[1] Finak, G., McDavid, A., Yajima, M., Deng, J., Gersuk, V., Shalek, A. K., ... & Linsley, P. S. (2015). MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. *Genome biology*, 16(1), 278.

[2] MAST topic branch that contains the regression coefficient shrinkage fix:

<https://github.com/RGLab/MAST/tree/fix/bayesglm>

Additional Assistance

If you need additional assistance, please visit [our support page](#) to submit a help ticket or find phone numbers for regional support.



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