3 Enrichment Analysis of Hypoxia Pathway Data

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In this notebook, we will isolate the hypoxia response (defined as the set of genes that fulfill the genetic equalities *egl-9* = *egl9;vhl-1* and *hif-1* = *egl-9 hif-1*), and we will perform enrichment analysis on the hypoxia response. We will also perform enrichment analyses on each mutant transcriptomes, to try to understand how different each transcriptome actually is.

```
In [1]: # important stuff:
        import os
        import pandas as pd
        import numpy as np

        # TEA and morgan
        import tissue_enrichment_analysis as tea
        import morgan as morgan
        import gvars
        import epistasis as epi

        # Graphics
        import matplotlib as mpl
        import matplotlib.pyplot as plt
        import seaborn as sns
        from matplotlib import rc
        rc('text', usetex=True)
        rc('text.latex', preamble=r'\usepackage{cmbright}')
        rc('font', **{'family': 'sans-serif',
                      'sans-serif': ['Helvetica']})

        # Magic function to make matplotlib inline;
        %matplotlib inline

        # This enables SVG graphics inline.
        %config InlineBackend.figure_formats = {'png','retina'}

        # JB's favorite Seaborn settings for notebooks
        rc = {'lines.linewidth': 2,
              'axes.labelsize': 18,
              'axes.titlesize': 18,
              'axes.facecolor': 'DFDFE5'}
        sns.set_context('notebook', rc=rc)
        sns.set_style("dark")

        mpl.rcParams['xtick.labelsize'] = 16
        mpl.rcParams['ytick.labelsize'] = 16
        mpl.rcParams['legend.fontsize'] = 14
```

```
In [2]: q = 0.1
        # this loads all the labels we need
        genvar = gvars.genvars()

        tissue_df = tea.fetch_dictionary()
        phenotype_df = tea.fetch_dictionary('phenotype')
        go_df = tea.fetch_dictionary('go')
        respiratory_complexes = pd.read_excel('../input/respiratory_complexes.xlsx')
```

```
In [3]: tidy = pd.read_csv('../output/temp_files/DE_genes.csv')
        tidy.sort_values('target_id', inplace=True)
        tidy.dropna(subset=['ens_gene'], inplace=True)
```

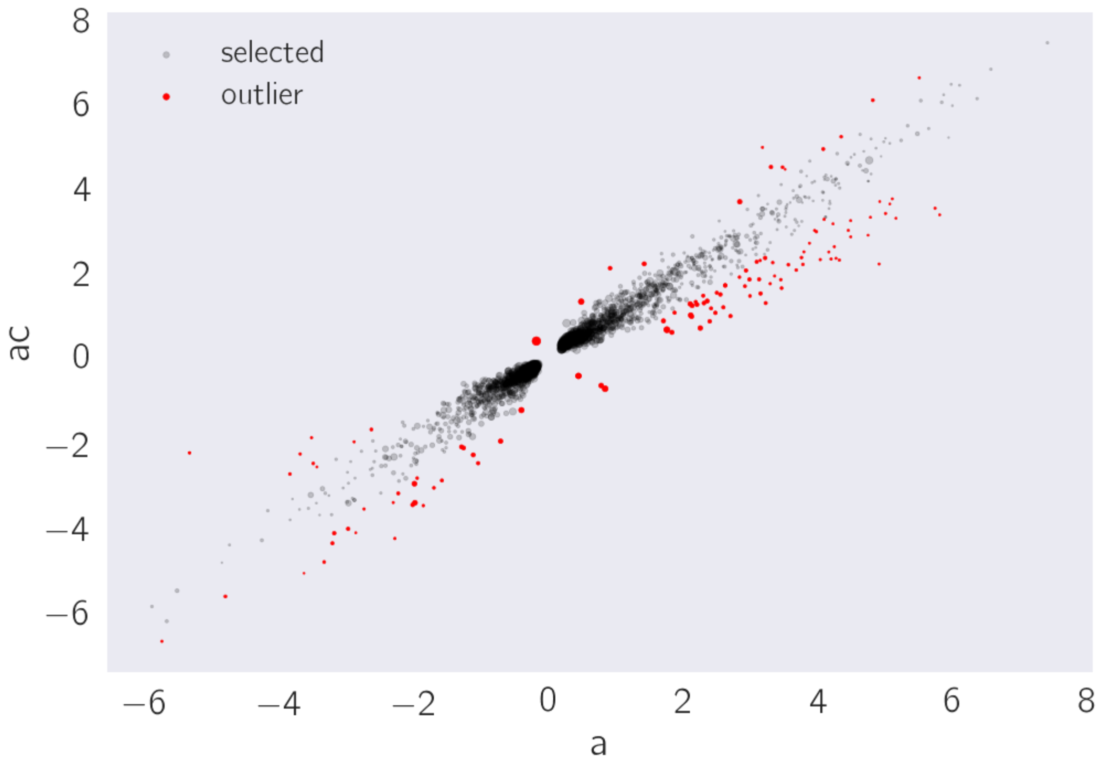
2 Defining the hypoxia response

The hypoxia response can be defined in genetic terms as those genes that obey the two epistasis relationships, *egl-9* = *egl-9;vhl-1* and *hif-1* = *egl-9 hif-1*.

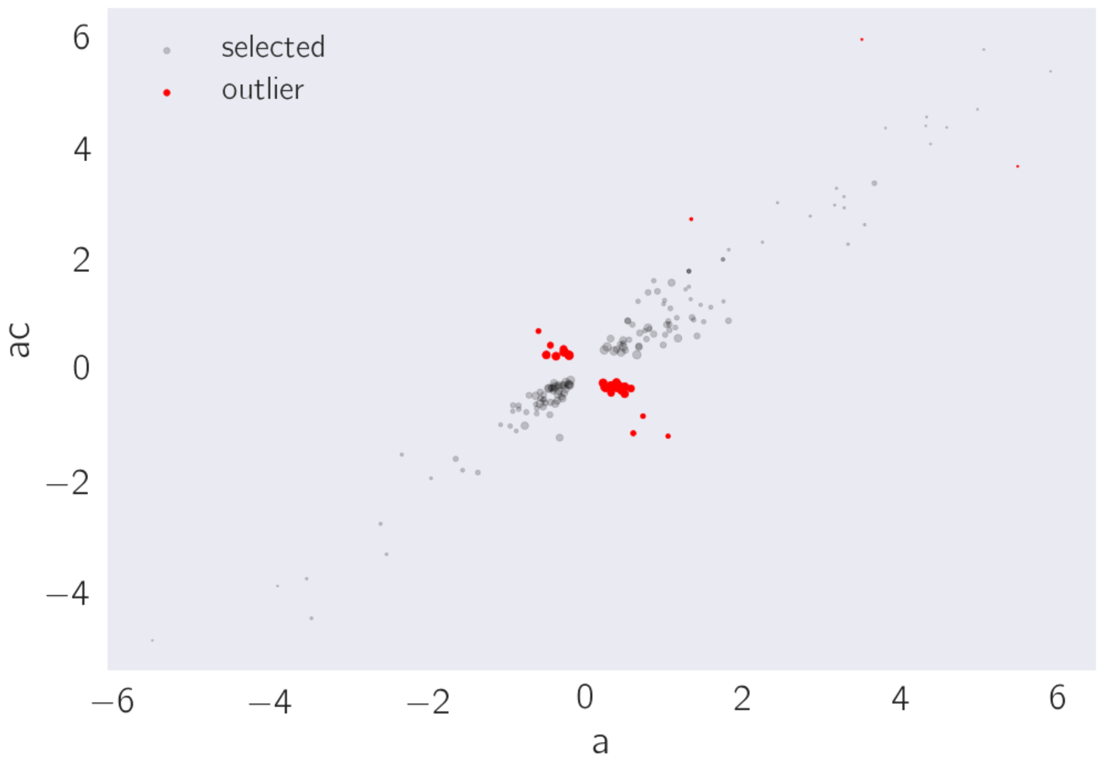
```
In [4]: def test_equality(equal_genotypes, third_genotype, df, col='code', q=0.1, n_std=2):
            """
            A function to test epistasis equality.

            For a set of genotypes, `a`, `b`, and `ab`, suppose that we want to find those gen
            that obey the rule `a`=`ab`. To identify genes with this expression pattern, we fi
            calculate the epistasis coefficient for transcripts within the STP(`a`, `ab`). The
            we find those transcripts that are <2sigma deviations away from the line of best f

            Params:
            equal_genotypes: the two genotypes that we want to set equal to each other
            third_genotype: the third genotype to be considered (needed to calculate epistasis
            coeff.).
            df - dataframe to use. Must contain `target_id` column
            col - column that encodes the genotypes
            q - q-value to be used
            n_std - number of standard deviations to use as cutoff

            Output:
```

```
        A list of target_ids
        """
        a, ac = equal_genotypes
        c = third_genotype

        # make sure the dataframe only contains the desired genotypes
        all_genotypes = [a, ac, c]

        df = df[df[col].isin(all_genotypes)]

        overlap = epi.find_overlap(equal_genotypes, df, col=col, q=q)

        df = df[df.target_id.isin(overlap)]
        a_df = df[df[col] == a].copy()
        c_df = df[df[col] == c]
        ac_df = df[df[col] == ac]

#     # the code below works only if the variance is invariant to the expected values
        normed_deltas = (ac_df.b.values - a_df.b.values)
        normed_deltas = normed_deltas/np.std(normed_deltas)

        # first condition guarantees we're not too far from the line y=x
        # second condition guarantees we are not on the line y=-x
        inside = (np.abs(normed_deltas) < n_std) & (ac_df.b.values*a_df.b.values > 0)

        # print a diagnostic plot:
        plt.scatter(a_df[inside].b, ac_df[inside].b, s=1/ac_df[inside].se_b, color='black'
        plt.scatter(a_df[~inside].b, ac_df[~inside].b, s=1/ac_df[~inside].se_b, color='red
        plt.xlabel('a')
        plt.ylabel('ac')
        plt.legend()

        # return list of target ids that meet criteria
        return a_df[inside].target_id.values
```

```
In [5]: # find the genes that obey egl-9 = egl-9;vhl-1
        filtered_egl = test_equality(['b', 'a'], 'd', tidy, n_std=2)
```



```
In [6]: # find the genes that obey hif-1 = egl-9 hif-1
        filtered_hif = test_equality(['c', 'f'], 'b', tidy, q=.1, n_std=2)
```



```
In [7]: # find those genes that are not DE in either hif-1 or egl-9 hif-1
        not_DE_hif = (tidy.code.isin(['c', 'f'])) & (tidy.qval > q)
        # a neat trick:
        not_DE = epi.find_overlap(['c', 'f'], tidy[not_DE_hif], q=1)

In [8]: # genes that are DE in hif-1 and egl-9, and obey both equations:
        equal_and_DE = (tidy.target_id.isin(filtered_egl)) & (tidy.target_id.isin(filtered_hif
        # genes that are DE in egl-9, but not in hif-1 genotypes and also obey both equations:
        equal_no_hif = (tidy.target_id.isin(filtered_egl)) & (tidy.target_id.isin(not_DE))

        # get the lists of both, then concatenate them for a hypoxia response:
        # most of the genes will come from the equal_no_hif condition
        de_both = tidy[(equal_and_DE)].target_id.unique()
        de_one = tidy[(equal_no_hif)].target_id.unique()
        overlap = list(set(np.append(de_both, de_one)))

        # find the hypoxia response
        hyp_response = tidy[tidy.target_id.isin(overlap)].copy()

In [9]: # annotate whether they are candidates for direct or
        # indirect regulation.
        def annotate(x):
            if x > 0:
```

```
            return 'candidate for direct regulation'
        else:
            return 'candidate for indirect regulation'

    # annotate
    hyp_response['regulation'] = hyp_response.b.apply(annotate)
```

```
In [10]: # save to file
         cols = ['genotype','target_id', 'ens_gene', 'ext_gene', 'b', 'se_b', 'qval', 'regulat
         hyp_response[cols].to_csv('../output/temp_files/hypoxia_response.csv', index=False)

         print('There are {0} genes in the predicted hypoxia response'.format(len(hyp_response
```

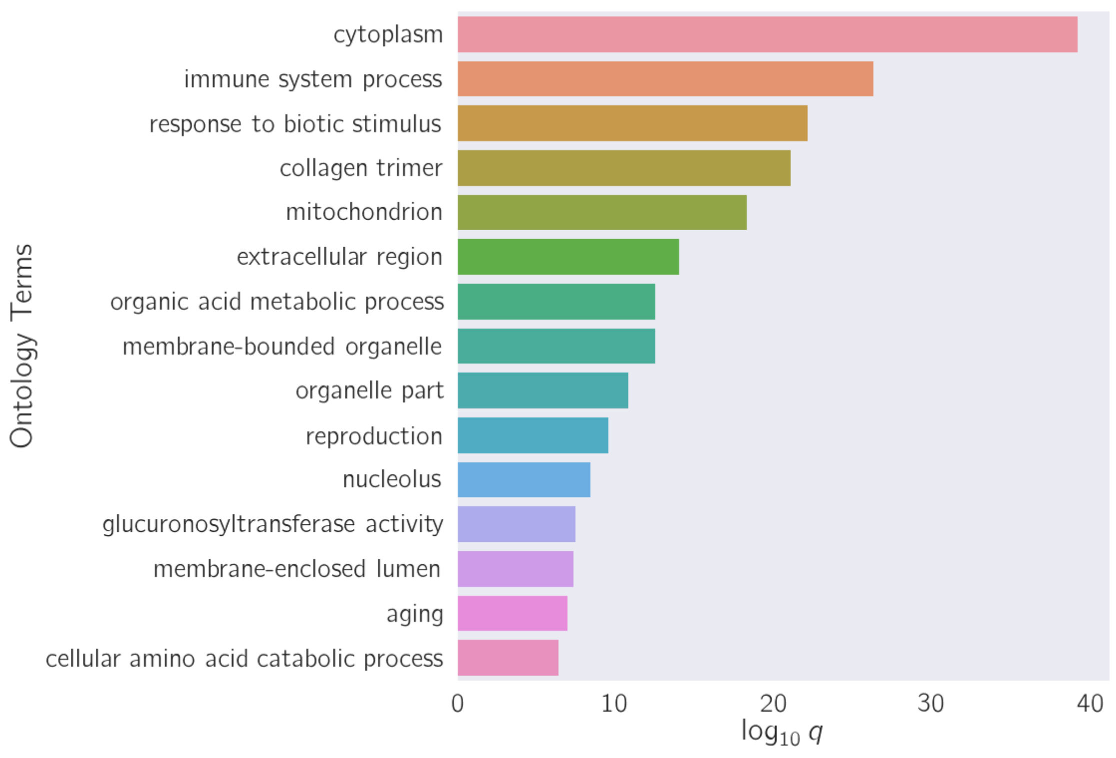
```
There are 1258 genes in the predicted hypoxia response
```

3 Enrichment Analysis of the Global HIF-1 response

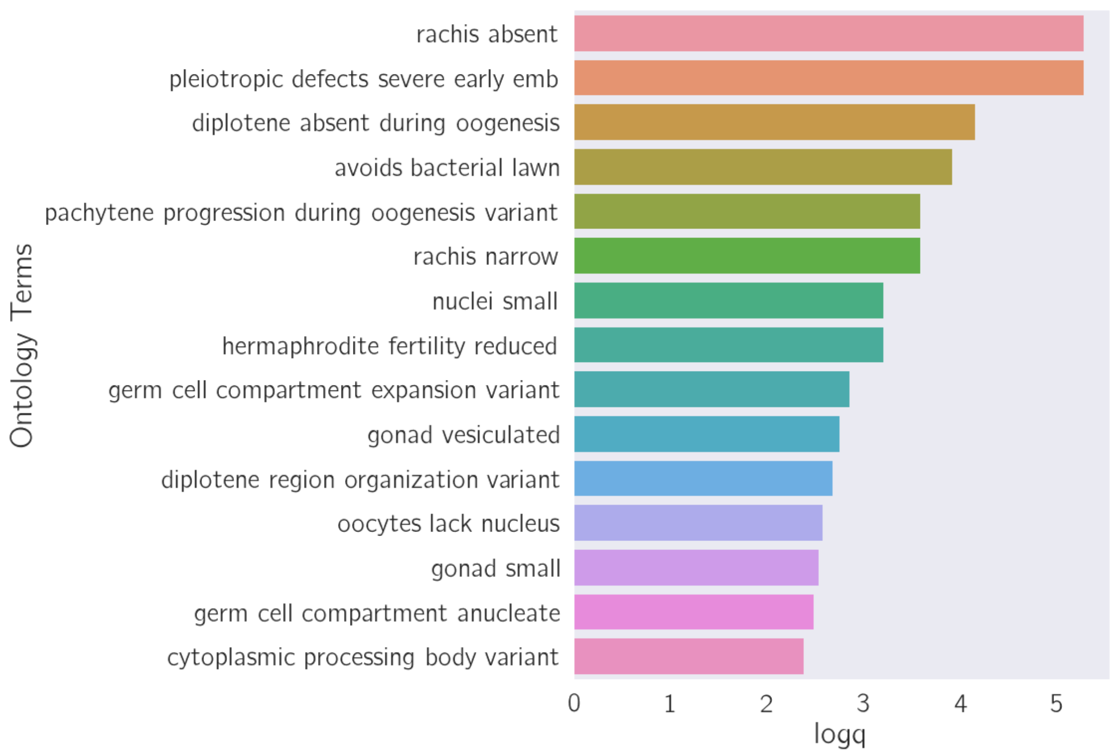
Now that we have found the hypoxia response, we can perform tissue, phenotype and gene ontology enrichment analysis on this gene battery. Note that we don't show all possibilities. When a particular analysis is not present, it is because the enrichment results were empty.

```
In [11]: teaH = tea.enrichment_analysis(hyp_response.ens_gene.unique(), tissue_df, show=False)
         peaH = tea.enrichment_analysis(hyp_response.ens_gene.unique(), phenotype_df, show=Fals
         geaH = tea.enrichment_analysis(hyp_response.ens_gene.unique(), go_df, show=False)

         for df in [teaH, peaH, geaH]:
             df['logq'] = -df['Q value'].apply(np.log10)
```

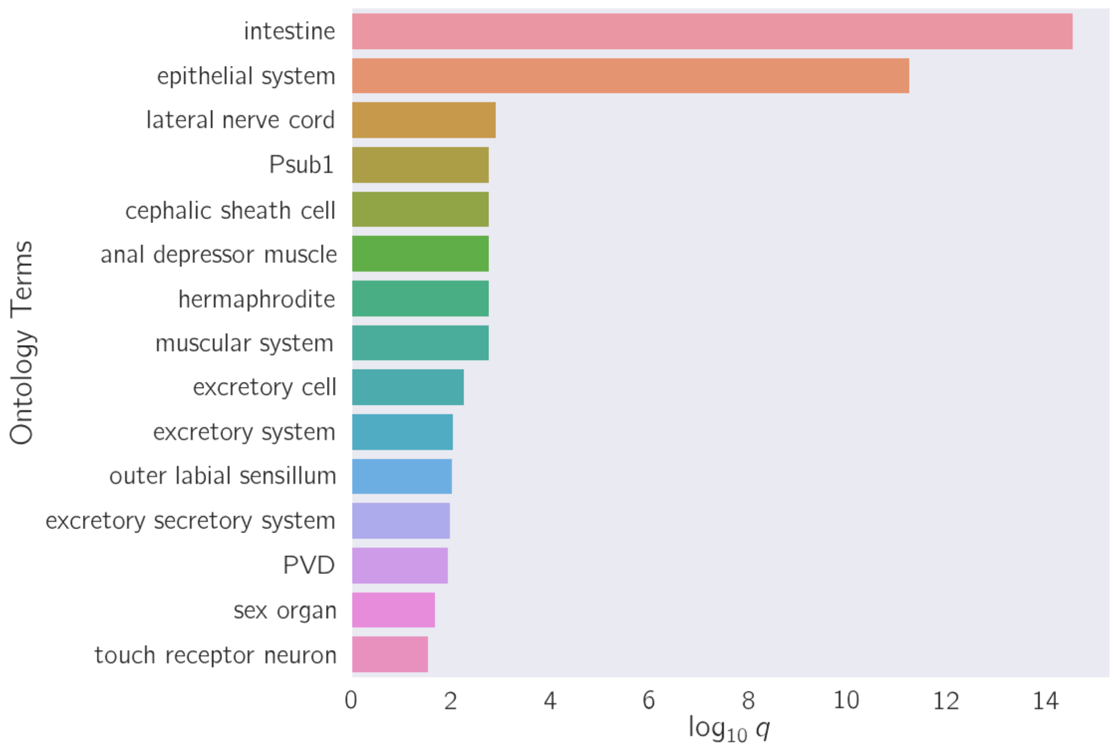
```
In [12]: ax = tea.plot_enrichment_results(geaH, analysis='go', y='logq')
         plt.xlabel('$\log_{10}{q}$')
         plt.savefig('../output/supp_figures/supplementary_figure_3.pdf')
```



```
In [13]: tea.plot_enrichment_results(peaH, analysis='phenotype', y='logq')

Out[13]: <matplotlib.axes._subplots.AxesSubplot at 0x112ce8898>
```



```
In [15]: ax = tea.plot_enrichment_results(teaH, analysis='tissue', y='logq')
         plt.xlabel('$\log_{10}{q}$')
         plt.savefig('../output/supp_figures/supplementary_figure_4.pdf', bbox_inches='tight')
```



intestine
epithelial system
lateral nerve cord
Psub1
cephalic sheath cell
anal depressor muscle
hermaphrodite
muscular system
excretory cell
excretory system
outer labial sensillum
excretory secretory system
PVD
sex organ
touch receptor neuron
Ontology Terms
0
2
4
6
8
10
12
14
$\log_{10} q$

```
In [ ]:
```