GENERAL INFORMATION AND CREDITS:

This software solution contains mass spectrometry data from ERE/pGREB1 DNA pulldown experiments and was created to be a tangible part of the following manuscript:

“Proteomic Analysis of Coregulators Bound to ERα on DNA and Nucleosomes Reveals Coregulator Dynamics”
(Molecular Cell; final submission May 22, 2013)

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Anna Malovannaya developed the software solution presented here and the in-house database software that supports infrastructure of this project. This application intends to serve as a portal for querying mass spectrometry results of paired experiments.

INSTALLATION/OPENING:

ERE Resource is packaged as two platform-specific (for Macintosh and Windows) FileMaker Pro (FMP) 12 runtime solutions. There is no install/uninstall process *per se* - the files can be simply opened/run. Below are the directions and recommendations for opening the ERE Resource files.

For Macintosh/Apple users:

1. Open 'ERE Resource_4Mac' Folder. It contains 3 files (ERE Resource_4Mac; doNOTuse_SupportingFile.fmpur; FMP_Acknowledgments.pdf) and an Extensions Folder with supporting libraries - do NOT delete any files.

2. Choose and open the ERE Resource_4Mac file.

For PC/Windows users:

1. Open 'ERE Resource_4Win' Folder. It contains multiple files and folders with supporting data - do NOT delete any files.

- To omit navigation through the supporting files in 4Win folder, we recommend that a shortcut of 'ERE Resource_4Win.exe' is made and placed in a convenient location for file opening.

2. Find and open the 'ERE Resource_4Win.exe' file (initial open is slower than subsequent runs).

3. When asked to enter your user name, any name can be used. This is an inherent prompt of the FMP Runtime for Windows; it will not appear again in subsequent runs.

USER INSTRUCTIONS:

The majority of Resource functionalities have in-solution mouse-over annotations that can be called by holding a pointer over the right bottom corner of column headers and data fields. GeneIDs in the results viewer are hyperlinked to the NCBI gene portal.

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1. The “list view” layout is a listing of all experiment clusters that correspond to the manuscript figures. The “list view” has the following buttons:

- red square with “x” - closes Demo solution;

- “ReadMe” - takes users to the ReadMe page;

- left-most column with cluster numbers - clicking on the cluster number in the left-hand-side column will produce the alignment of mass spectrometry protein identifications for the corresponding experiments in a second “cluster view” layout.

2. The “cluster view” layout has the following buttons:

- red square with “x” - closes Demo solution;

- “ReadMe” - takes users to the ReadMe page;

- green back arrow - returns user to the cluster listings;

- double clipboard - shows all identified proteins in the cluster;

- looking class - allows user to search the data - use tab to move between fields; hit Enter/Return after typing in queries; choose “Find”, “Constrain”, or “Extend” to manipulate found sets;

- “abs/norm/FOT” - toggle button for different methods of quantity normalization:
“abs” = “absolute” - will show peptide spectral matches (PSMs, spectral counts) and total summed area under the curve (AUC7, in 10^7 units; shared peptide areas were distributed equally between gene products) for all peptides belonging to a given protein
“norm” = “normalized to peptide capacity” - will show modified spectral abundance factor (SAF1 = PSMs/number of available protein peptides) and intensity-based absolute quantification values (iBAQ3, in 10^3 units; iBAQ=AUC/number of available peptides)
“FOT” = “fraction of total” - will show input-normalized SAF and intensity-based FOT (NSAF and iFOT defined as SAF/SAF(total) and iBAQ/iBAQ(total), respectively)

- “relaxed/strict” - toggle button for different levels of data confidence:
“relaxed” confidence level contains all identifications that have IDSet of 1-2 and IDG(IDGroup) of 1-5 in at least one of the experiments for a particular cluster (see parameter explanations below)
“strict” confidence level contains all identifications that have IDSet of 1-2 and IDG(IDGroup) of 1-3 in at least one of the experiments for a particular cluster (see parameter explanations below)

3. Explanation of mass spectrometry parameters:

- IDSets refer to the protein inference/homology issues as follows:
IDSet 1 (best, unambiguous) = gene products that have unique-to-gene peptides identified
IDSet 2 (good, but ambiguous) = gene products that have a distinct and largest set of peptides assigned, but no peptides are unique to those gene products (all peptides are shared with other gene products)
IDSet 3 (poor evidence, but cannot be fully excluded) = gene products represented by a smaller subset of peptides that could belong to IDSet 1 or IDSet 2 gene products (all peptides are shared with other gene products)

- IDGs (IDGroups) refer to the “quality” of the best peptide for a given gene product as judged by Mascot ion scores and Percolator q-values:
IDG 1 (best) = ion score equal or more than 30 AND q-value equal or less than 0.1
IDG 2 (good) = ion score equal or more than 30 AND q-value between 0.1 and 0.5
IDG 3 (good) = ion score between 20 and 30 AND q-value equal or less than 0.1
IDG 4 (OK) = ion score between 20 and 30 AND q-value between 0.1 and 0.5
IDG 5 (OK) = ion score between 10 and 20 AND q-value equal or less than 0.1
IDG 6 (bad) = ion score between 10 and 20 AND q-value between 0.1 and 0.5
IDG 7 (bad) = ion score between 0 and 10 AND q-value equal or less than 0.1
IDG 8 (worst) = ion score between 0 and 10 AND q-value between 0.1 and 0.5

- uIDGs (unique IDGroups) - same as IDGs, but judged on unique-to-gene peptides only (only available for IDSet 1 gene products)

- Pepts = number of distinct peptides assigned to a given gene product

- uPepts = number of unique-to-gene peptides assigned to a given gene product (only available for IDSet 1 gene products)

- PSM-based quantity columns (PSMs; SAF1; NSAF5; according to normalization type) show values (”abs”) and ratios (”ratio”, in comparison to control as listed in the column header; control itself does not show ratios)

- area-based quantity columns (AUC7; iBAQ3; iFOT5; according to normalization type) show values (”abs”) and ratios (”ratio”, in comparison to control as listed in the column header; control itself does not show ratios)

4. The “ReadMe” view has the following buttons:

- red square with “x” - closes Demo solution;
- green back arrow - returns user to the previous layout.