Using shRNA experiments to validate gene regulatory networks

Catharina Olsen, Kathleen Fleming, Niall Prendergast, Renee Rubio, Frank Emmert-Streib, Gianluca Bontempi, John Quackenbusch, Benjamin Haibe-Kains

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A B S T R A C T
Quantitative validation of gene regulatory networks (GRNs) inferred from observational expression data is a difficult task usually involving time intensive and costly laboratory experiments. We were able to show that gene knock-down experiments can be used to quantitatively assess the quality of large-scale GRNs via a purely data-driven approach (Olsen et al. 2014). Our new validation framework also enables the statistical comparison of multiple network inference techniques, which was a long-standing challenge in the field.

In this Data in Brief we detail the contents and quality controls for the gene expression data (available from NCBI Gene Expression Omnibus repository with accession number GSE53091) associated with our study published in Genomics (Olsen et al. 2014). We also provide R code to access the data and reproduce the analysis presented in this article.

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Experimental design, materials and methods

Short hairpin RNA experiments

We selected eight genes known to be involved in the RAS pathway, namely CDK5, HRAS, MAPK1, MAPK3, MAP2K1, MAP2K2, NGFR and RAF1. They are hereafter referred to as ‘core genes’ due to their relevance in the RAS pathway [2] and their consequential importance in colorectal cancer [8].

The knock-down experiments were performed on the eight core genes using short hairpin RNA (shRNA) in two colorectal cancer cell lines SW480 and SW620 [4]. For each knock-down there are six replicates (except CDK5 in SW620 with five replicates) with three different types of controls (empty vector, nontarget and non-transduced), totaling 125 samples. We used the Affymetrix GeneChip HG-U133PLUS2 platform to profile the gene expression of each sample.

Quality control

We used the simpleaffy Bioconductor package [7] to check the quality of each individual CEL file. Fig. 1 shows that a majority of files contains a sufficiently large percentage of present calls (>40%, except for biological replicates one and two and HRAS biological replicate three with 39.58%) and all scale factors lie within a 3-fold range which complies with the good quality guidelines from Affymetrix [1]. In more
Normalization

The raw and normalized data are available from NCBI Gene Expression Omnibus repository [3] with accession number GSE53091.

Basic analysis

A successful knock-down experiment should result in significantly lower expression compared to the unperturbed samples. Here, we assess the quality of a knock-down experiments by testing the difference between matched knock-down samples versus nontarget control samples using a Wilcoxon signed rank test [6].

In Fig. 3, we show the difference between knock-down and control sample expression for each of the eight knock-downs for both cell-lines together. In each plot, the knocked-down gene is highlighted in blue and the obtained p-values are represented by symbols in the bottom of each plot. The significance levels are represented as follows: '***' for p < 0.001, '**' for p < 0.01, '*' for p < 0.05 and '-' for p < 0.1.

From the eight plots in Fig. 3, we can observe that the difference in expression between knock-down and control samples is significant for all of the eight knock-downs (the differential expression of the blue boxes is significantly lower than zero). In our study [5], we determined the set of significantly affected genes for each of the eight knock-downs. For example, the knock-down of RAF1 significantly changes the expression of MAP2K2 and NGFR with p-values < 0.001 (only considering the eight core genes). We then used the set of significantly affected genes to quantitatively validate inferred gene regulatory interactions.

Discussion

In this article we described a unique data set containing RAS pathway-related gene knock-down experiments in two different colon cancer cell-lines. It contains the expression values from the knock-down of eight genes as well as three different controls in six biological replicates. The genome-wide gene expression was measured using the Human Genome U133 Plus 2.0 Array. This data was recently used in a published study on the validation of regulatory gene networks [5].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.03.011.

Fig. 1. Quality controls for the Affymetrix Raw data generated in [5]. CEL file names for each experiment is provided on the left side, followed by the percentage of present and absent calls (in red) following the Affymetrix guidelines. The blue region in the middle of the plot represents the 3-fold region for scale factor as this region is considered as acceptable according to Affymetrix guidelines; any scale factor outside this region is drawn in red as it is considered an indicator of poor quality. Beta-actin and GAPDH 3′–5′ ratios are also represented on the right side by triangles and circles, respectively; ratio higher than 1.25 are drawn in red as they are considered indicators of poor quality.
Fig. 2. Call percentage for each CEL file. The colors correspond to the biological replicate number. The quality of the first two replicates is lower than for the remaining five replicates.

Table 1
For each biological replicate, the time of data generation is specified. There are three main batches: 2008 (biological replicates 1), 2009 (biological replicates 2) and 2011 (biological replicates 3–7).

| Biological replicate | Date        | 2008-12-16 | 2008-12-17 | 2009-07-15 | 2011-07-19 | 2011-07-20 |
|----------------------|-------------|------------|------------|------------|------------|------------|
| 1                    | 11          | 10         | 0          | 0          | 0          | 0          |
| 2                    | 0           | 0          | 22         | 0          | 0          | 0          |
| 3                    | 0           | 0          | 0          | 15         | 1          | 0          |
| 4                    | 0           | 0          | 0          | 19         | 1          | 0          |
| 5                    | 0           | 0          | 0          | 17         | 3          | 0          |
| 6                    | 0           | 0          | 0          | 18         | 2          | 0          |
| 7                    | 0           | 0          | 0          | 0          | 2          | 0          |
Fig. 3. Each plot shows the difference of expression for the eight core genes. The knocked down gene highlighted in light blue. The significance level is indicated by ‘-’ for $p < 0.1$, ‘*’ for $p < 0.05$, ‘**’ for $p < 0.01$ and ‘***’ for $p < 0.001$ using a Wilcoxon signed rank test.