Network-based analysis of stem cells differentiation

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Motivation and Objectives
Understanding the real developmental stage of reprogrammed stem cells is still a demanding task for researchers in regenerative medicine. In fact, developmental biology is in need of methods that would accurately predict the developmental stage reached by cells cultured in non-standard conditions, such as the induced Pluripotent Stem Cells (iPSCs). Bioinformatics approaches would be extremely useful for assessing the pluripotency status of the cells, and thus, their potential use in the clinics for repairing malfunctioning tissues and organs.

To this aim, several works have recently demonstrated the utility of applying dimensionality reduction techniques to genome-wide expression data (Aiba et al., 2009). As we have shown (Zagar et al., 2011), these methods can be successfully used to predict the developmental stage of cells by mapping their transcriptional profile to a one-dimensional ruler, that we named differentiation scale. The proposed approach was also useful for identifying reduced subsets of genes that drive each developmental stage (Mulas et al., 2012).

A crucial issue in this field is the integration of the findings extracted from the data with the available knowledge coming from biological databases. For instance, genes that are surrounded by a high number of selected genes in the protein-protein interaction networks should be included in the analysis (Nitsch et al., 2010). STRING imports and combines data gathered from heterogeneous sources to provide information about known and predicted protein-protein associations. A confidence score is also assigned to each predicted association.

In this work, we developed and analyzed a set of STRING-based networks, one for each stage of development, by applying the following procedure:

1. Network building. For each stage-specific list of genes, we mapped the gene symbols to their corresponding protein ids provided by the UNIPROT database. A pair of genes in each network was connected if the confidence score of their proteins association exceeded a selected global threshold.
2. Topological analysis. The developmental stages measured in the considered experiments were grouped into three phases according to the clusters of projections that we observed in the differentiation scale. We analyzed the similarity of the networks obtained for each phase in terms of their topological properties. First, in order to take into account the number of common gene links in the networks, we computed the median Jaccard index between networks of different phases. Moreover, to quantitatively characterize the importance of the nodes in our networks, we considered a topological index known as betweenness centrality. This measure is defined as the number of shortest paths that go through a considered node, and represents the influence of that node in the flow of information within the network. Nodes with a high betweenness typically make possible the communications in the network among clusters of nodes characterized by high internal connectivity. The betweenness values of the genes present in each phase were used to compare the different developmental phases and to identify the most relevant genes in each network.

3. Biological analysis. The expanded lists of genes obtained with STRING were further analyzed in light of the knowledge on developmental processes reported in the literature. Different works have recently pointed out the existence of a set of specific genes that are responsible for a particular pluripotency status of the cell (Zuccotti et al., 2011). First, in order to evaluate the effect of the network-based procedure on the gene selection, we compared the list of genes retrieved with our approach with the known markers. The analysis then focused on identifying the most biologically significant genes for each differentiation phase. The importance of a gene in a developmental stage is represented by its role in the transcriptional regulatory circuitry of the process and is best quantified by its betweenness centrality value. We therefore looked for sets of significant genes among the bottlenecks of the developed networks. Phase-specific important genes were identified applying a selection procedure based on a global threshold value. For each phase, we determined a list of characterizing genes by extracting those that were contained in more than the 60% of the total number of networks present in the phase. All genes included in at least one phase-specific list were assigned the median of all their betweenness centrality values. We then considered the distribution of such median values and computed the 95th percentile, which was assumed as the threshold. Finally, from each phase-specific gene list all genes whose betweenness centrality value exceeded the threshold were extracted.

Results and Discussion
The results of the presented procedure confirmed that a transcriptional wave is active during differentiation and influences the topological properties of the networks. While the analysis of the Jaccard index showed no significant phase characterization in terms of common edges, a phase comparison based on betweenness similarity highlighted instead a distance between the first phase and the last stages (Table 1). The biological analysis, i.e. the study of the expanded lists of genes in light of knowledge on developmental processes reported in the literature, identified 53 known markers included in at least one network. In particular, a number of known key genes retrieved with this method, such as Pou5f1, Nanog, Klf4, Sox2, were not previously selected by the data-driven procedure based on their expression profiles. This result confirmed how network-based approaches can integrate experimental findings contributing to the identification of significant genes, whose importance is due to the crucial role they play in the regulation of the global network.

Gene characterization based on betweenness centrality selected a higher number of genes (24) in the first phase if compared to the others (11 and 9 genes for the second and the last phase, respectively), confirming a distinction of the early stages, where the majority of transcriptional changes are known to occur. Known markers as well as novel yet uncharacterized genes were identified. Starting from these results, future experiments will be focused on the application of network-based prioritization procedures, that would help to automatically retrieve the most significant genes in the networks.

### Table 1: Topological similarity of the networks for the three phases of differentiation.

<table>
<thead>
<tr>
<th>Phases</th>
<th>1:2</th>
<th>1:3</th>
<th>2:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaccard</td>
<td>0.78</td>
<td>0.8</td>
<td>0.75</td>
</tr>
<tr>
<td>Betweenness</td>
<td>0.58</td>
<td>0.58</td>
<td>0.91</td>
</tr>
</tbody>
</table>
Acknowledgements
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References