

Sequence analysis

omiXcore: a web server for prediction of protein interactions with large RNA

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Abstract

Summary: Here we introduce *omiXcore*, a server for calculations of protein binding to large RNAs (> 500 nucleotides). Our webserver allows (i) use of both protein and RNA sequences without size restriction, (ii) pre-compiled library for exploration of human long intergenic RNAs interactions and (iii) prediction of binding sites.

Results: *omiXcore* was trained and tested on enhanced UV Cross-Linking and ImmunoPrecipitation data. The method discriminates interacting and non-interacting protein-RNA pairs and identifies RNA binding sites with Areas under the ROC curve > 0.80, which suggests that the tool is particularly useful to prioritize candidates for further experimental validation.

Availability and implementation: *omiXcore* is freely accessed on the web at http://service.tartaglia-lab.com/grant_submission/omixcore.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

RNA-binding proteins (RBPs) amount to a large number of heterogeneous molecules encompassing a vast array of biological functions and binding modalities (Marchese *et al.*, 2016). The identification of RNA targets is important to characterize RBPs roles in physiological (Tartaglia, 2016) and pathological (Bolognesi *et al.*, 2016) conditions. Considerable attention has been given to long non-coding RNAs that are implicated in important cell functions (Guttman and Rinn, 2012) but are difficult to characterize because of their tissue-dependent expression (Chen *et al.*, 2016). Indeed, RNA interactions with RBPs require laborious experimental procedures such as chromatin isolation by RNA purification to detect protein networks bound to the RNA of interest (Chu *et al.*, 2015). The development of enhanced UV Cross-Linking and ImmunoPrecipitation (eCLIP) has recently provided a wealth of information on RBPs-binding sites at the transcriptomic level (Van Nostrand *et al.*, 2016). The large and homogeneous amount of data provided by eCLIP experiments represents an ideal dataset to

train methods for prediction of protein interactions with long non-coding RNAs. Indeed, despite considerable efforts in RNA crystallography (Zhang and Ferré-D'amaré, 2014), the paucity of structural information leads to an urgency in the implementation of high-throughput approaches for identification of protein-RNA interactions. Using the *catRAPID* approach (Bellucci *et al.*, 2011), we developed the uniform fragmentation procedure to predict interaction propensities between protein and RNA fragments (Cirillo *et al.*, 2017). Here, we introduce *omiXcore* to perform predictions of long RNAs (500 nt and larger). Calibrated on eCLIP data, *omiXcore* allows fast and quantitative prediction of RBP interactions with human long intergenic RNAs (lincRNAs), facilitating experimental design and analysis.

2 Workflow and implementation

The *omiXcore* server allows calculation of the interaction propensities of a protein sequence against i) human lincRNAs (14 717

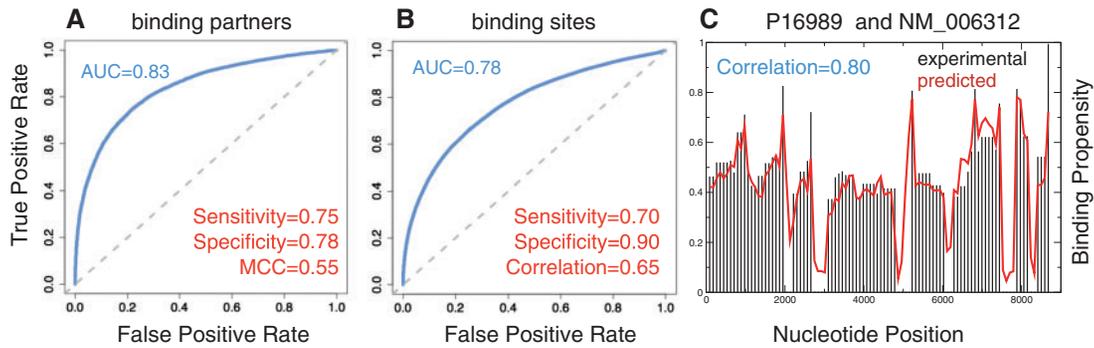


Fig. 1. *omiXcore* performances. **(A)** Binding partner prediction. For each RBP, the algorithm discriminates between interacting and non-interacting RNA pairs (A^{pred} cut-off of 0.25). **(B)** Within each RNA sequence, binding sites can be identified in a binary way (α^{pred} cut-off of 0.1) or in the continuum range (average correlation of 0.65). **(C)** Example of correlation between experimental and predicted binding sites: Y-box-binding protein 3 and nuclear receptor corepressor transcript (correlation of 0.80)

entries available in <http://www.ensembl.org/>) or ii) a custom list of transcripts (maximum of 30 K characters). Once the user submits a protein of interest, the *catRAPID signature* algorithm (Livi *et al.*, 2015) estimates the RNA-binding ability. If the protein is predicted to interact with RNA, its partners are calculated and the binding sites visualized.

- To train the algorithm, we used the eCLIP interactomes of 96 RBPs (56 studied in HepG2 and 78 in K562; downloaded from <https://www.encodeproject.org/> in July 2016). We mapped targets of RBPs to their canonical transcript isoforms. For each RNA, we measured the overall affinity A^{exp} defined as the number of reads (average of two replicas) divided by isoforms abundance (Trapnell *et al.*, 2012).
- For each RBP, we ranked the transcripts by A^{exp} and computed the local affinities α^{exp} at each RNA site. To build the negative set, we compiled a list of transcripts that do not interact with the RBP of interest (i.e. they are not reported in the two eCLIP replicas) but bind to at least one of the other RBPs. In total we used 12 234 positive and 12 717 negative interactions (balanced set with 100 RNAs per RBPs).
- For each protein-RNA pair, we used the *uniform fragmentation* procedure to calculate interaction propensities between protein and RNA fragments (Cirillo *et al.*, 2017). The uniform fragmentation approach is based on the division of protein and RNA sequences into overlapping segments [100 fragments for each molecule] (Cirillo *et al.*, 2013). This analysis is particularly useful to identify protein and RNA regions involved in the binding.
- We computed mean μ and SD σ of the interaction propensities between each RNA fragment i and the protein fragments, which we combined in the position-dependent vector $F_i = (\mu, \sigma)_i$.
- To predict the binding sites α_i^{exp} of a specific RNA fragment i , we integrated the interaction propensities F_i using the formula $h_k = \tanh(\omega_k^i F_i)$ and calculating $\alpha_i^{\text{pred}} = \tanh(\Omega_k^k h_k)$. Similarly, A^{exp} is computed using $h_k = \tanh(\omega_k^i F_i)$ and $A^{\text{pred}} = \tanh(\Omega_k^k h_k)$. Both α_i^{pred} and A^{pred} are defined in the range [0,1] and fitted to the experimental α_i^{exp} and A^{exp} optimizing the internal weights ω_k^i and Ω_k^k (neural network architecture with $i = 100$ and $k = 50$; total of 1.2×10^6 binding regions used).

3 Performances

omiXcore builds on top of *catRAPID* algorithms that have been previously validated on a large number of interactions (Agostini *et al.*, 2013;

Cirillo *et al.*, 2017; Livi *et al.*, 2015): to evaluate *omiXcore* performances, we employed a leave-one-out procedure on the 96 individual subsets, each one corresponding to one RBP with its positive and negative interactors. Performances on RBP partners (Area under the ROC curve AUC = 0.83; Sensitivity = 0.75; Specificity = 0.78; Matthews correlation coefficient of 0.55; Fig. 1A) and RNA binding sites (AUC = 0.78; Sensitivity = 0.70; Specificity = 0.90; Fig. 1B) were assessed using a binary classification of interacting versus non-interacting pairs (α^{exp} and A^{exp} cut-offs at 0.25). Cut-off points for A^{pred} and α^{pred} (0.5 and 0.1, respectively) were set maximizing the distance of the ROC curve from diagonal line (Fig. 1A and B). The 0.65 correlation (Spearman's Rho) between α^{exp} and α^{pred} allows to quantify binding sites in the continuum range (Fig. 1B and C), which is useful to detect low-affinity interactions (Jankowsky and Harris, 2015). On the testing set, *omiXcore* shows higher AUCs (in the range of 0.93–0.99) than binary classifiers such as *RPISeq* [*RPISeq*-RF:0.50–0.60; *RPISeq*-SVM:0.46–0.66] (Muppilala *et al.*, 2011) and *Global Score* [0.55–0.88; see also Supplementary Material for other performances] (Cirillo *et al.*, 2017).

4 Conclusions

In this work, we introduced the *omiXcore* tool for predicting RBP interactions with large RNAs. The algorithm allows detection of RNA binding sites by evaluating local physicochemical properties of polypeptide and nucleotide sequences (Bellucci *et al.*, 2011). *omiXcore* was calibrated on eCLIP data (Van Nostrand *et al.*, 2016) and is useful to prioritize coding and non-coding RNA targets for further experimental validation. We optimized the webserver to perform fast calculations of lincRNAs, for which we provide a pre-compiled library. Indeed, lincRNAs are poorly abundant and regulated in a precise spatiotemporal manner, which makes their characterization particularly difficult in the wet lab.

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Conflict of Interest: none declared.

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