BackCLIP: a tool to identify common background presence in PAR-CLIP datasets

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2. Research problem
3. Proposed approach
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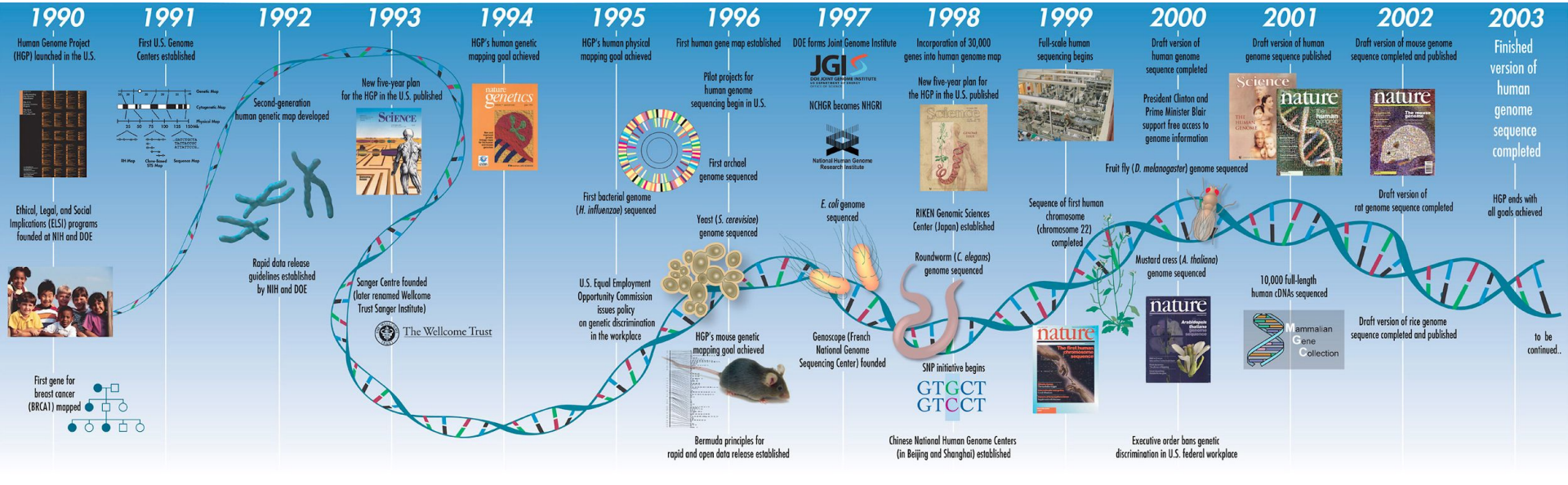
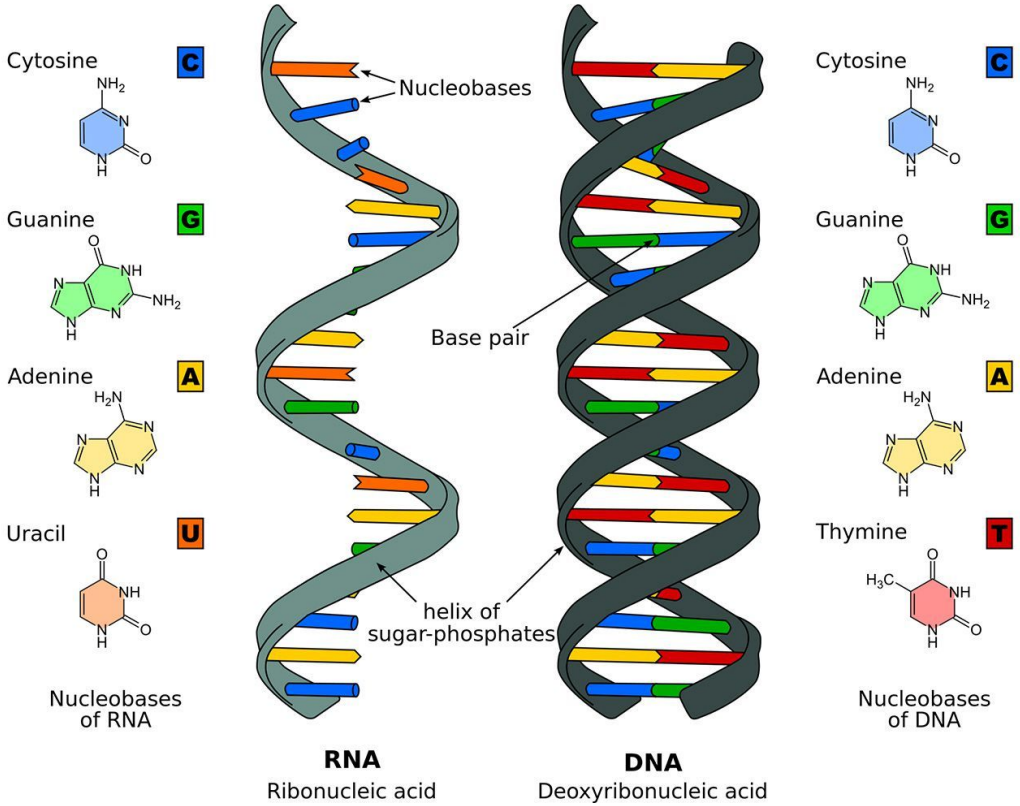
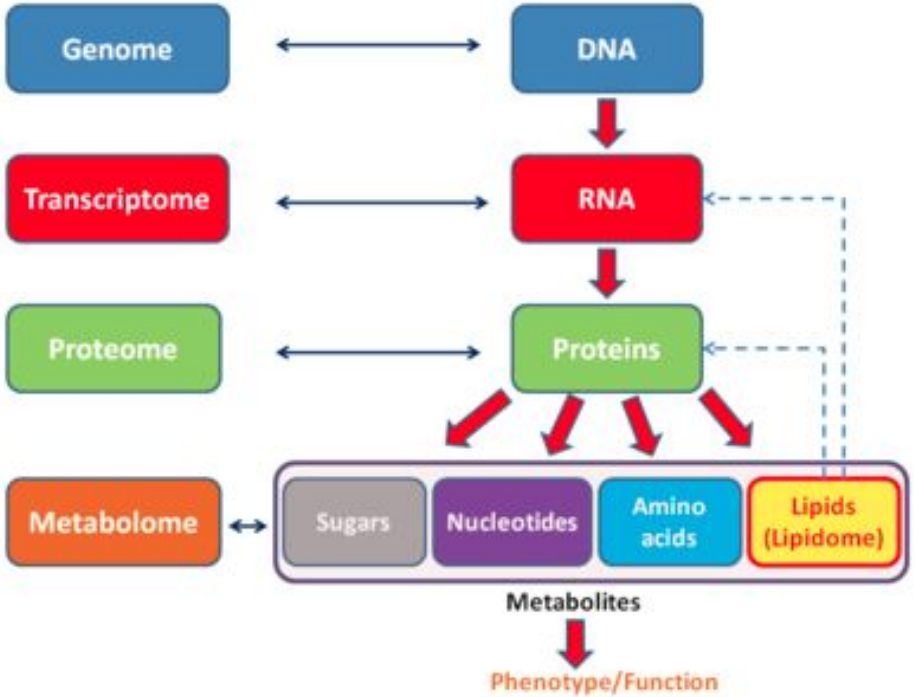
Human Genome



DNA & RNA


Cytosine
C
Guanine
G
Adenine
A
Uracil
U
Nucleobases of RNA
Nucleobases
Base pair
helix of sugar-phosphates
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
Cytosine
C
Guanine
G
Adenine
A
Thymine
T
Nucleobases of DNA

Transcriptome

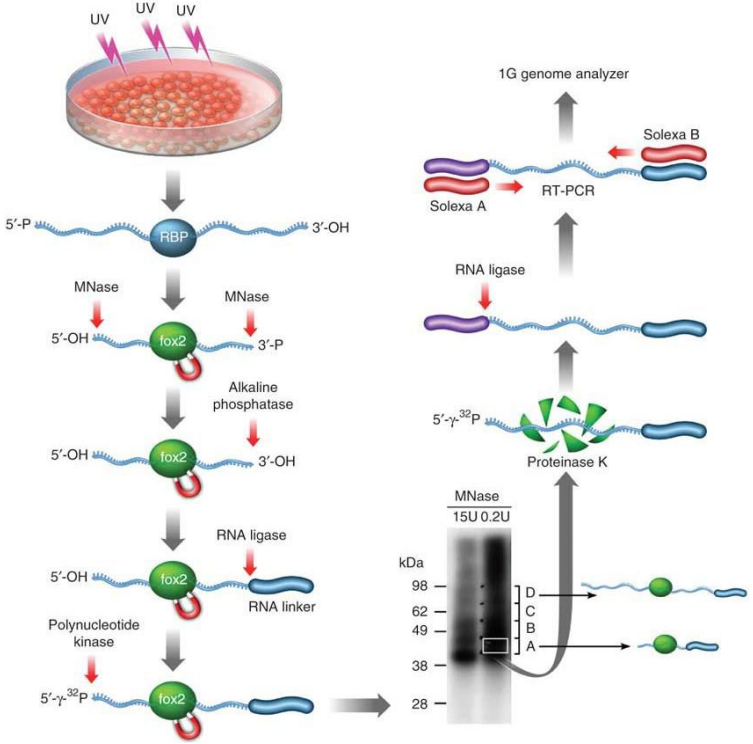


CLIP-seq protocol

Check interactions between RNA and proteins.

It uses antobiotics to affect protein binding sites.

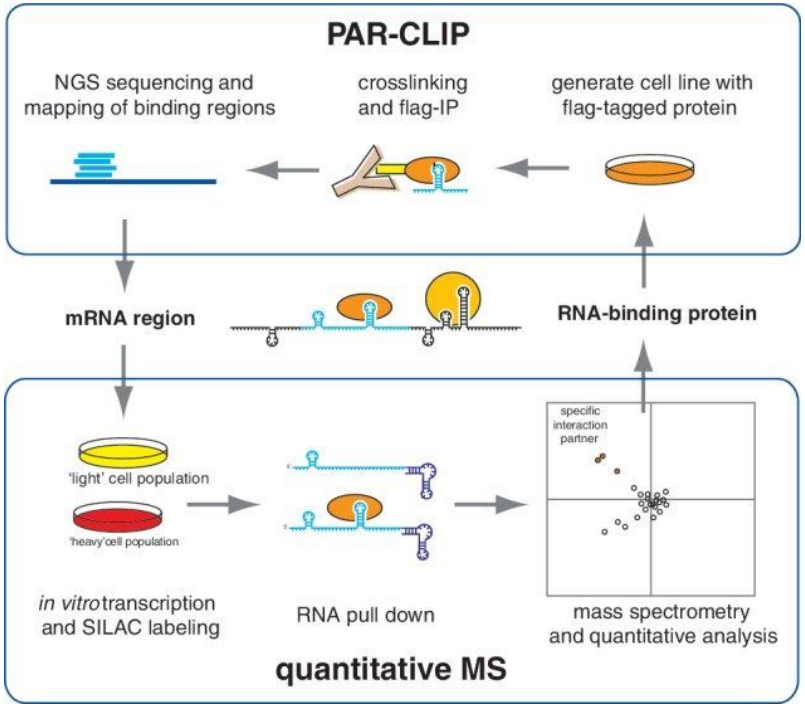
Immunoprecipitation helps to obtain specific genetic sequences.



PAR-CLIP data

PAR-CLIP, a frequently used CLIP-seq protocol, uses **photo activatable** nucleosides to label the transcripts in addition to an enhanced crosslinking (*Hafner et al., 2010*).

These modifications induce specific nucleotides transitions that facilitate the recognition of the cross-linked sites.



RBP (RNA Binding Proteins)

RNA-binding proteins (RBPs) have important roles in RNA regulation. The first step to understand RBPs' specific functions is to identify the RNA targets for each RBP.

The introduction of CLIP-seq protocols have made it possible to obtain sets of binding sites for RBPs at a transcriptome-wide scale (*Licatalosi et al., 2008*).

However, each CLIP-seq protocol introduces distinct modifications to reduce the presence of background (non-crosslinked RNA).

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Motivation

PAR-CLIP derives a transcriptome wide set of binding sites for RNA-binding proteins. Even though the protocol uses stringent washing to remove experimental noise, some of it remains.

A recent study measured three sets of non-specific RNA backgrounds which are present in several PAR-CLIP datasets (*Sievers et al., 2012*).

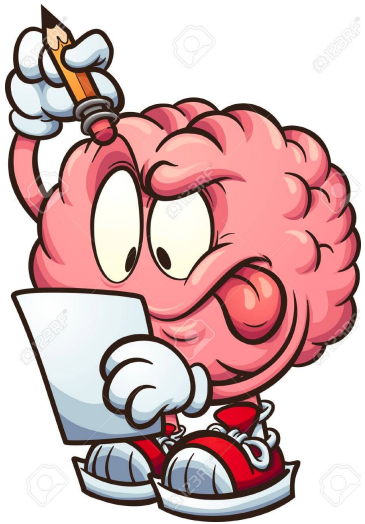
Motivation

However, a tool to identify the presence of common background in PAR-CLIP datasets is not yet available.

Non-specific RNA background must be taken into account when processing PAR-CLIP data because it can interfere with the distinction of the specific characteristics recognized by the RBPs, and therefore the identification and understanding of binding targets and protein function (*Friedersdorf and Keene, 2014*).

Research Question

Is it possible to create a tool to identify common background presence in PAR-CLIP datasets and remove it?



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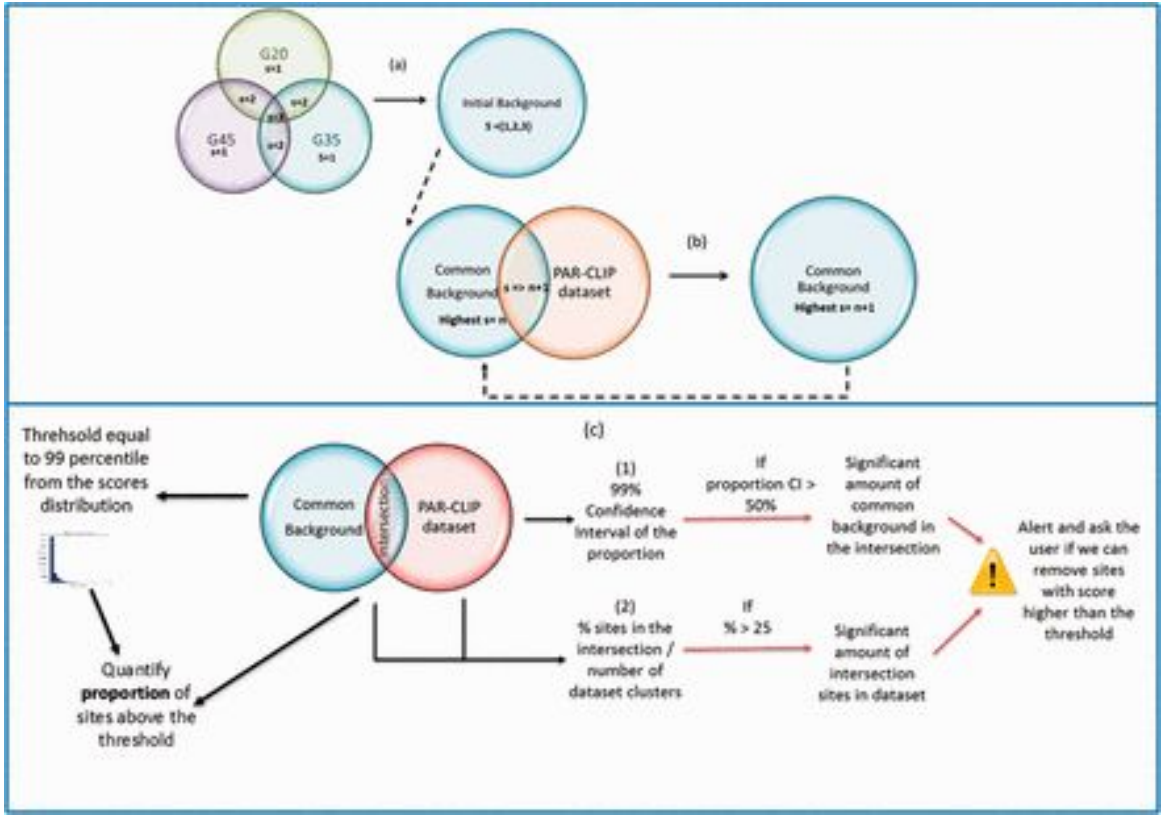
Proposed Approach



Score Measure

Definition of possible motif candidates.

Clusters detection based on position, chromosome, and motif candidate.

Count occurrences of motifs into clusters based on clustering profiles.

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Results

We used the proposed strategy in 30 PAR-CLIP datasets from nine proteins.

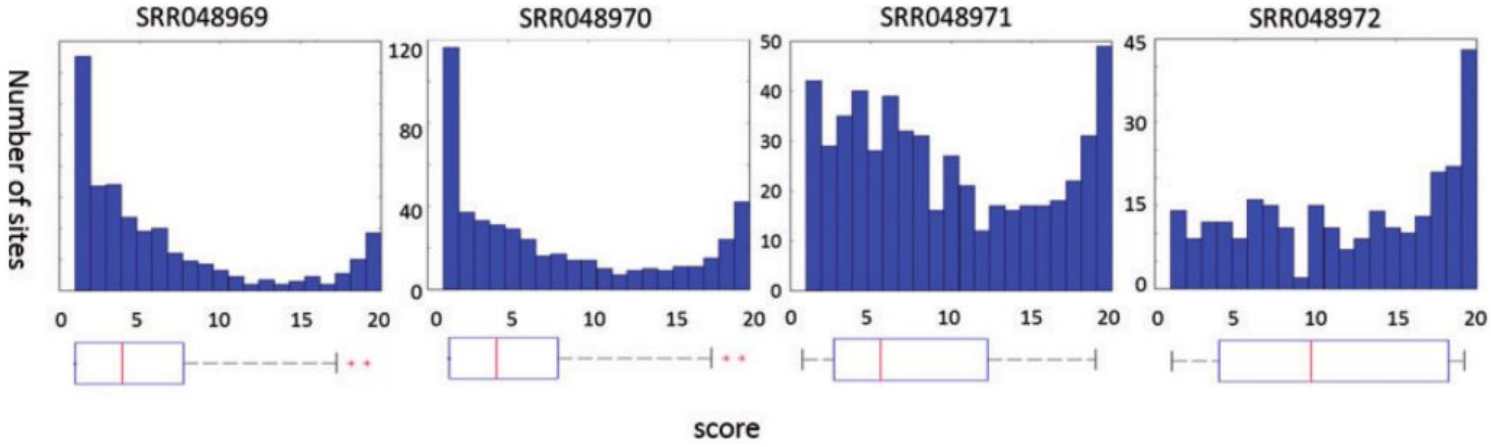
As an example, we selected Quaking (QKI) protein from the nine RBPs (30 PAR-CLIP datasetsand four of its PAR-CLIP datasets.

Results

Table 1. QKI datasets results for the PAR-CLIP dataset and for the background intersection

Dataset	Clusters	Clusters with motif (%)	Background intersection Sites	Background intersection sites with motif (%)	Background intersection sites / Clusters (%)	motifs in intersection / motifs in dataset (%)	Proportion of sites above threshold (score = 8)	BackCLIP sites identified (score ≤ 8)	BackCLIP sites with motif (%)
SRR048969	5286	44	654	11	12	3.1	[32%, 41%]	260	2
SRR048970	5091	48	479	14	9	2.7	[29%, 39%]	176	2
SRR048971	1688	23	539	5	32	7.0	[41%, 52%]	294	2
SRR048972	590	13	276	3	47	10.8	[55%, 59%]	178	1

Results



Histogram and boxplot of the scores in the intersection between the common background and four QKI PAR-CLIP datasets (Tophat alignment)

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It is possible to identify the presence of common backgrounds in a dataset and identify differences in datasets for the same protein.

BackCLIP is a useful tool to identify the amount of common background in any dataset. This method is the first step in the process of completely removing such backgrounds.

GitHub link: ***https://github.com/phrh/BackCLIP***

Thanks!

Questions?

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