Xinjian Mao Linheng Li Lab

CLIP-Seq
(from Zhenrui's 2018 'Cell Research' paper)

1. irCLIP was performed on ~3 $\times 10^8$ Flag-Ythdf2 HPC7 cells by UV crosslinking the cells at 0.4 J/cm2 with three treatments.
2. Whole-cell lysates were generated in lysis buffer (150mM KCl, 10mM HEPES pH 7.6, 2mM EDTA, 0.5% NP-40, 0.5mM dithiothreitol (DTT), 1:100 protease inhibitor cocktail, 400 U/ml RNase inhibitor; 1ml cell pellet and 2ml lysis buffer).
3. The lysates were pipetted up and down several times, and then incubated on ice for 5 mins and shock-frozen at −80 °C with liquid nitrogen. The mRNP lysate was thawed on ice and centrifuged at 15,000 g for 15 min to clear the lysate.
4. Flag-Ythdf2 was isolated with 30µl of protein-G magnetic beads per 1ml lysate, previously bound to 2µg of anti-Flag monoclonal antibody (Sigma) for 2 h at 4 °C on rotation. The beads were collected, washed eight times with 1ml ice-cold NT2 buffer (200mM NaCl, 50mM HEPES pH 7.6, 2mM EDTA, 0.05% NP-40, 0.5mM DTT, 200 U/ml RNase inhibitor) and once with 200µl irCLIP NT2 buffer (50mM Tris, pH 7.5; 150 mM NaCl; 1mM MgCl2; 0.0005% NP-40).
5. mRNP complex was digested with RNase 1 (Thermo Fisher #AM2294) at 0.4 U/µl in irCLIP NT2 buffer (aqueous volume of 30µl and supplemented with 6µl of PEG400 (16.7% final)).
6. The nuclease reaction was incubated at 30 °C for 15 min in an Eppendorf Thermomixer, 15s 1400 r.p.m., 90s rest. Nuclease digestions were stopped by addition of 0.5 mL of ice-cold high-stringency buffer (20mM Tris, pH 7.5; 120mM NaCl; 25mM KCl; 5mM EDTA; 1% Trition X-100; 1% Na-deoxycholate).
7. Immuno-precipitates were then quickly rinsed with 0.25 mL then with 0.05 mL of ice-cold irCLIP NT2 buffer.
8. The irCLIP adaptor ligation and library construction followed a previously reported protocol.[1]

Data analysis

1. Data were demultiplexed using FAST-iCLIP version 0.9.3 and aligned to mouse genome mm10 from UCSC using STAR (2.4.2a) with parameters "–outFilterScoreMinOverLread 0 –outFilter-MatchNminOverLread 0 –outFilterMatchNmin 0".
2. RPM normalized genome browser tracks were created in R (3.4.1) and plotted using the Gviz package (1.20.0).

3. Enriched motifs were identified by taking midpoints of each binding site found in all three replicates, adding 20 bases up and downstream, and running MEME (4.11.1) with parameters "-dna -mod zoops -revcomp -minw 5 -maxw 10 -nmotifs 10 -maxsize 1000000".
4. After motifs were identified, tomtom (4.11.1) against transfac (1–2017) was run to identify known binding sites.
5. GO enrichment analysis was performed using a hypergeometric test in R. GO terms were considered enriched if they had a BH-adjusted p-value < 0.05.
6. Selected terms of interest are shown in the bar plot. Bars in the bar plot indicate percentage of genes in the list being tested having the term divided by the percentage of genes in the genome having the term.
7. Peaks found by FAST-iCLIP in all three replicates were assigned to various features in the genome. Promoters were defined as upstream 150 bases from the TSS. "trans_stop" was defined as upstream and downstream 200 bases from the transcript start site.

(References)

1. Zarnegar, B. J. et al. irCLIP platform for efficient characterization of protein-RNA interactions. Nat. Methods 13, 489–492 (2016).