RNA Bind-N-Seq (RBNS) Experimental Protocol, updated Nov. 2015

Preparation of random RNA

RBNS input random RNA was prepared by in vitro transcription using the RBNS T7 template, a DNA oligo containing a random 20mer sequence flanked by priming sites for the addition of Illumina adapters and the T7 promoter sequence. To artificially create a double-stranded T7 promoter, the T7 oligo was annealed to the region of the RBNS T7 template corresponding to the T7 promoter sequence by heating the template, T7 oligo and water at 65 degrees for 5 minutes and then allowing the solution to cool at room temperature for 2 minutes. The RBNS input RNA pool was then in vitro transcribed with T7 polymerase using Ampliscribe (Epicentre) or HiScribe T7 In vitro transcription kits (NEB). The in vitro transcribed RNA was then purified with RNA Clean & Concentrator-100 kits (Zymo Research). The resulting RBNS input RNA pool is:

GAGUUCUACAGUCCGACGAUC$(N)_{20}$UGGAAUUCUCGGGUGUCAAGG.

RBNS T7 template:

CCTTGACACCCGAGAATTCCA$(N)_{20}$GATCGTCGGACTGTAGAACTCCCTATAGTGAGTCGTATTA

T7 oligo: TAATACGACTCACTATAGGG

RNA Bind-n-seq (RBNS)

RBNS was performed after purification of the RBP and in vitro transcription of RBNS input RNA. Five concentrations of RBP (5, 20, 80, 320, 1300 nM), as well as a no RBP condition, were each equilibrated in 250ul of binding buffer (25mM tris pH 7.5, 150 mM KCl, 3mM MgCl2, 0.01% tween, 500 ug/mL BSA, 1 mM DTT) for 30 minutes at 37°C or 4°C. RBNS input random RNA was then added to a final concentration of 1uM with 40 U of Superasin (Ambion). RBP and RNA were incubated for 1 hour at 37°C or 4°C. During this incubation, streptavidin magnetic beads (Invitrogen) were washed 3 times with 1 mL of binding buffer and then equilibrated in binding buffer until needed. To pull down tagged RBP and interacting RNA, each RNA/protein solution was then added to 1 mg of washed magnetic beads and incubated for one hour at 37°C or 4°C. Unbound RNA was removed from the beads and the beads were washed 3 times with 1 mL of

wash buffer (25mM tris pH 7.5, 150 mM KCl, 0.5 mM EDTA, 0.01% tween). To elute bound RBP/RNA complexes, beads were incubated at at 37°C or 4°C for 15 minutes in 50 uL of elution buffer (4mM biotin, 1x PBS); the eluate was collected; the elution step was repeated; and eluates were pooled. Bound RNA was extracted from the eluate with the RNA Clean & Concentrator-5 kit (Zymo Research). The extracted RNA from each condition was reverse transcribed into cDNA using Superscript III (Invitrogen) according to manufacturer's instructions using the RBNS RT primer. To control for any nucleotide biases in the input random library, 0.5 pmol of the RBNS input RNA pool was also reverse transcribed and Illumina sequencing library prep followed for all experimental conditions as outlined below. To make Illumina sequencing libraries, primers with Illumina adapters and sequencing barcodes were used to amplify the cDNA by PCR using high fidelity Phusion (NEB) with 12-14 amplification cycles. PCR primers always included RNA PCR 1 (RP1) and one the indexed primers listed below. PCR products were then gel-purified from 8% TBE polyacrylamide gels or 3% agarose gels. Sequencing libraries corresponding to all concentrations of a given RBP were pooled in a single lane and the random 20mer was sequenced on the HiSeq2000.

RBNS RT primer: GCCTTGGCACCCGAGAATTCCA

RNA PCR (RP1)

AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGACGATC

Index 1 (RPI1)

CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 2 (RPI2)

CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 3 (RPI3)

CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 4 (RPI4)

CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 5 (RPI5)

CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 6 (RPI6)

CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 7 (RPI7)

CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 8 (RPI8)

CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 9 (RPI9)

CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA

Index 10 (RPI10)

CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCCTTGGCACCCGAGAATT
CCA