ChIP-Seq:

#Quality control

fastqc –o ./output\_dir \*.fastq

#Trim adapter sequences

trim\_galore –o ./output\_dir \*.fastq

#Build genome

bowtie2-build NRC1.fna NRC1

#Align

bowtie2 –x NRC1 –U \*\_trimmed.fq –S \*.sam

#Convert and index

samtools view –bS \*.sam > \*.bam

samtools sort \*.bam –o \*\_sorted.bam

samtools index \*\_sorted.bam \*\_sorted.bam.bai

#Generate WIG and perbase-depth text files

samtools mpileup sorted.bam | perl -ne 'BEGIN{print "track type=wiggle\_0 name=sorted description=fileName\n"};($c, $start, undef, $depth) = split; if ($c ne $lastC) { print "variableStep chrom=$c\n"; };$lastC=$c;next unless $. % 10 ==0;print "$start\t$depth\n" unless $depth<3;' > sorted.wig

bedtools genomecov -ibam sorted.bam -d > perbase.txt

#Peak calling

macs2 callpeak -t IP\_sorted.bam -c WCE\_sorted.bam -f BAM -n Control -B -q 0.01 --nomodel

RNA-Seq: (paired-end sequencing)

#Quality control

fastqc –o ./output\_dir \*.fastq

#Trim adapter sequences

trim\_galore –o ./output\_dir --paired \*.fastq

#Build genome

bowtie2-build NRC1.fna NRC1

#Align

bowtie2 –x NRC1 –1 \*\_1\_val\_1.fq.gz –2 \*\_2\_val\_2.fq.gz –S \*.sam

#Convert and index

samtools view –bS \*.sam > \*.bam

samtools sort \*.bam –o \*\_sorted.bam

samtools index \*\_sorted.bam

#Generate count files using gff file downloaded from NCBI

htseq-count –t gene –i Name –s reverse –f bam \*.bam NRC1.gff > \*.count

RNA-Seq (differential expression analysis):

Count files were generated as above. It was found that ~90% of reads corresponded to rRNA in the first batch (NEB-suppled rRNA probes) despite their apparent removal as seen on the Bioanalyzer. It was hence decided, based on analysis using Scotty (<http://scotty.genetics.utah.edu/>) that another batch of 3 more replicates should be sequenced to get adequate representation of differentially expressed genes. This second batch was cleaned using custom-designed probes, and were found to be an improvement with ~70-90% reads mapping to ribosomal RNA. These rRNA reads were manually removed before differential expression analysis.

Outliers were detected and removed using strong PCA (as described in Chen et. al. 2020 BMC Bioinformatics) on: (a) all 24 samples as a whole to detect cross-condition outliers. Three outlier samples were detected; (b) sets of 12 samples separately (each batch resulted from the different rRNA removal methods). Four outlier samples were detected (including 2 of the 3 found by the other method). Separate differential gene expression analyses were carried out on each of these sample sets using DeSeq2 (i.e. 21 samples with 3 outliers removed, and 20 samples with 4 outliers removed). The 170 resultant differentially expressed genes present in the intersection of gene lists from each of these two analyses are given in Supplementary Table S5 and discussed in the text. **The Rmd file “2021Combined\_hpyAsalt\_newcounts\_all-AKS**“ shows analysis with the 21 samples. The same code was used for the 20 sample set. Differential expression is measured as (∆*hpyA*/WT), and is looked for in optimal salt samples only, reduced salt samples only, and in all samples as a combinatorial effect (i.e. which genes have a different (∆*hpyA*/WT) genotypic ratio in the two salt concentration). For each analysis, dispersion, MA, and volcano plots were made to check for any biases in the data. Differentially-expressed genes were noted at a significance threshold of a Benjamini-Hochberg adjusted p-value of 0.05 using the Wald test (default within DeSeq2). Results from this analysis were manually combined into the table S4.