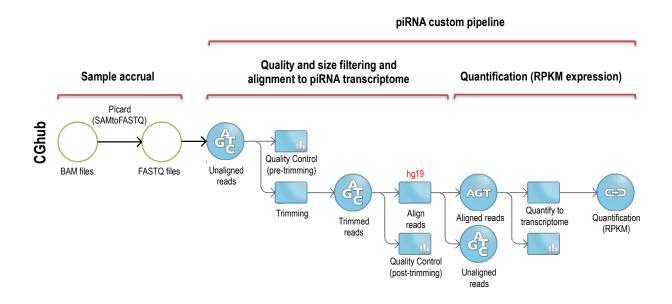
Article Title: An atlas of gastric PIWI-Interacting RNA transcriptomes and their utility for identifying signatures of gastric cancer recurrence Journal: Gastric Cancer Authors: Victor D Martinez, Katey SS Enfield, David A. Rowbotham, Wan L Lam

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Supplementary Figure 1. Custom sequence analysis pipeline to characterize piRNA expression in non-malignant stomach tissue and gastric adenocarcinoma. Flow diagram depicting the three main steps of our custom analysis pipeline (sample accrual, quality control/size-based piRNA enrichment, and data quantification). Circles represent analyzed data, squares represent data processing steps. Green colouring represents files downloaded from The Cancer Genomics Hub (CGHub); blue colouring represents in-house generated data. Raw read sequences and quality scores were extracted using the "SamToFastq" tool in the Picard analysis package (http://picard.sourceforge.net). Resulting FASTQ files were trimmed based on quality (Phred quality score  $\geq 20$ ) and size (read length  $\geq 24$  bp), and then remapped to human genome (hg19) using STAR aligner (1). Quantification was performed using PartekFlow<sup>TM</sup> (Partek Inc., MO, USA). Read counts were scaled using the Reads Per Kilobase of exon model per Million mapped reads (RPKM) method (2). We built a custom piRNA reference transcriptome using genomic coordinates obtained from the functional RNA database (v 3.4) (3, 4) available through an fRNAdb track in the UCSC genome browser. MicroRNA sequences (miRBase version 20) had minimal overlap ( $\leq 0.5\%$ ) with piRNA sequences. Circular representation of genome-wide expression in non-malignant and tumour tissue was performed using CircosPlot (5). GEO submission in progress.

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