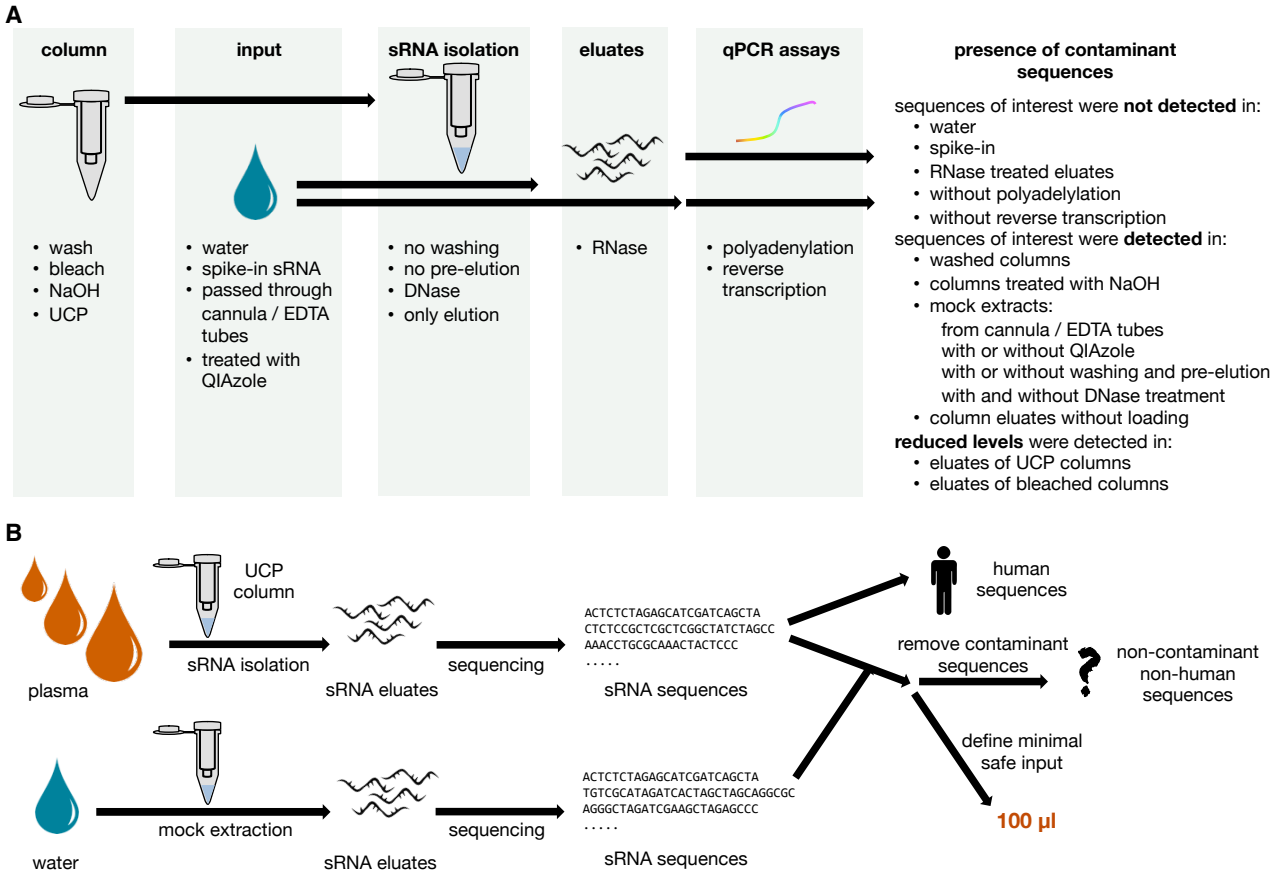
A
column
input
sRNA isolation
eluates
qPCR assays
presence of contaminant sequences
• wash
• bleach
• NaOH
• UCP
• water
• spike-in sRNA
• passed through cannula / EDTA tubes
• treated with QIAzole
• no washing
• no pre-elution
• DNase
• only elution
• RNase
• polyadenylation
• reverse transcription
sequences of interest were **not detected** in:
• water
• spike-in
• RNase treated eluates
• without polyadelylation
• without reverse transcription
sequences of interest were **detected** in:
• washed columns
• columns treated with NaOH
• mock extracts:
from cannula / EDTA tubes
with or without QIAzole
with or without washing and pre-elution
with and without DNase treatment
• column eluates without loading
reduced levels were detected in:
• eluates of UCP columns
• eluates of bleached columns
B
plasma
UCP column
sRNA isolation
sRNA eluates
sequencing
ACTCTCTAGAGCATCGATCAGCTA
CTCTCCGCTCGCTCGGCTATCTAGCC
AAACCTGCGCAAACTACTCCC
.....
sRNA sequences
water
mock extraction
sRNA eluates
sequencing
ACTCTCTAGAGCATCGATCAGCTA
TGTCGCATAGATCACTAGCTAGCAGGCGC
AGGGCTAGATCGAAGCTAGAGCCC
.....
sRNA sequences
human sequences
remove contaminant sequences
?
non-contaminant non-human sequences
define minimal safe input
100 µl