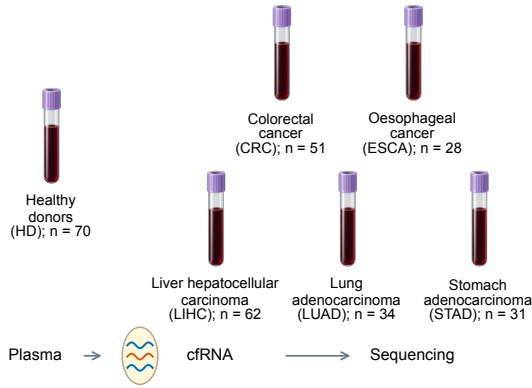


a Plasma cfRNA cohort

70 healthy donors · 206 cancer patients

**b Preserve the assay signal**

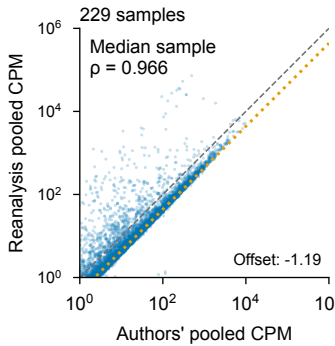
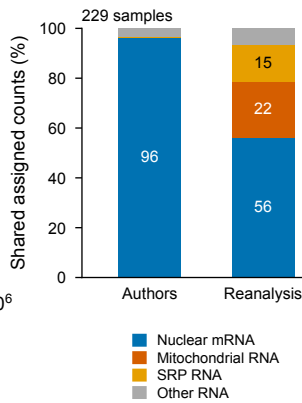
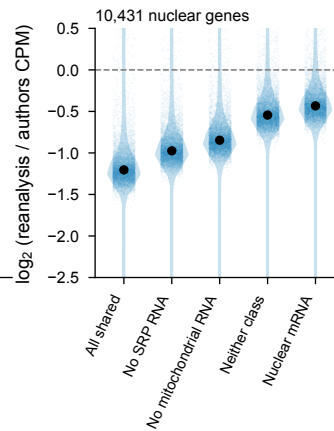
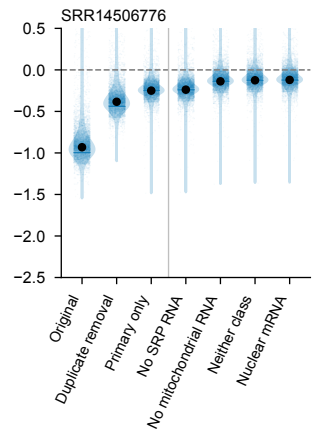
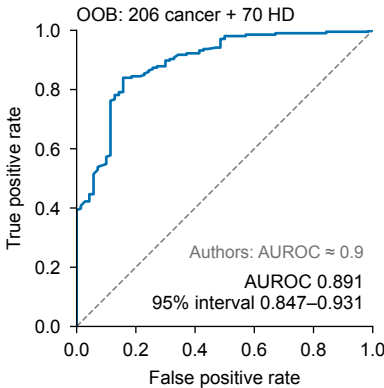
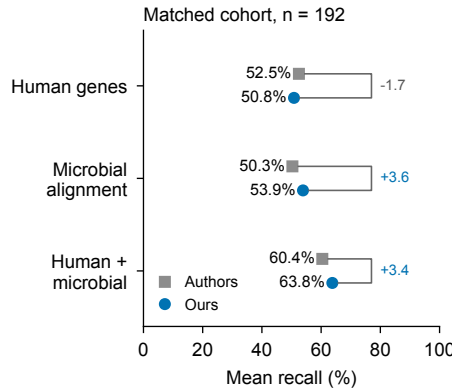
nf-core/maseq
4 preprocessing submissions

- Keep microbial reads
--skip_bbsplit true
--save_unaligned true
- Remove human rRNA only
SortMeRNA; human-only rRNA manifest
- Retain negative controls
--min_mapped_reads 0
- Match author gene counts
GENCODE v27; featureCounts 1.6.2
Exclude marked duplicates

c Downstream stages

Flyte: 2 processing +
6 analysis submissions

- Process reads · 2 runs
Counts, circular RNA, microbes
Limit parallel downloads
1 fails → 1 succeeds
- Analyse data · 6 runs
Exclude near-zero libraries
to fix normalisation
1 fails; 5 succeed
- Compare outputs
Quality filters and classifiers
Human and microbial features
Includes alternative analyses

d Matched counts**e RNA composition****f Normalisation sensitivity****g Single-sample sensitivity****h Human cancer versus HD****i Cancer-type classification****j Human + microbial: cancer type**

	n = 192				
	CRC	ESCA	LIHC	LUAD	STAD
True / recall					
CRC 66%	66%	2%	2%	16%	13%
ESCA 70%	3%	70%	14%	8%	5%
LIHC 92%	1%	3%	92%	2%	2%
LUAD 41%	25%	11%	4%	41%	18%
STAD 49%	28%	7%	4%	12%	49%
	CRC	ESCA	LIHC	LUAD	STAD
	Cancer type (predicted)				