

Figure S1. Linearity of response for a variety of capture and detection schemes in monovalent mRNA samples, with HA in left column and NA in right column. a) Coding region capture with polyT label as shown in Figure 1e(i), (b) Coding region capture and labeling with 5' cap antibody label as shown in Figure 1e(ii). Data point are the average of three replicates and error bars indicate ± 1 standard deviation ($n=3$). R^2 in upper left of each plot is based on a single linear regression.

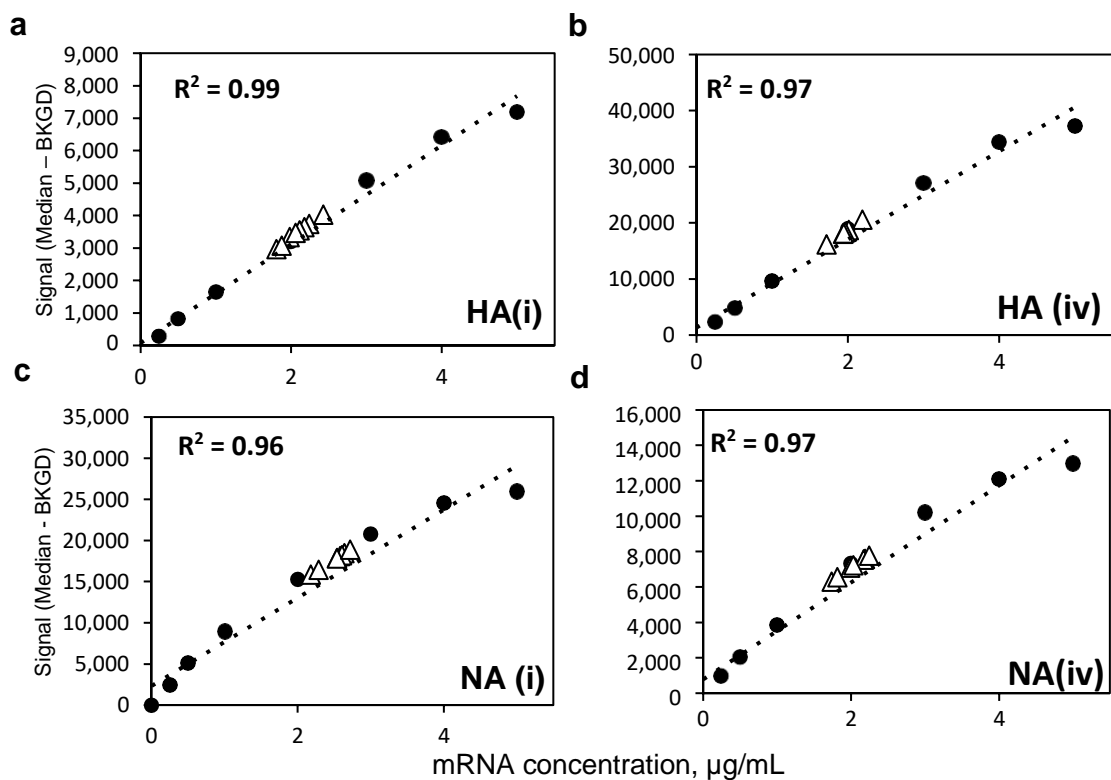


Figure S2. Signal Response curves for monovalent LNP-mRNA samples, labeled with polyT label. 8-pt response curves generated comparing a monovalent HA standard curve (●) to eight replicates of lysed LNP-mRNA (Δ) using (a) HA(i) coding region capture oligo, (b) HA(iv) coding region capture oligo, and for NA mRNA using (c) NA(i) coding region capture oligo, and (d) NA(iv) coding region capture oligo.

Table S1. HA and NA construct sequences

mRNA	Sequence
HA	ATGAAGGTGAAGCTGCTGGTGCTGCTGTGCACCTTCACCGCCACCTACGCCGACACCATCTGCATCGG CTACCACGCCAACAACTCCACCGACACCGTGACACCGTGCTGGAGAAGAACGTGACCGTGACCCACT CCGTGAACCTGCTGGAGAACGGCGGGCGGGCGGCAAGTACGTGTGCTCCGCCAAGCTGCGCATGGTGAC CGGCCTGCGCAACAAGCCCTCCAAGCAGTCCCAGGGCCTGTTGCGCGCCATCGCCGGCTTCACCGAG GGCGGCTGGACCGGCATGGTGGACGGCTGGTACGGCTACCACCACCAGAACGAGCAGGGCTCCGGC TACGCCGCCGACCAGAAGTCCACCCAGAACGCCATCAACGGCATCACCACAAGGTGAACTCCGTGAT CGAGAAGATGAACACCCAGTACACCGCCATCGGCTGCGAGTACAACAAGTCCGAGCG ₉ TGCATGAAGC AGATCGAGGACAAGATCGAGGAGATCGAGTCCAAGATCTGGTGCTACAACGCCGAGCTGCTGGTGCTG CTGGAGAACGAGCGCACCCCTGGACTTCCACGACTCCAACGTGAAGAACCTGTACGAGAAGGTGAAGT CCAGCTGAAGAACAACGCCAAGGAGATCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCAACGACG AGTGCATGGAGTCCGTGAAGAACGGCACCTACGACTACCCCAAGTACTCCGAGGAGTCCAAGCTGAAC CGCGAGAAGATCGACGGCGTGAAGCTGGAGTCCATGGGCGTGTACCAGATCGAGGGCCGC
NA	ATGAACCCCAACCAGAAGATCATCACCATCGGCTCCATCTGCATGACCATCGGCATGGCCAACCTGATC CTGCAGATCGGCAACATCATCTCCATCTGGGTGTCCCACTCCATCCAGATCGGCAACCAGTCCCAGATC GAGACCTGCAACCAGTCCGTGATCACCTACGAGAACAACACCTGGGTGAACCAGACCTACGTGAACAT CTCCAACACCAACTTCGCCCGCCGGCCAGTCCGTGGTGCTCCGTGAAGCTGGCCGGCAACTCCTCCCTGT GCCCCGTGTCCGGCTGGGCCATCTACTCAAGGACAACCTCCGTGCGCATCGGCTCCAAGGGCGACGT GTTTCGTGATCCGCGAGCCCTTCATCTCCTGCTCCCCCTGGAGTGCCGCGACCTTCTTCCTGACCCAGG GCGCCCTGCTGAACGACAAGCACTCCAACGGCACCATCAAGGACCGCTCCCCCTACCGCACCCCTGATG TCCTGCCCCATCGGCGAGGTGCCCTCCCCCTACAACCTCCCGCTTCGAGTCCGTGGCCTGGTCCGCCTC CGCCTGCCACGACGGCATCAACTGGCTGACCATCGGCATCTCCGGCCCCGACTCCGGCGCCGTGGCC GTGCTGAAGTACAACGGCATCATCACCAGACCATCAAGTCCTGGCGCAACAACATCCTGCGCACCCA GGAGTCCGAGTGCGCCTGCGTGAACGGCTCCTGCTTCACCATCATGACCGACGGCCCCCTCCGACGGC CAGGCCTCCTACAAGATCTTCCGCATCGAGAAGGGCAAGATCATCAAGTCCGTGGAGATGAAGGCCCC CAACTACCACTACGAGGAGTGCTCCTGCTACCCCGACTCCTCCGAGATCACCTGCGTGTGCCGCGACA ACTGGCACGGCTCCAACCGCCCCCTGGGTGTCTTCAACCAGAACCTGGAGTACCAGATGGGCTACATC TGCTCCGGCGTGTTTCGGCGACAACCCCCGCCCCAACGACAAGACCGGCTCCTGCGGCCCCGTGTCCT CCAACGGCGCCAACGGCGTGAAGGGCTTCTCCTTCAAGTACGGCAACGGCGTGTGGATCGGCCGAC CAAGTCCATCTCCTCCCGCAAGGGCTTCGAGATGATCTGGGACCCCAACGGCTGGACCGGCACCGACA ACAAGTTCTCCATCAAGCAGGACATCGTGGGCATCAACGAGTGGTCCGGCTACTCCGGCTCCTTCGTG CAGCACCCCGAGCTGACCGGCCCTGGACTGCATCCGCCCTGCTTCTGGGTGGAGCTGATCCGCGGCC GCCCCGAGGAGAACACCATCTGGACCTCCGGCTCCTCCATCTCCTTCTGCGGCGTGAACCTCCGACACC GTGGGCTGGTCTGGCCCCACGGCGCCGAGCTGCCCTTACCATCGACAAG

Table S2. Oligonucleotide sequences designed

Type	Name	Sequence (5' → 3')
Capture Oligo	HA(i)	TGTTGGCGTGGTAGCCGATGC
	HA(ii)	CCGCTCGGACTTGTTGTACTC
	HA(iii)	TGCACTCGTCGTTGCACTTGTG
	HA(iv)	CGTCGATCTTCTCGCGGTTTC
	NA(i)	GATGTTGCCGATCTGCAGGATC
	NA(ii)	GTTGTTGCGCCAGGACTTGAT
	NA(iii)	GTTCTGGTTGAAGGACACCCAGG
	NA(iv)	TGATGGAGAACTTGTGTGCG
	polyT	TTTTTTTTTTTTTTTTTTTTTTTTTTTT
Label	polyT	TTTTTTTTTTTTTTTTTTTTTTTTTTTT
	HA nt741-760	CGTCGATCTTCTCGCGGTTTC
	NA nt1149-1168	TGATGGAGAACTTGTGTGCG