Molecular Cell, Volume *80*

Supplemental Information

Phosphoregulation of Phase Separation by the SARS-CoV-2 N Protein Suggests a Biophysical Basis for its Dual Functions

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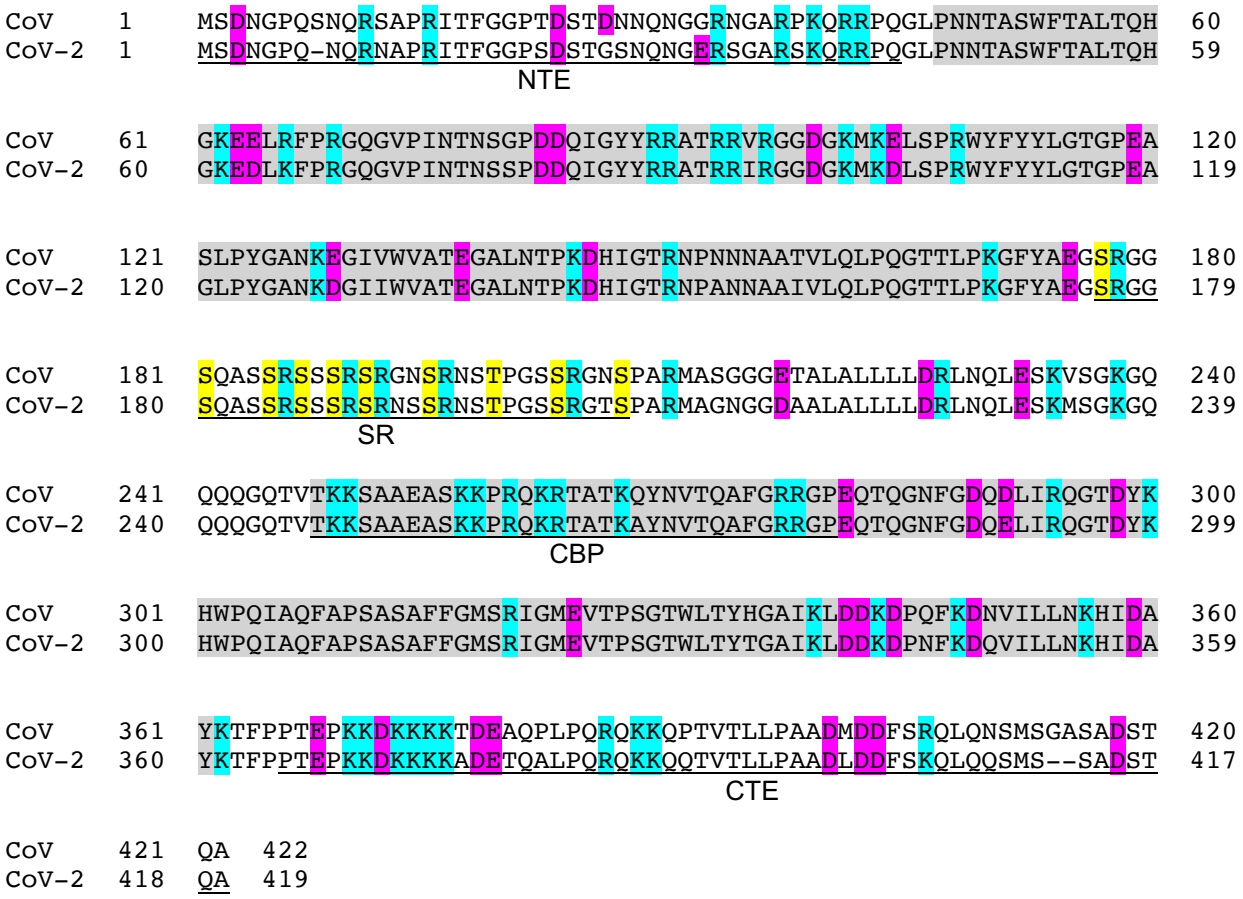

Figure S1. Amino acid sequences of N proteins from SARS-CoV and SARS-CoV-2, related to Figure 1A.

The two globular domains, NTD and CTD, are highlighted in gray. Underlining indicates the four regions analyzed by deletion mutants. Acidic residues highlighted in pink, basic residues highlighted in blue, and phosphorylation sites of the SR region highlighted in yellow.

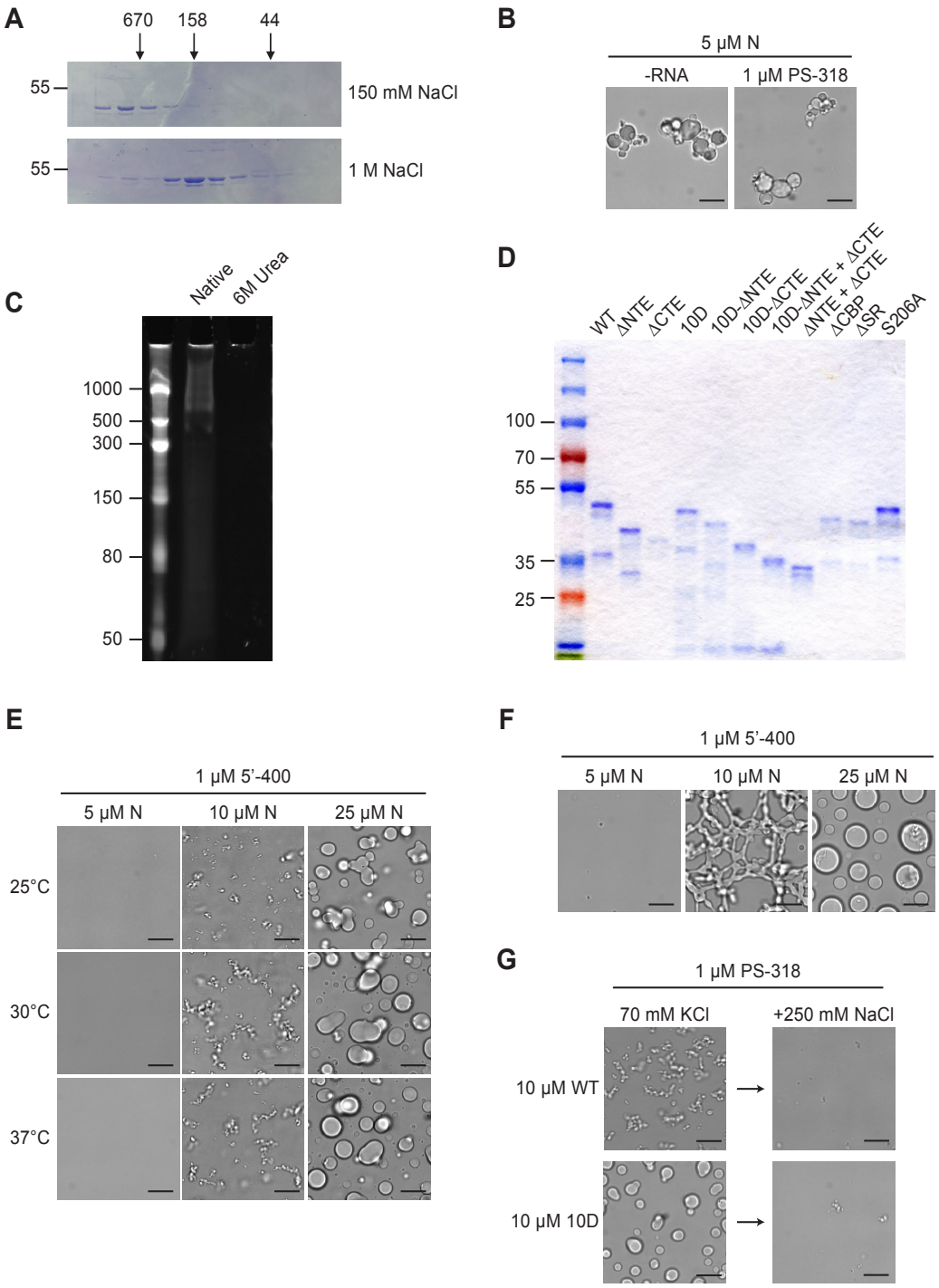

Figure S2. Characterization of N protein behavior, related to Figure 1B.

(A) Superdex 200 gel filtration analysis of native N protein at 150 mM or 1 M NaCl, purified under non-denaturing conditions. Arrows at top indicate migration of molecular weight standards (kDa). Fractions were analyzed by SDS-PAGE and Coomassie Blue staining.

(B) Representative images of 5 μM native N protein after incubation in the presence or absence of 1 μM PS-318 RNA. Scale bar, 10 μm.

(C) 5 μl of 10 μM N protein (purified under native or denaturing [6M Urea] conditions) was mixed with urea loading buffer, incubated at 65°C for 5 min, and separated on a 10% polyacrylamide TBE-Urea gel, followed by staining with SYBR Gold. RNA length standards (nt) in left lane.
(D) SDS-PAGE analysis of all N protein mutants used in this study, stained with Coomassie Blue.
(E) N protein was incubated at the indicated temperature for 30 min in the presence of 1 μM 5'-400 RNA. Scale bar, 10 μm.
(F) N protein was incubated with 1 μM 5'-400 for 16 h at room temperature. Scale bar, 10 μm.
(G) Condensates of 10 μM WT or 10D N protein were formed in droplet buffer (70 mM KCl) by incubation with 1 μM PS-318 RNA for 30 min and imaged. NaCl was then added to a final concentration of 250 mM for 15 min before imaging again.

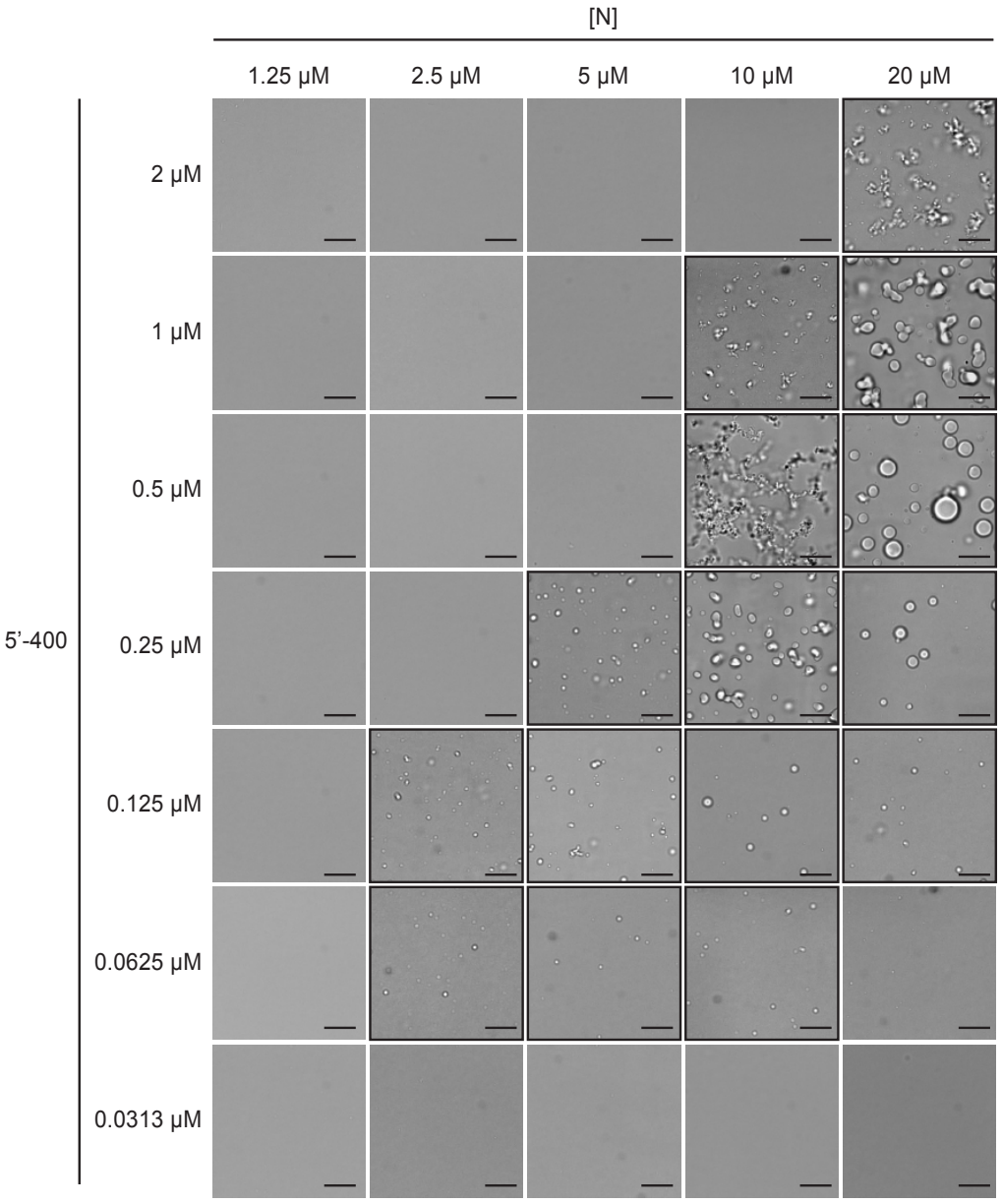

Figure S3. Condensate formation at a range of N protein and 5'-400 RNA concentrations, related to Figure 1C.

Light microscopy images of N protein condensates after 30 min incubation at room temperature with the indicated concentrations of 5'-400 RNA. Images that contain visible condensates are highlighted with a black border. Scale bar, 10 μm.

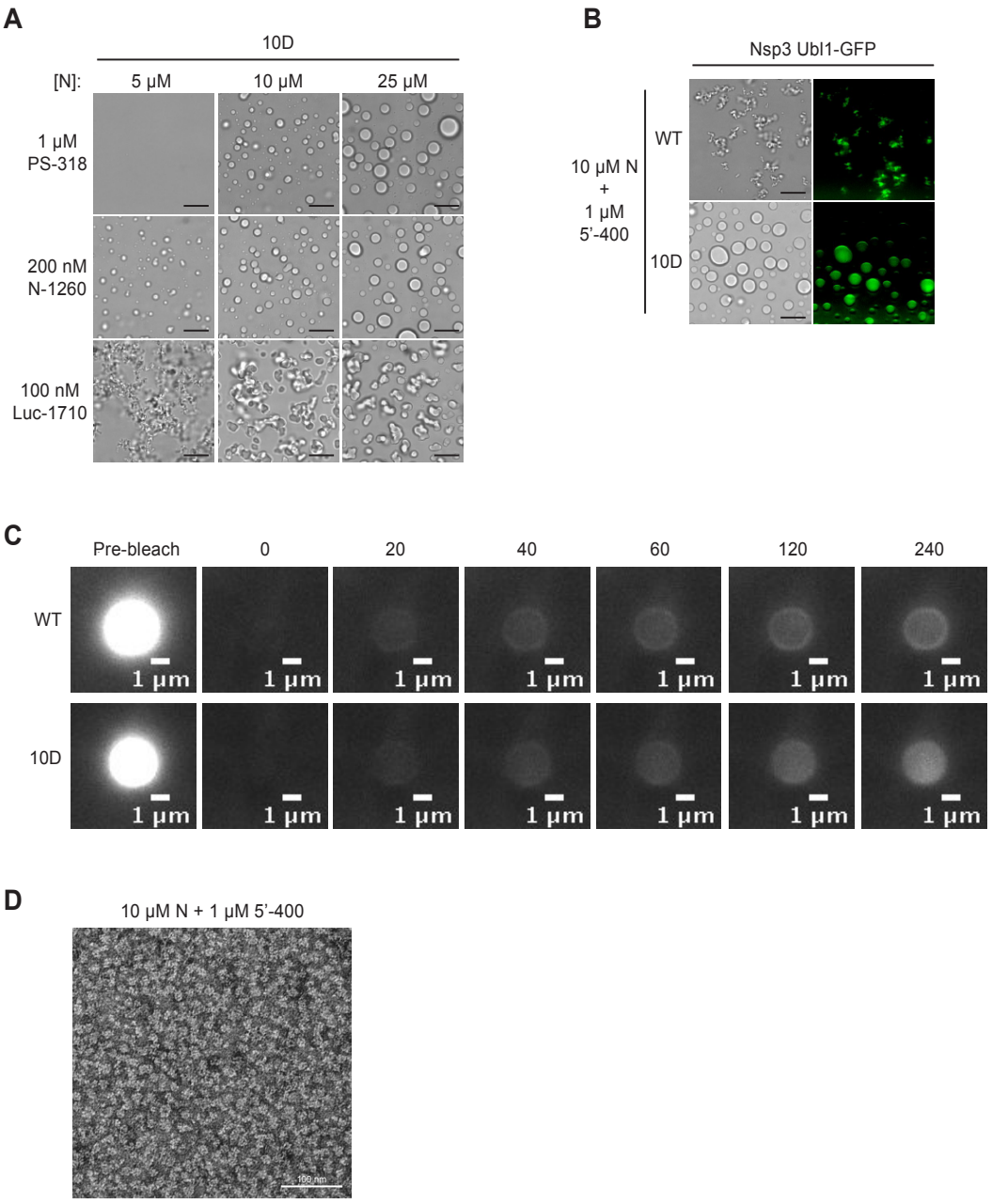

Figure S4. Properties of wild-type N protein and 10D mutant, related to Figures 3 and 4.
(A) Images of N protein 10D mutant following 30 min incubation with the indicated RNAs. Scale bar, 10 μm.
(B) 10 μM wild-type (WT) or 10D N protein was incubated with 1 μM 5'-400 RNA for 10 min. Nsp3 Ubl1-GFP was then added to a concentration of 1 μM and incubated for an additional 15 min before imaging in brightfield (left) or fluorescence (right).
(C) Images of droplets formed with 20 μM fluorescent N protein (WT or 10D) and 1 μM 5'-400 RNA, before photobleaching and at the indicated times (s) after bleaching. Scale bar, 1 μm.
(D) 10 μM wild-type N protein was incubated with 1 μM 5'-400 RNA for 15 min prior to analysis by negative-stain electron microscopy. Scale bar, 100 nm.

Table S1. RNA sequences used in this study, related to Figures 1-4.

5'-400 5' 400 nt from SARS-CoV-2 (Wuhan Hu-1 strain; nt 1-400).	AUUAAAGGUUUAUACCUUCCCAGGUAACAAACCAACCAACUUUCGAU CUCUUGUAGAUCUGUUCUCUAAACGAACUUUAAAAUCUGUGUGGCU GUCACUCGGCUGCAUGCUUAGUGCACUCACGCAGUAUAAUUAAUAA CUAAUUACUGUCGUUGACAGGACACGAGUAACUCGUCUAUCUUCUG CAGGCUGCUUACGGUUUCGUCCGUGUUGCAGCCGAUCAUCAGCACA UCUAGGUUUCGUCCGGGUGUGACCGAAAGGUAAGAUGGAGAGCCUU GUCCCUGGUUUCAACGAGAAAACACACGUCCAACUCAGUUUGCCUG UUUUACAGGUUCGCGACGUGCUCGUACGUGGCUUUGGAGACUCCG UGGAGGAGGUCUUAUCAGAGGCACGUCAACAU
PS-318 318 nt from SARS CoV (Tor2 strain; nt 19715-20031), with an extra C (red) after A19802 as in (Woo et al., 2019).	UGAGCUUUGGGCUAAGCGUAACAUUAAACCAGUGCCAGAGAUUAAG AUACUCAAUAAUUUGGGUGUUGAUAUCGCUGCUAAUACUGUA**C**AUC UGGGACUACAAAAGAGAAGCCCCAGCACAUGUAUCUACAAUAGGUG UCUGCACAAUGACUGACAUUGCCAAGAAACCUACUGAGAGUGCUUG UUCUUCACUUACUGUCUUGUUUGAUGGUAGAGUGGAAGGACAGGUA GACCUUUUUAGAAACGCCCGUAAUGGUGUUUUAAUAACAGAAGGUU CAGUCAAAGGUCUAACACCUUCAAAGGGACCAGCACAAGCUA
N-1260 RNA containing the 1260 nt open reading frame (gray highlight) of the N gene from SARS-CoV-2 (Wuhan Hu-1 strain; nt 28274-29533), plus flanking plasmid sequence.	GGGAAUUGUGAGCGGAUAACAAUUCCCCUCUAGAAAUAAUUUUGUU UAACUUUAAGAAGGAGAUAUACCAUGGGCAGCAGCCAUCAUCAUCAU CAUCACAGCAGCGGCCUGGUGCCGCGCGGUACCACGGAAAACCUGU AUUUUCAGGGAUCCAUGUCUGAUAAUGGACCCCAAAAUCAGCGAAAU GCACCCCGCAUUACGUUUGGUGGACCCUCAGAUUCAACUGGCAGUA ACCAGAAUGGAGAACGCAGUGGGGCGCGAUCAAAACAACGUCGGCC CCAAGGUUUACCCAAUAAUACUGCGUCUUGGUUCACCGCUCUCACU CAACAUGGCAAGGAAGACCUUAAAUUCCCUCGAGGACAAGGCGUUC CAAUUAACACCAAUAGCAGUCCAGAUGACCAAAUUGGCUACUACCGA AGAGCUACCAGACGAAUUCGUGGUGGUGACGGUAAAAUGAAAGAUC UCAGUCCAAGAUGGUAUUUCUACUACCUAGGAACUGGGCCAGAAGC UGGACUUCCCUAUGGUGCUAACAAAGACGGCAUCAUAUGGGUUGCA ACUGAGGGAGCCUUGAAUACACCAAAAGAUCACAUUGGCACCCGCAA UCCUGCUAACAAUGCUGCAAUCGUGCUACAACUUCCUCAAGGAACAA CAUUGCCAAAAGGCUUCUACGCAGAAGGGAGCAGAGGCGGCAGUCA AGCCUCUUCUCGUUCCUCAUCACGUAGUCGCAACAGUUCAAGAAAU UCAACUCCAGGCAGCAGUAGGGGAACUUCUCCUGCUAGAAUGGCUG GCAAUGGCGGUGAUGCUGCUCUUGCUUUGCUGCUGCUUGACAGAU UGAACCAGCUUGAGAGCAAAAUGUCUGGUAAAGGCCAACAACAACAA GGCCAAACUGUCACUAAGAAAUCUGCUGCUGAGGCUUCUAAGAAGC CUCGGCAAAAACGUACUGCCACUAAAGCAUACAAUGUAACACAAGCU UUCGGCAGACGUGGUCCAGAACAAACCCAAGGAAAUUUUGGGGACC AGGAACUAAUCAGACAAGGAACUGAUUACAAACAUUGGCCGCAAAUU GCACAAUUUGCCCCCAGCGCUUCAGCGUUCUUCGGAAUGUCGCGCA UUGGCAUGGAAGUCACACCUUCGGGAACGUGGUUGACCUACACAGG UGCCAUCAAAUUGGAUGACAAAGAUCCAAAUUUCAAAGAUCAAGUCA UUUUGCUGAAUAAGCAUAUUGACGCAUACAAAACAUUCCCACCAACA GAGCCUAAAAAGGACAAAAAGAAGAAGGCUGAUGAAACUCAAGCCUU ACCGCAGAGACAGAAGAAACAGCAAACUGUGACUCUUCUUCCUGCU GCAGAUUUGGAUGAUUUCUCCAAACAAUUGCAACAAUCCAUGAGCAG UGCUGACUCAACUCAGGCCUAAGAAUUCGAGCUCCGUCGACA

Luc-1710 RNA containing the 1710 nt firefly luciferase open reading frame (gray highlight) plus flanking plasmid sequence.	GGUCUAGAAAUAAUUUUGUUUAACUUUAAGAAGGAGAUAUAACCAUG AAAAUCGAAGAAGGUAAAGGUCACCAUCACCAUCACCACGGAUCCAU GGAAGACGCCAAAAACAUAAAGAAAGGCCCGGCGCCAUUCUAUCCUC UAGAGGAUGGAACCGCUGGAGAGCAACUGCAUAAGGCUAUGAAGAG AUACGCCCUGGUUCCUGGAACAAUUGCUUUUACAGAUGCACAUAUC GAGGUGAACAUCACGUACGCGGAAUACUUCGAAAUGUCCGUUCGGU UGGCAGAAGCUAUGAAACGAUAUGGGCUGAAUACAAAUCACAGAAUC GUCGUAUGCAGUGAAAACUCUCUUCAAUUCUUUAUGCCGGUGUUGG GCGCGUUAUUUAUCGGAGUUGCAGUUGCGCCCGCGAACGACAUUUA UAAUGAACGUGAAUUGCUCAACAGUAUGAACAUUUCGCAGCCUACC GUAGUGUUUGUUUCCAAAAAGGGGUUGCAAAAAAUUUUGAACGUGC AAAAAAAAUUACCAAUAAUCCAGAAAAUUAUUAUCAUGGAUUCUAAAA CGGAUUACCAGGGAUUUCAGUCGAUGUACACGUUCGUCACAUCUCA UCUACCUCCCGGUUUUAAUGAAUACGAUUUUGUACCAGAGUCCUUU GAUCGUGACAAAACAAUUGCACUGAUAAUGAAUUCCUCUGGAUCUAC UGGGUUACCUAAGGGUGUGGCCCUUCCGCAUAGAACUGCCUGCGU CAGAUUCUCGCAUGCCAGAGAUCCUAUUUUUGGCAAUCAAAUCAUU CCGGAUACUGCGAUUUUAAGUGUUGUUCCAUUCCAUCACGGUUUUG GAAUGUUUACUACACUCGGAUAUUUGAUAUGUGGAUUUCGAGUCGU CUUAAUGUAUAGAUUUGAAGAAGAGCUGUUUUUACGAUCCCUUCAG GAUUACAAAAUUCAAAGUGCGUUGCUAGUACCAACCCUAUUUUCAUU CUUCGCCAAAAGCACUCUGAUUGACAAAUACGAUUUAUCUAAUUUAC ACGAAAUUGCUUCUGGGGGCGCACCUCUUUCGAAAGAAGUCGGGGA AGCGGUUGCAAAACGCUUCCAUCUUCCAGGGAUACGACAAGGAUAU GGGCUCACUGAGACUACAUCAGCUAUUCUGAUUACACCCGAGGGGG AUGAUAAACCGGGCGCGGUCGGUAAAGUUGUUCCAUUUUUUGAAGC GAAGGUUGUGGAUCUGGAUACCGGGAAAACGCUGGGCGUUAAUCAG AGAGGCGAAUUAUGUGUCAGAGGACCUAUGAUUAUGUCCGGUUAUG UAAACAAUCCGGAAGCGACCAACGCCUUGAUUGACAAGGAUGGAUG GCUACAUUCUGGAGACAUAGCUUACUGGGACGAAGACGAACACUUC UUCAUAGUUGACCGCUUGAAGUCUUUAAUUAAAUACAAAGGAUAUCA GGUGGCCCCCGCUGAAUUGGAAUCGAUAUUGUUACAACACCCCAAC AUCUUCGACGCGGGCGUGGCAGGUCUUCCCGACGAUGACGCCGGU GAACUUCCCGCCGCCGUUGUUGUUUUGGAGCACGGAAAGACGAUGA CGGAAAAAGAGAUCGUGGAUUACGUCGCCAGUCAAGUAACAACCGC GAAAAAGUUGCGCGGAGGAGUUGUGUUUGUGGACGAAGUACCGAAA GGUCUUACCGGAAAACUCGACGCAAGAAAAAUCAGAGAGAUCCUCAU AAAGGCCAAGAAGGGCGGAAAGUCCAAACUCGAGUAAGGUUAACCU GCAGGAGG