Instruction for using ProbeDealer for conventional single-molecule RNA FISH

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Besides probe design for multiplexed FISH experiments, ProbeDealer can be used to design FISH probes targeting individual RNA species for conventional single-molecule RNA FISH. This functionality may offer a cheaper alternative to commercial options of custom-target single-molecule RNA FISH.

When designing single-molecule RNA FISH probes targeting just one RNA species, we recommend choosing "sequential RNA FISH" as the probe type in ProbeDealer, designing 36 probes for the single target sequence, and choosing "primary probe sequences" as the output type.

When purchasing such probes, one of the most economical strategies is to order 36 individual primary probes from Integrated DNA Technologies (IDT) using the "ssDNA oligo plate, 25 nmole scale, standard desalting" option, and order one dye-labeled secondary probe from IDT using the "ssDNA oligo, 250 nmole scale, HPLC purification" option. The typical yield of the primary probes ordered this way can support more than 10,000 FISH experiments. The typical yield of the secondary probe ordered this way can support more than 700 FISH experiments. The excess amount of secondary probe can be saved for future experiments targeting different RNA species.

One important note regarding the strategy above is that the IDT 25-nmole-scale synthesis has a length limit of 60 nucleotides (nt), while the output primary probe sequences from ProbeDealer by default is 70-nt long. This 70-nt sequence contains two 20-nt secondary probe binding sequences flanking a 30-nt RNA targeting sequence. To reduce the oligo length to below 60 nt for the low cost of the 25-nmole-scale synthesis, we suggest users to _append secondary sequence at only one end of the primary targeting region_. To do so, open "primers and secondary sequences/RNA secondaries.xlsx", and go to Sheet "sequential RNA FISH". Between the two columns, "sequence at 5' end" and "sequence at 3' end", delete all the sequences in one of the two columns and leave the sequence entries in that column BLANK. Only provide your secondary sequences in the other column. This change allows secondary sequence to be appended at only one end of the primary targeting region. To allow successful FISH, secondary sequence can be appended at either end. (Please note that the 5' end and 3' end here is relative to the template probe library, which is the reverse-complement of the primary probe sequences. Therefore, the secondary sequences listed in the "5' end" column will be appended to the 3' end in the primary probe sequences.)