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 deleting the last 20-nt of each 70-nt sequence.