Ordering dye-labeled secondary probes and primers

To visualize genomic loci or RNA foci in experiment, users should purchase dye-labeled secondary probes by appending fluorescent dyes to the 5' end of the sequences included in the DNA secondaries.xlsx and RNA secondaries.xlsx provided with ProbeDealer package, respectively. Examples of the 50 secondary probes for chromatin tracing were reported in Liu et al Supplementary Data File 6 in the "TAD tracing" tab[1]. Examples of the 16 secondary probes for RNA MERFISH were reported in Liu et al Supplementary Data File 6 in the "RNA MERFISH" tab[1].

To synthesize and amplify primary probes from a template oligo library, users should order primers according to the sequences used in Primers.xlsx. When ordering forward primers, users should append the T7 promoter sequence (GCCGTACGGATAATACGACTCACTATAGGG) at the 5' end of forward primers. When ordering the reverse primers, users should reverse-complement the reverse priming sequences in Primers.xlsx. The primers to order for the three default pairs of priming sequences are:

For chromatin tracing, forward primer:
GCCGTACGGATAATACGACTCACTATAGGG GTGGTAAAGCTCCGCGGCTT;
reverse primer: TCGTTCCGCATTGACCAATC.

For RNA MERFISH, forward primer:
GCCGTACGGATAATACGACTCACTATAGGG CCCGCGTTAACCATACACCG;
reverse primer: CATCGAAGCGTGTGGCTACC.

For sequential single-molecule RNA FISH, forward primer:
GCCGTACGGATAATACGACTCACTATAGGG GCGTCGTTATGGTGCAACGT;
reverse primer: TTGTCGCACGTTCGGTGTCG.

References:

1. Liu, M. *et al.* Multiplexed imaging of nucleome architectures in single cells of mammalian tissue. *Nat. Commun.* **11**, 1–14 (2020).