ARTICLES

NATURE NANOTECHNOLOGY



Fig. 1 Schematic of the NanoDeep method. **a**, A DNA nanoassembly (NanoComb) consisting of a double-stranded backbone with four barcoded protruding ssDNA strands (prongs) is preloaded with an oligonucleotide-conjugated binder specific for the reference protein and incubated with fixed cells. **b**, A library of binders is added, each conjugated to an ssDNA sequence bearing a barcode that identifies its target protein as well as a sequence that is partially complementary to the sequences of the detection prongs. **c**, During the incubation, affibody-oligonucleotide (oligo) conjugates are hybridized with a blocking strand. Unblocking is promoted by toehold-mediated displacement guided by an invading strand. **d**, The free 3' ends formed by the hybridization of the prongs with the binder oligonucleotides function as primers for DNA polymerase. **e**, DNA polymerase reaction creates dsDNA sequences that contain the barcodes both for the relative positions of the prongs within the NanoComb and for the prongs and the binder oligonucleotides, leading to the release of dsDNA sequences. **g**, dsDNA sequences are analysed by NGS. **h**, Schematic representation of prongs and oligonucleotides conjugated to the binder targeting the reference protein (left) this region is 20 nucleotides (nt) long. For detection prongs and the oligonucleotide conjugated to the library binders (right) the binding regions is 15 nt long; a 5-nt-long bulk region is added to the oligonucleotides or the prongs respectively. Further, both the prongs and the binder oligons are followed by a 6-nt barcode identifying the protein or the position, in the binder oligonucleotides or the prongs respectively. Further, both the prongs and the binder oligonucleotides to the binder oligonucleotides or the prongs respectively. Further, both the prongs and the binder oligonucleotides by a 6-nt barcode identifying the protein or the position, in the binder oligonucleotides or the prongs respectively. Further, both the prongs and the binder oligonucleotides contain nuclea