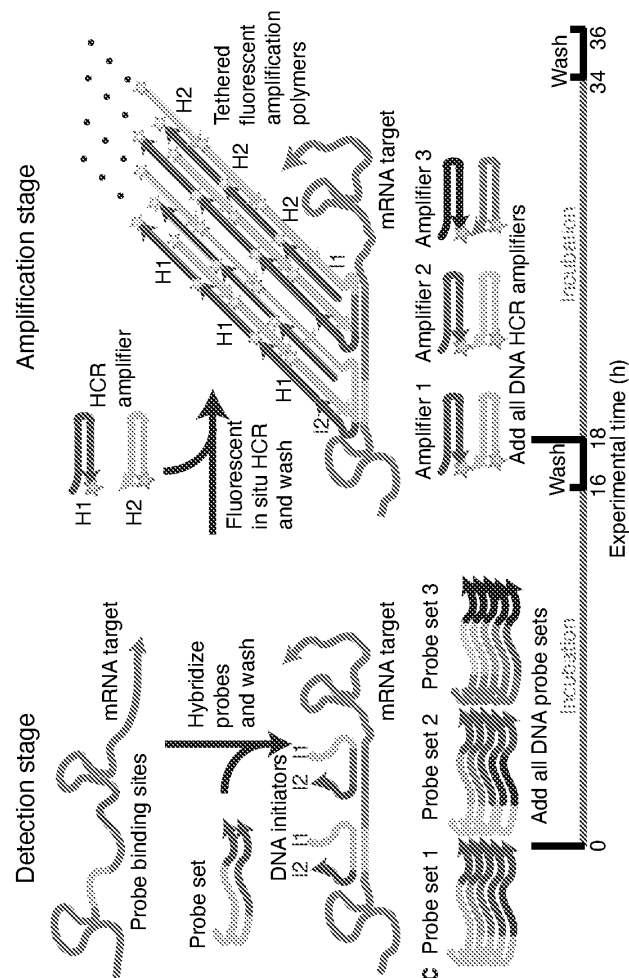
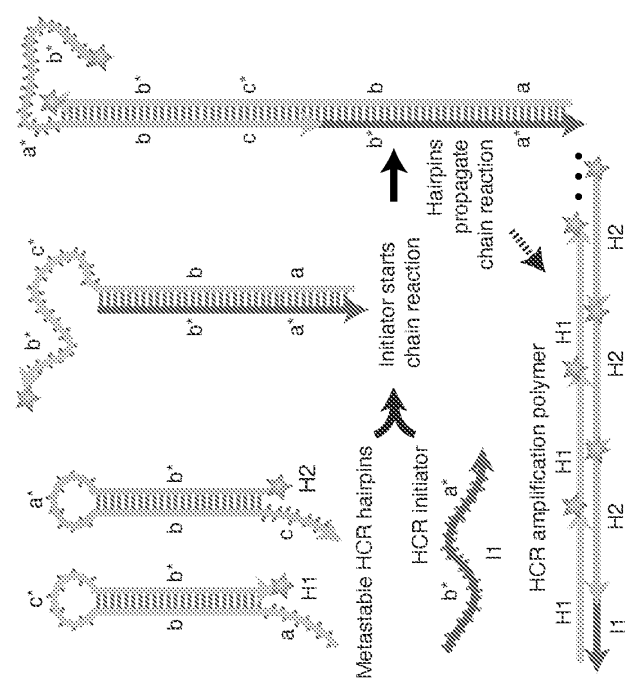


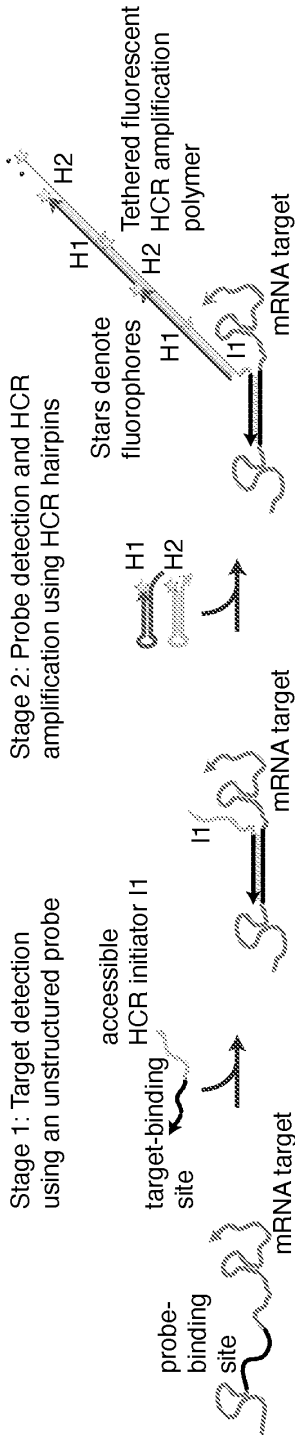


மேல்

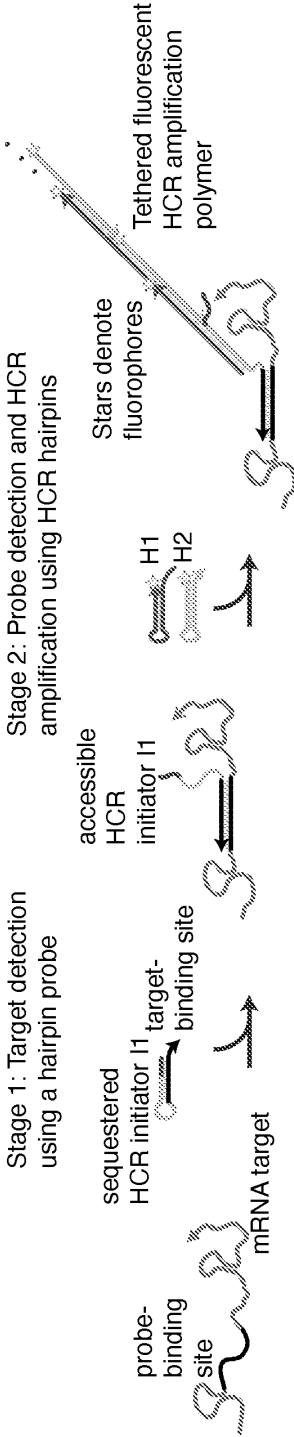


AGLE

Scheme A: Two-stage protocol



Scheme B: Two-stage protocol



Scheme C: Three-stage protocol

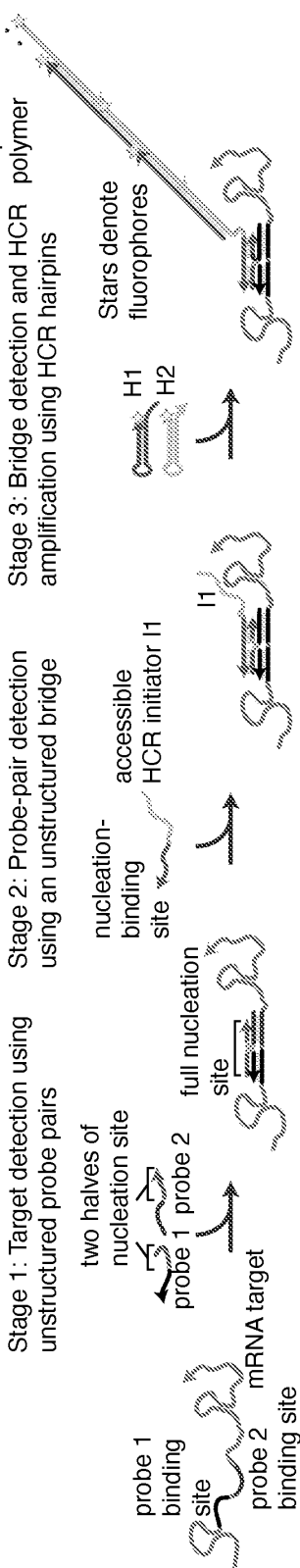
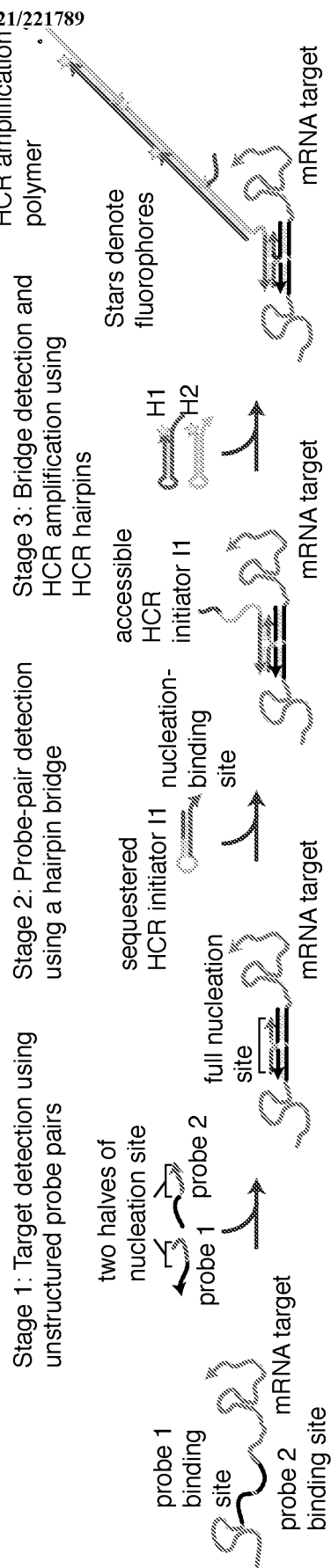


FIG. 2A

Scheme D: Three-stage protocol



Scheme E: Two-stage protocol

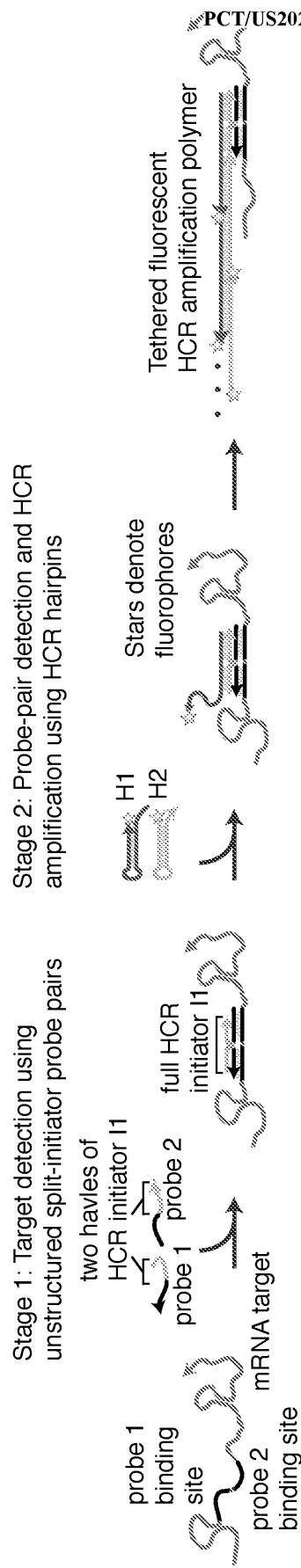
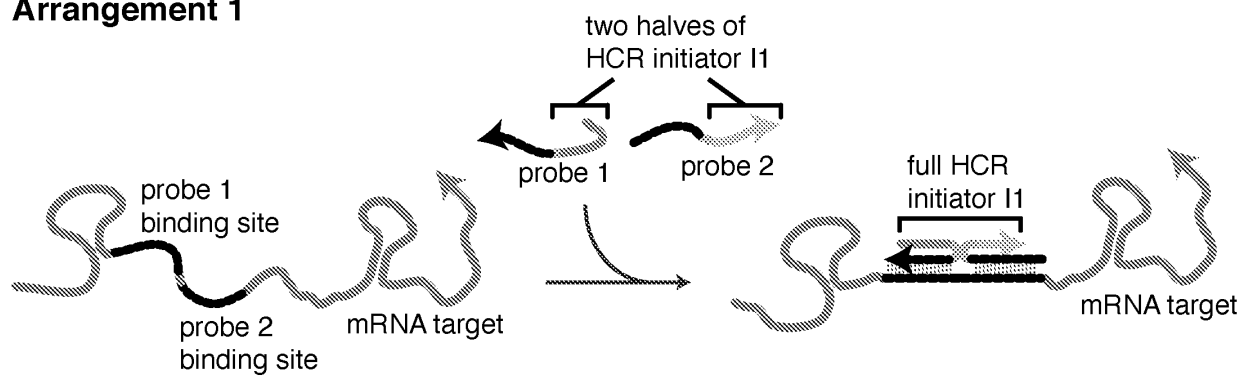
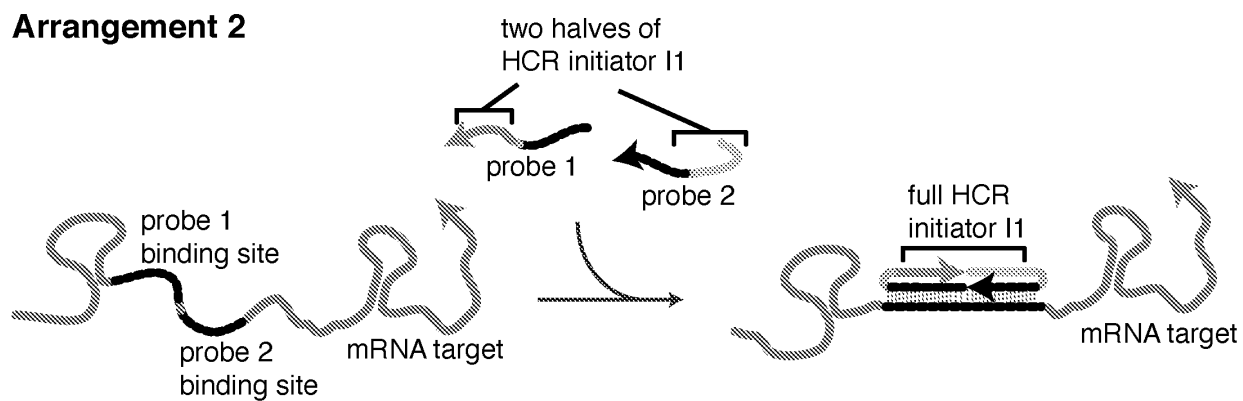
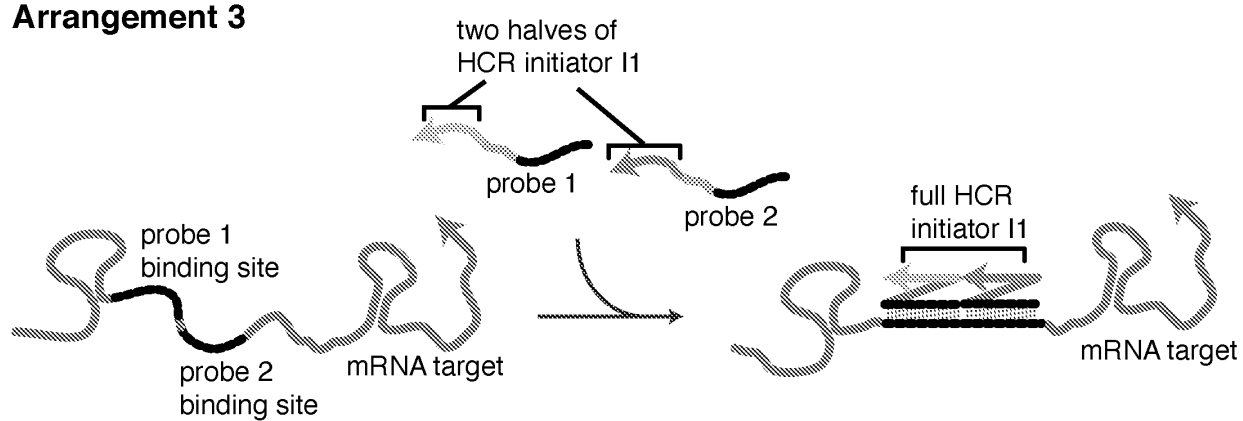
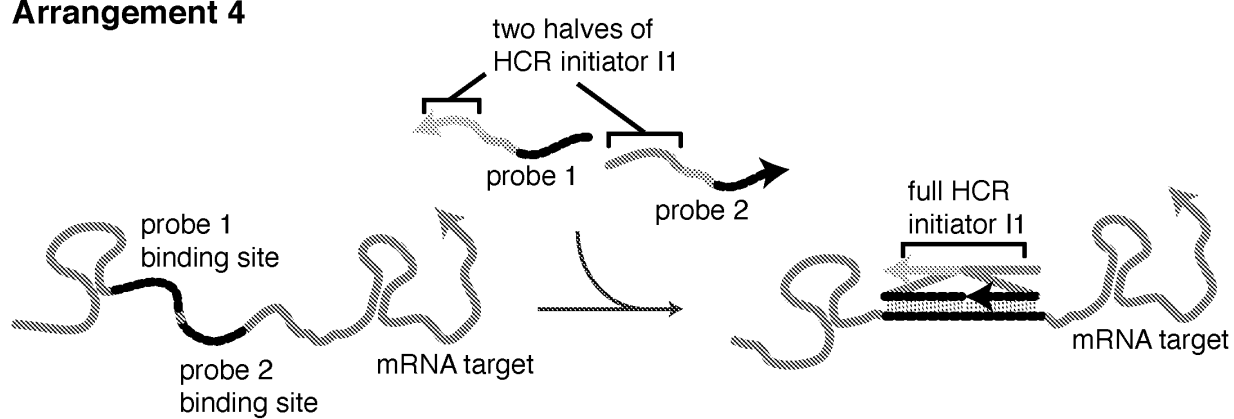
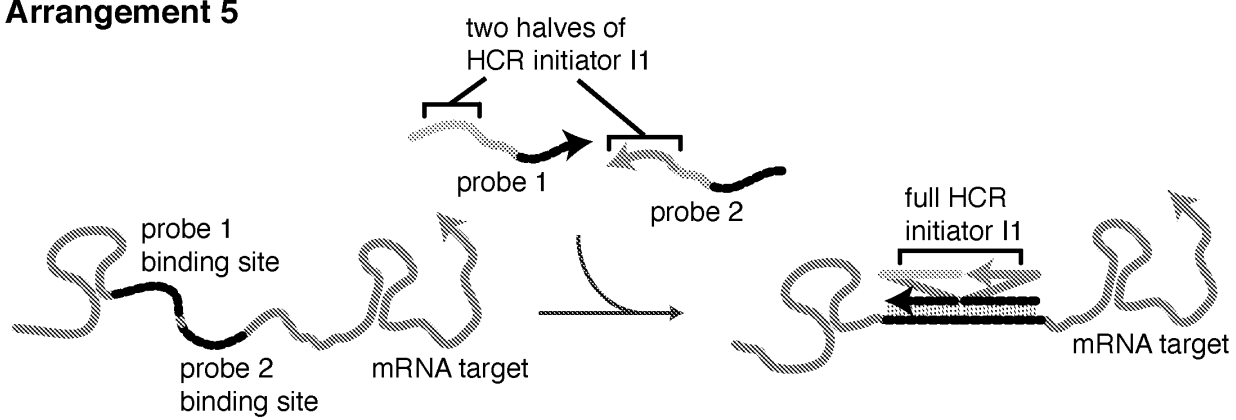
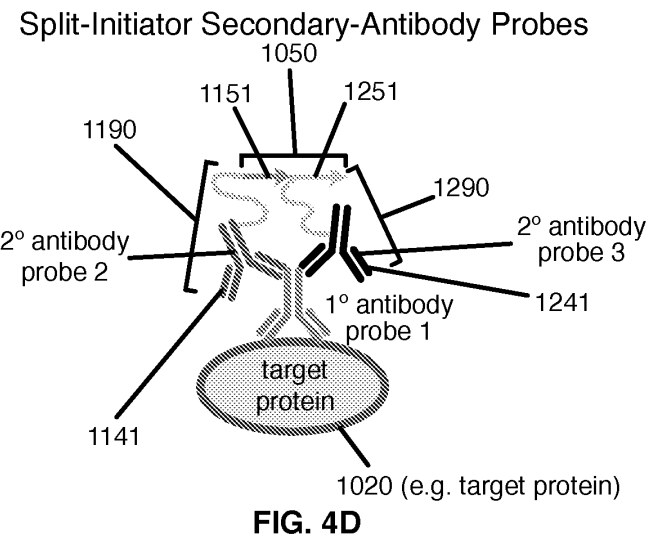
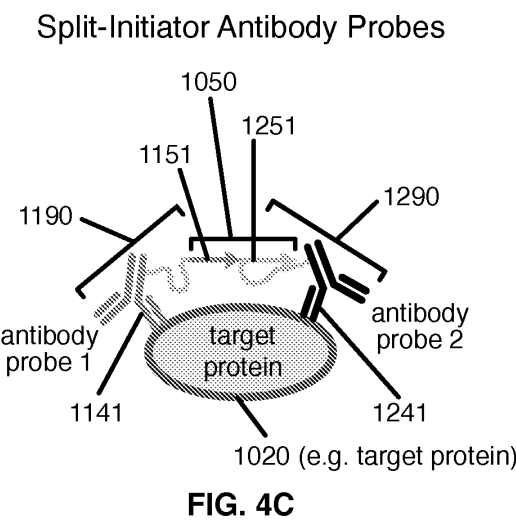
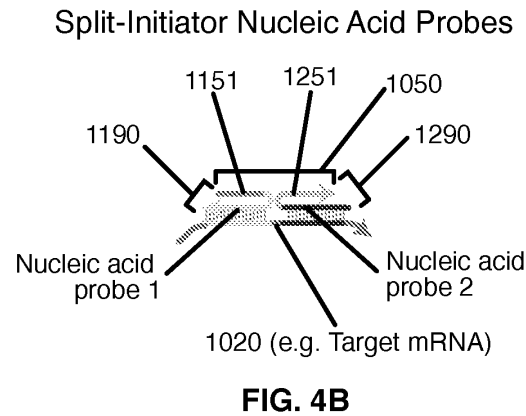
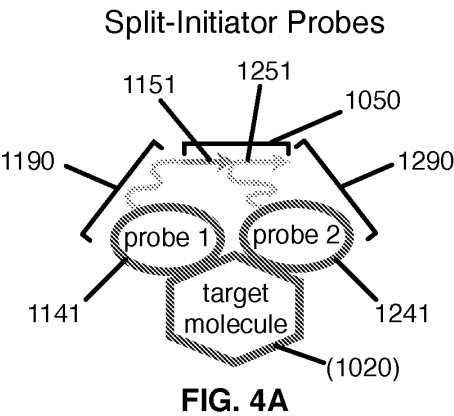
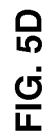
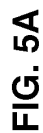


FIG. 2B

Arrangement 1**Arrangement 2****Arrangement 3****FIG. 3A**

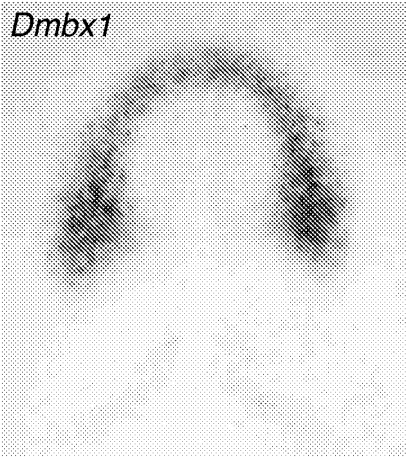
Arrangement 4**Arrangement 5****FIG. 3B**



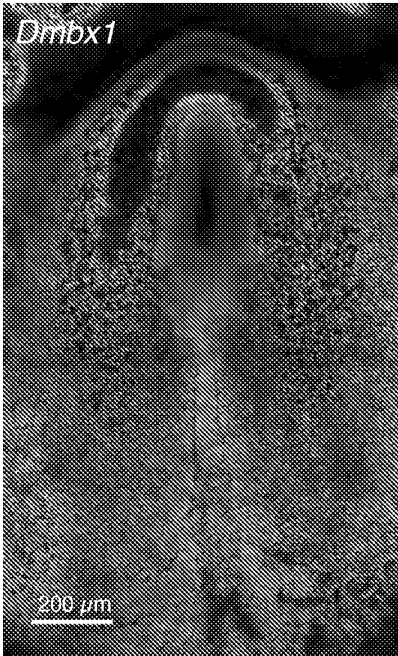


1020 (e.g. target complex
(target protein 1 bound to target protein 2))

Reference expression pattern
(traditional in situ hybridization)



Standard probes (Scheme A)
(unoptimized probe set)



Split-initiator probes (Scheme E)
(unoptimized probe set)

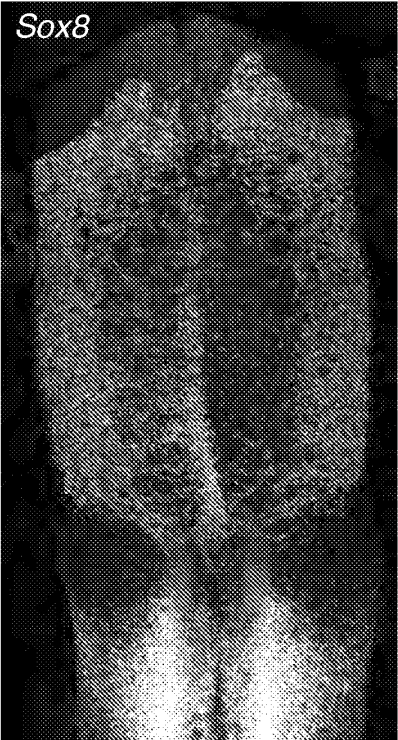
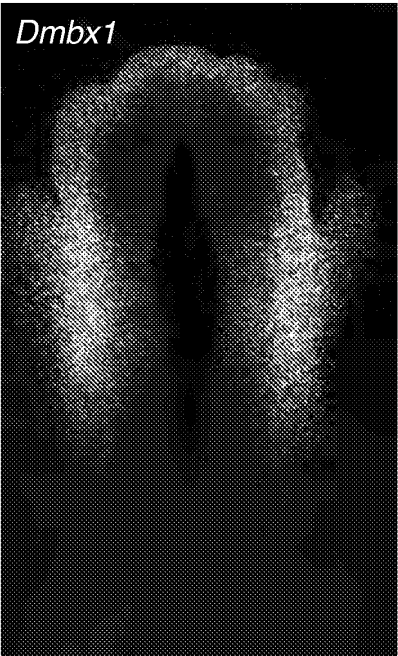


FIG. 6

Full HCR initiator I1
b* a*
P1 P2
Target mRNA

Split-initiator probes P1 and P2 each carry a fraction of HCR initiator I1; selective binding of probes P1 and P2 to the target mRNA colocalizes full HCR initiator I1

FIG. 7A

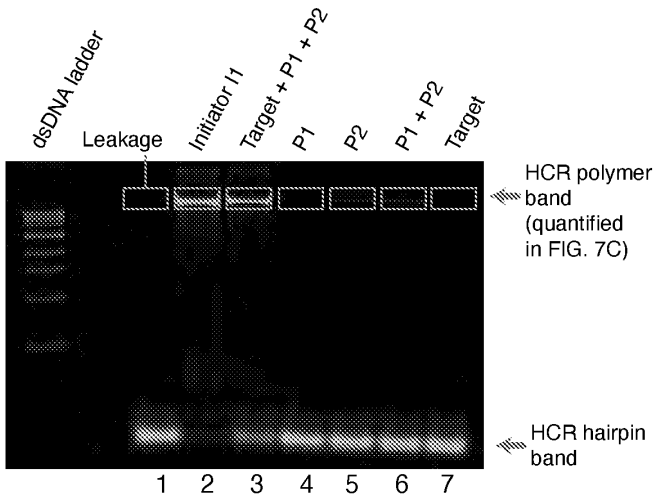


FIG. 7B

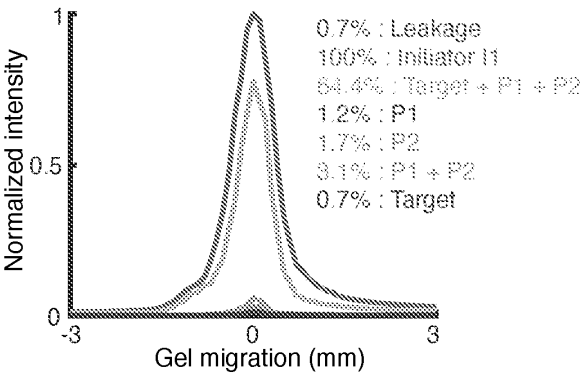


FIG. 7C

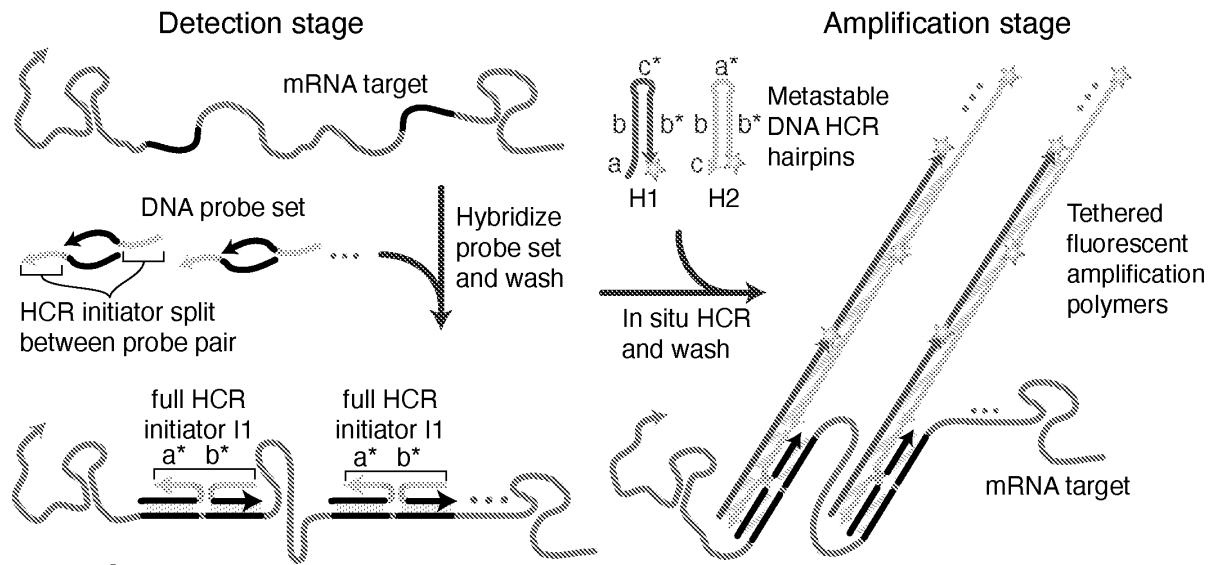


FIG. 8A

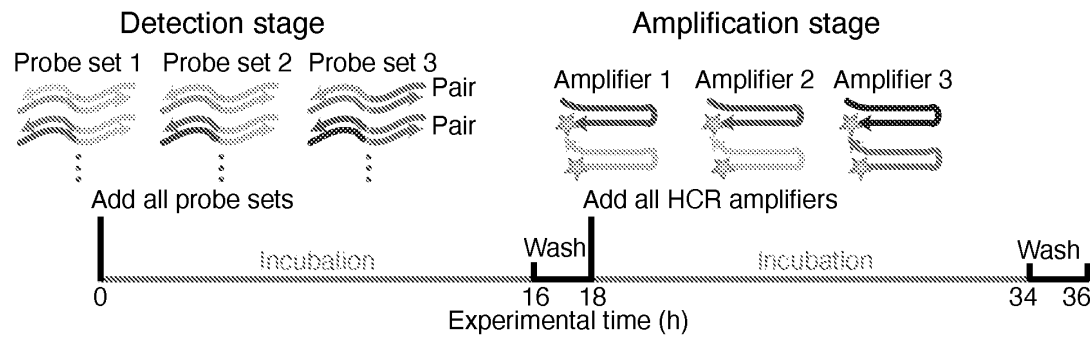


FIG. 8B

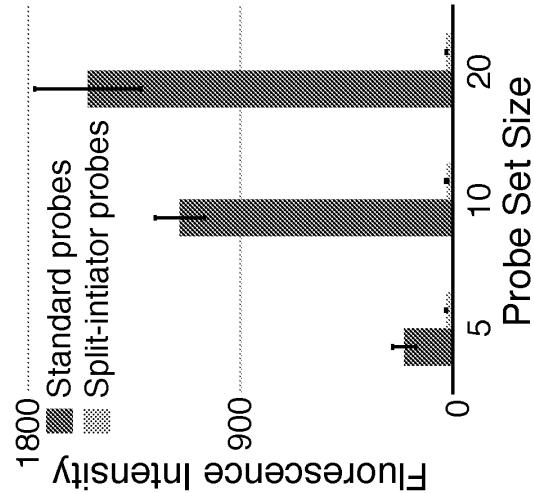


FIG. 9A

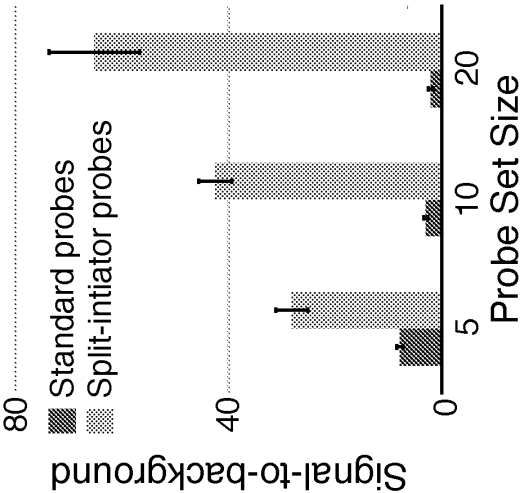


FIG. 9B

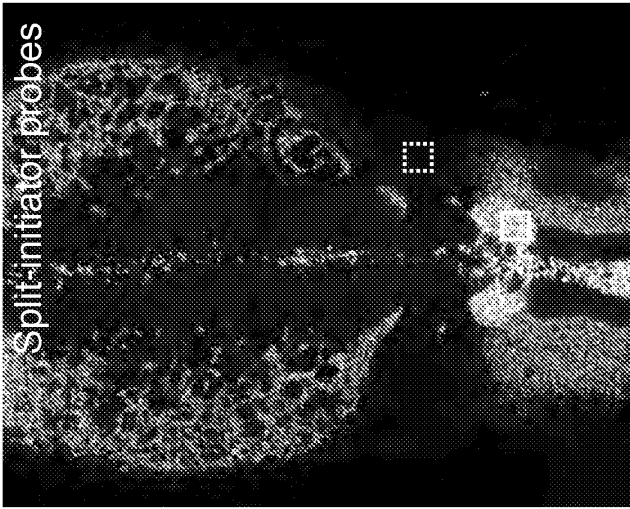
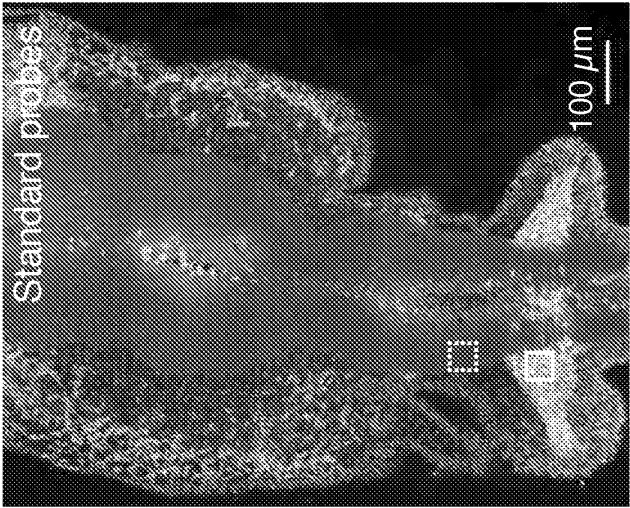


FIG. 9C

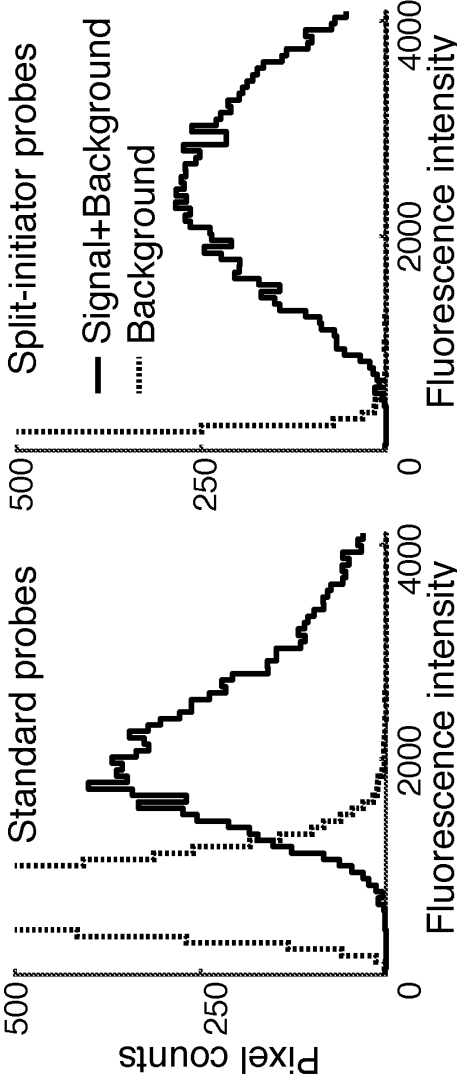


FIG. 9D

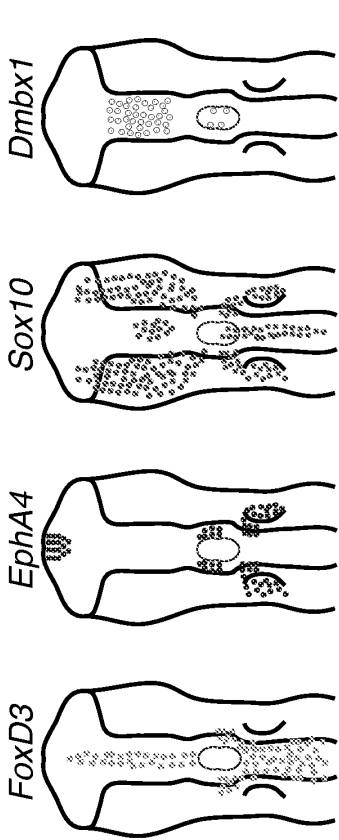


FIG. 10A

Signal-to-background
27 ± 6

43 ± 2

59 ± 4

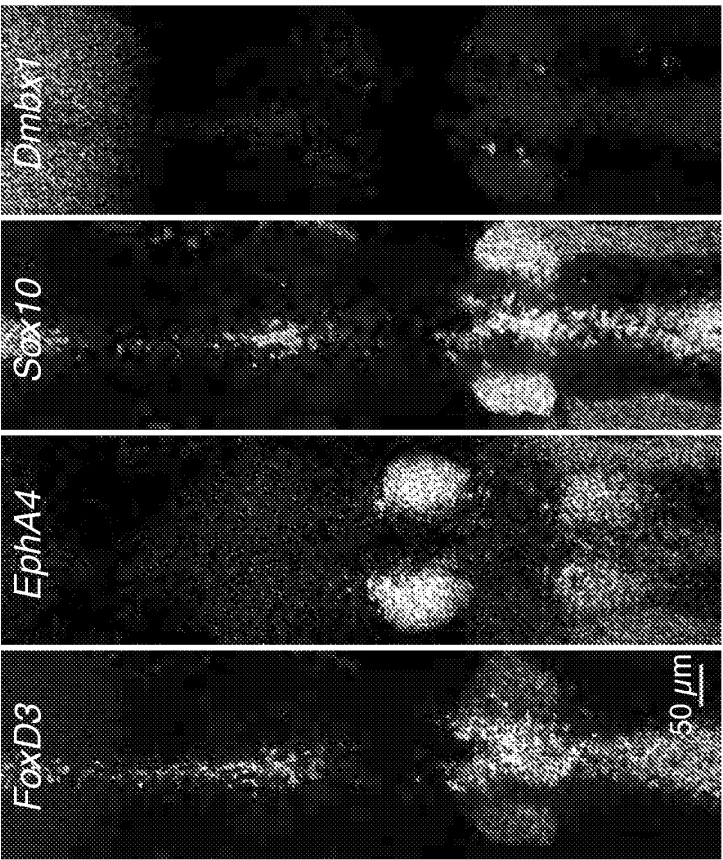


FIG. 10D

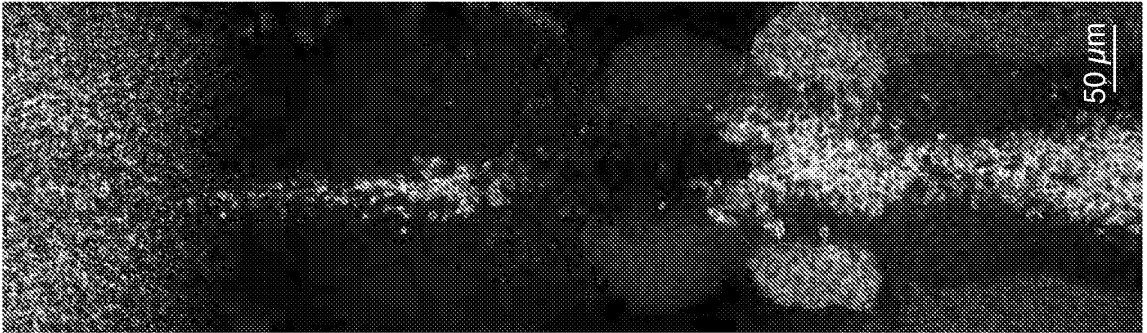


FIG. 10C

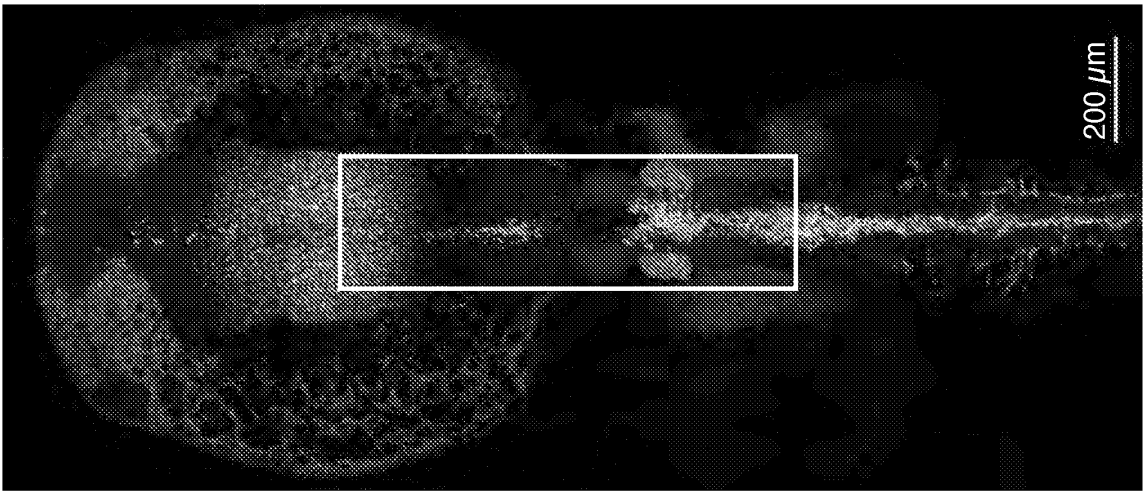


FIG. 10B

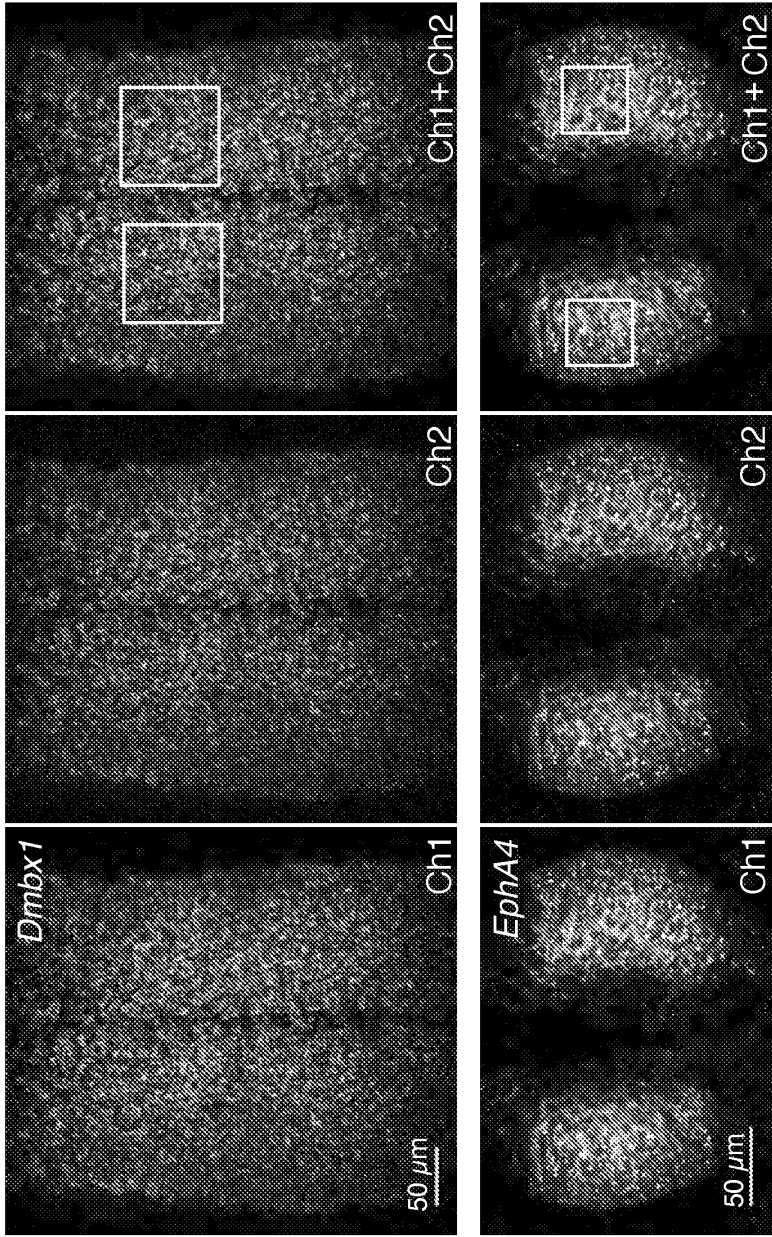


FIG. 11A

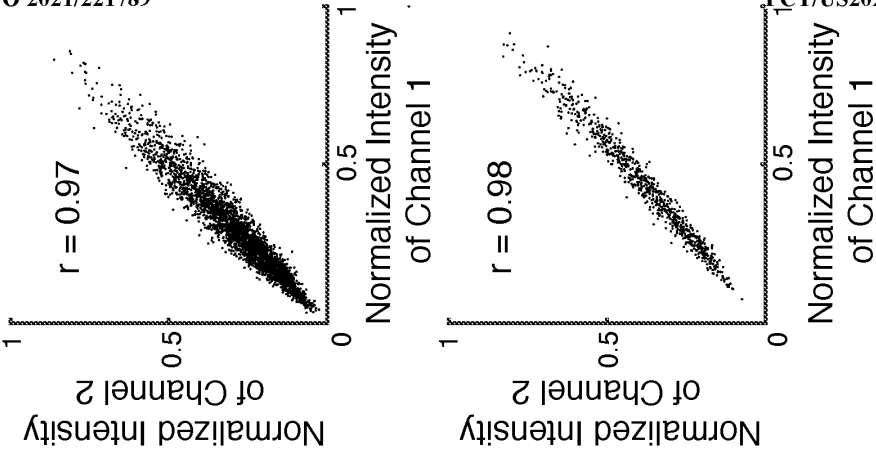


FIG. 11B

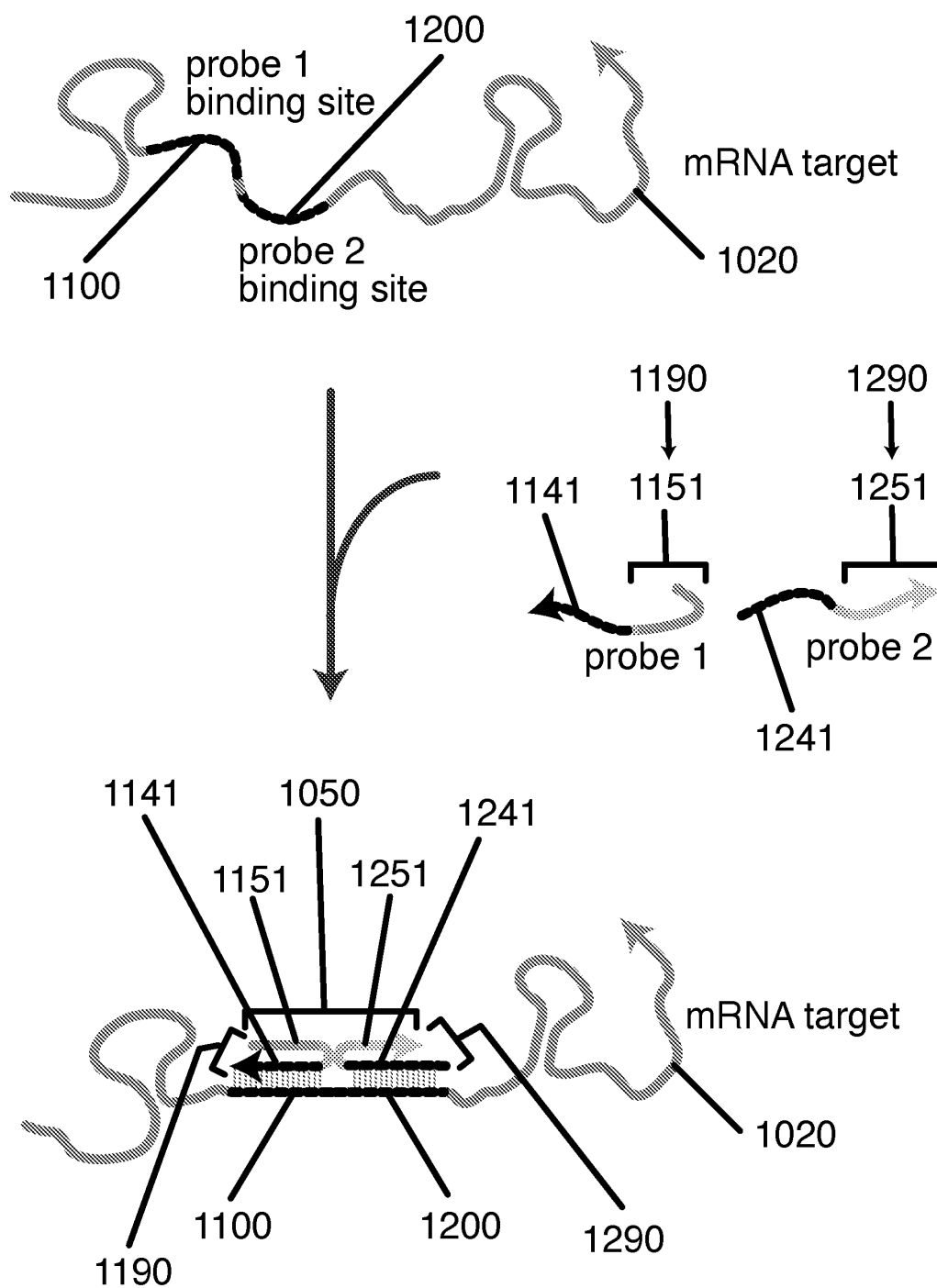


FIG. 12

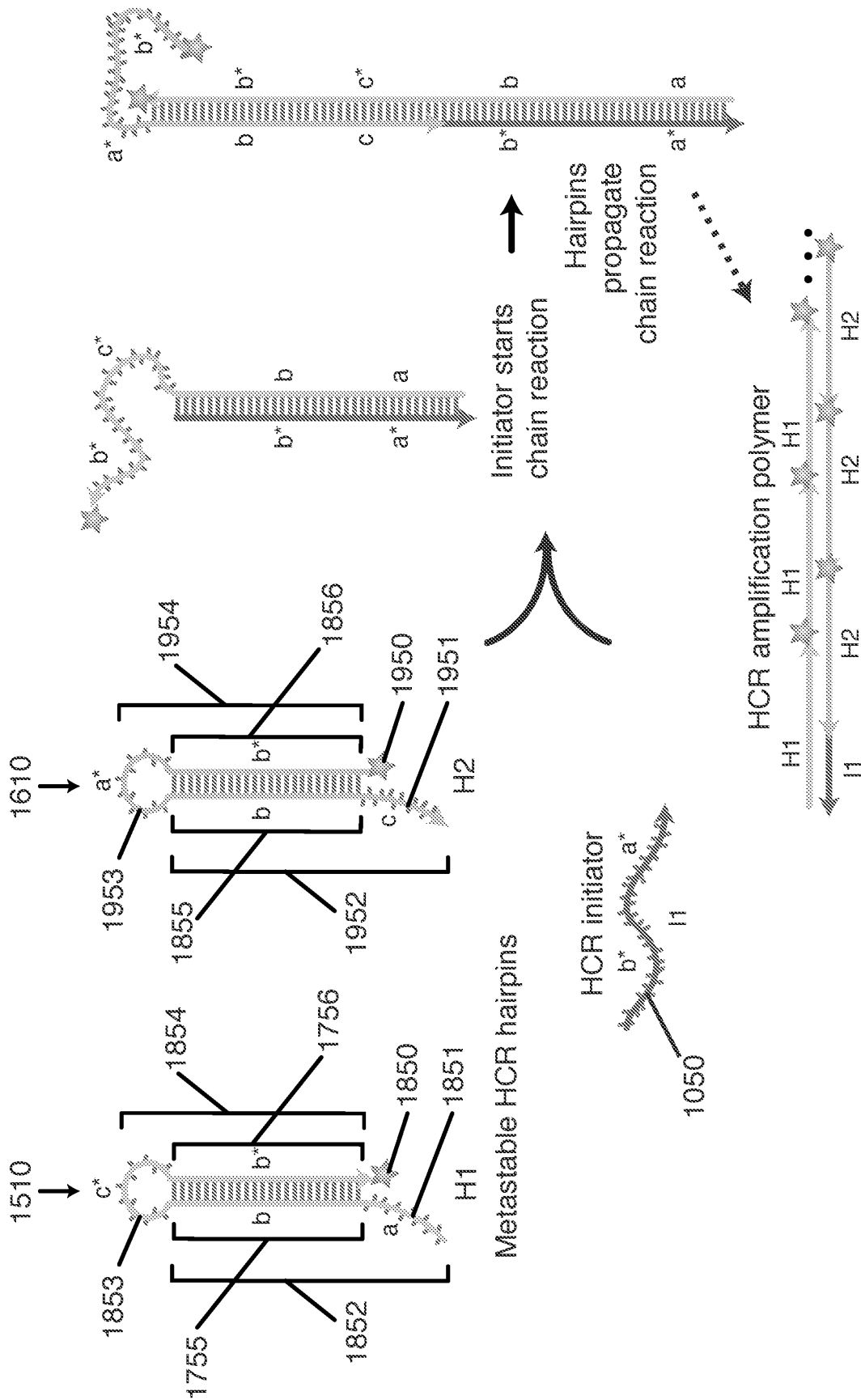
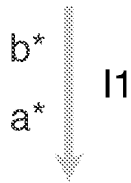
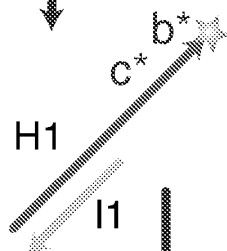


FIG. 13

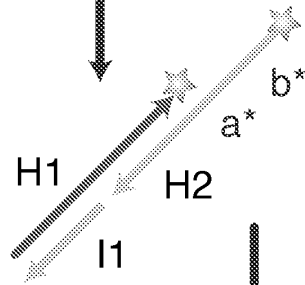
DNA HCR
initiator I



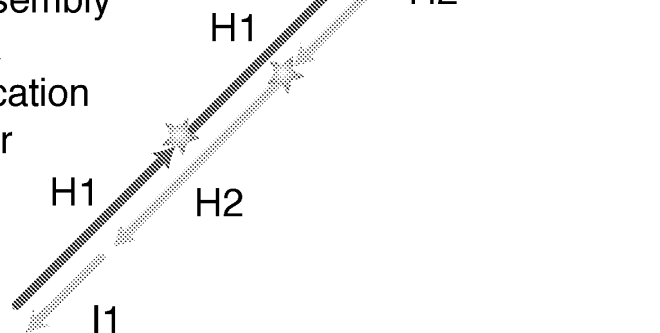
HCR initiator I
starts chain
reaction



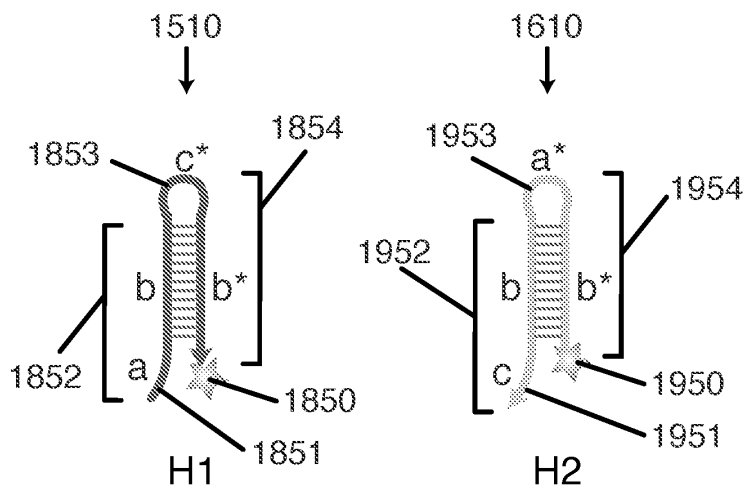
HCR hairpins
H1 and H2
propagate
chain reaction



Conditional
self-assembly
of HCR
amplification
polymer



Stars denote
fluorophores



Metastable DNA HCR
hairpins H1 and H2

FIG. 14

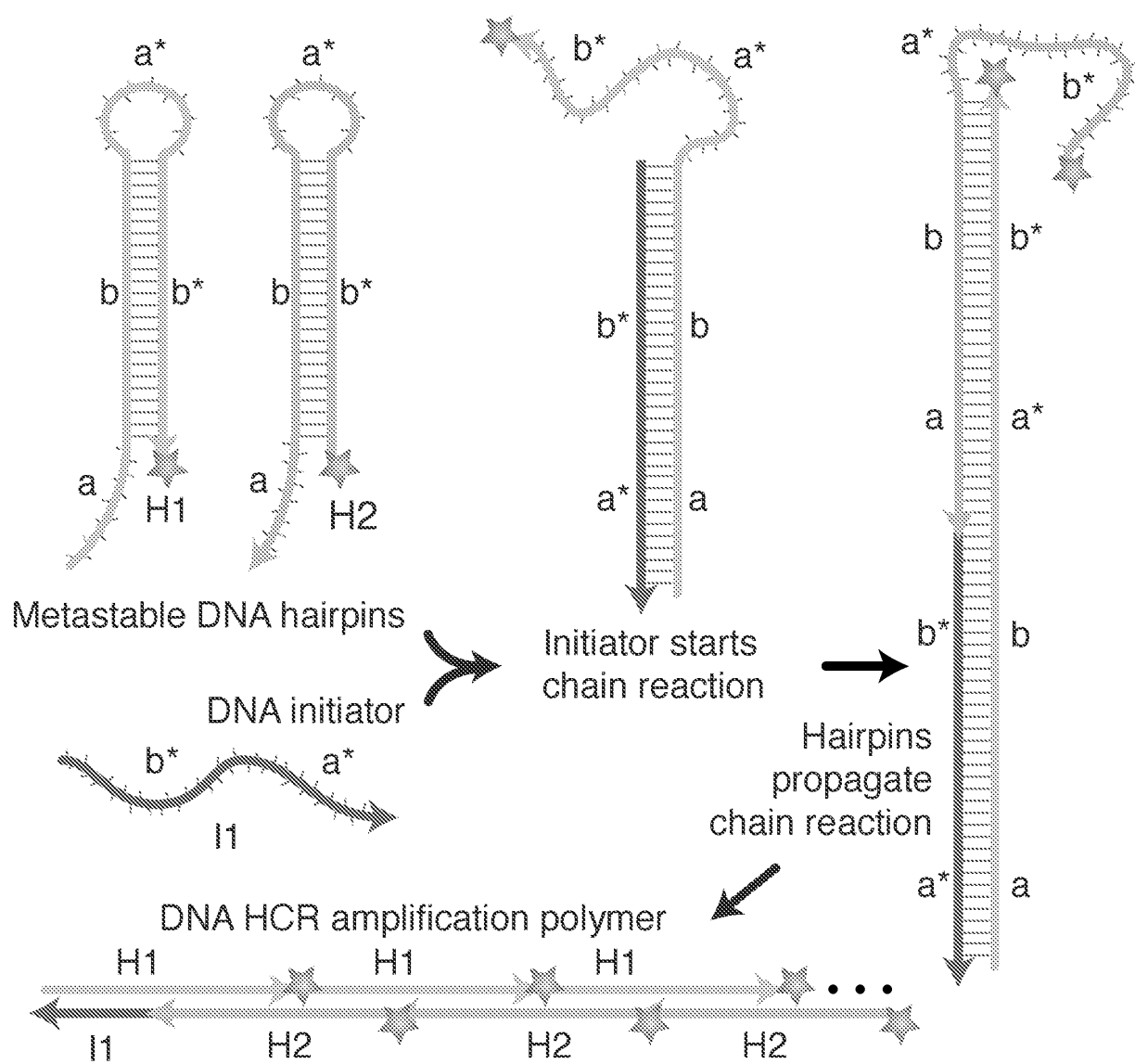
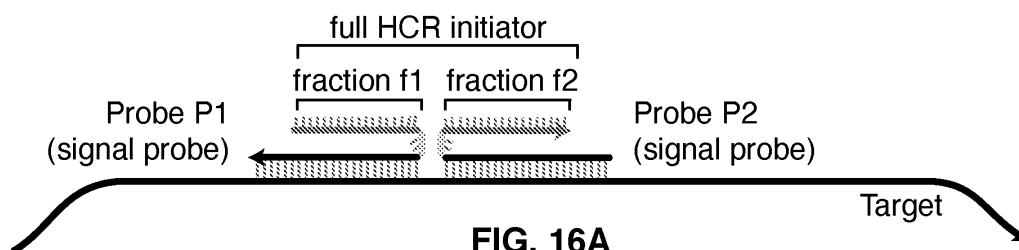
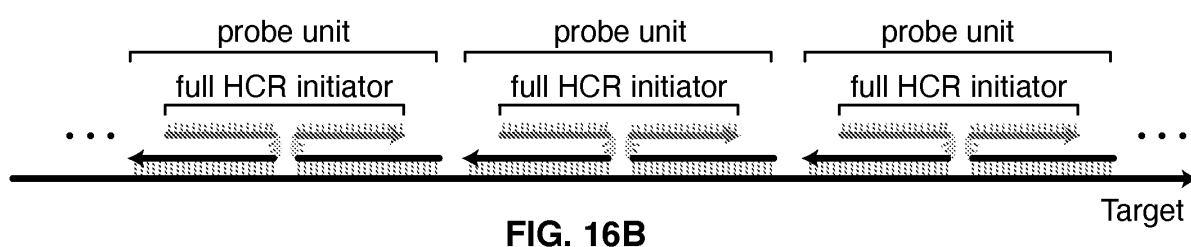


FIG. 15

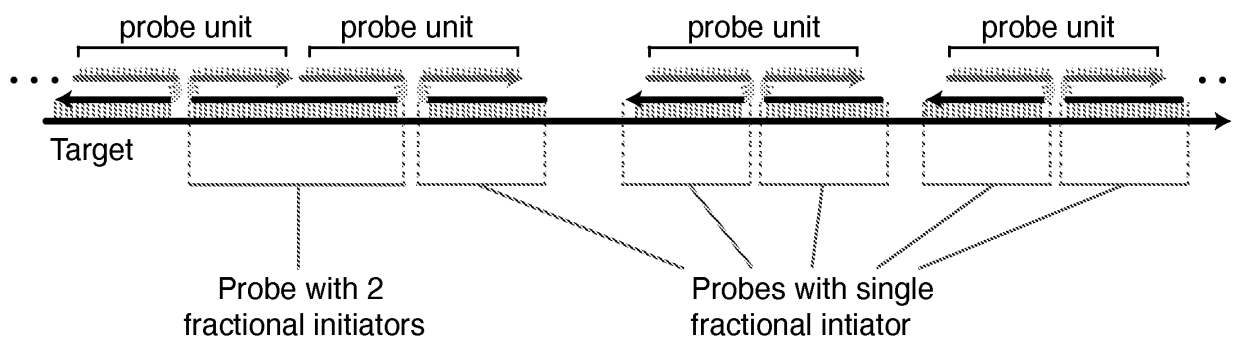
Probe set comprising one probe unit



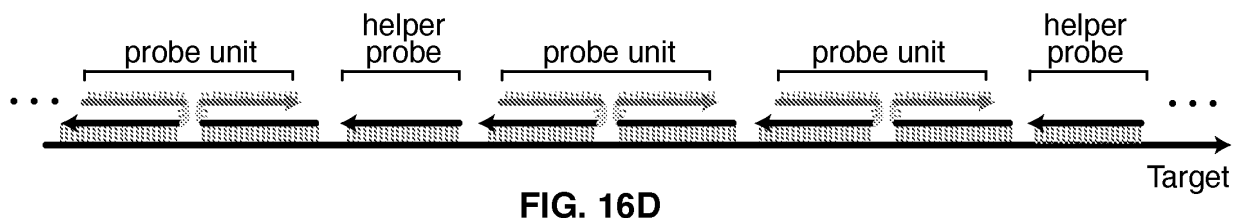
Probe set comprising multiple probe units



Probe set comprising multiple probe units including some probes that comprise two fractional initiators and participate in two probe units



Probe set comprising multiple probe units as well as helper probes



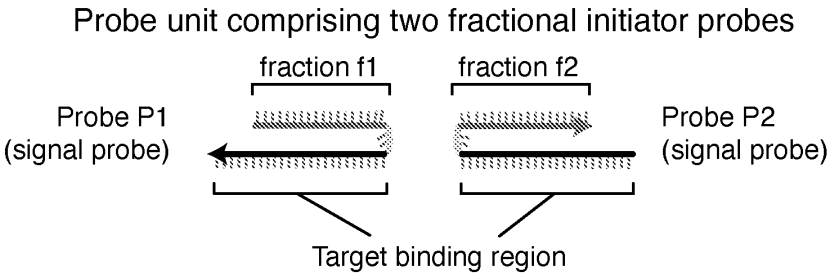


FIG. 17A

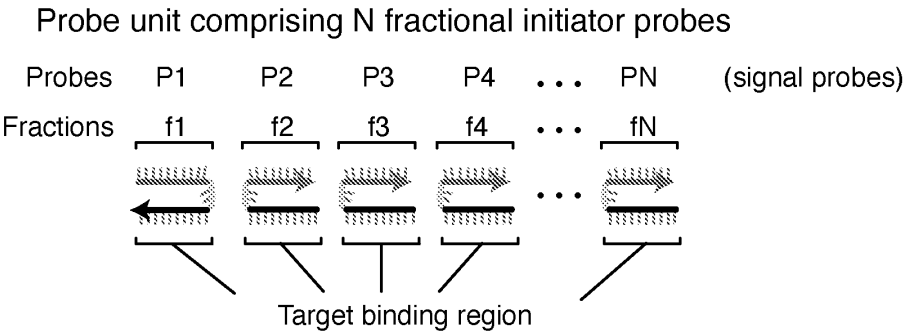


FIG. 17B

Probes that comprise two fractional initiators and participate in two probe units

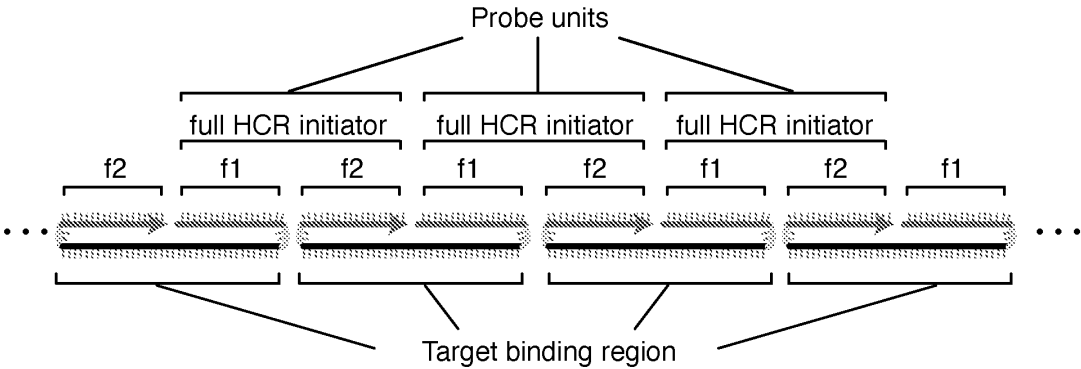
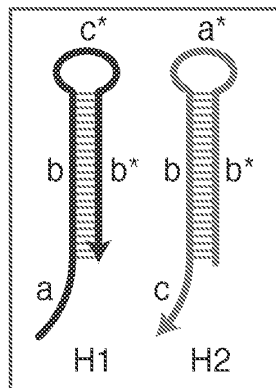


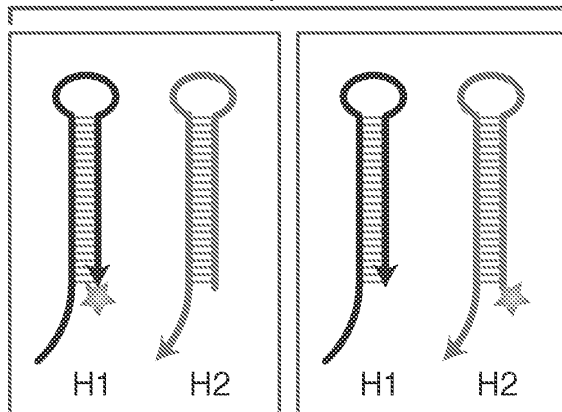
FIG. 17C

Unlabeled HCR hairpins

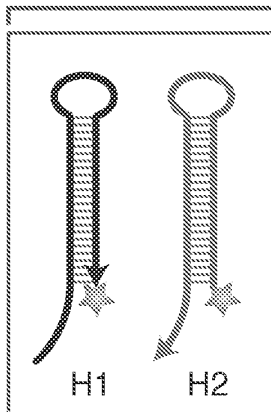
**FIG. 18A**

Reporter-labeled HCR hairpins

One hairpin is labeled

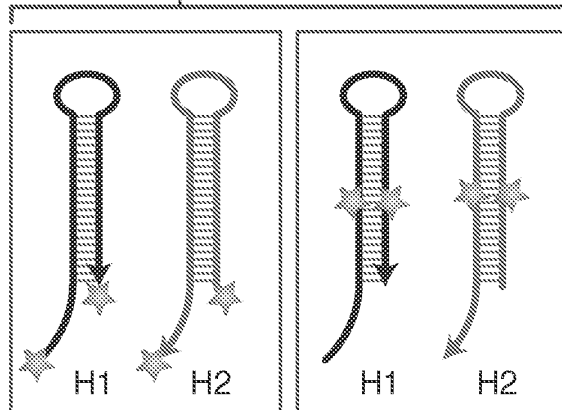


Both hairpins are labeled

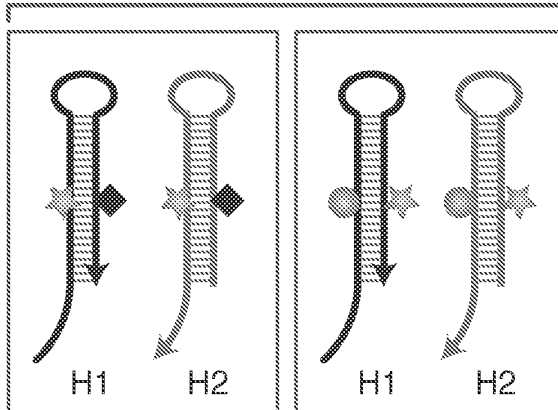


★ denotes reporter
 ◆ denotes quencher
 ● denotes FRET donor

Hairpins are dual-labeled



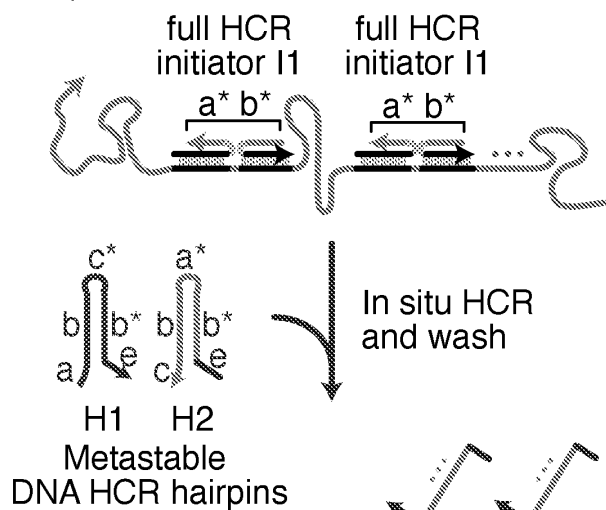
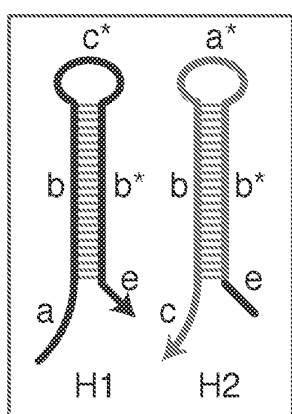
Internal-labeled hairpins



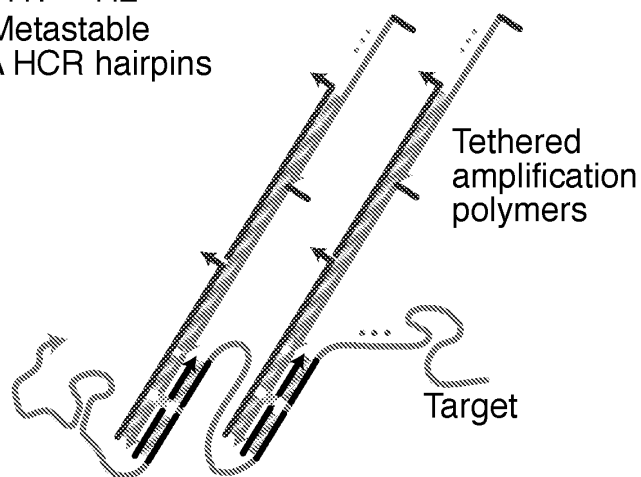
Each hairpin labeled with reporter/quencher pair

Each hairpin labeled with FRET pair

FIG. 18B



Amplification



Label probe carries reporter



Label and wash

Labeling

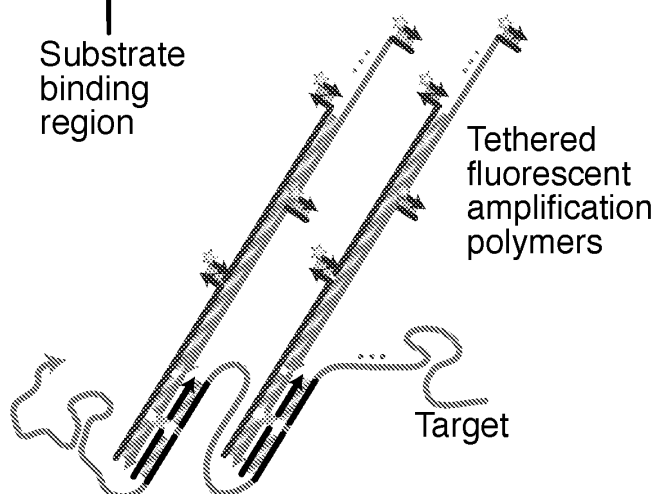
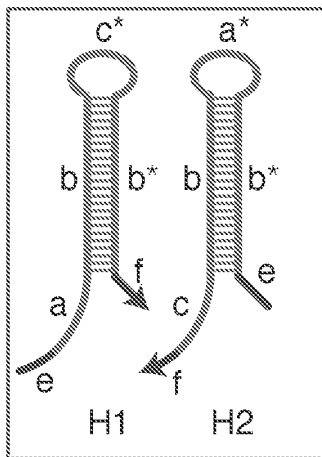
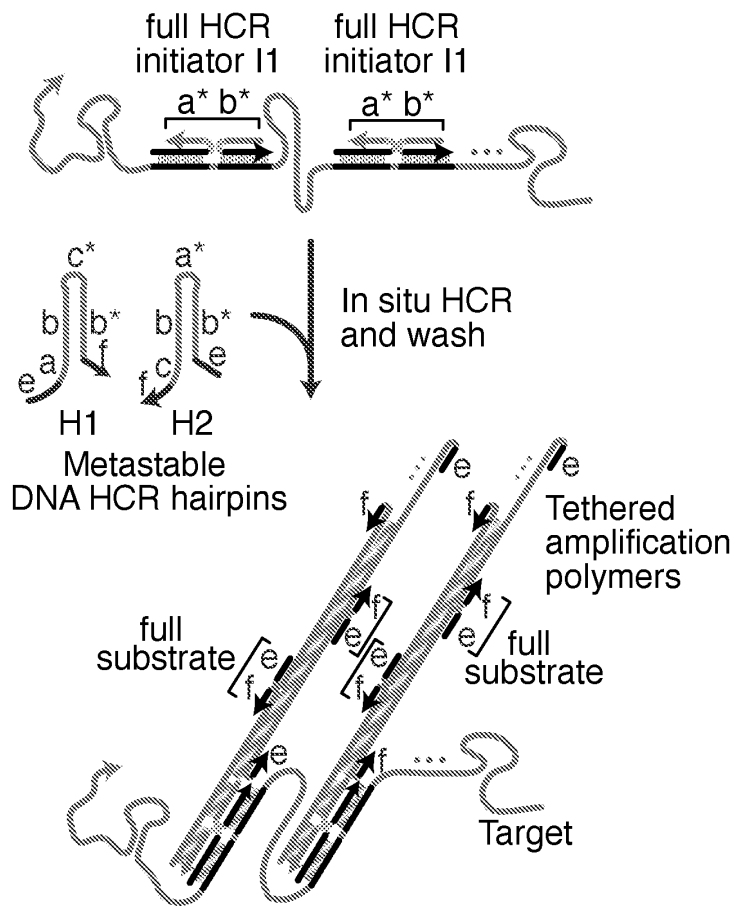


FIG. 18C



segments "e" and "f"
are fractional substrates

Amplification



Label probe
carries reporter

Label
and wash

Substrate
binding
region

Labeling

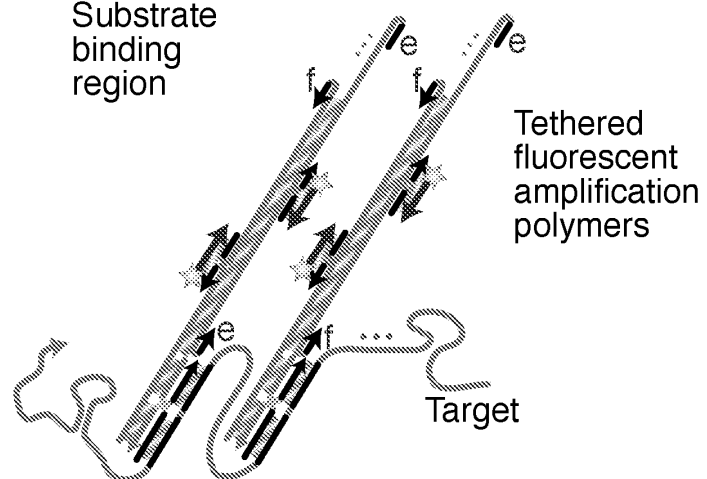


FIG. 18D

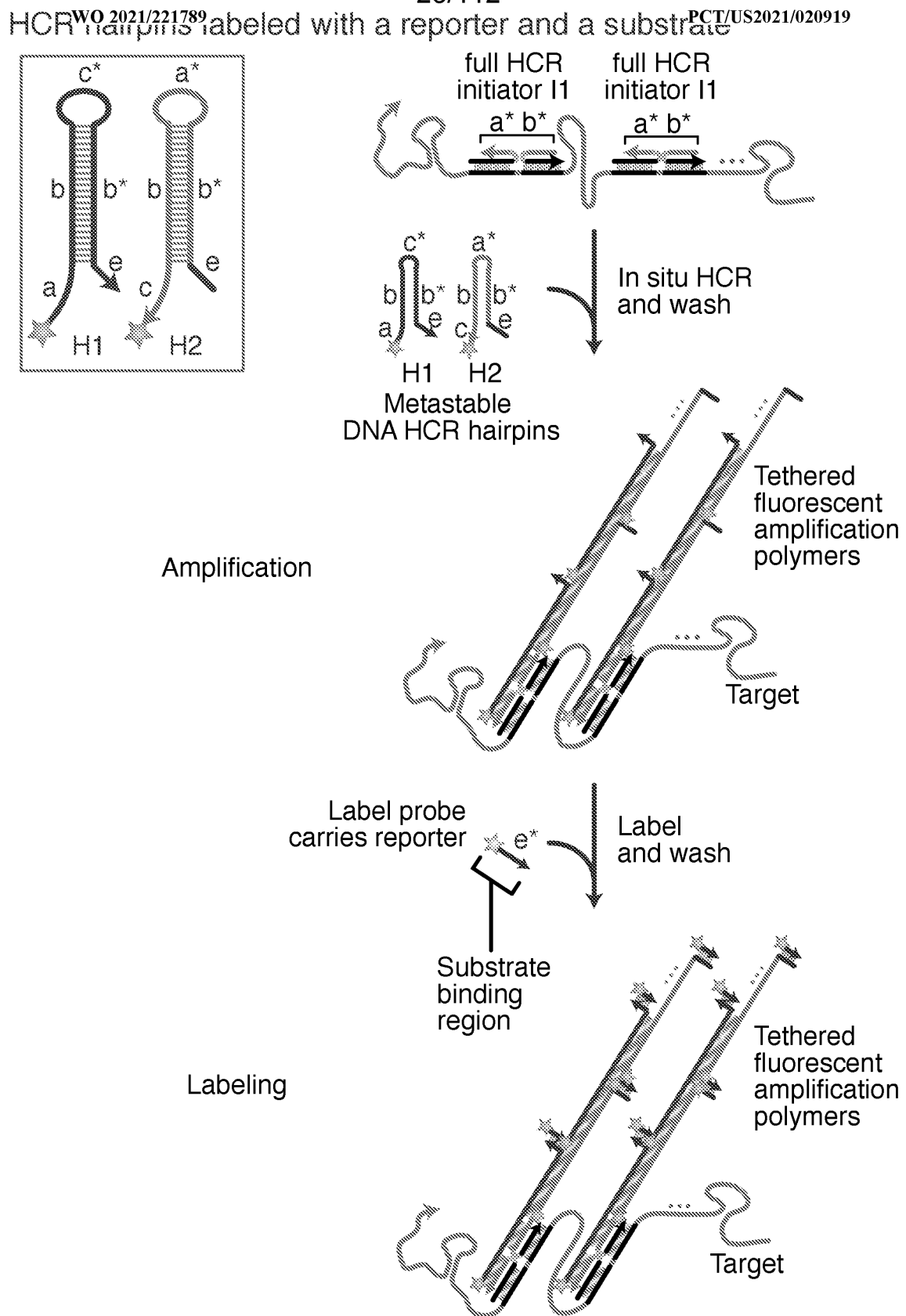
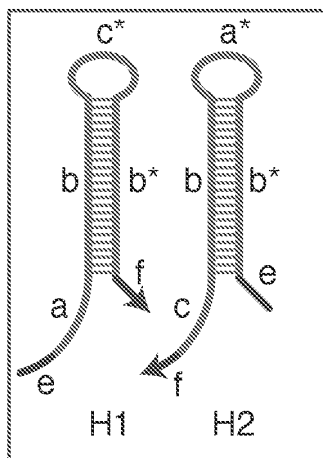


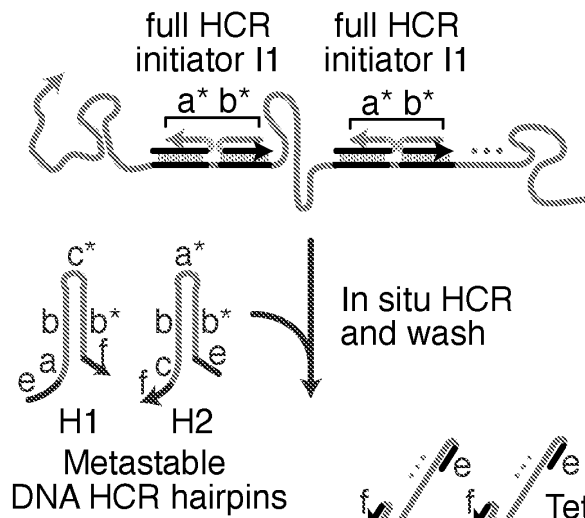
FIG. 18E

Wash-free signal generation with quenched label probe

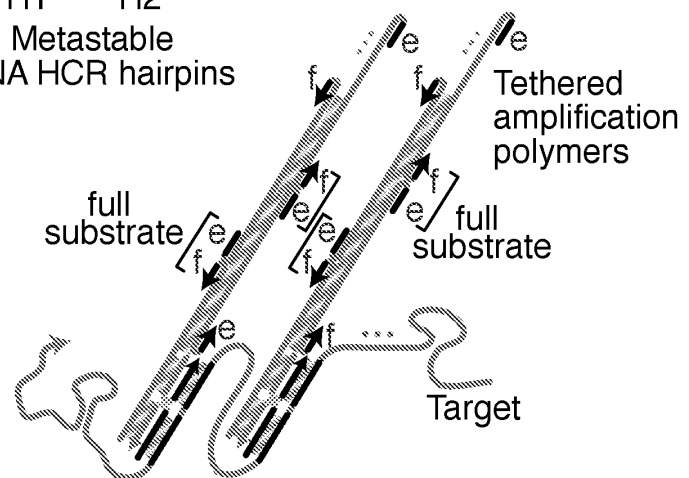


segments "e" and "f" are fractional substrates

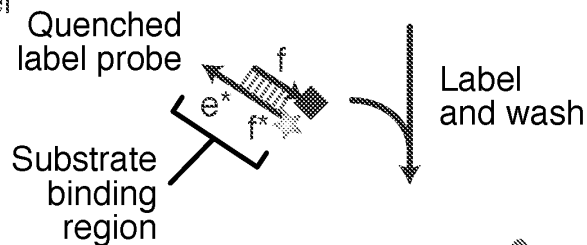
Amplification



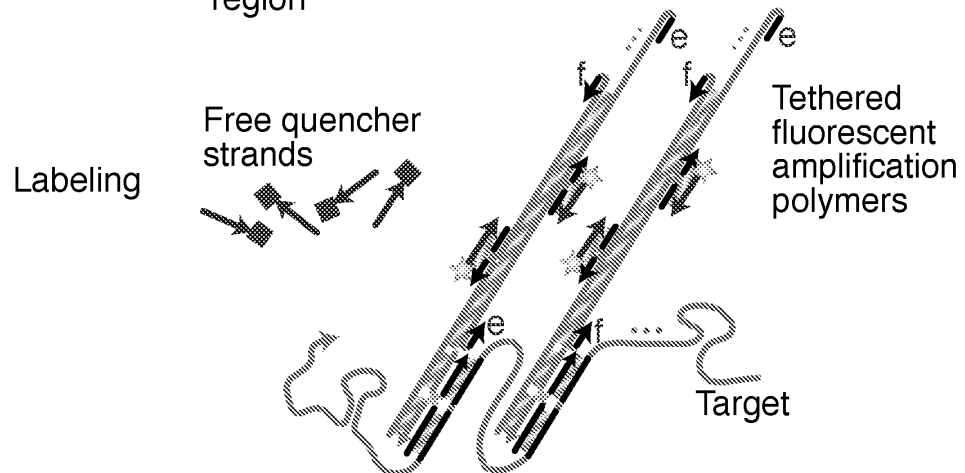
In situ HCR and wash



★ denotes reporter
◆ denotes quencher



Label and wash



Labeling

Free quencher strands

Tethered fluorescent amplification polymers

FIG. 18F

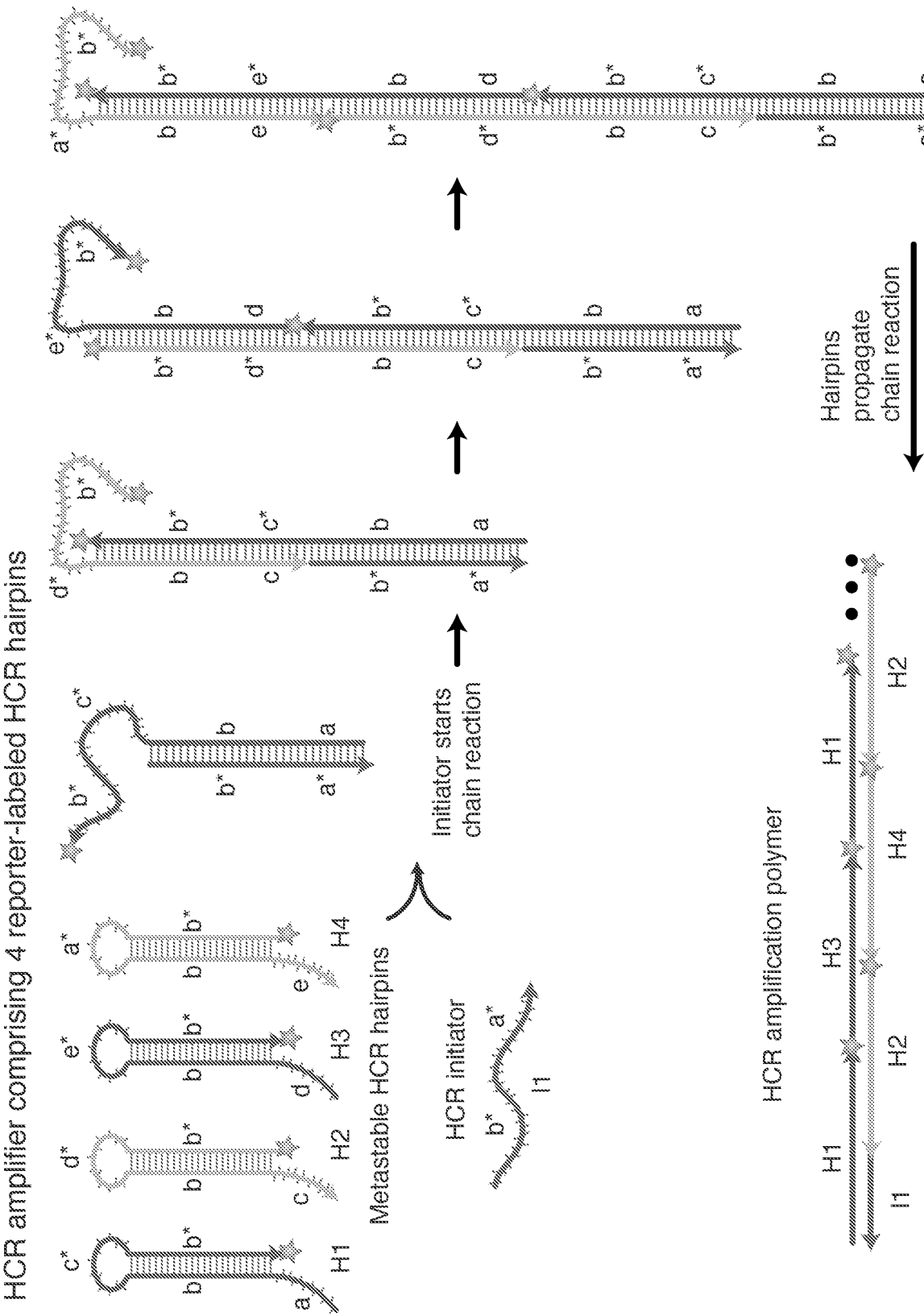


FIG. 19A

HCR amplifier comprising 4 HCR hairpins labeled with a FRET donor or acceptor

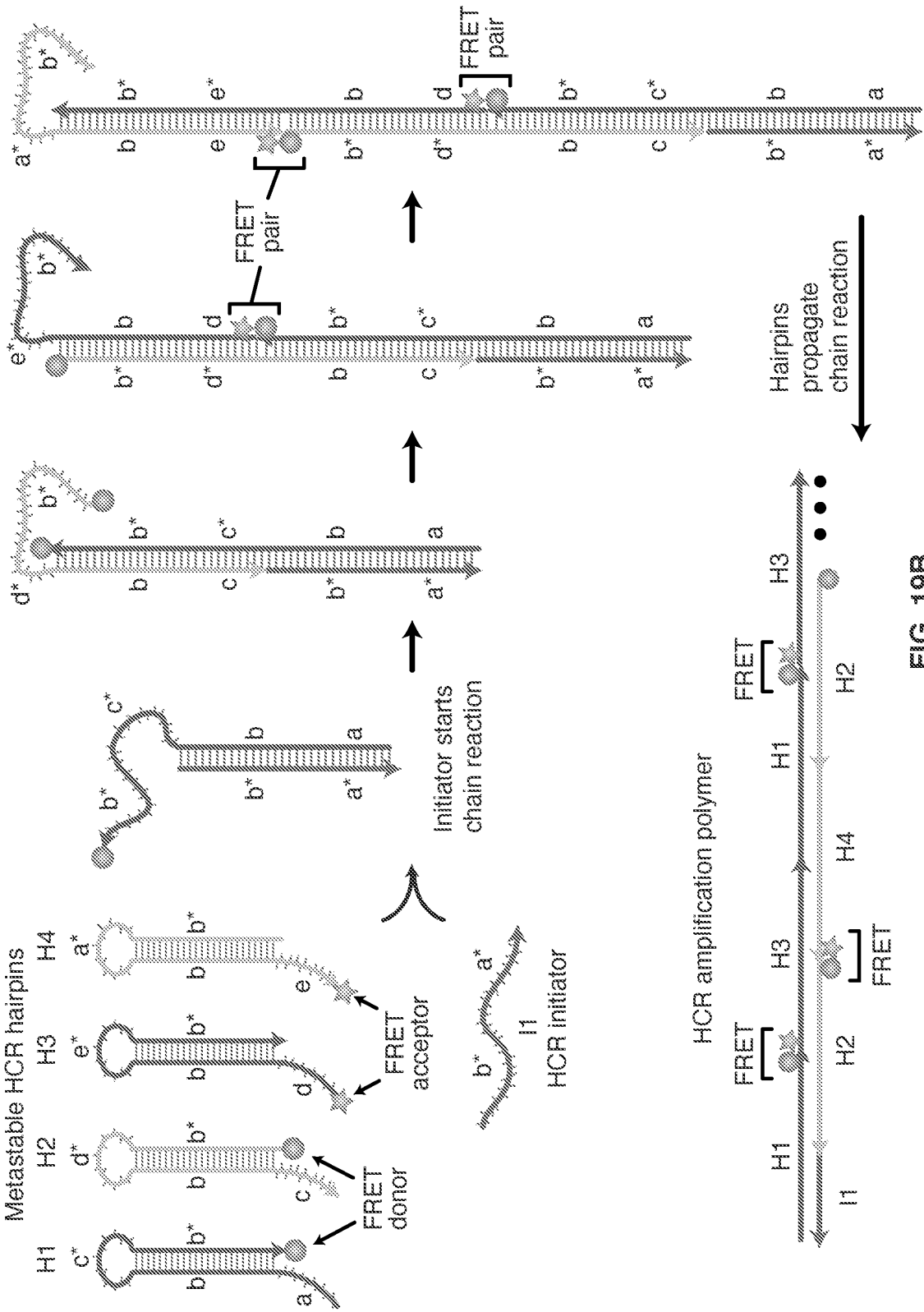
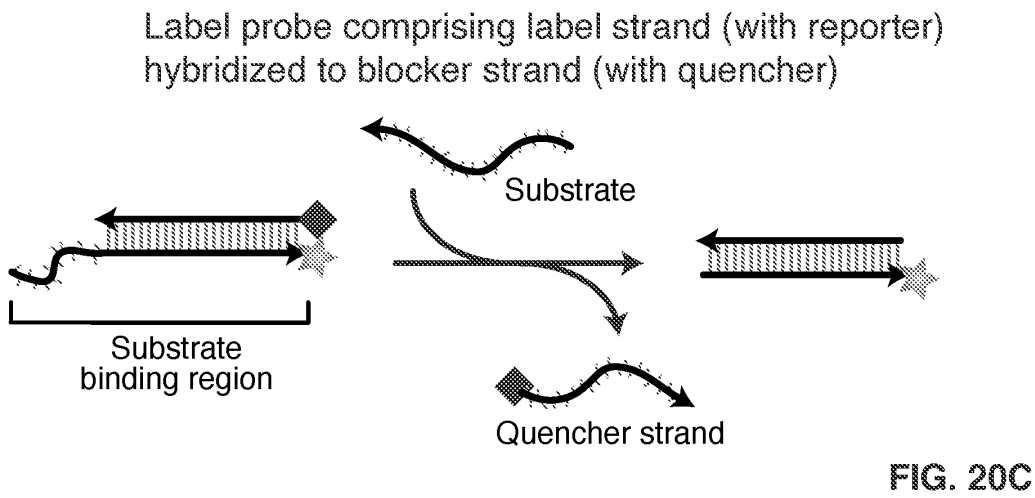
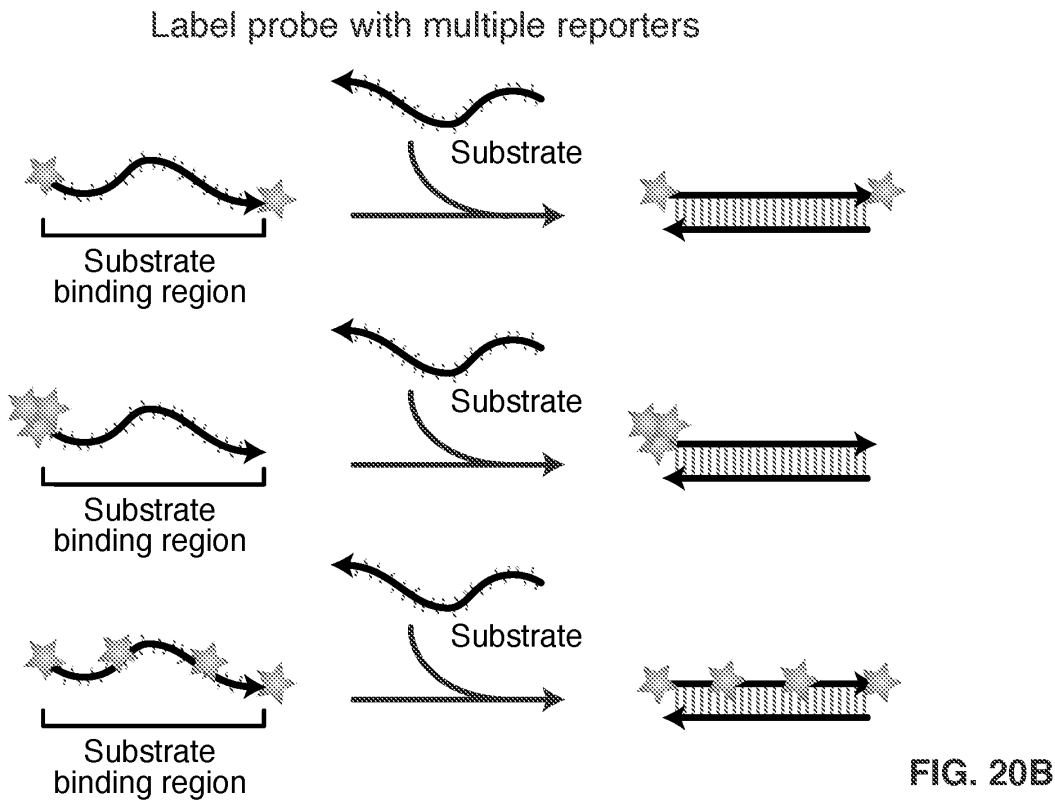
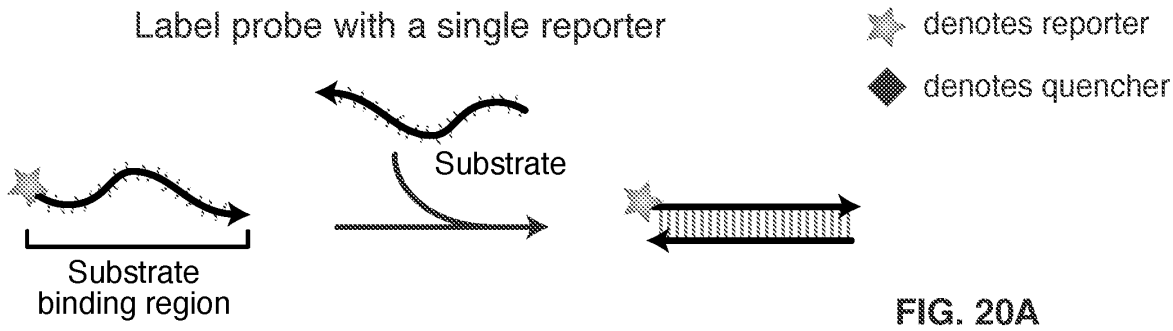
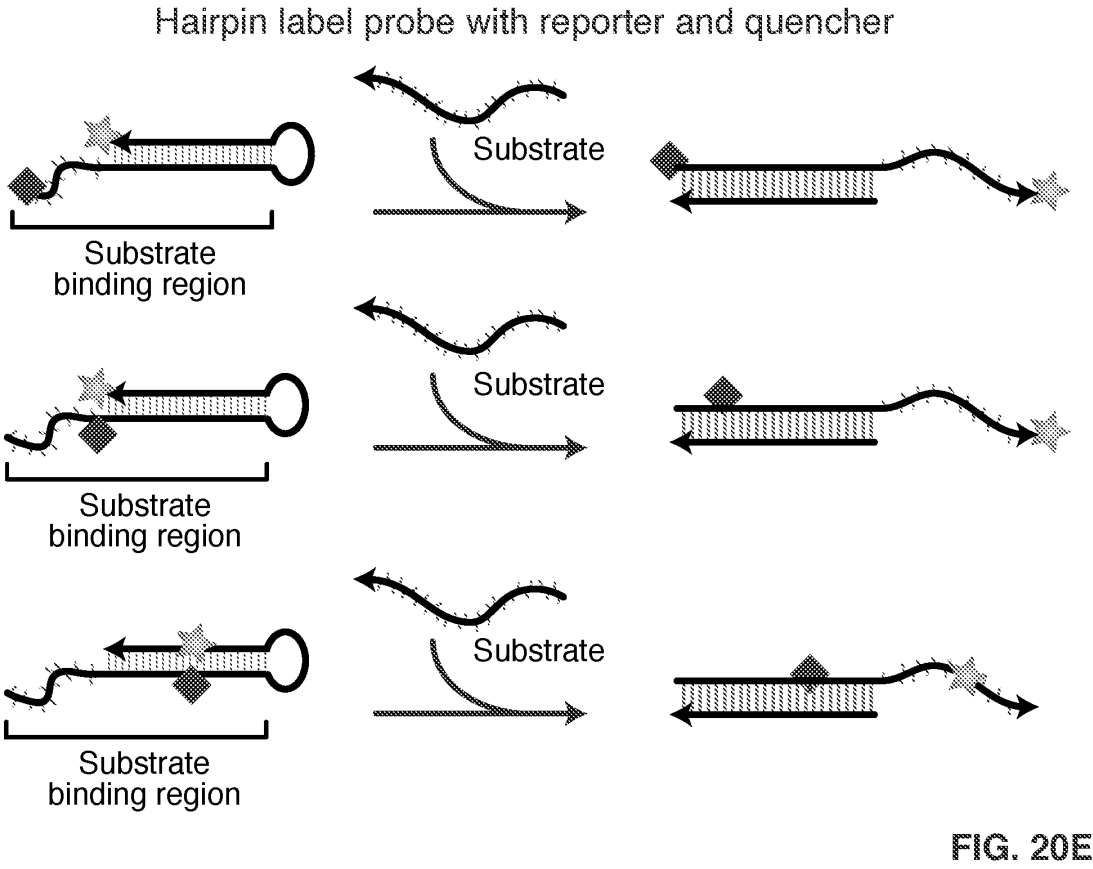
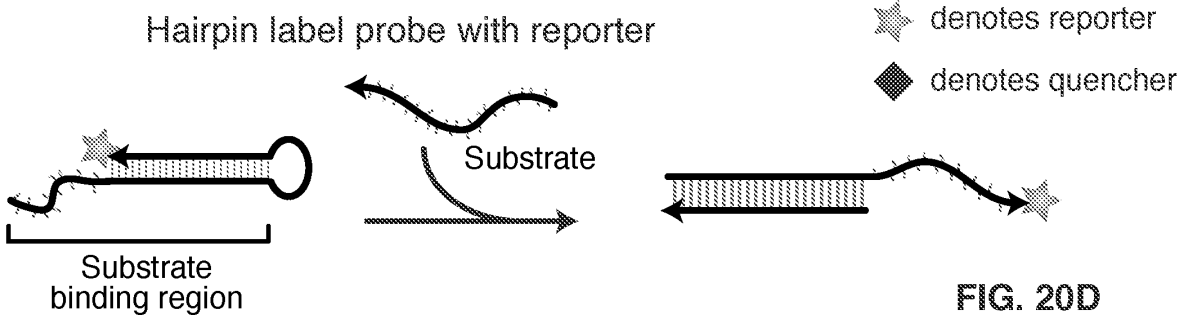


FIG. 19B





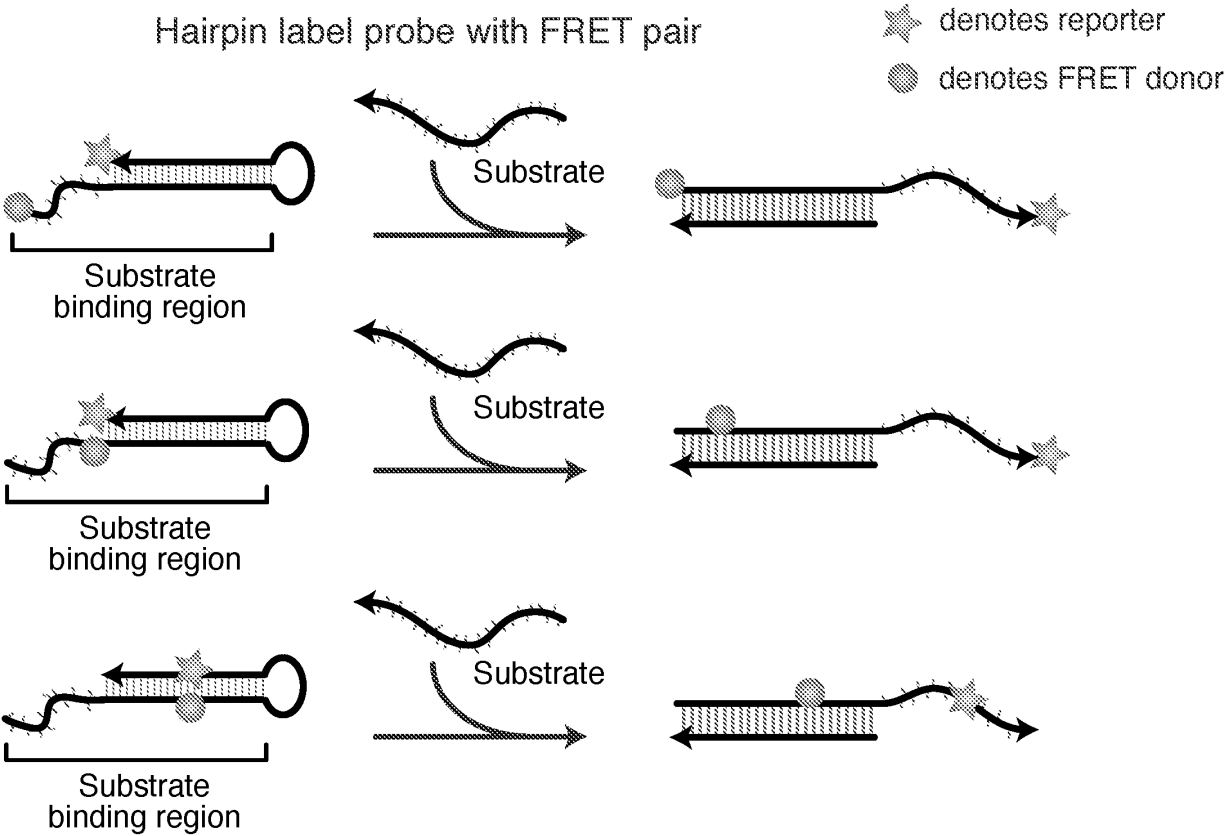
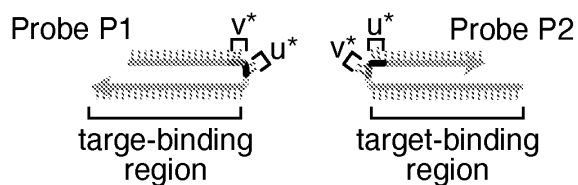
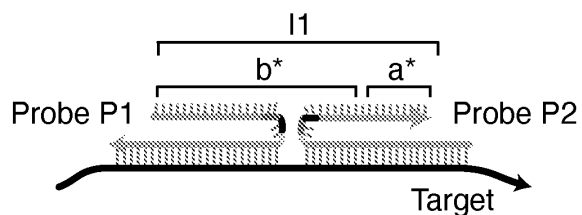


FIG. 20F

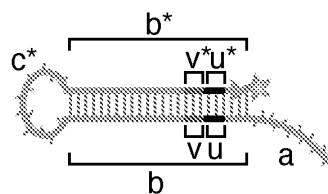
Fractional initiator probes P1 and P2 each carrying a fraction of full HCR initiator I1 such that neither probe alone initiates HCR signal amplification by opening HCR hairpin H1



HCR fractional initiator probes P1 and P2 hybridize to adjacent binding sites on the target to colocalize full HCR initiator I1



Reporter-labeled HCR hairpin H1



Colocalized full initiator I1 initiates HCR signal amplification by opening HCR hairpin H1

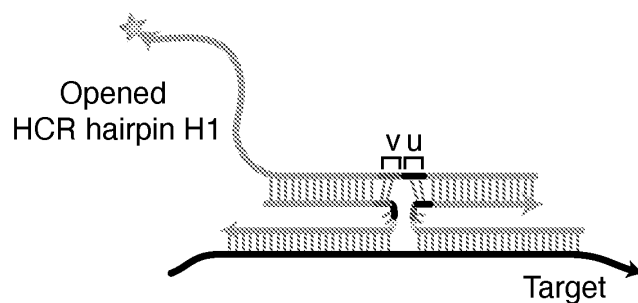
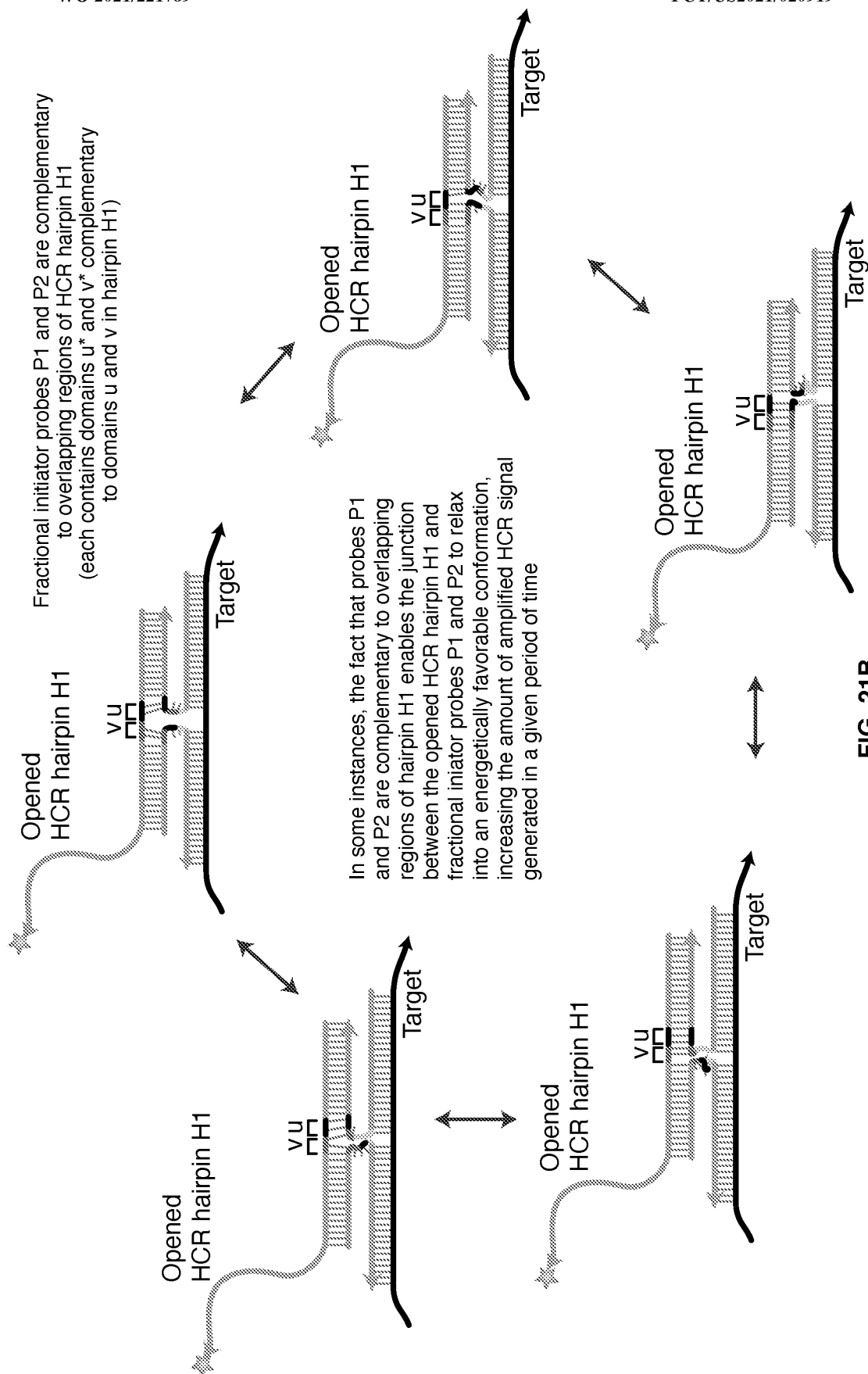
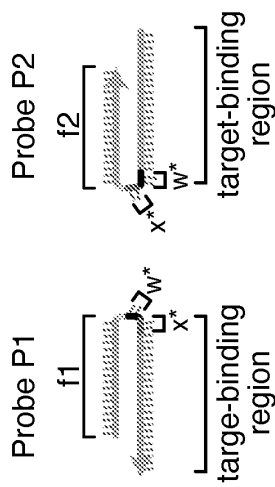


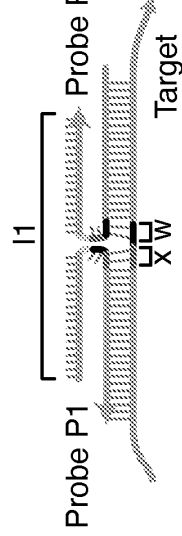
FIG. 21A



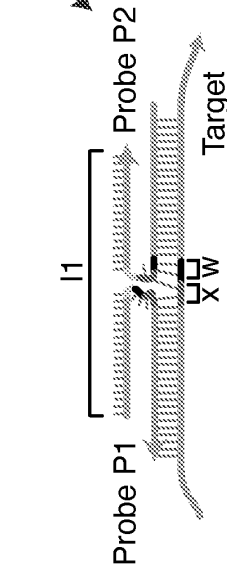
Fractional initiator probes P1 and P2



HCR fractional initiator probes P1 and P2 hybridize to adjacent binding sites on the target to colocalize full HCR initiator I1



Fractional initiator probes P1 and P2 are complementary to overlapping regions of the target (each contains domains w^* and x^* complementary to domains w and x in the target)



In some instances, the fact that probes P1 and P2 are complementary to overlapping regions of the target enables the junction between the target and fractional initiator probes P1 and P2 to relax into an energetically favorable conformation, increasing the hybridization efficiency of the probes in a given period of time

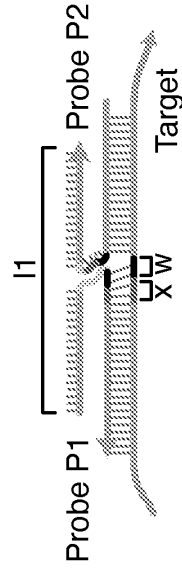
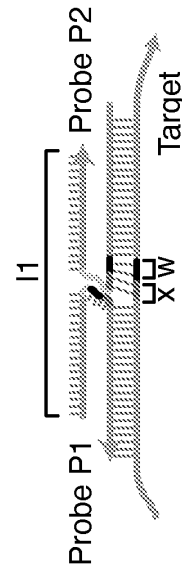
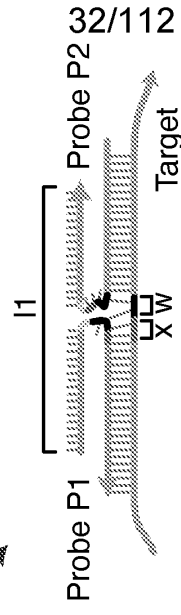
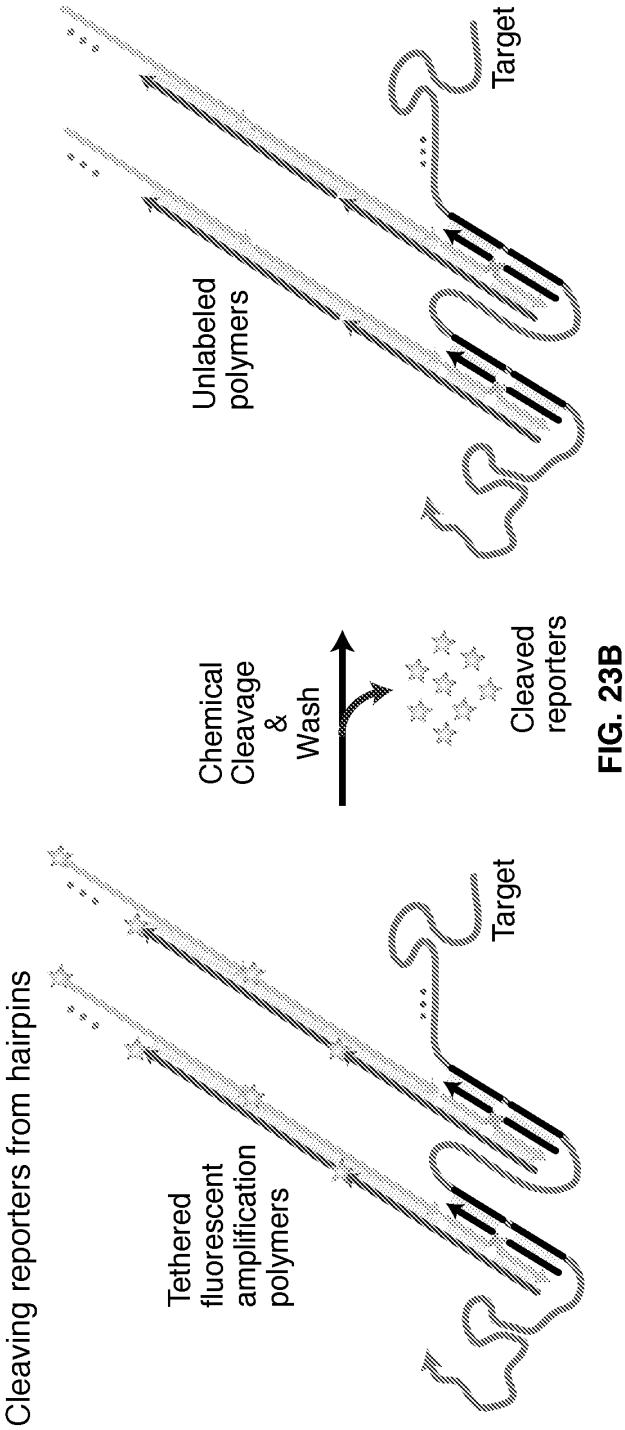
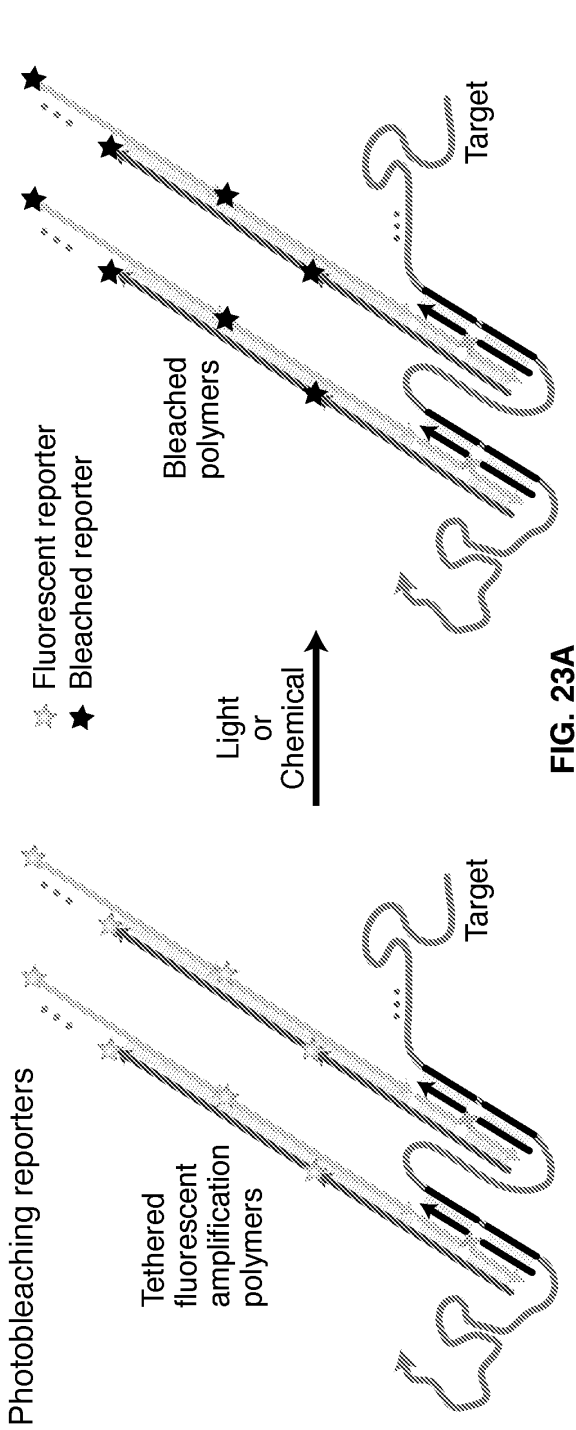


FIG. 22



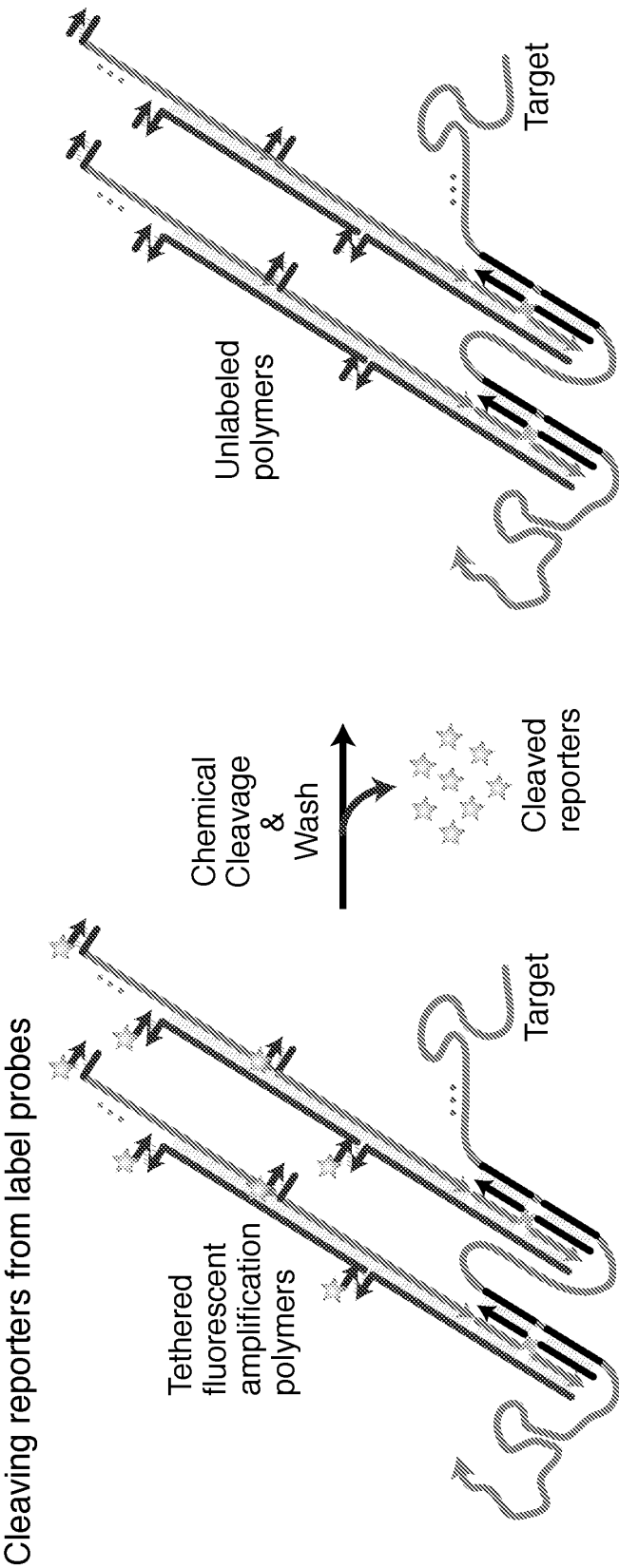


FIG. 23C

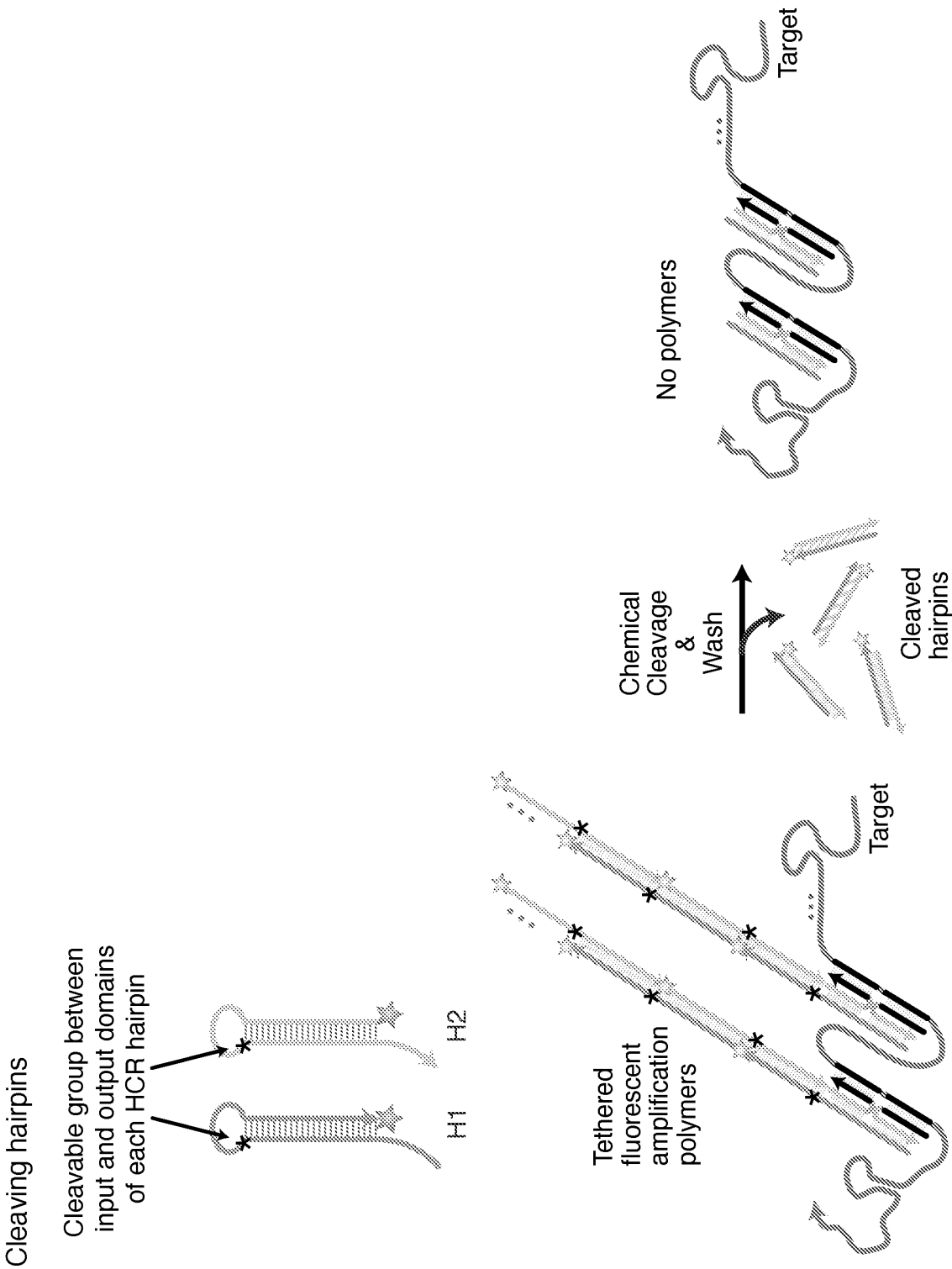


FIG. 23D

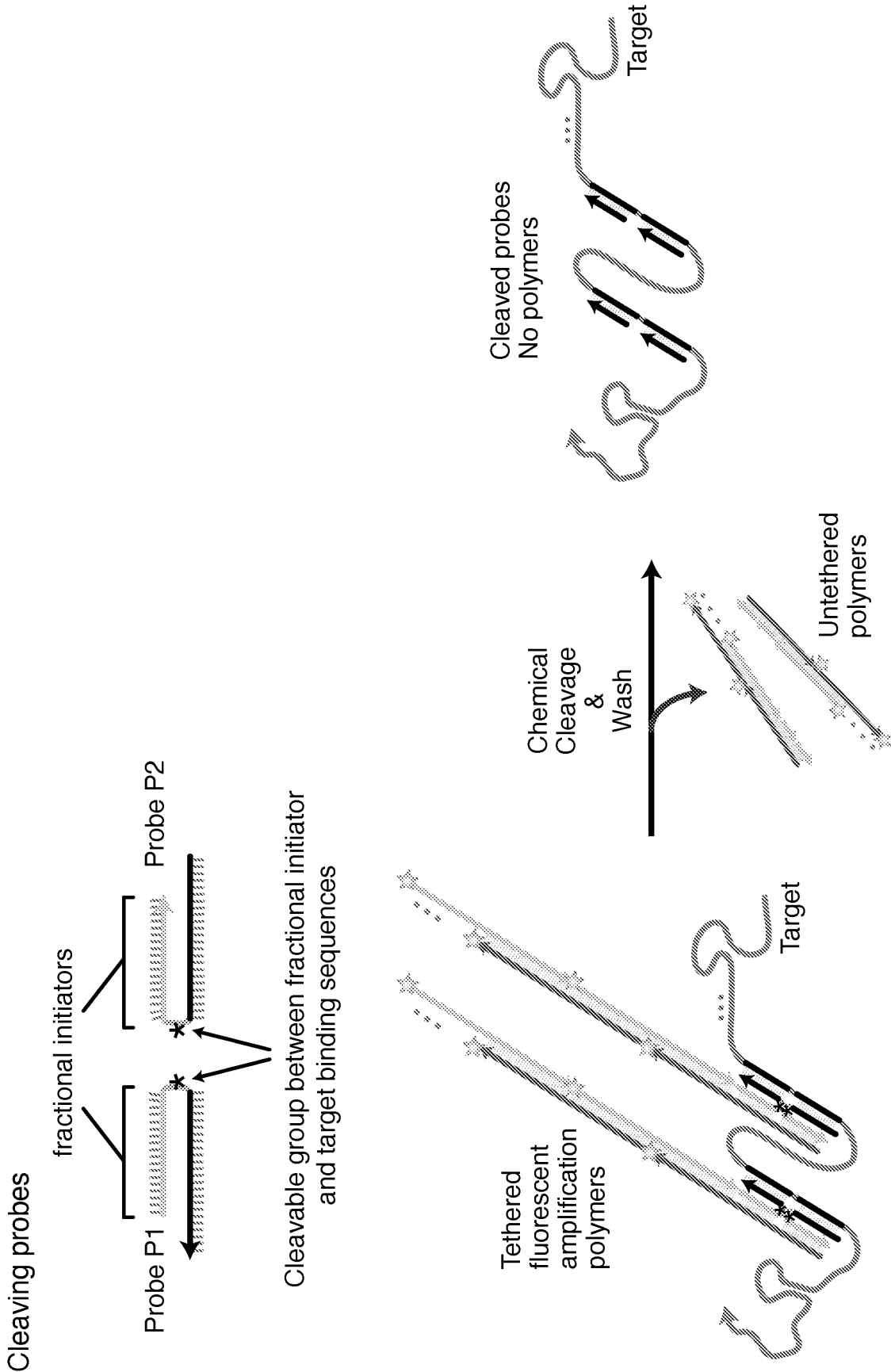


FIG. 23E

Dehybridizing hairpins with auxiliary strands

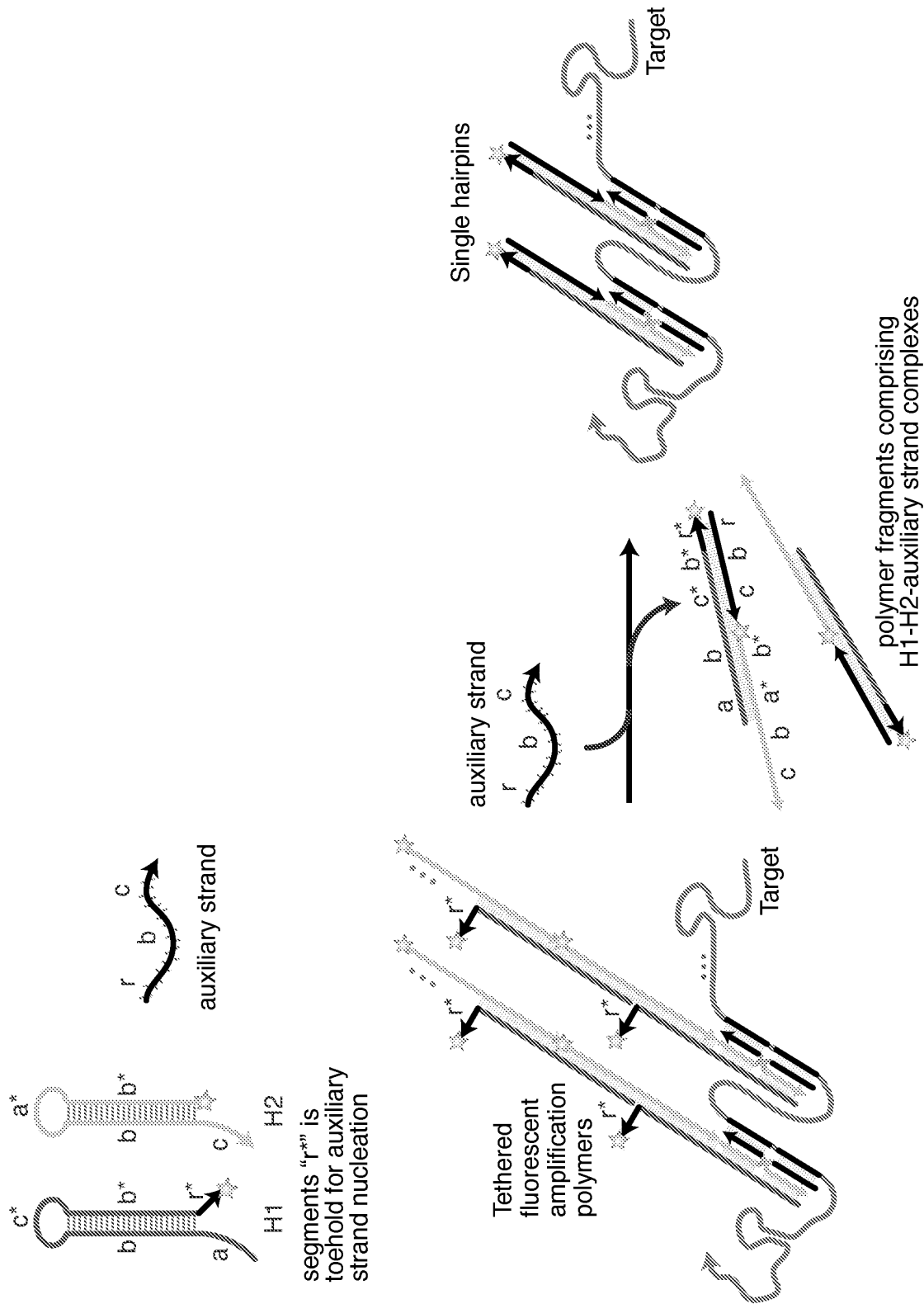


FIG. 23F

Dehybridizing label probes with auxiliary strands

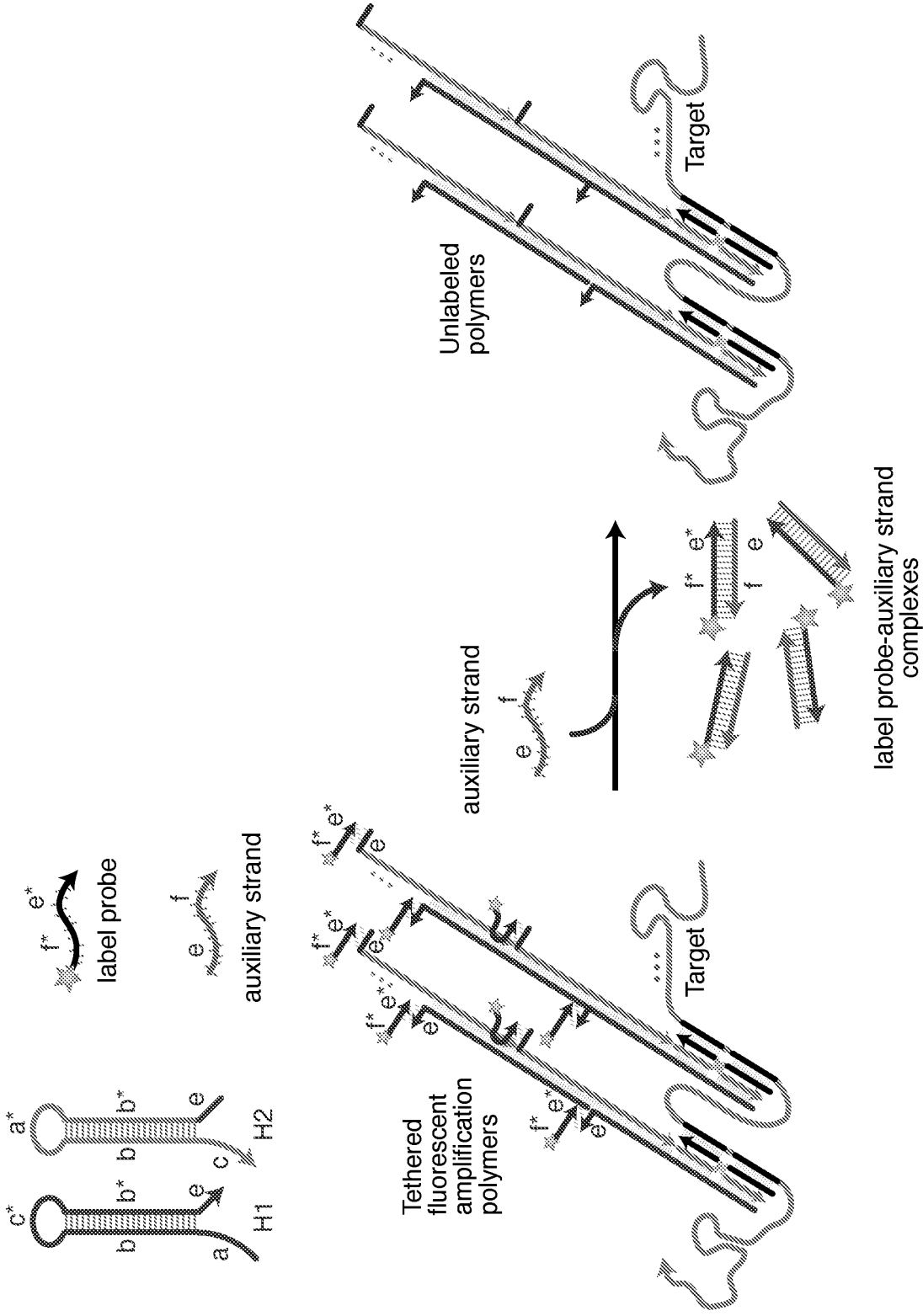


FIG. 23G

Destabilizing hairpins from polymers with chemical or elevated temperature

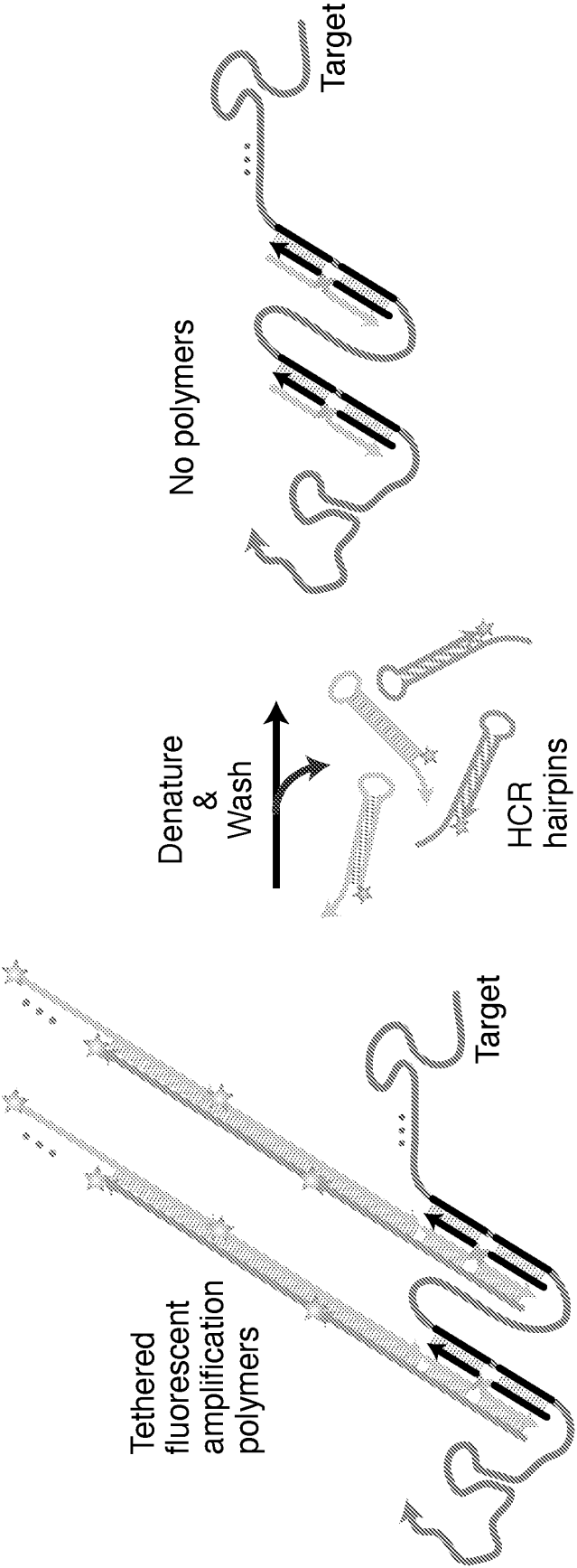


FIG. 23H

Destabilizing probes from target with chemical or elevated temperature

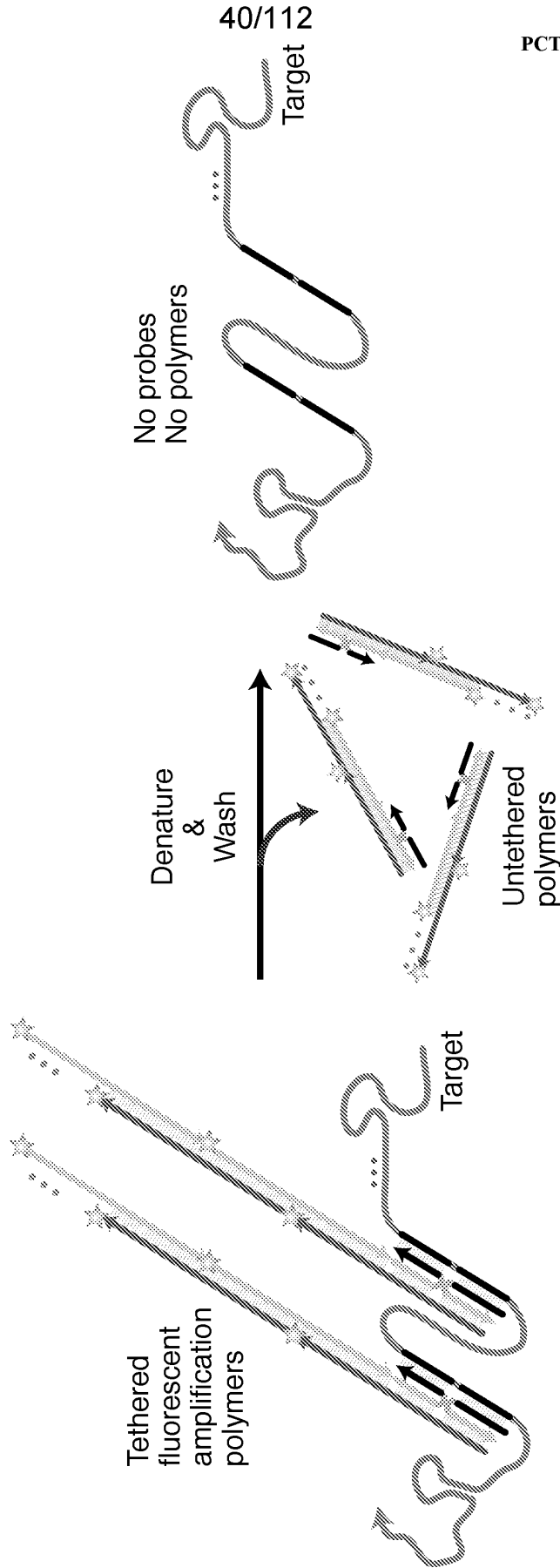


FIG. 23I

Destabilizing label probes from substrates with chemical or elevated temperature

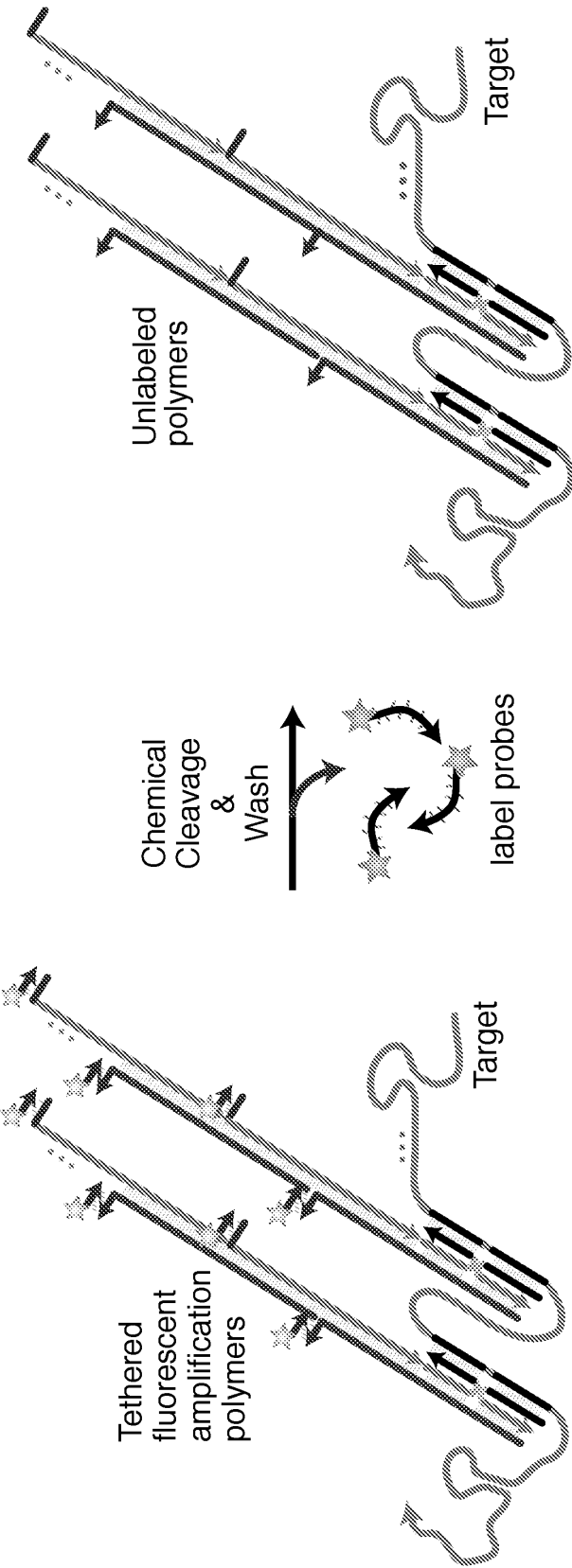


FIG. 23J

Degrading HCR probes and polymers with enzymes (e.g., DNase)

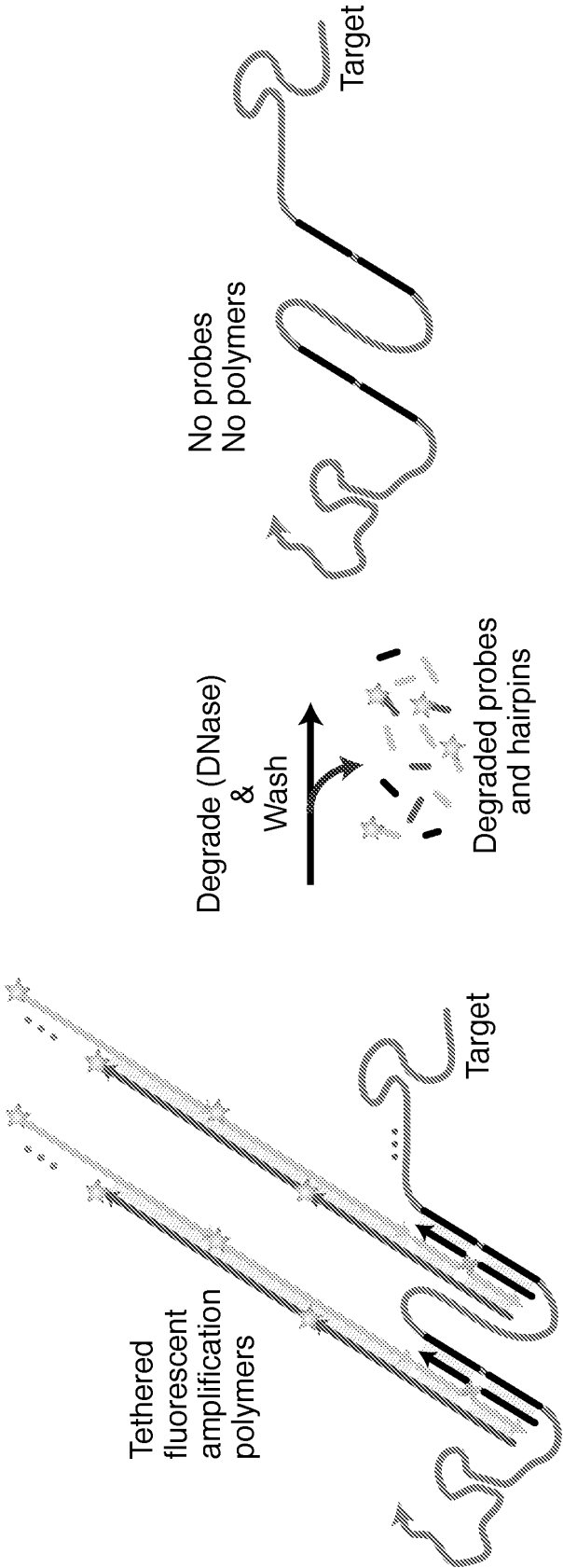


FIG. 23K

Degrading RNA targets with RNases

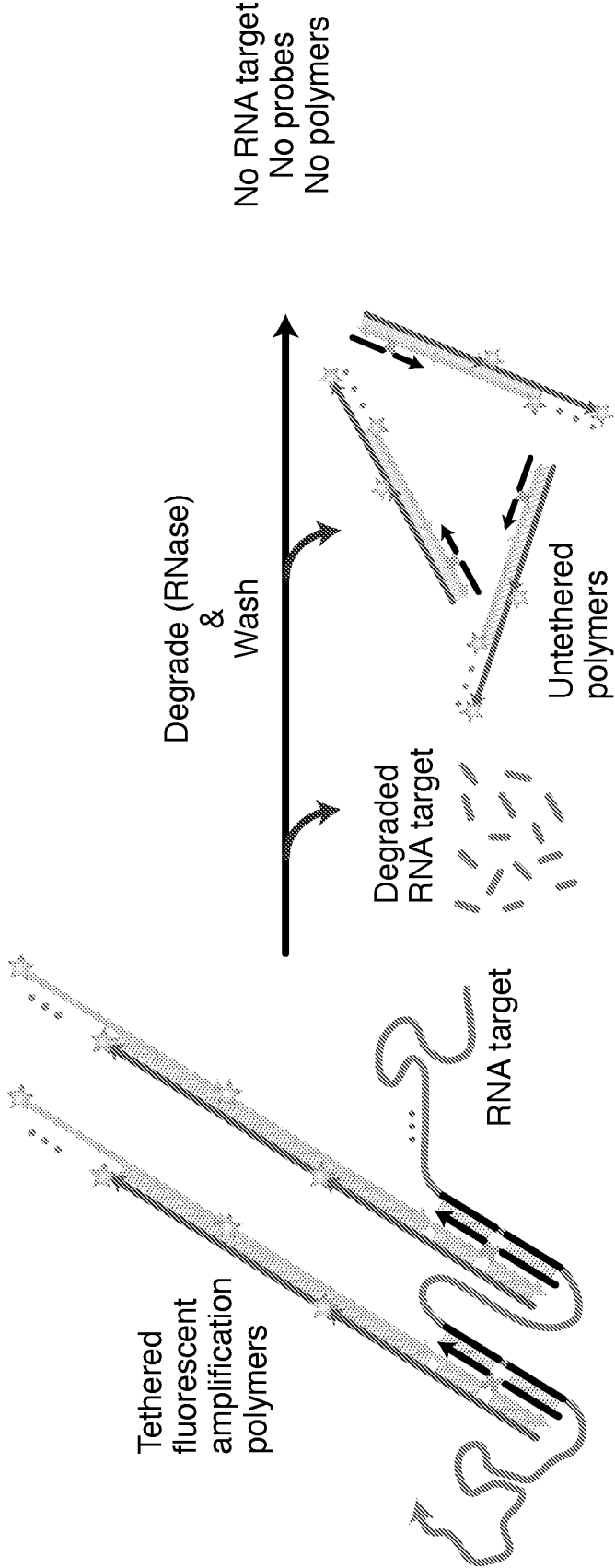


FIG. 23L

Degrading protein targets and antibodies with proteases

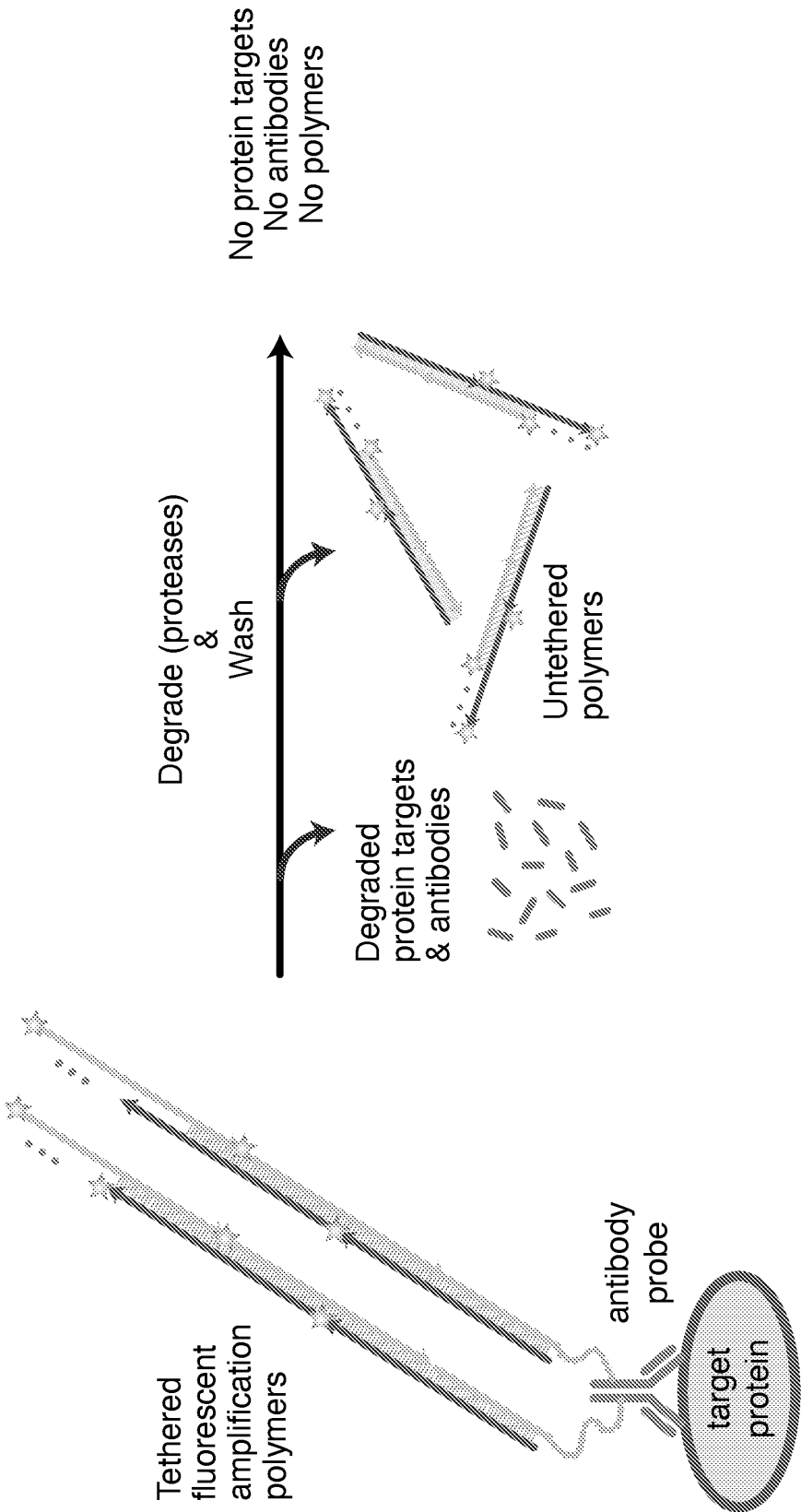


FIG. 23M

Degrading RNA targets with RNases and HCR probes and hairpins with DNases

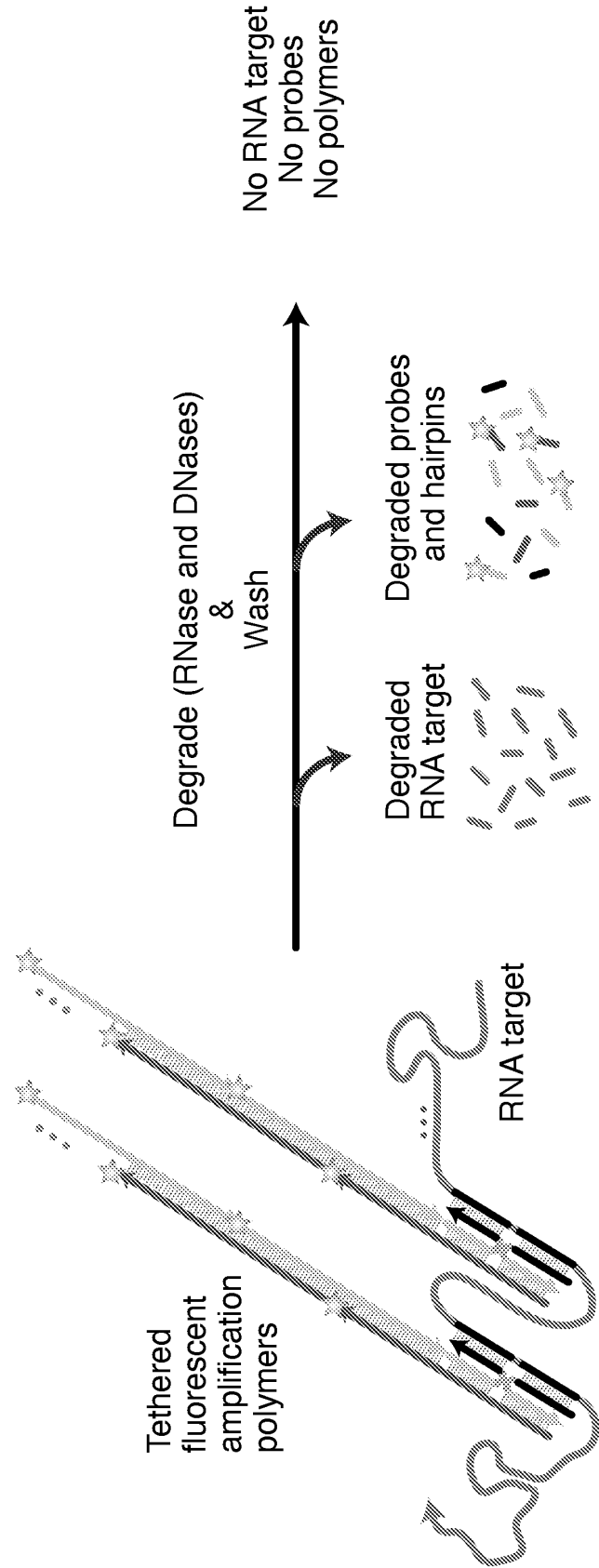


FIG. 23N

Degrading protein targets and antibodies with proteases and HCR probes and hairpins with DNases

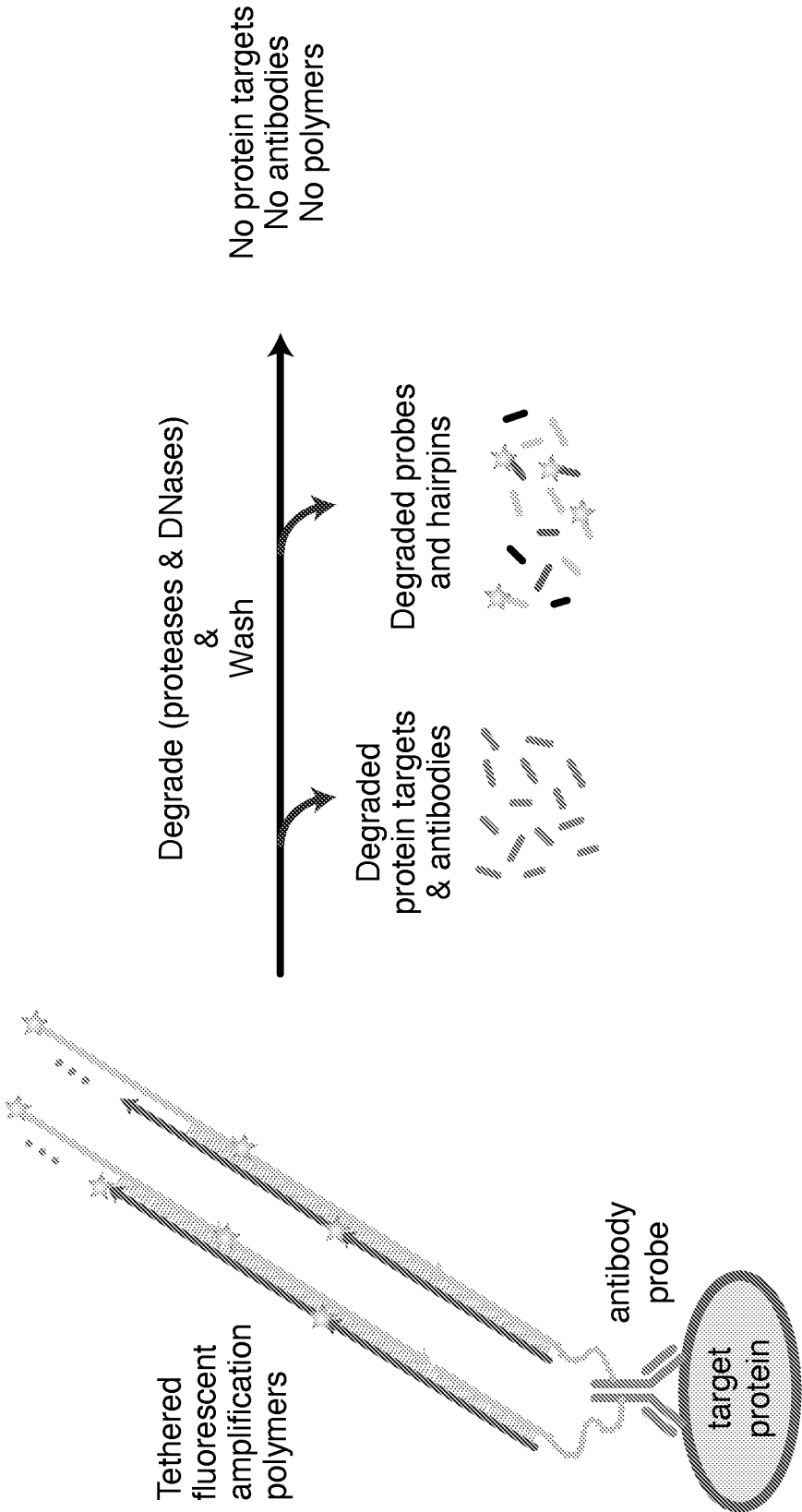
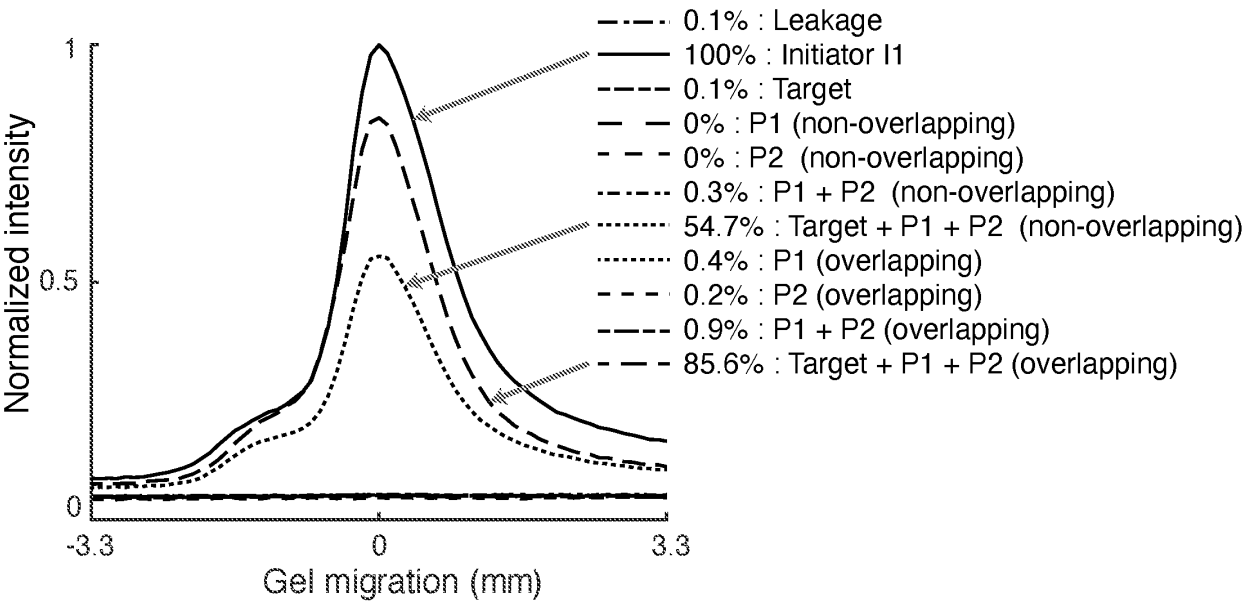
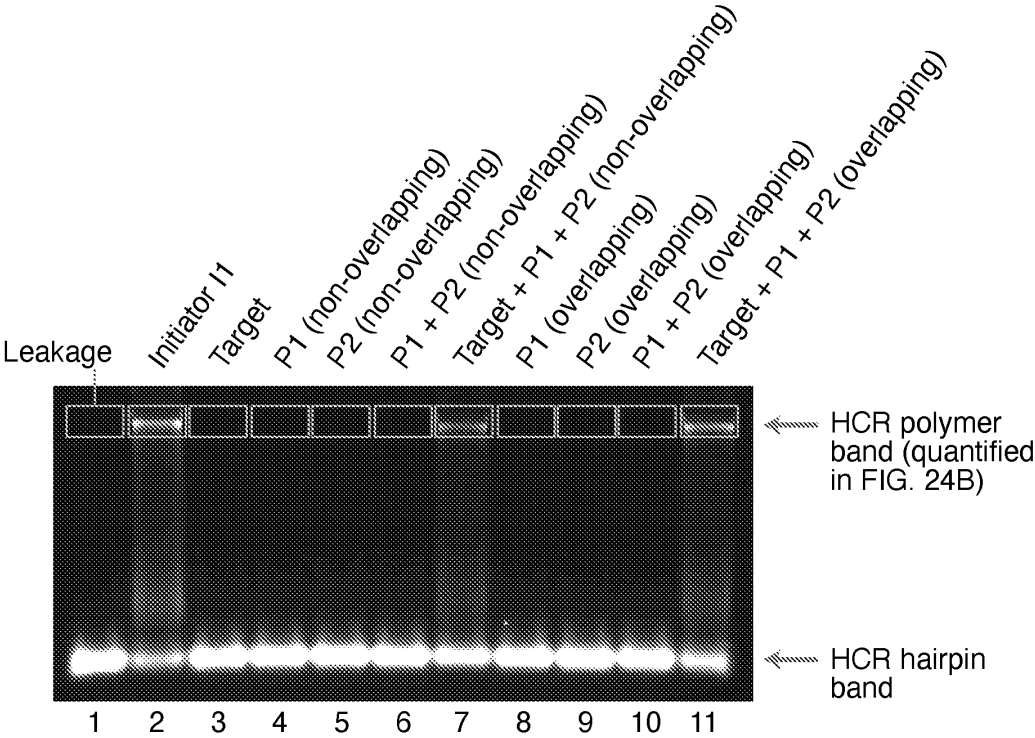


FIG. 230



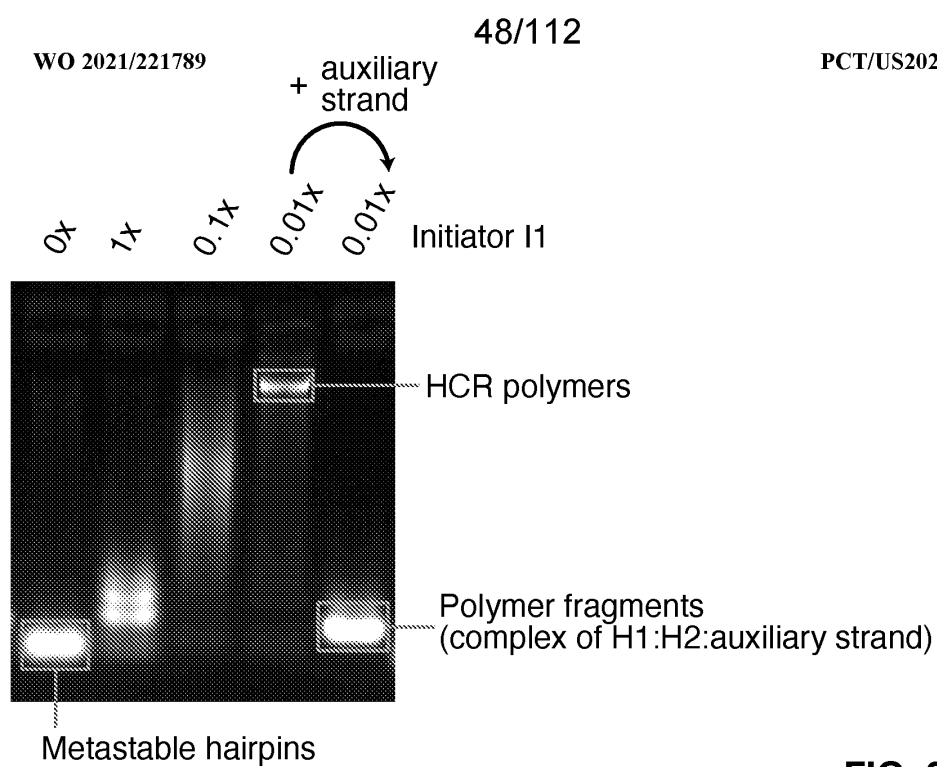


FIG. 25A

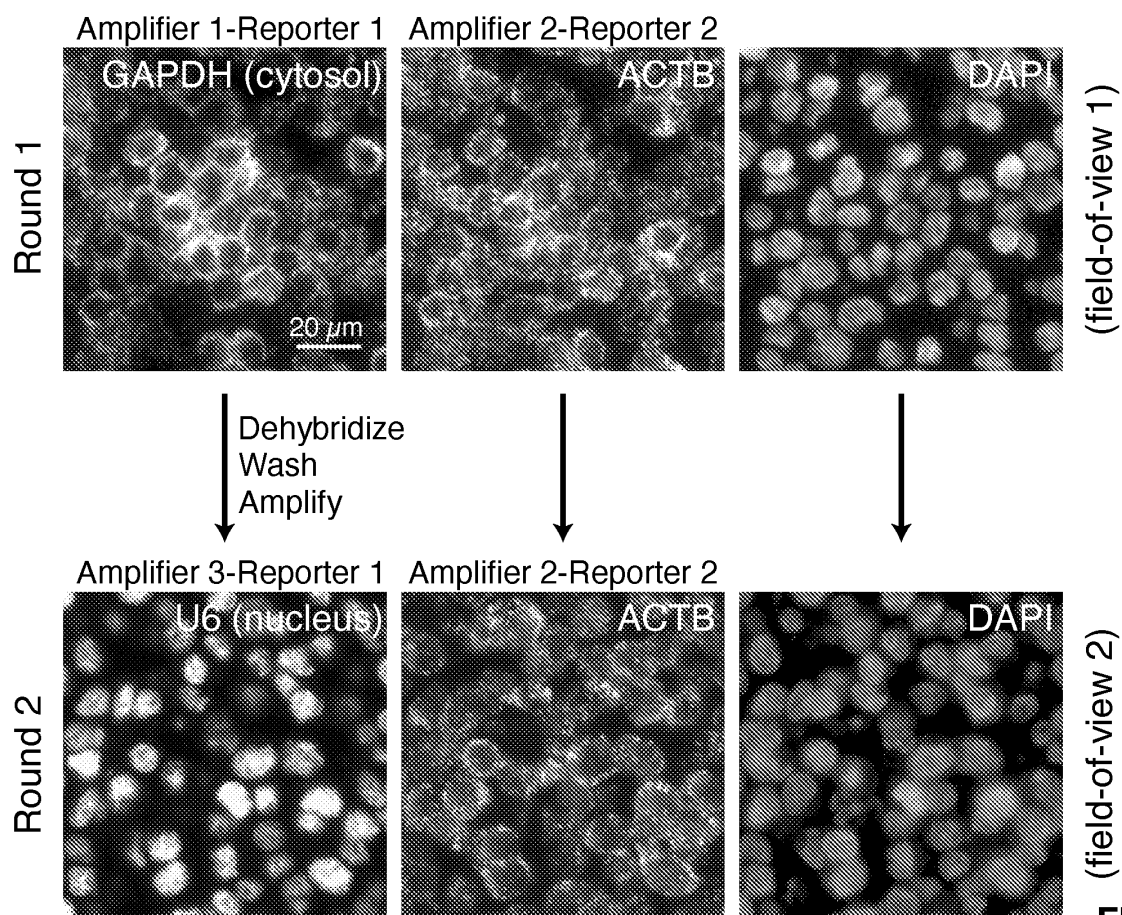
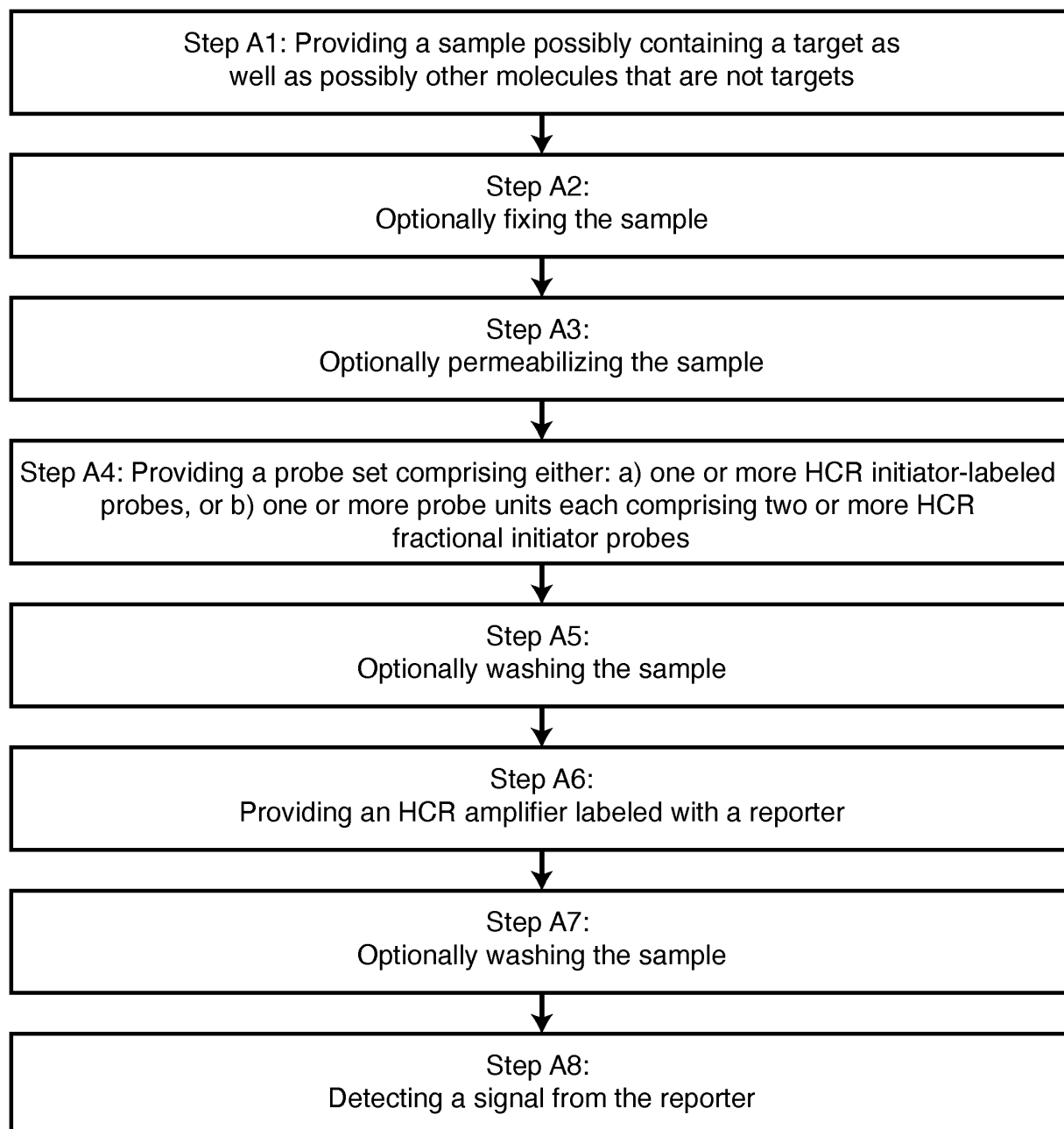
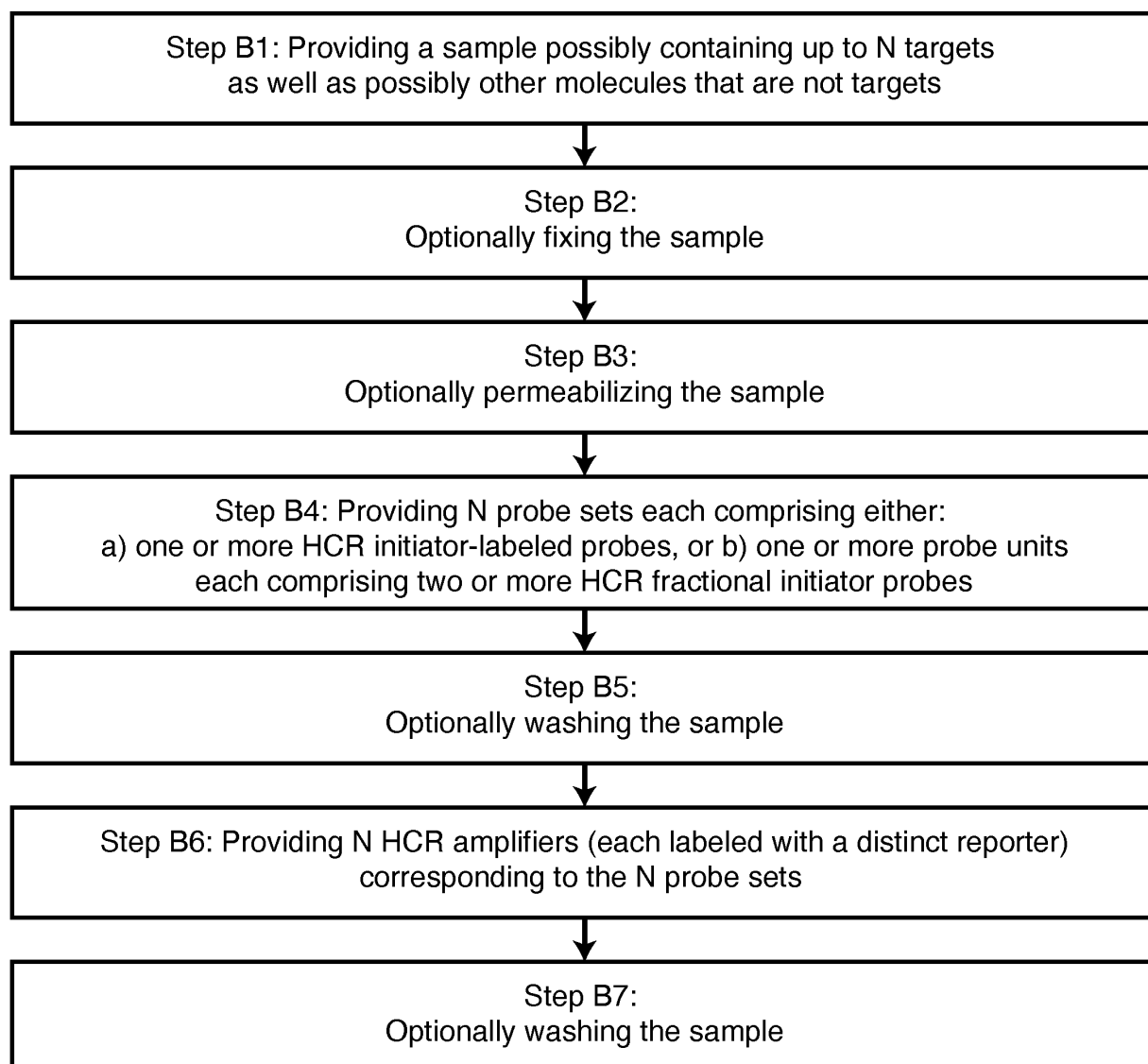


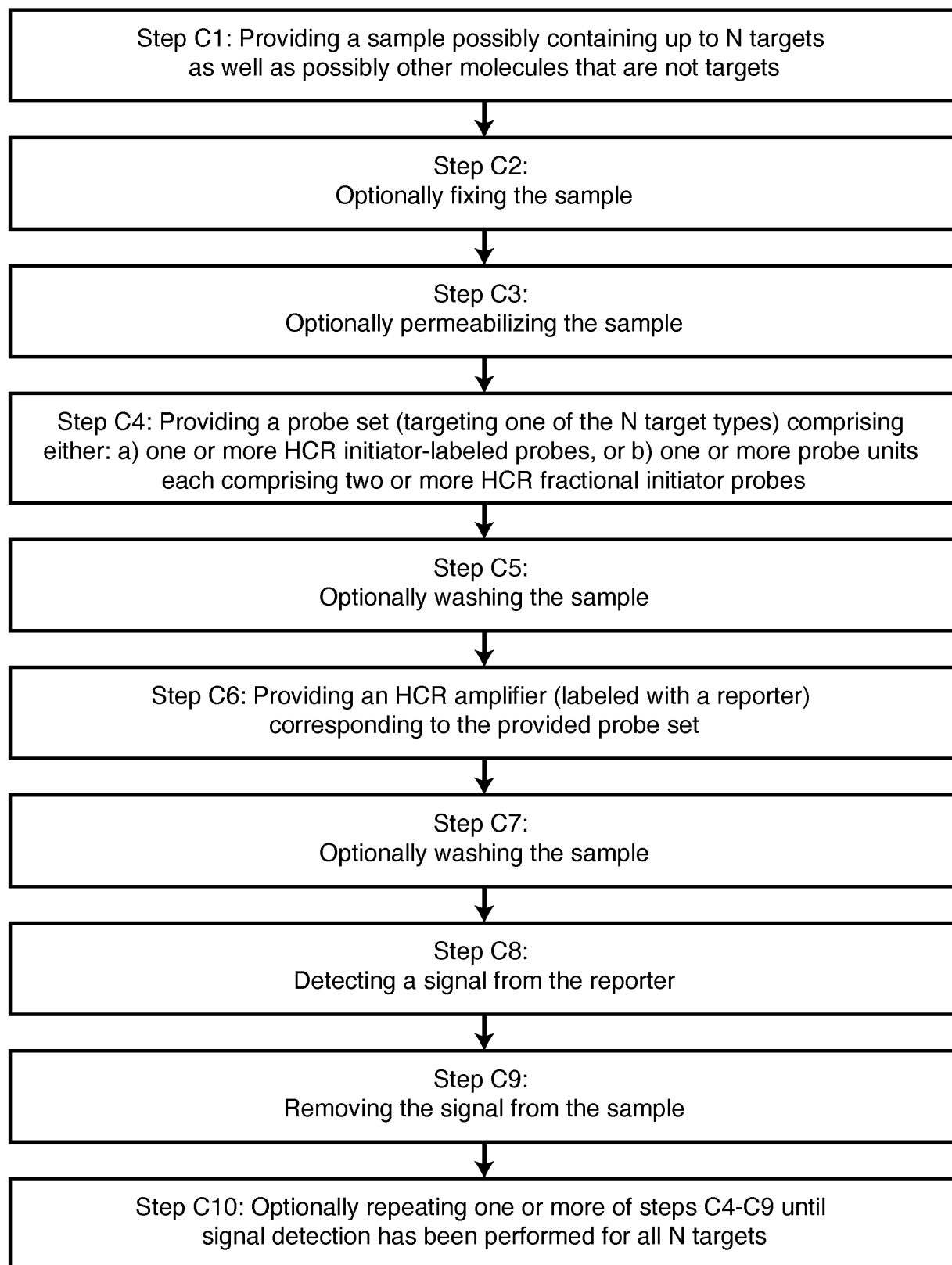
FIG. 25B

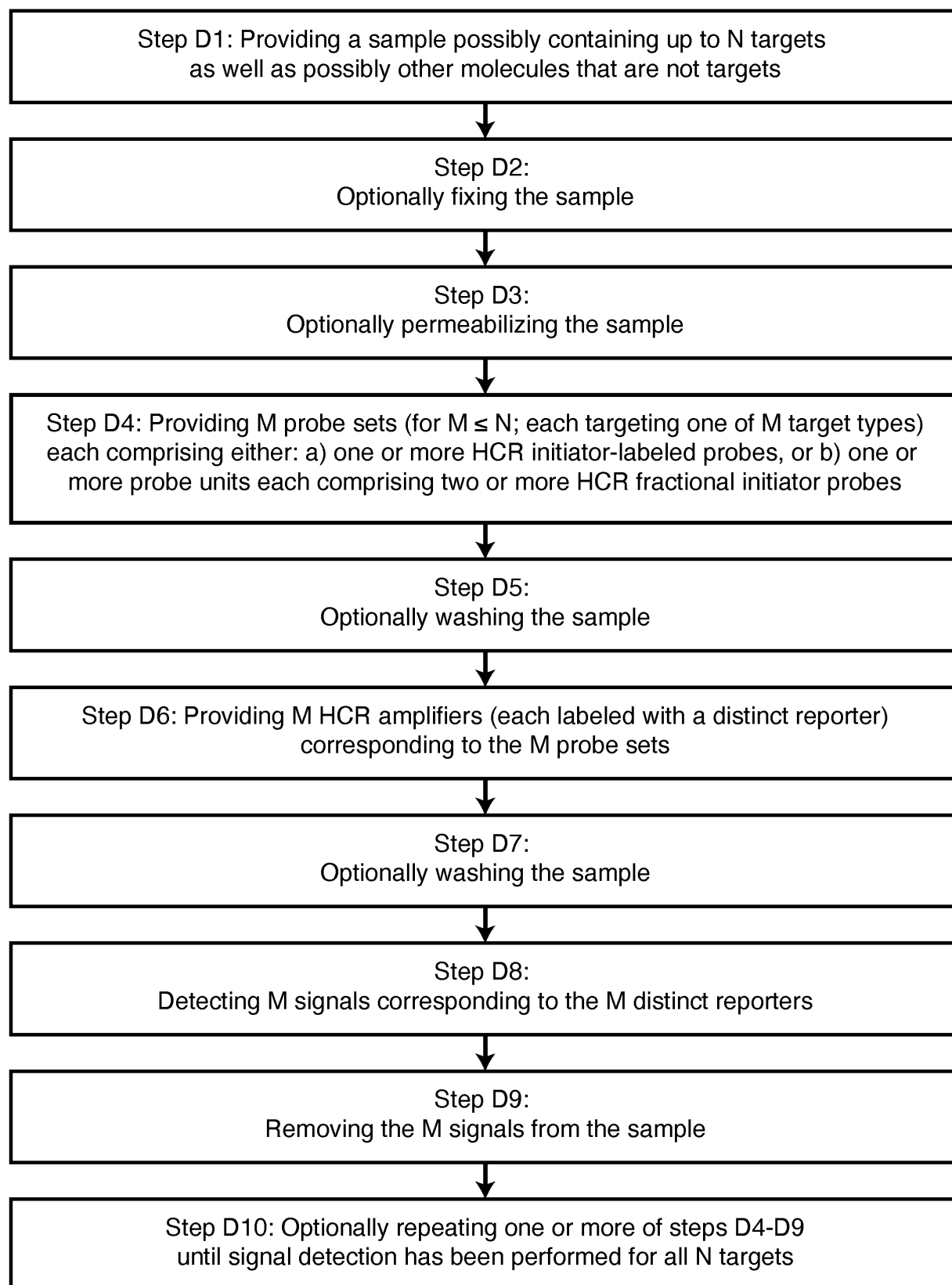
Method A

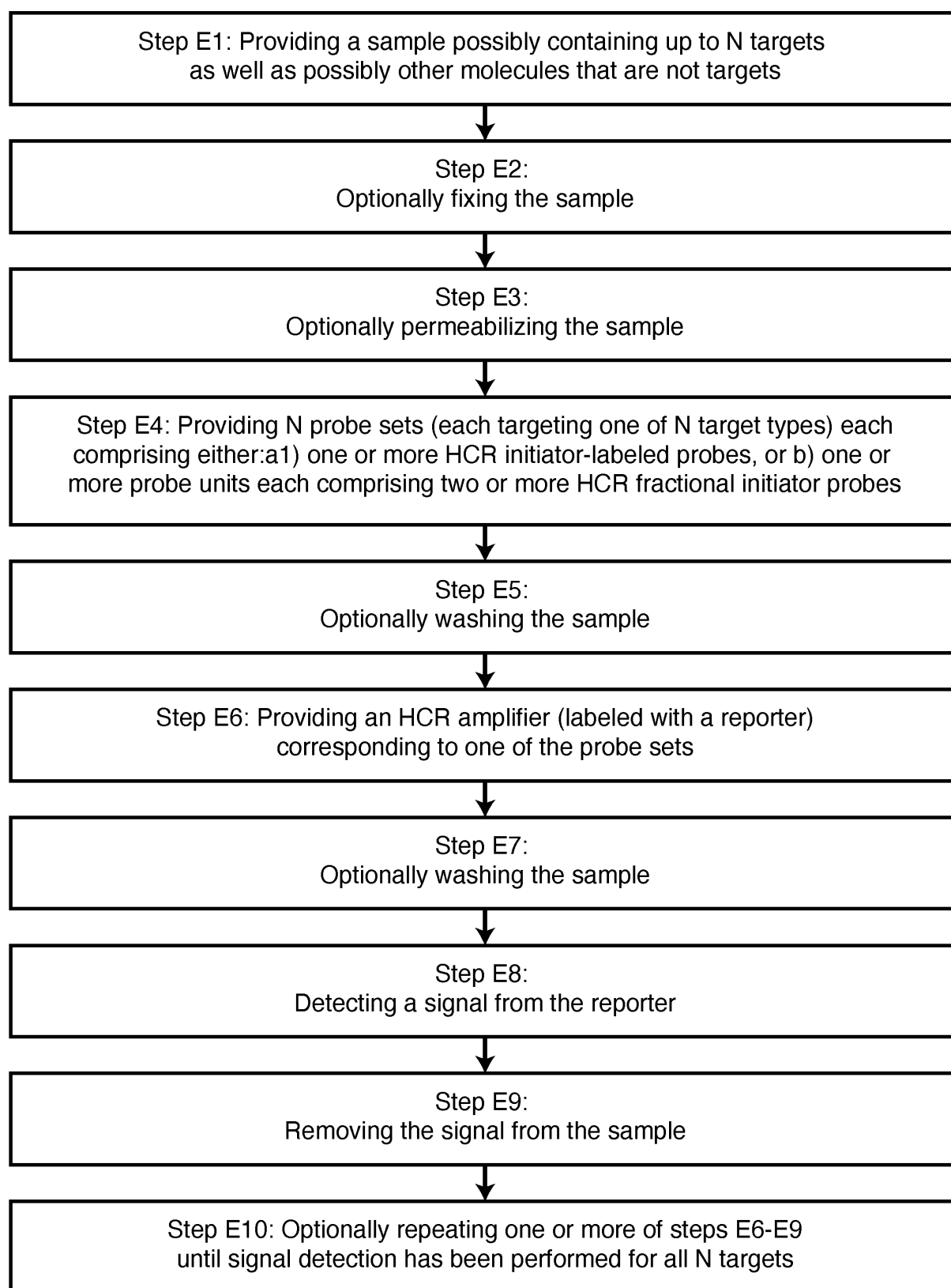
**FIG. 26A**

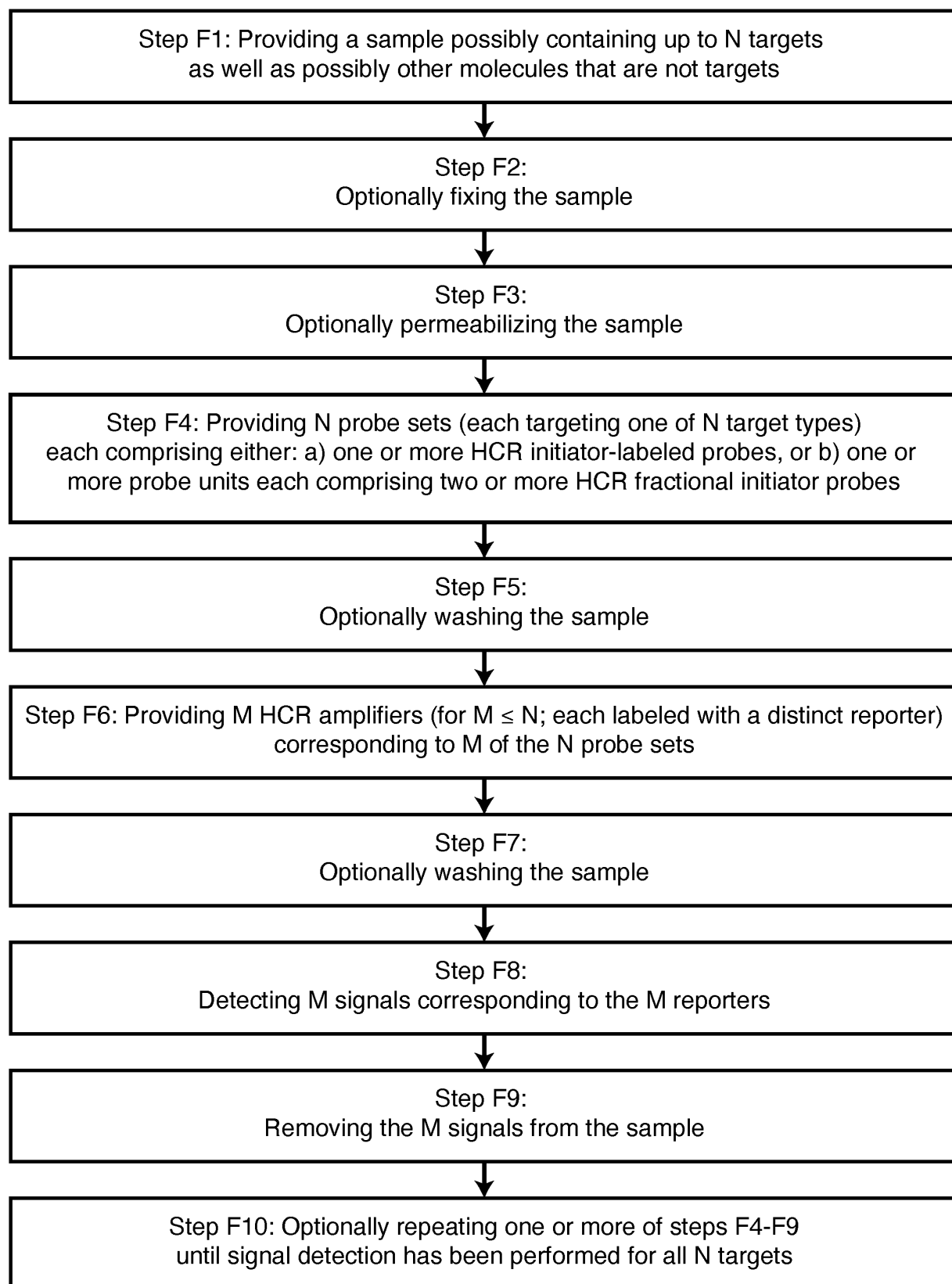
Method B

**FIG. 26B**

Method C**FIG. 26C**

**FIG. 26D**

**FIG. 26E**

**FIG. 26F**

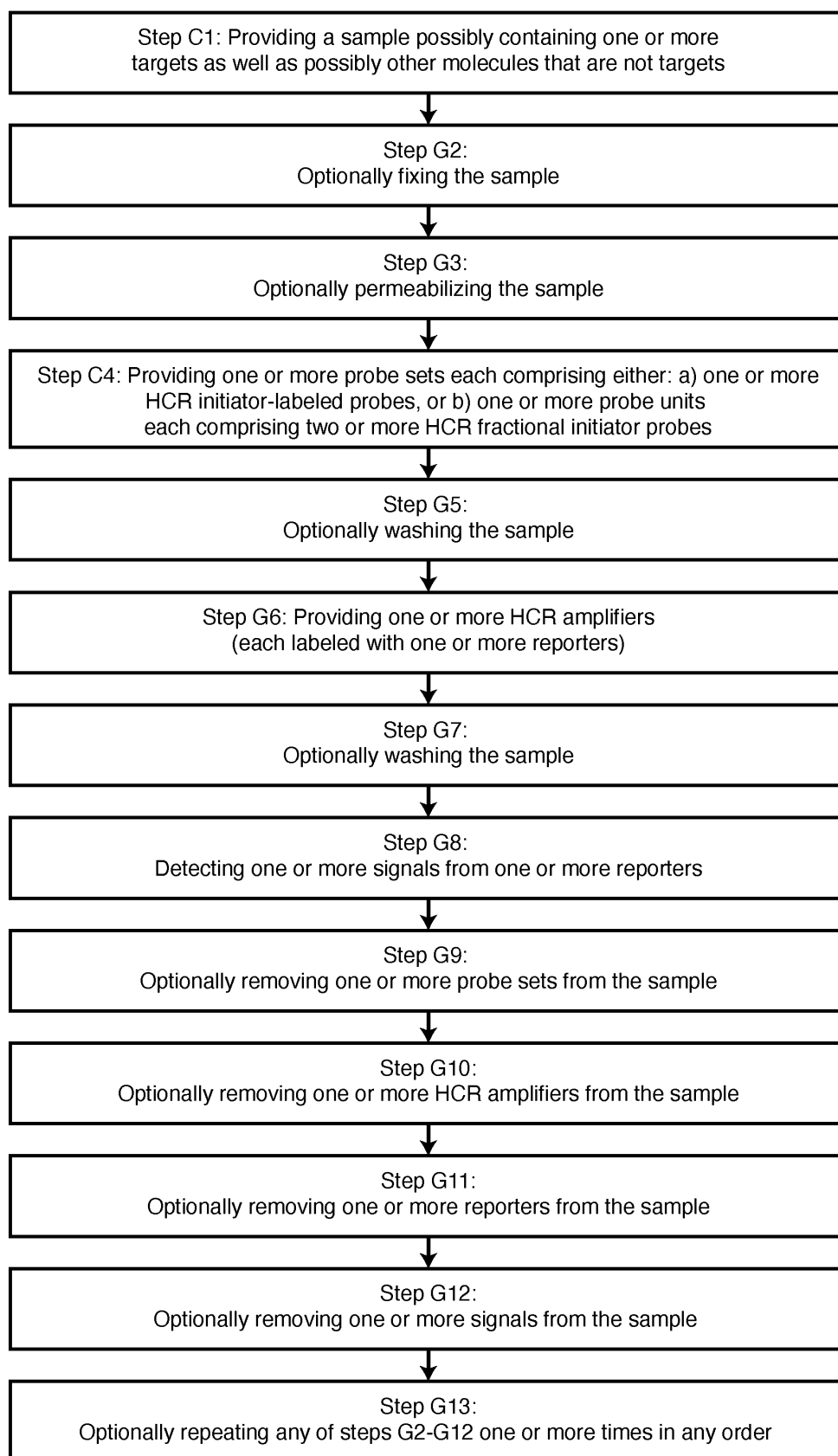
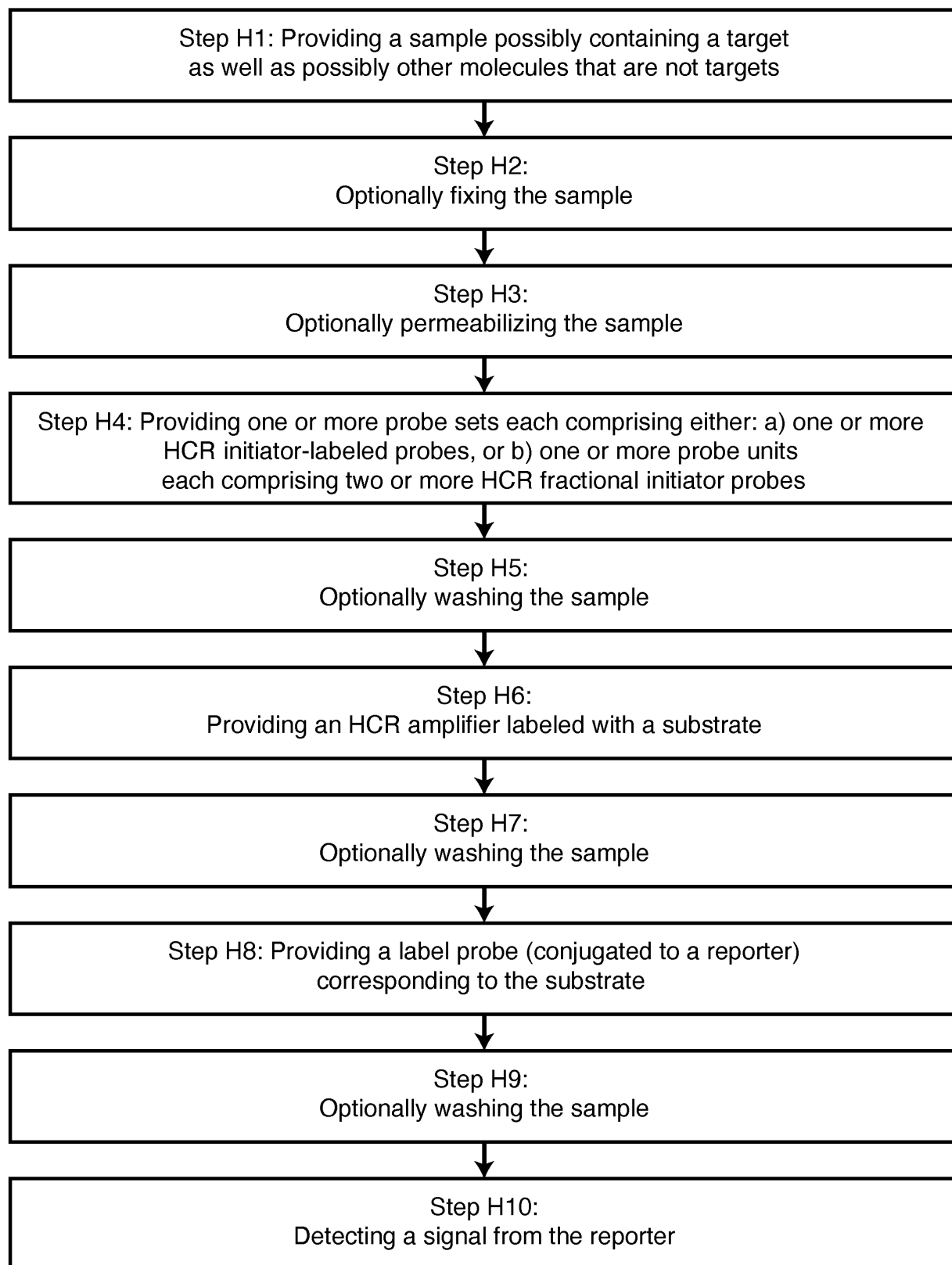
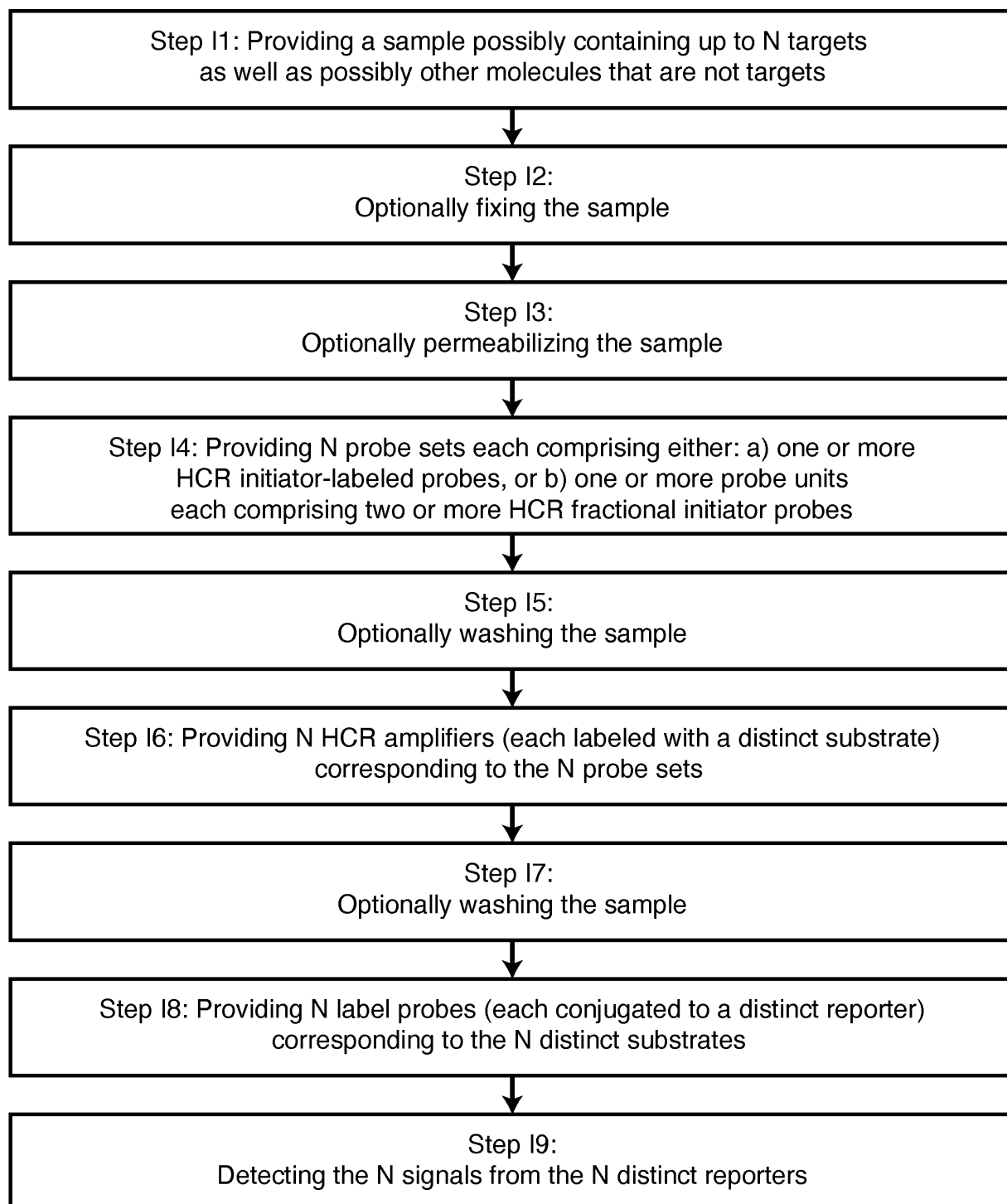
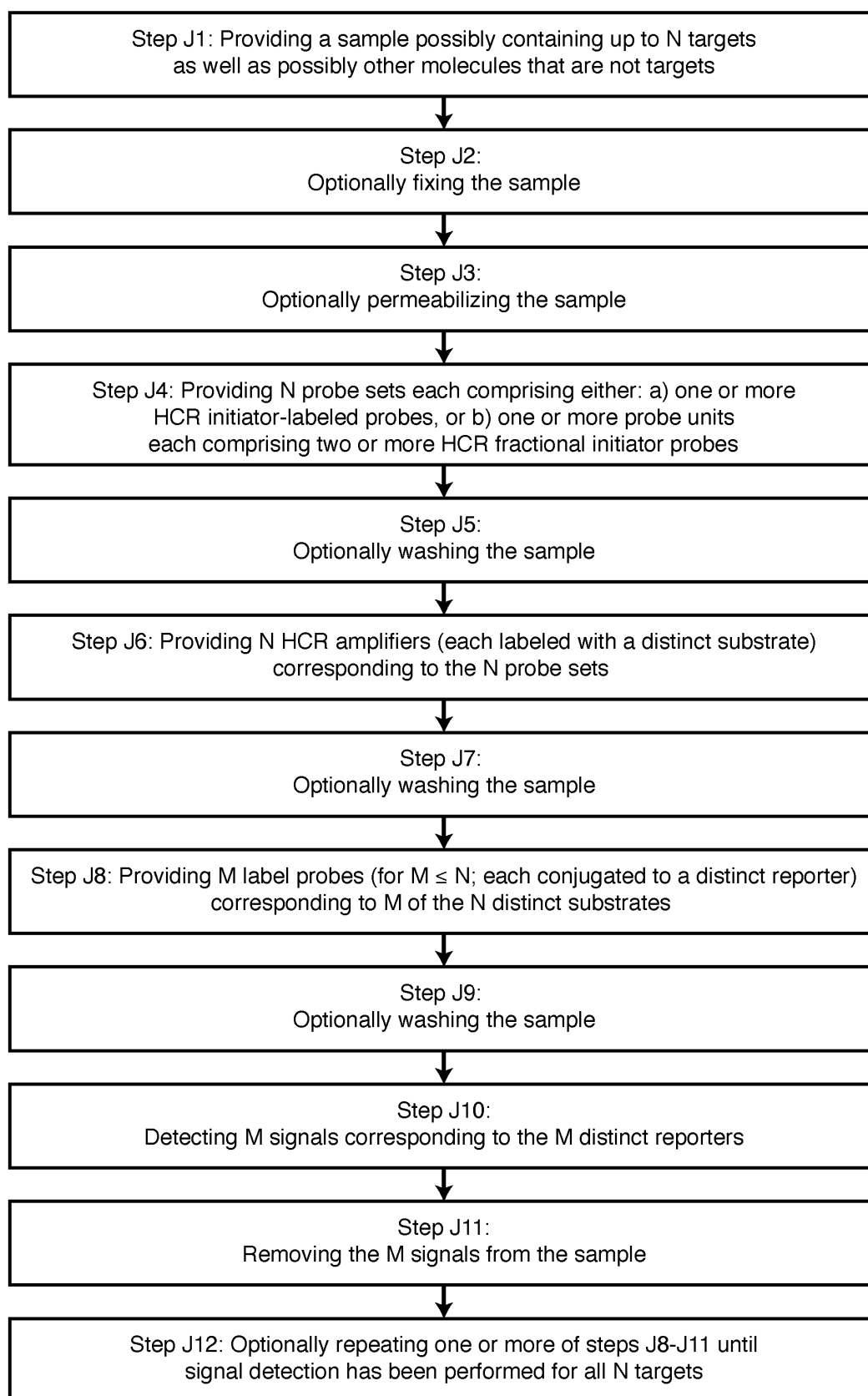


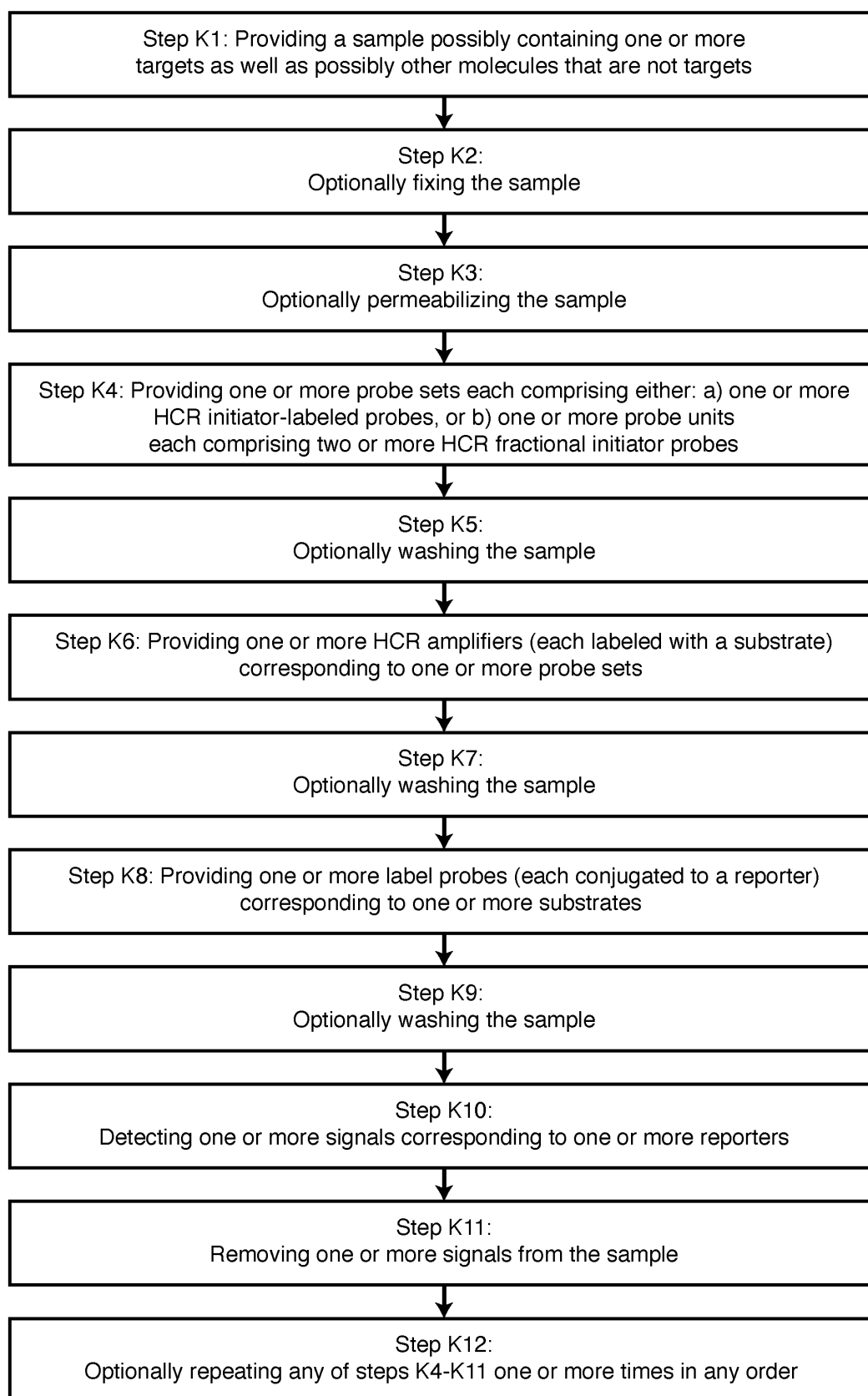
FIG. 26G

Method H**FIG. 26H**

Method I

**FIG. 26I**

**FIG. 26J**

**FIG. 26K**

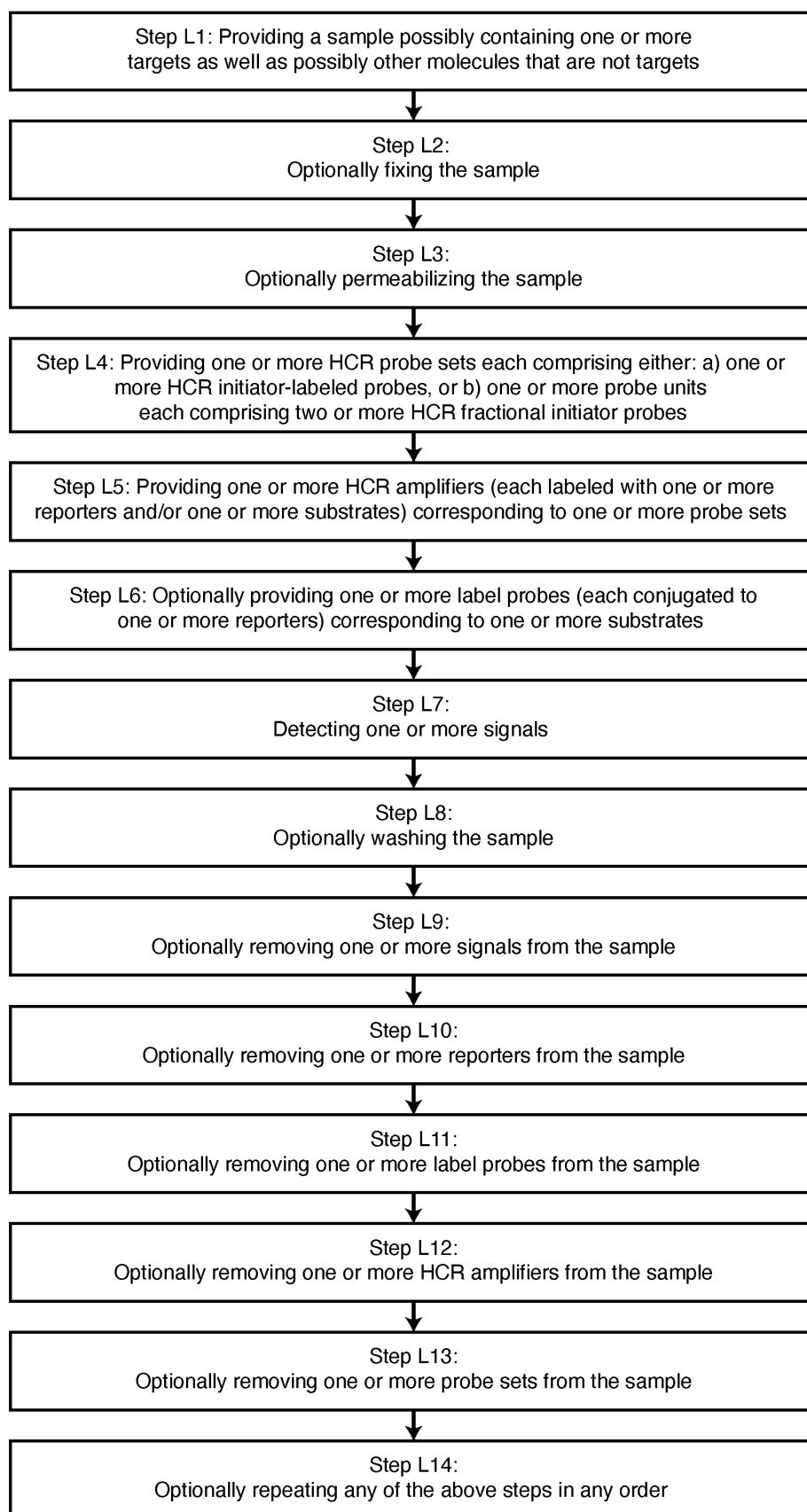
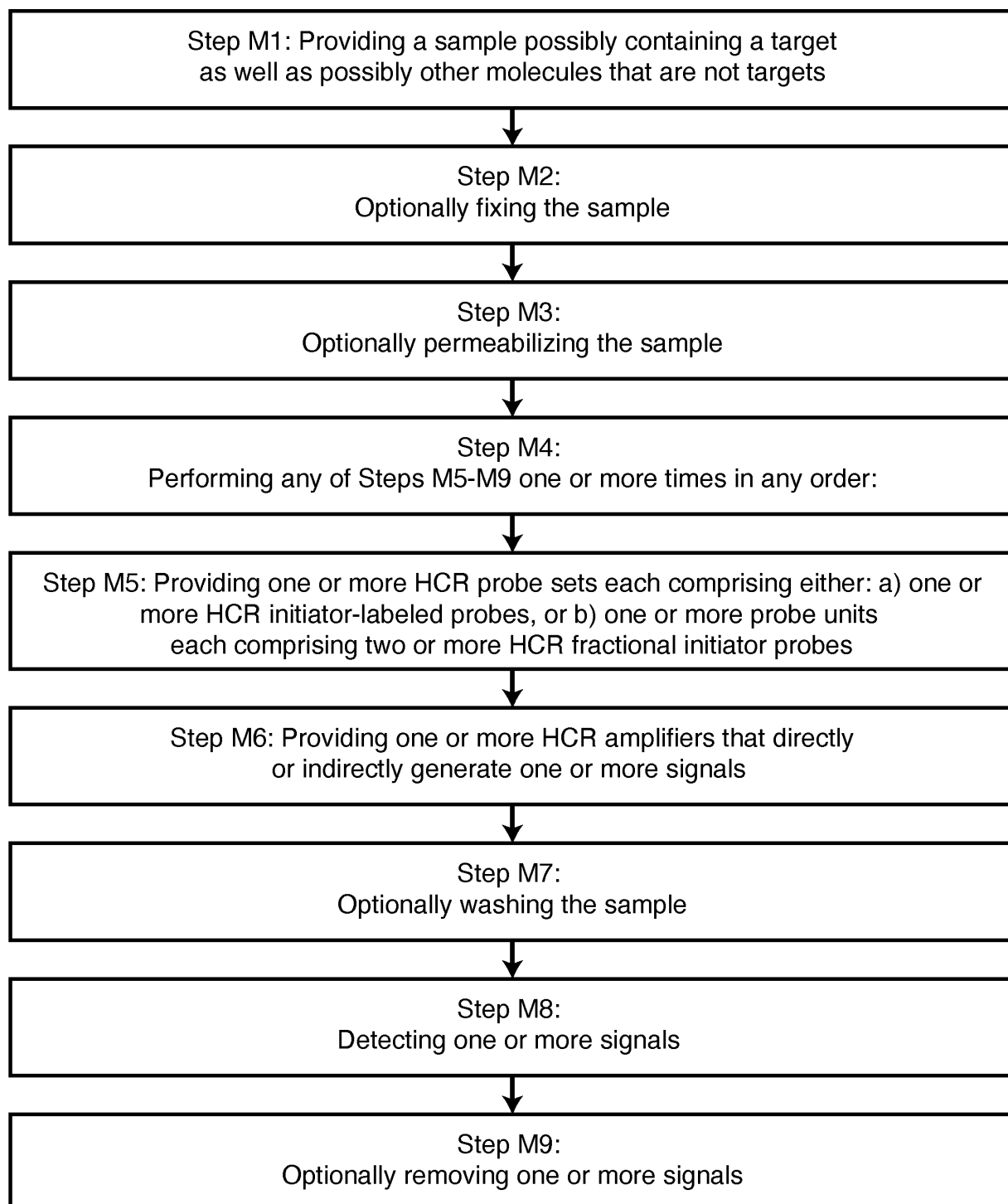
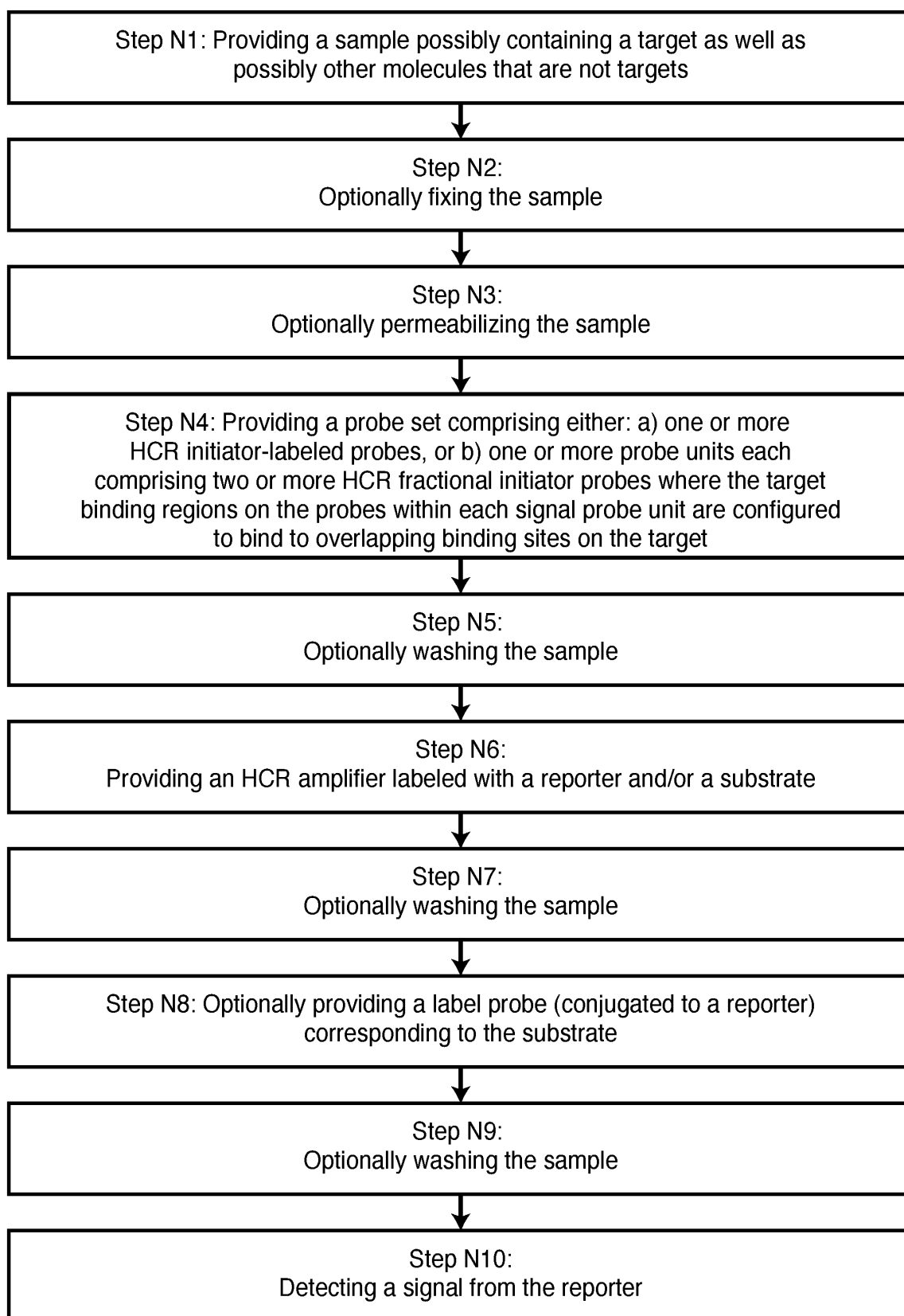
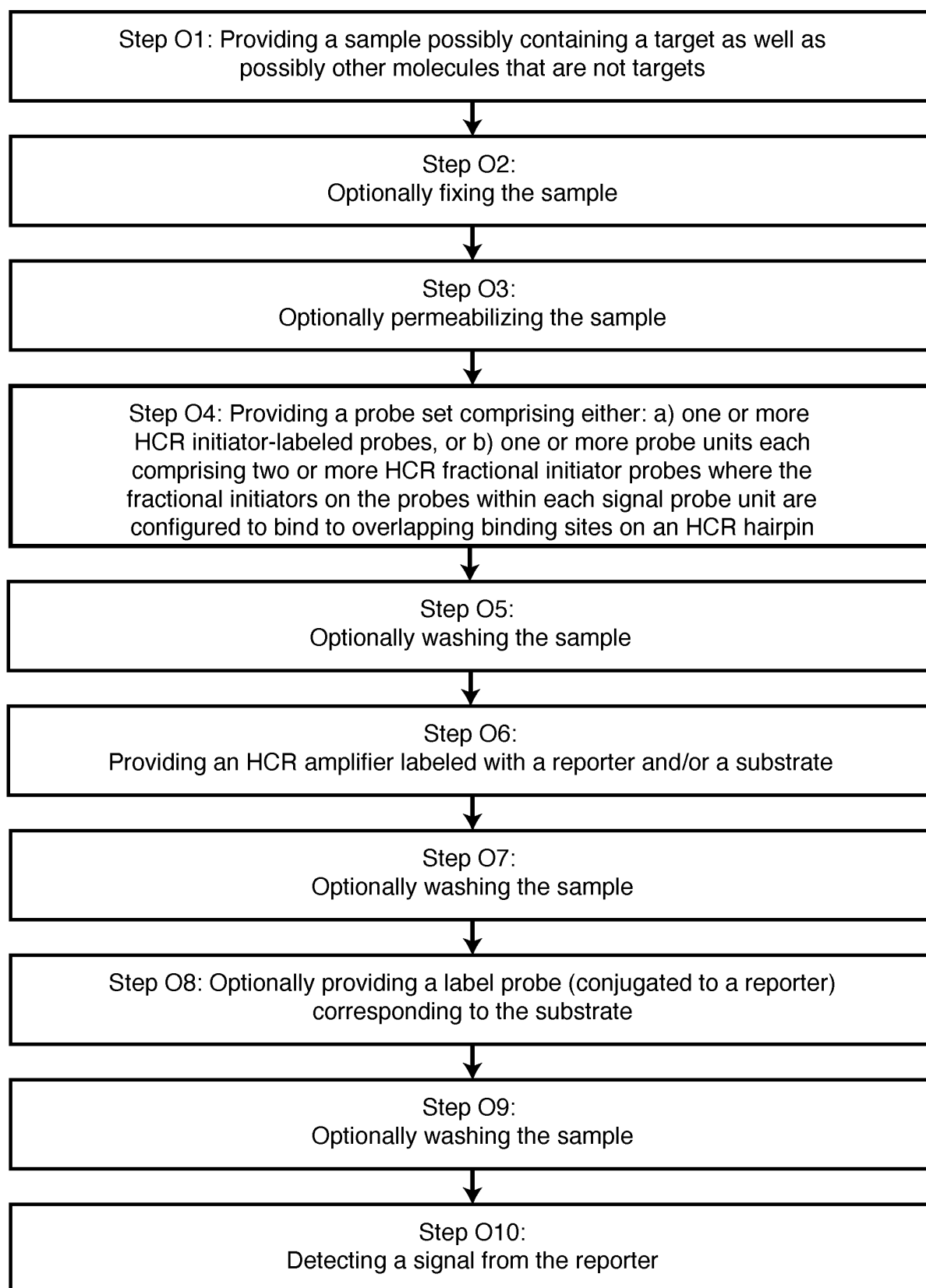


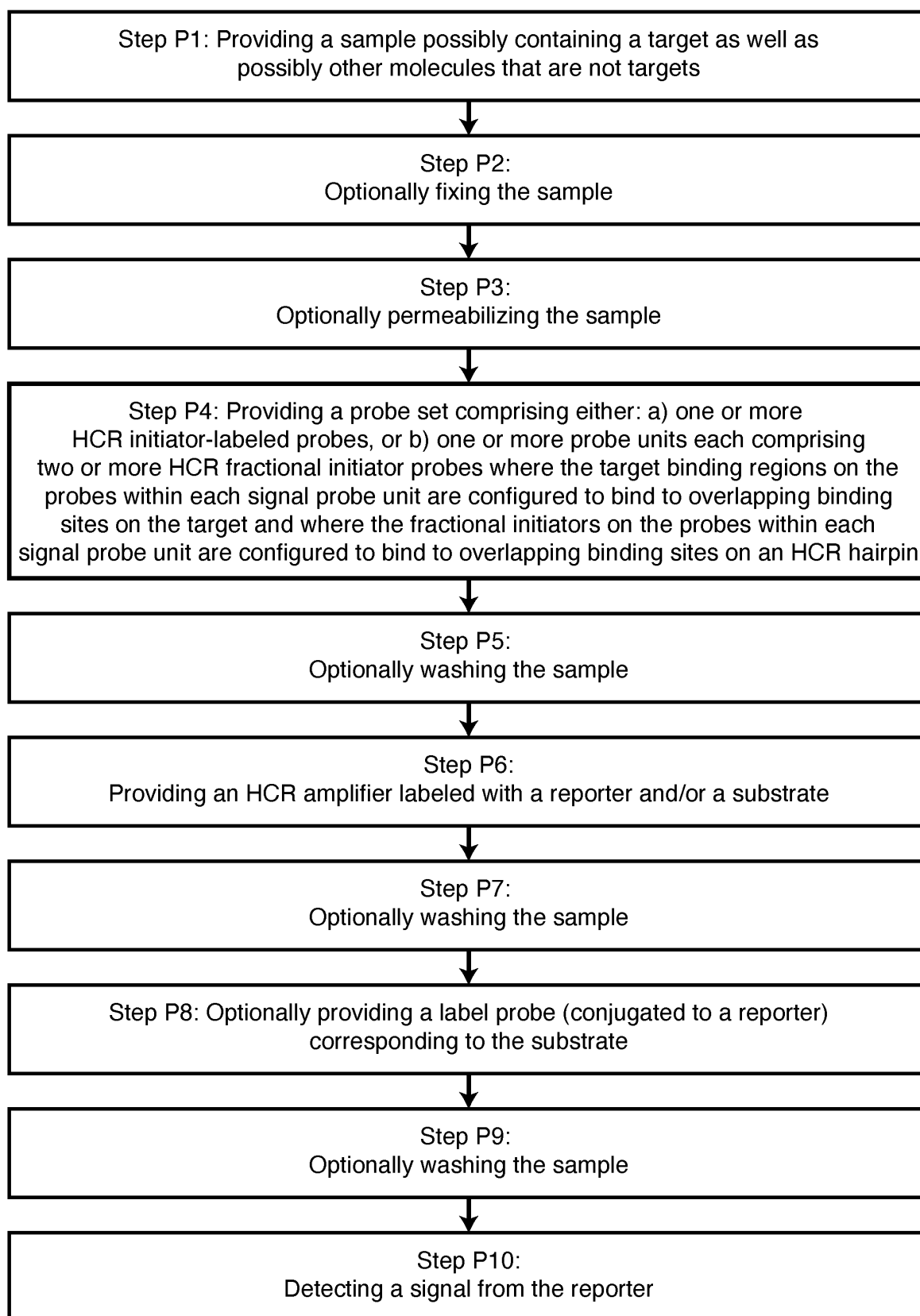
FIG. 26L

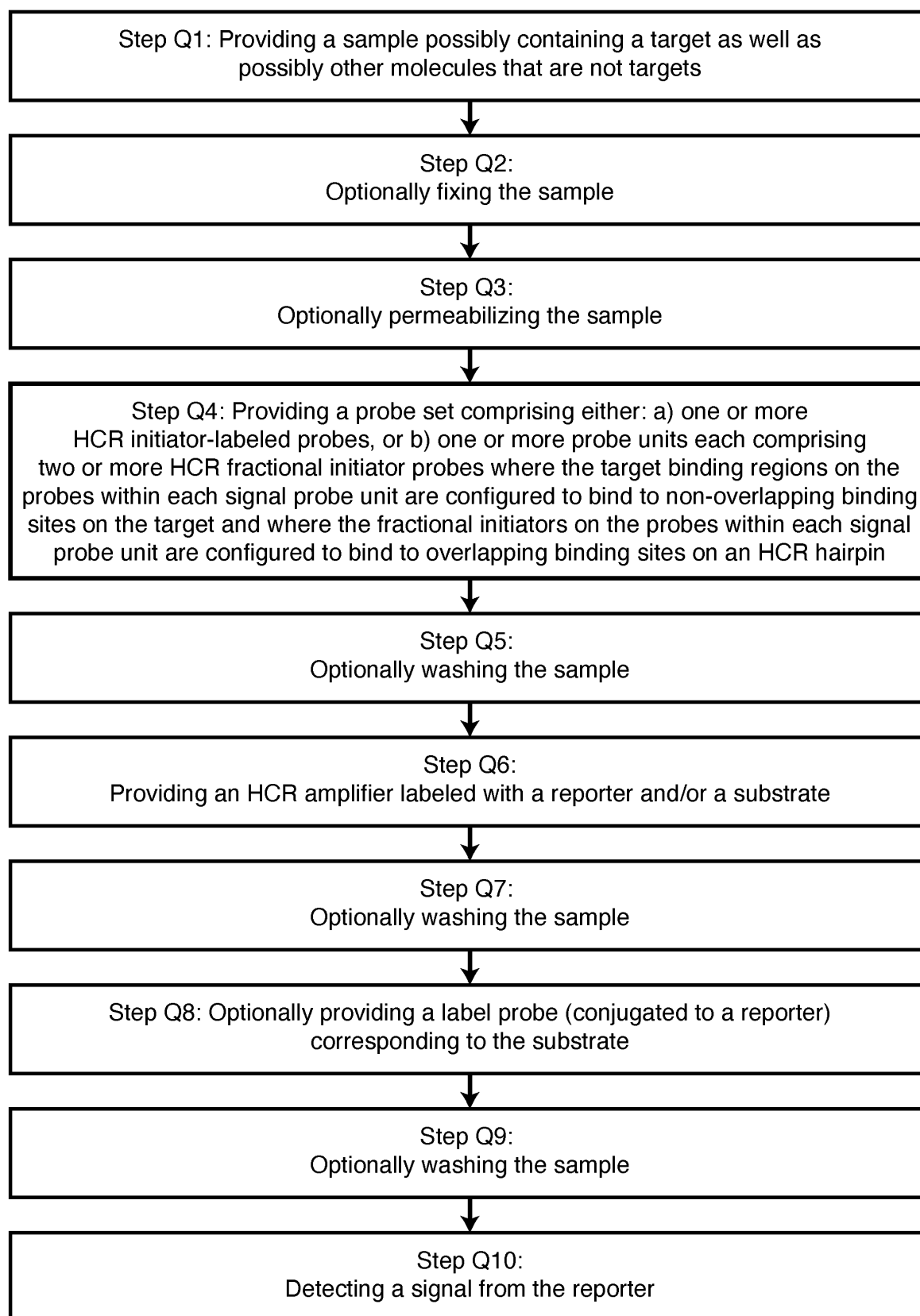
Method M

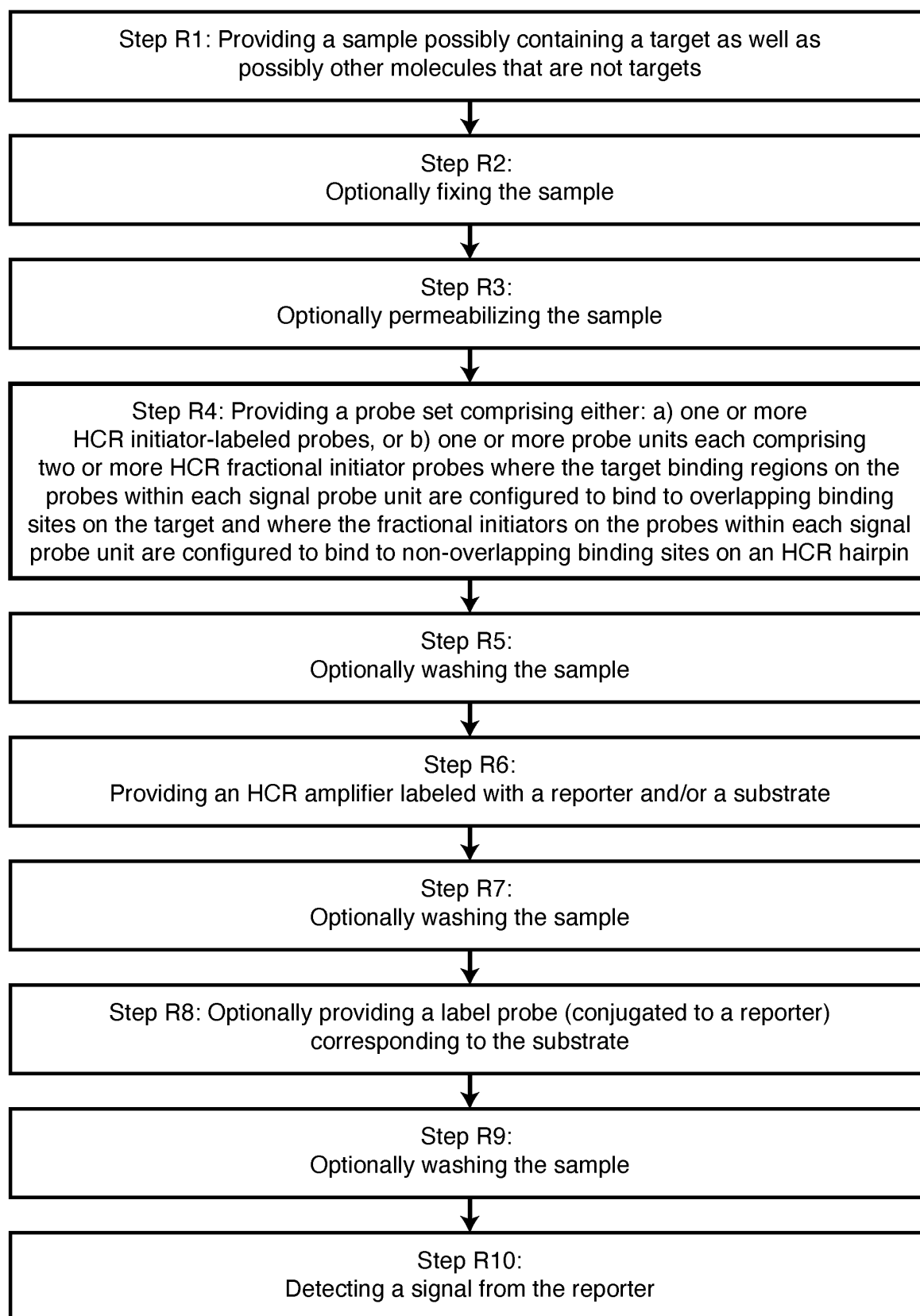
**FIG. 26M**

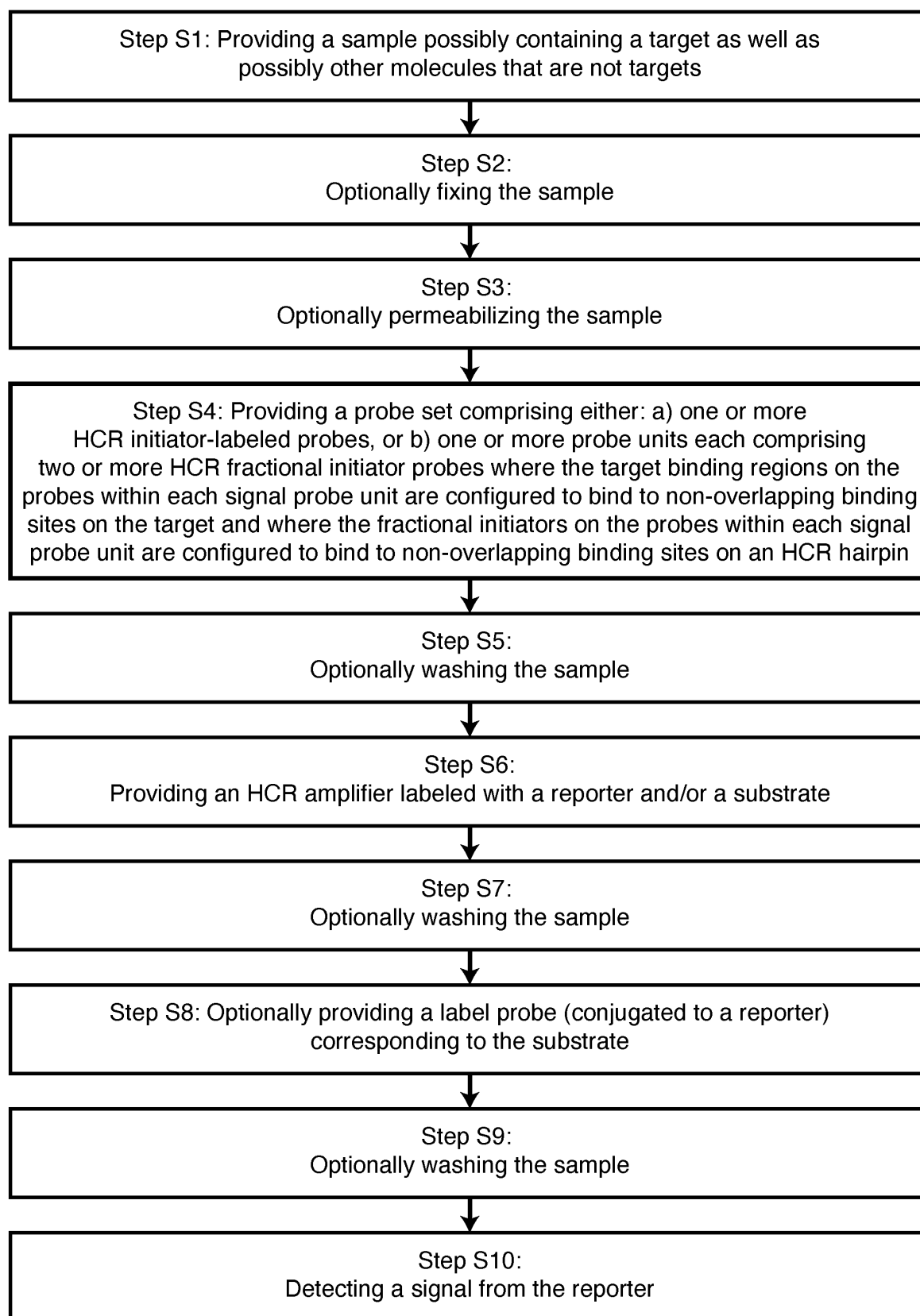
**FIG. 26N**

**FIG. 26O**

**FIG. 26P**

**FIG. 26Q**

**FIG. 26R**

**FIG. 26S**

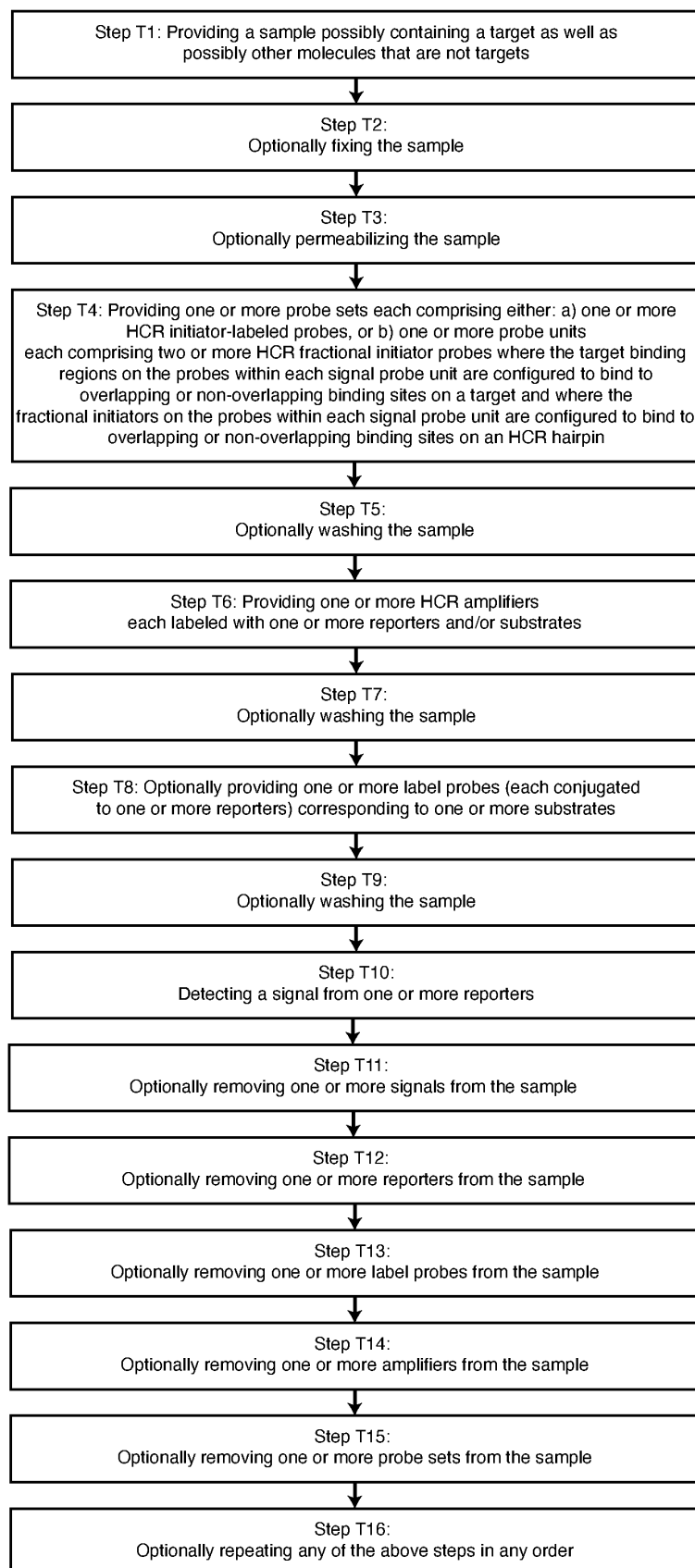
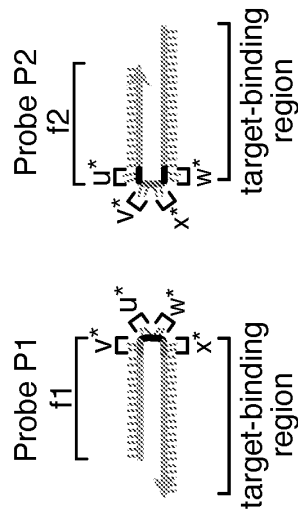


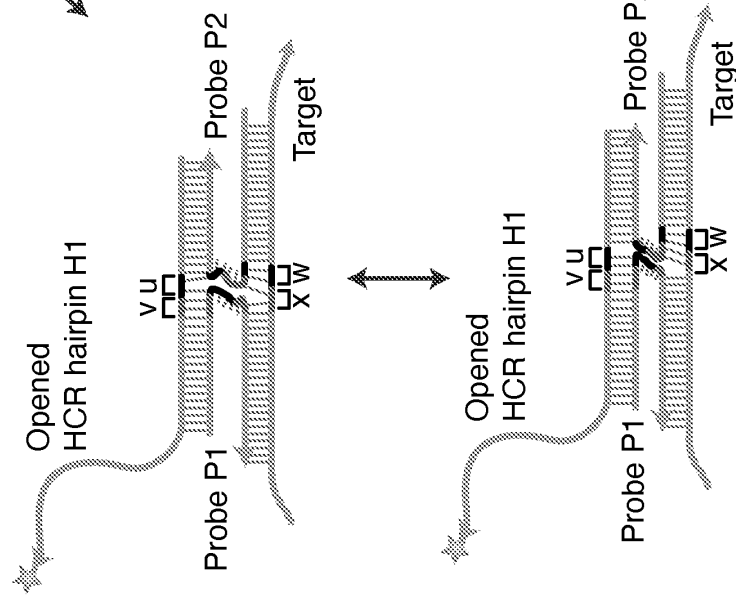
FIG. 26T

Fractional initiator probes P1 and P2

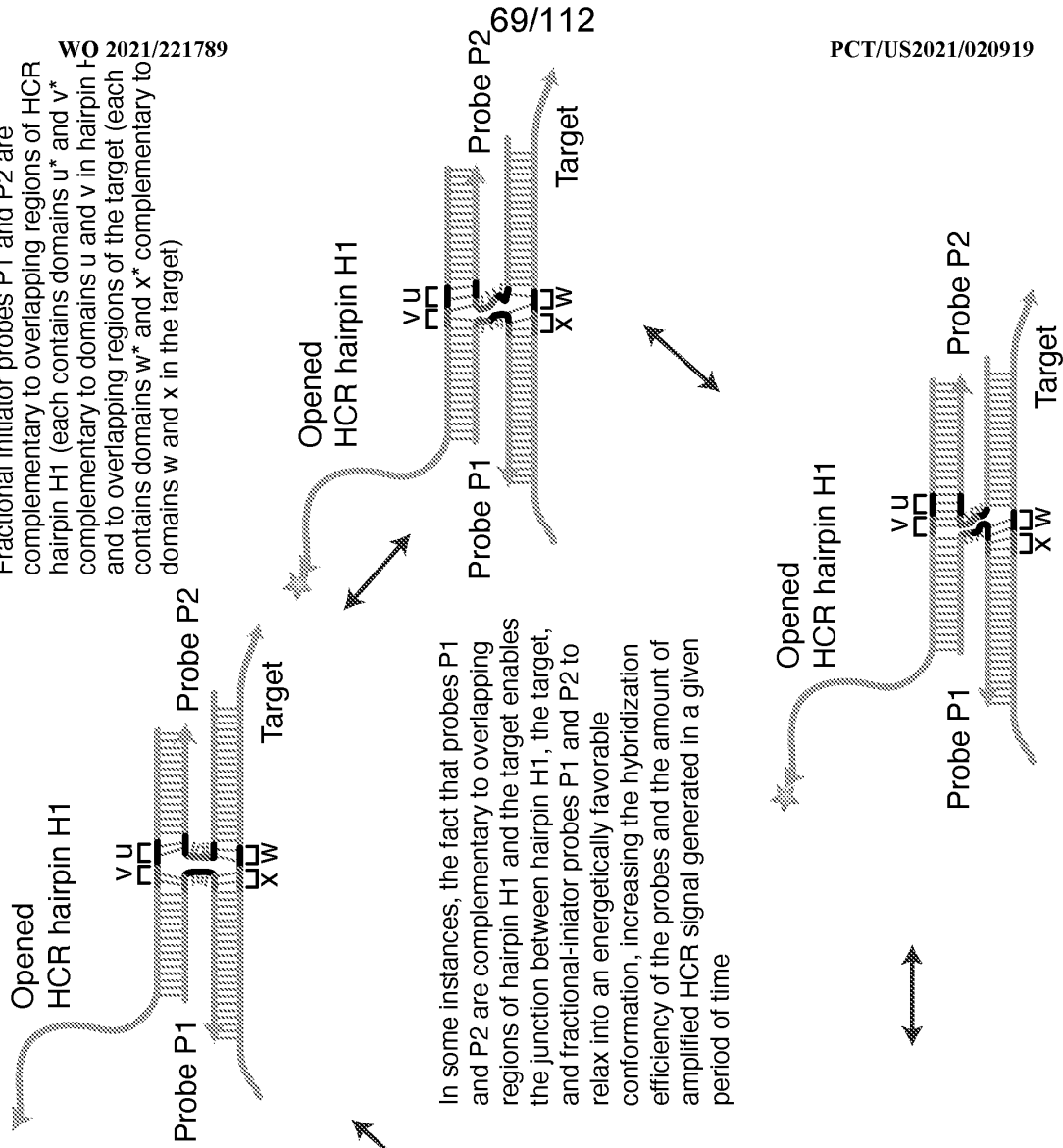


Fractional initiator probes P1 and P2 are complementary to overlapping regions of HCR hairpin H1 (each contains domains u* and v* complementary to domains u and v in hairpin H1 and to overlapping regions of the target (each contains domains w* and x* complementary to domains w and x in the target))

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In some instances, the fact that probes P1 and P2 are complementary to overlapping regions of hairpin H1 and the target enables the junction between hairpin H1, the target, and fractional-initiator probes P1 and P2 to relax into an energetically favorable conformation, increasing the hybridization efficiency of the probes and the amount of amplified HCR signal generated in a given period of time



PCT/US2021/020919

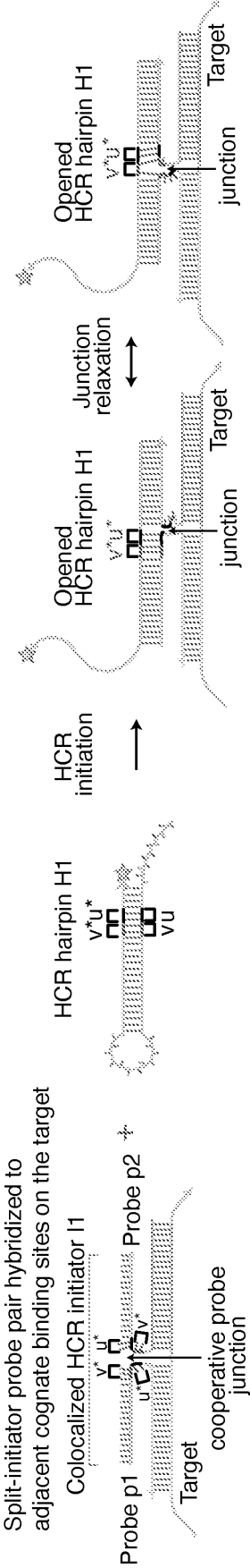


FIG. 28A

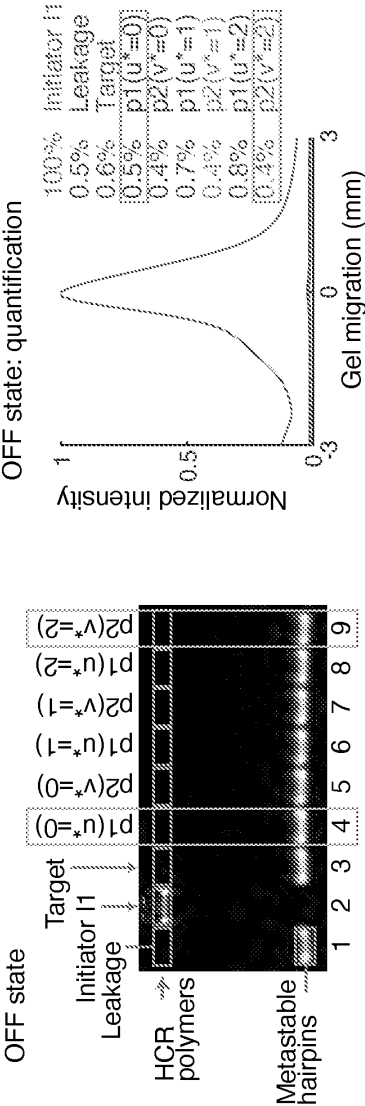


FIG. 28B

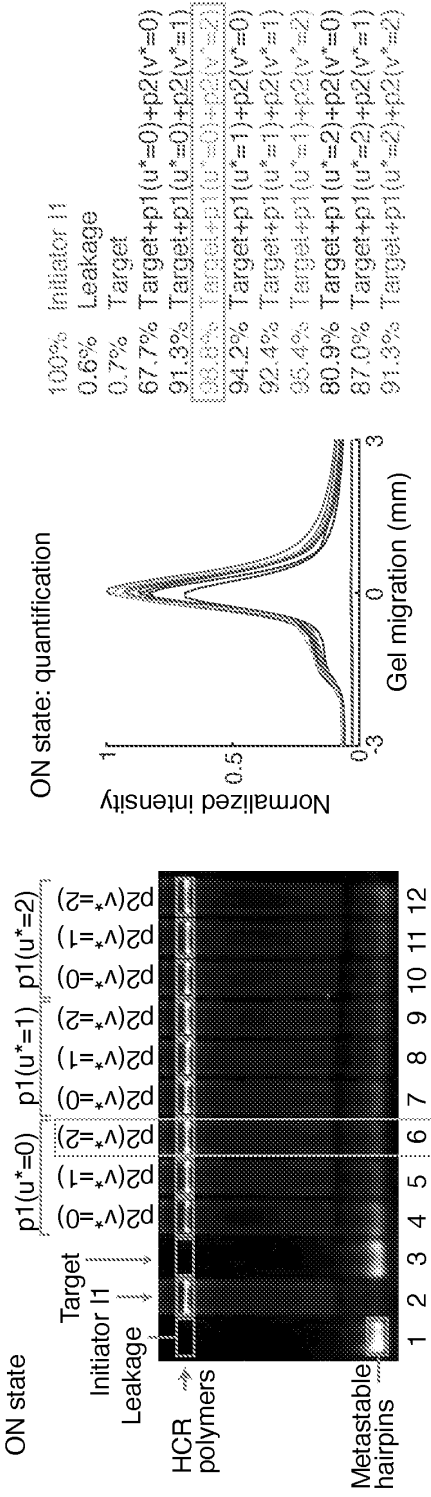


FIG. 28C

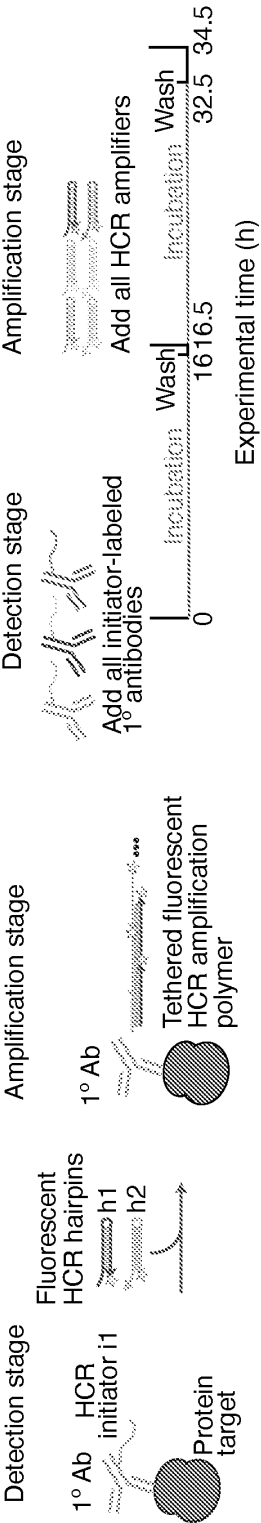


FIG. 29A

FIG. 29B

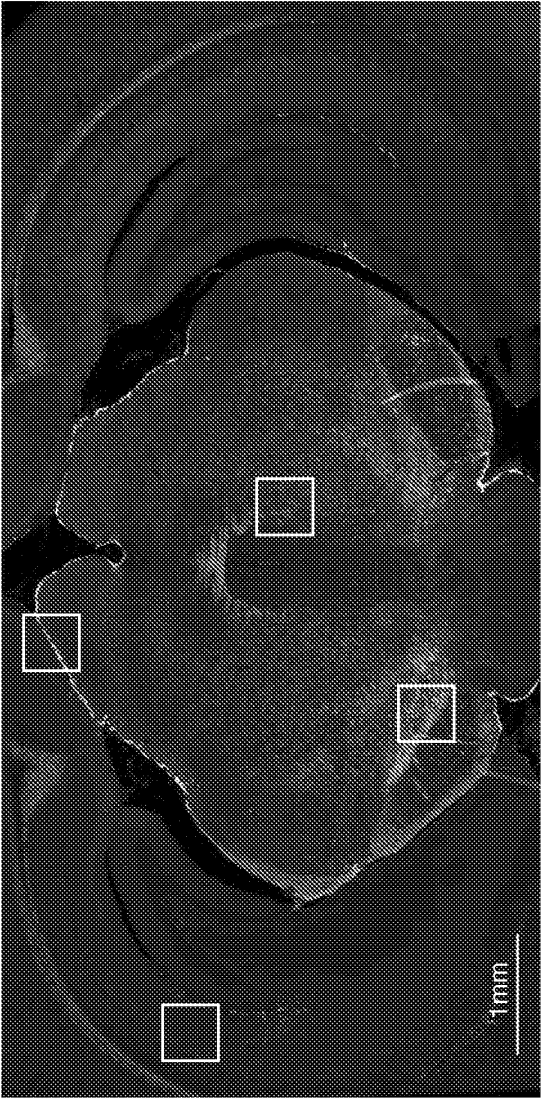


FIG. 29C

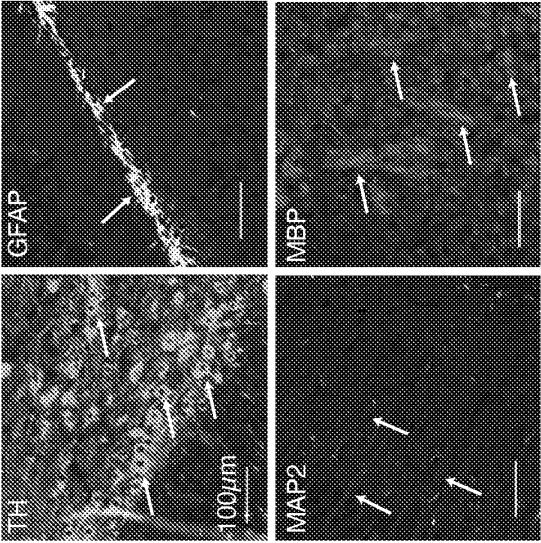


FIG. 29D

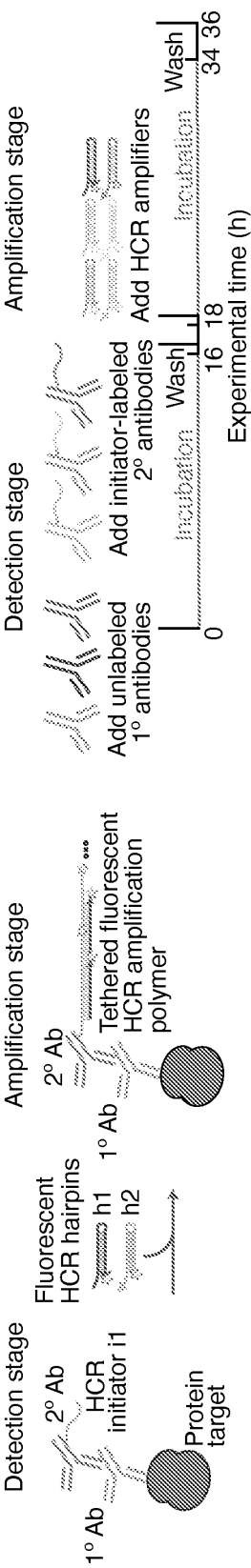


FIG. 30A

FIG. 30B

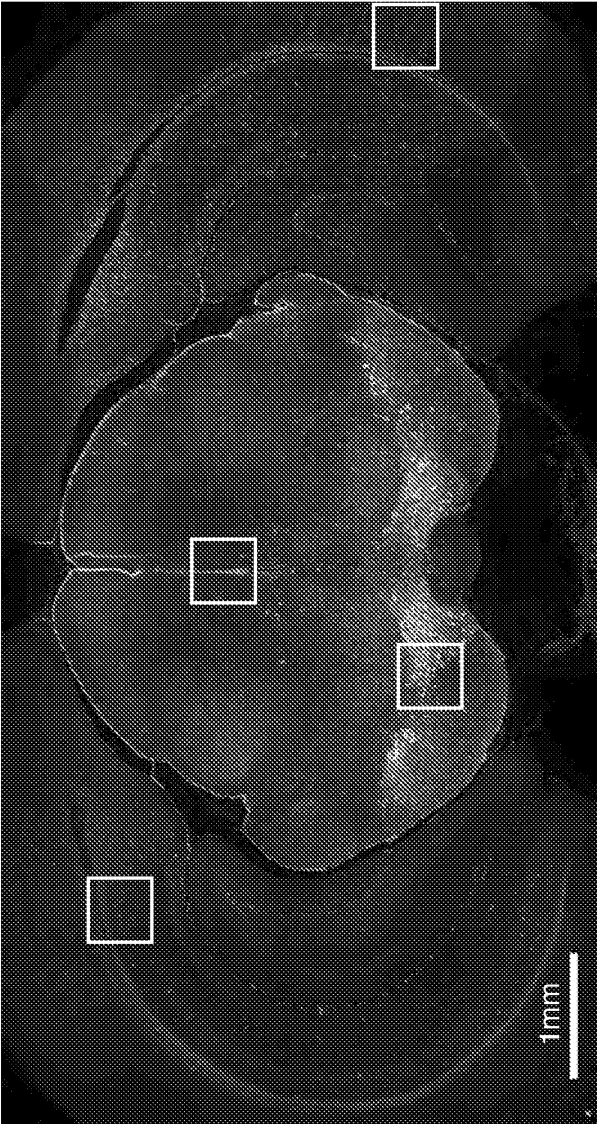


FIG. 30C

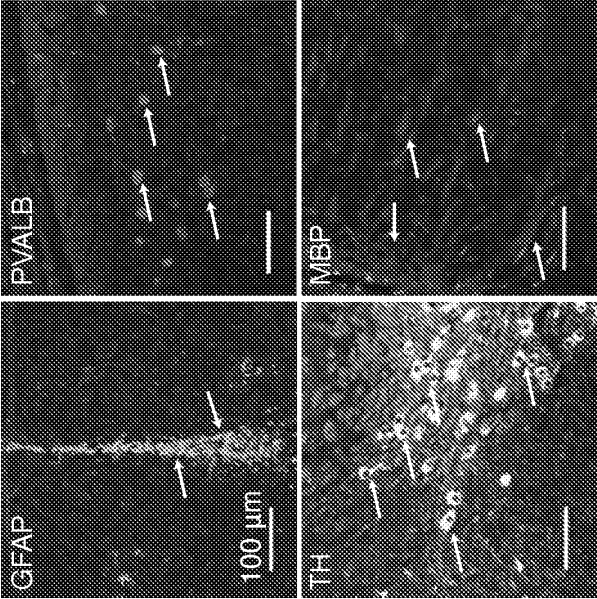


FIG. 30D

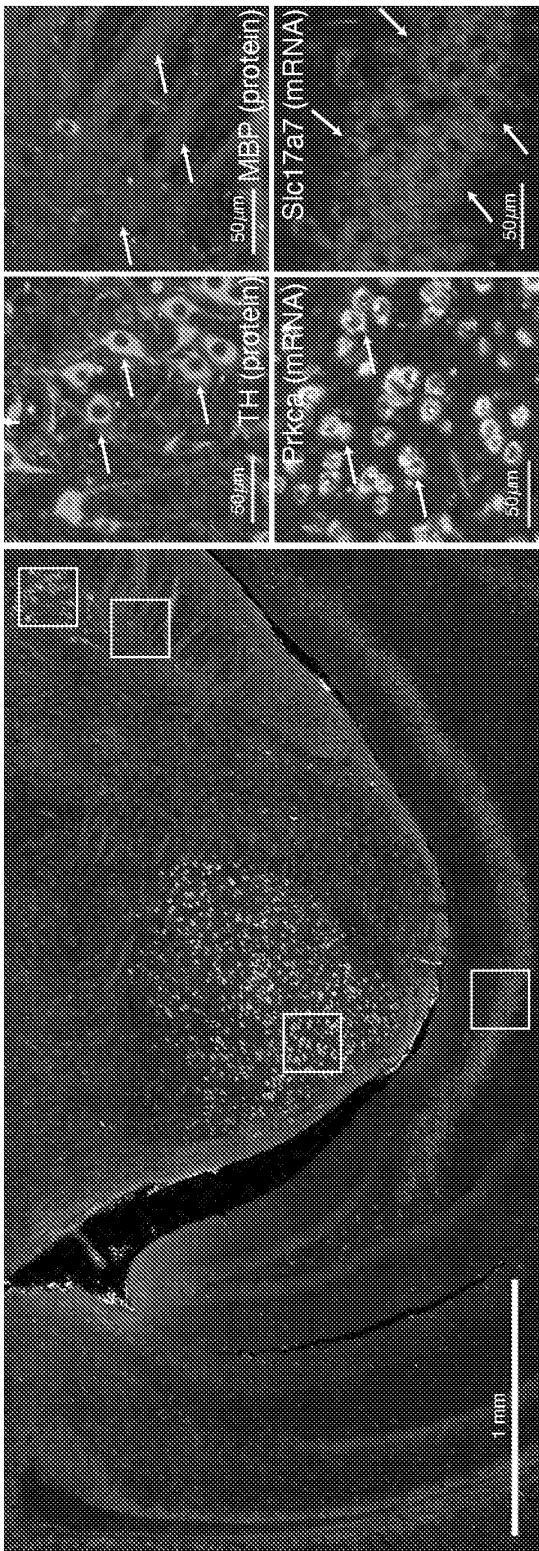
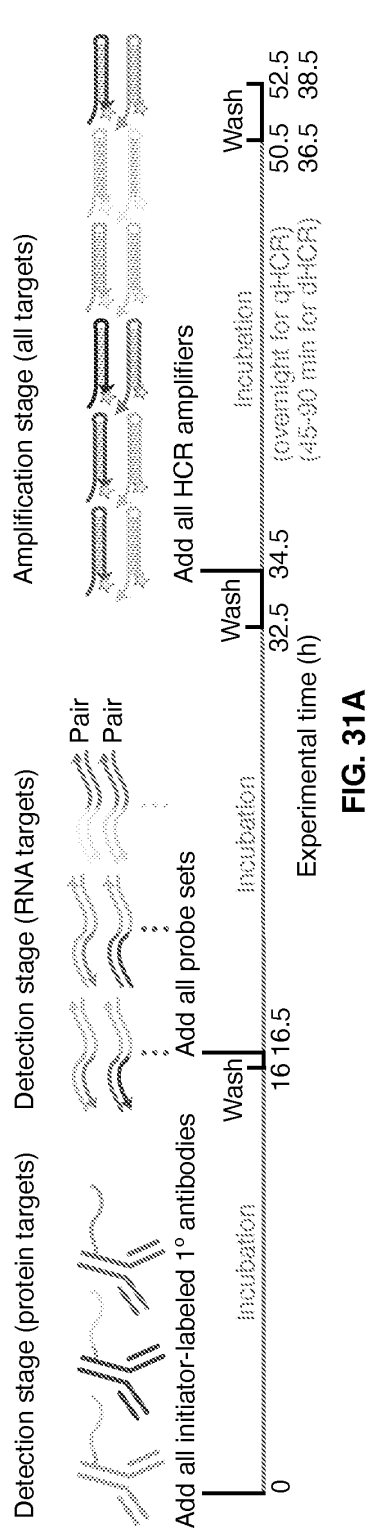
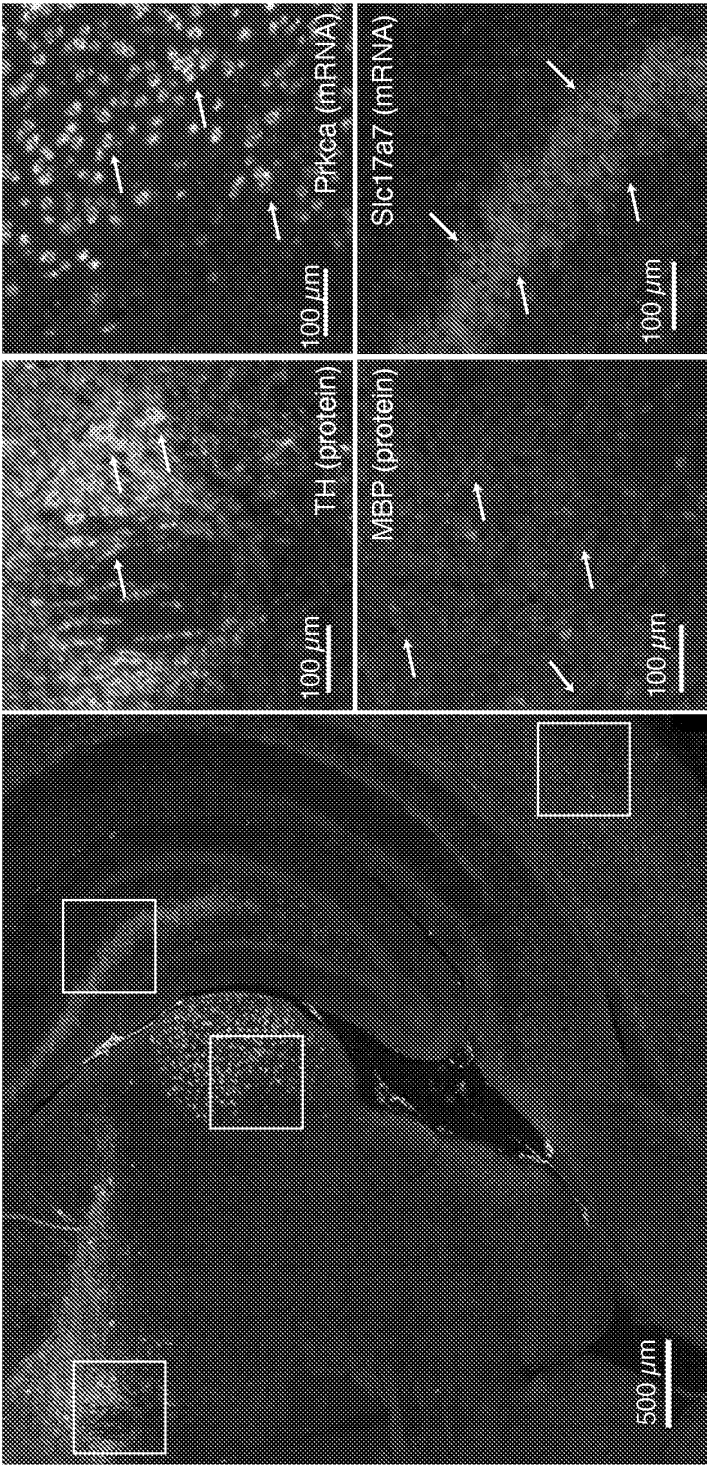
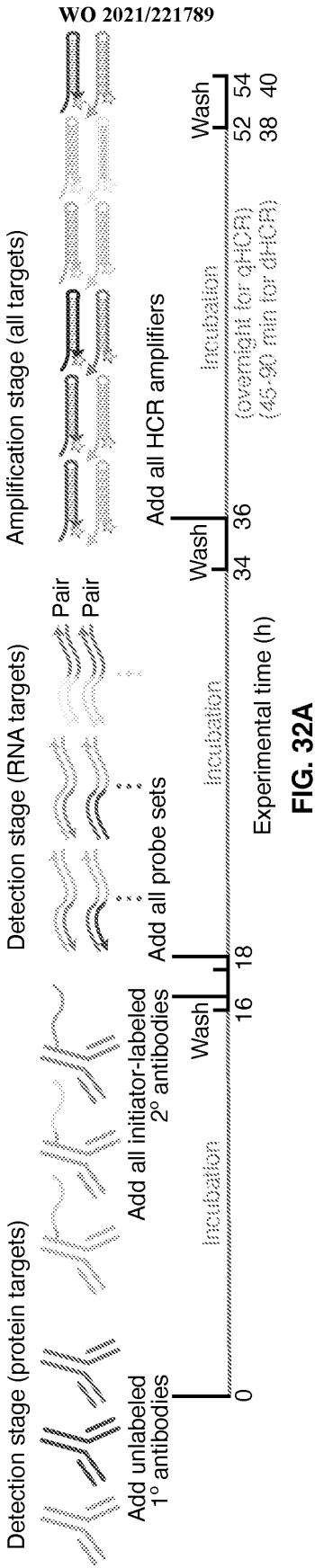


FIG. 31C

FIG. 31B



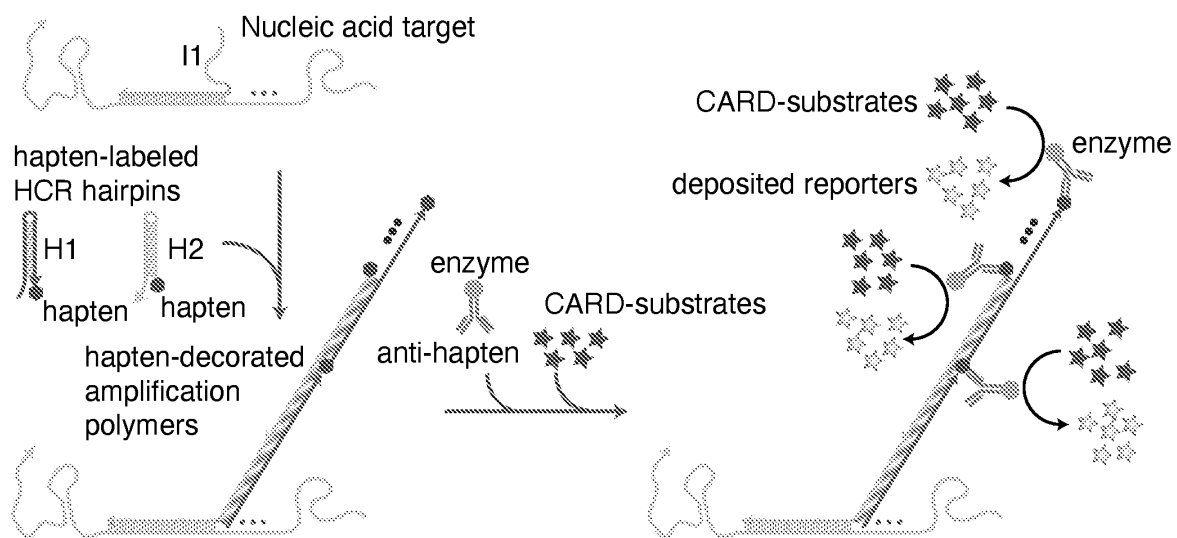


FIG.33A

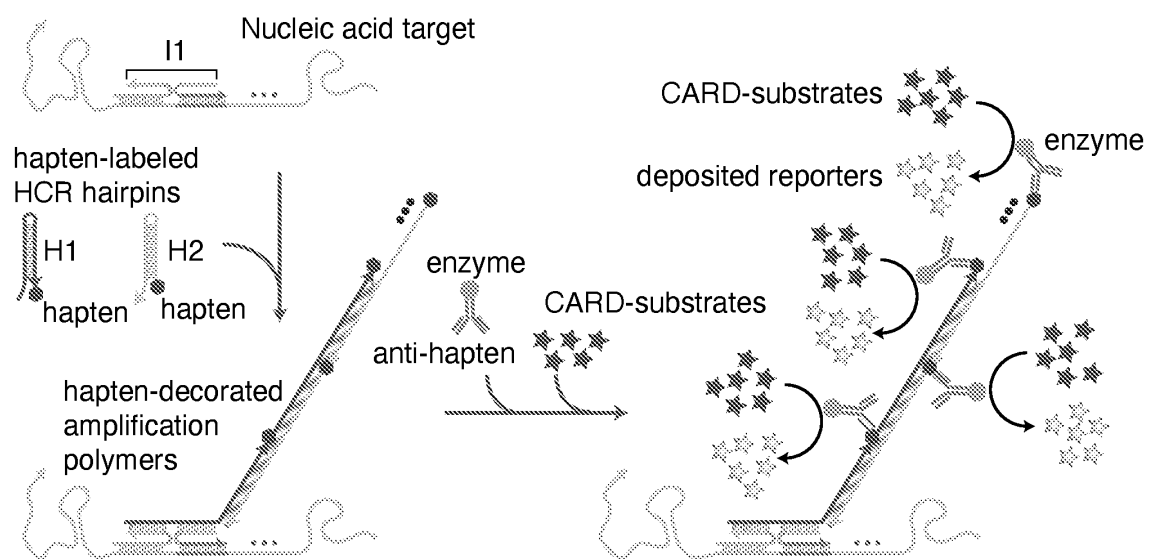


FIG.33B

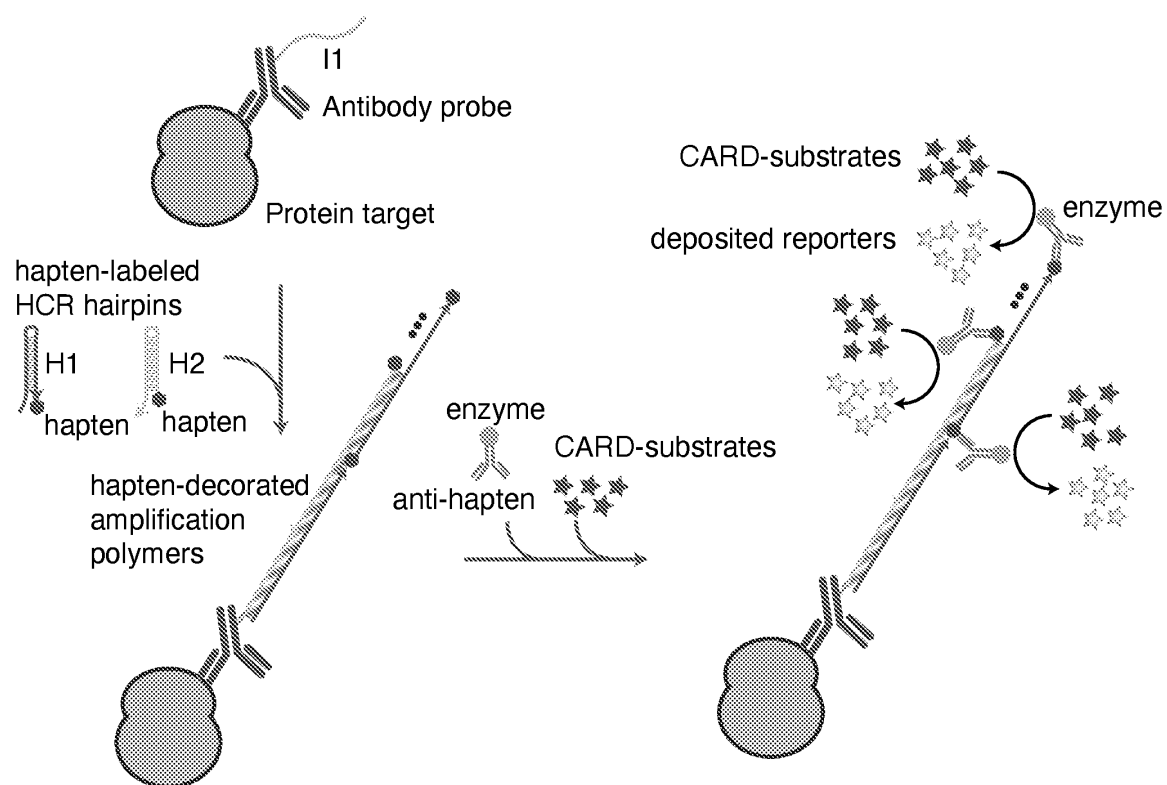


FIG. 33C

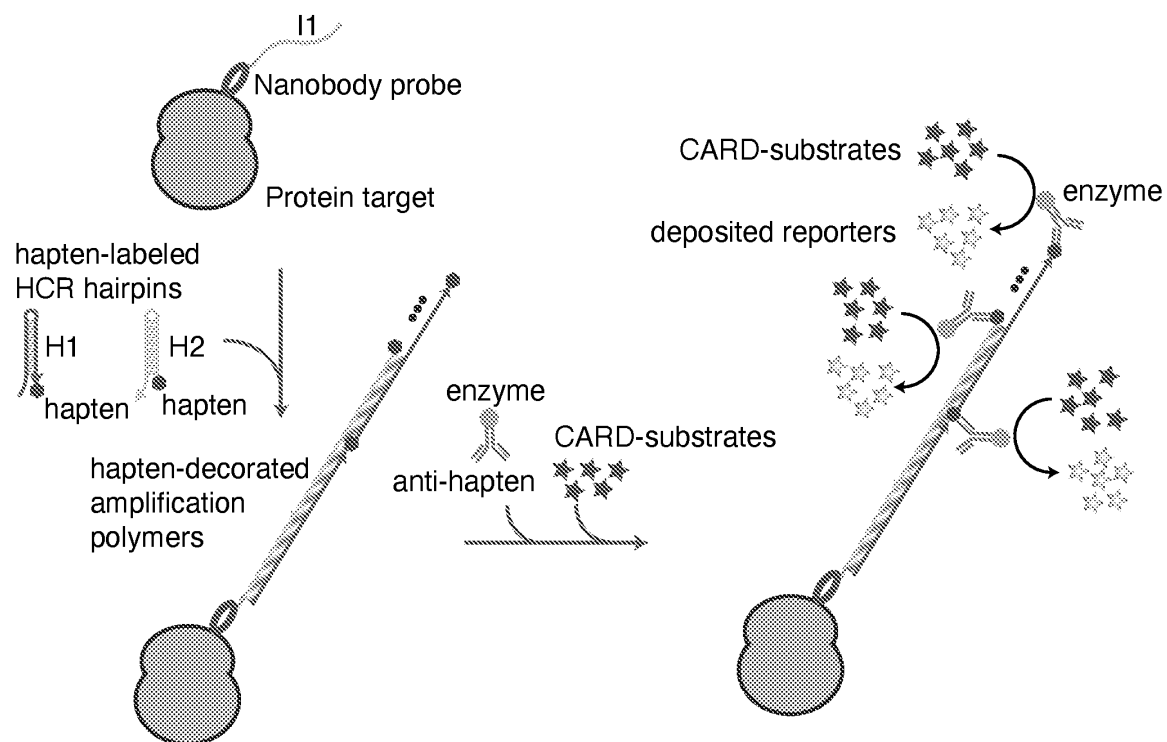


FIG. 33D

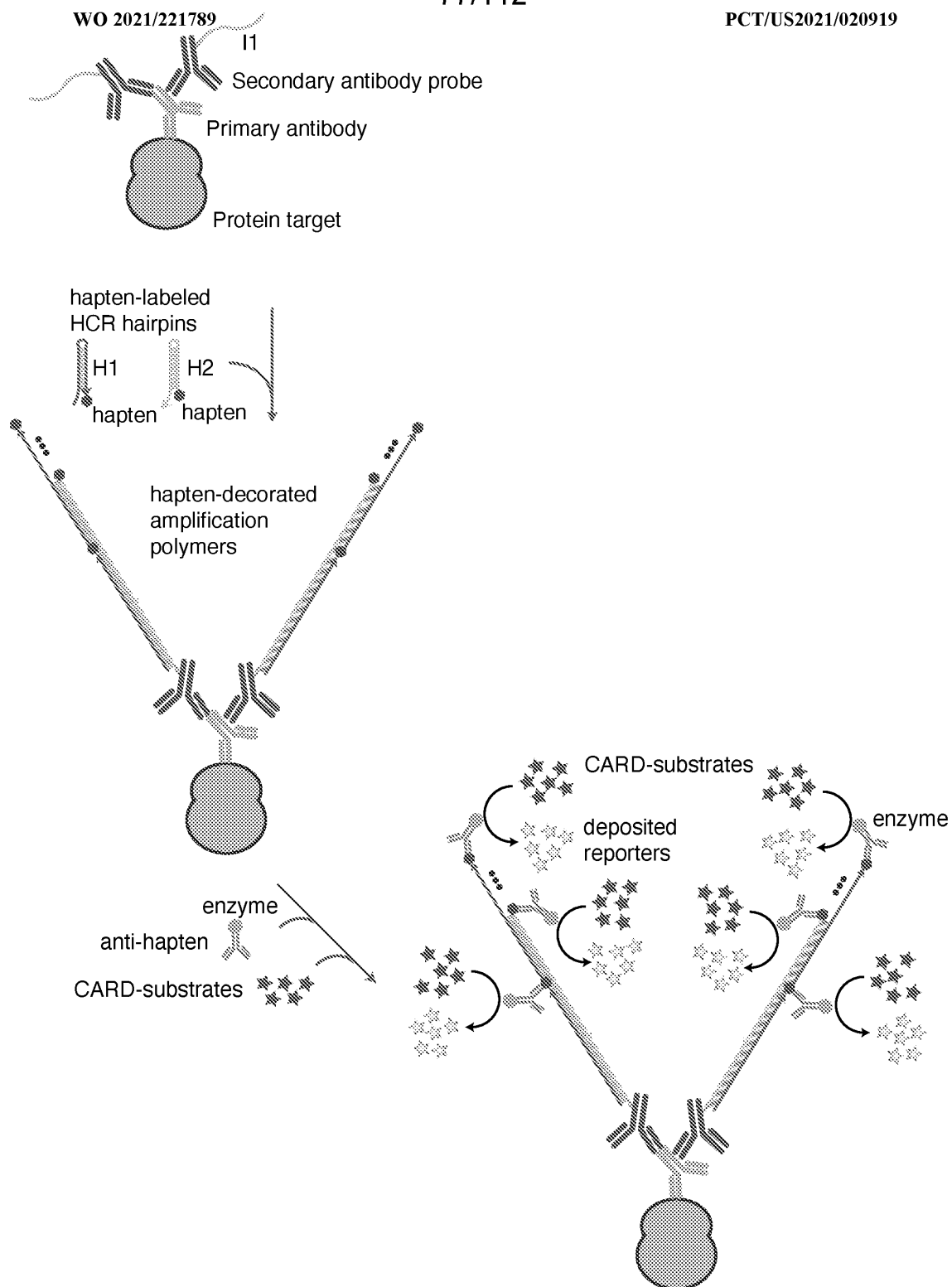


FIG. 33E

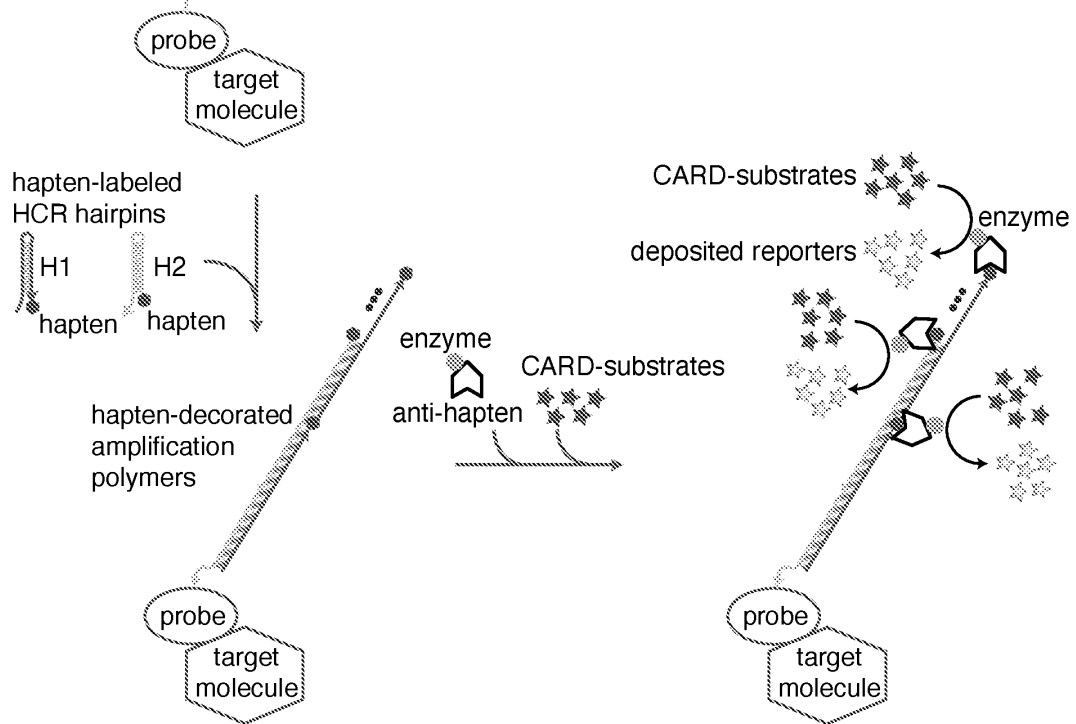


FIG. 34A

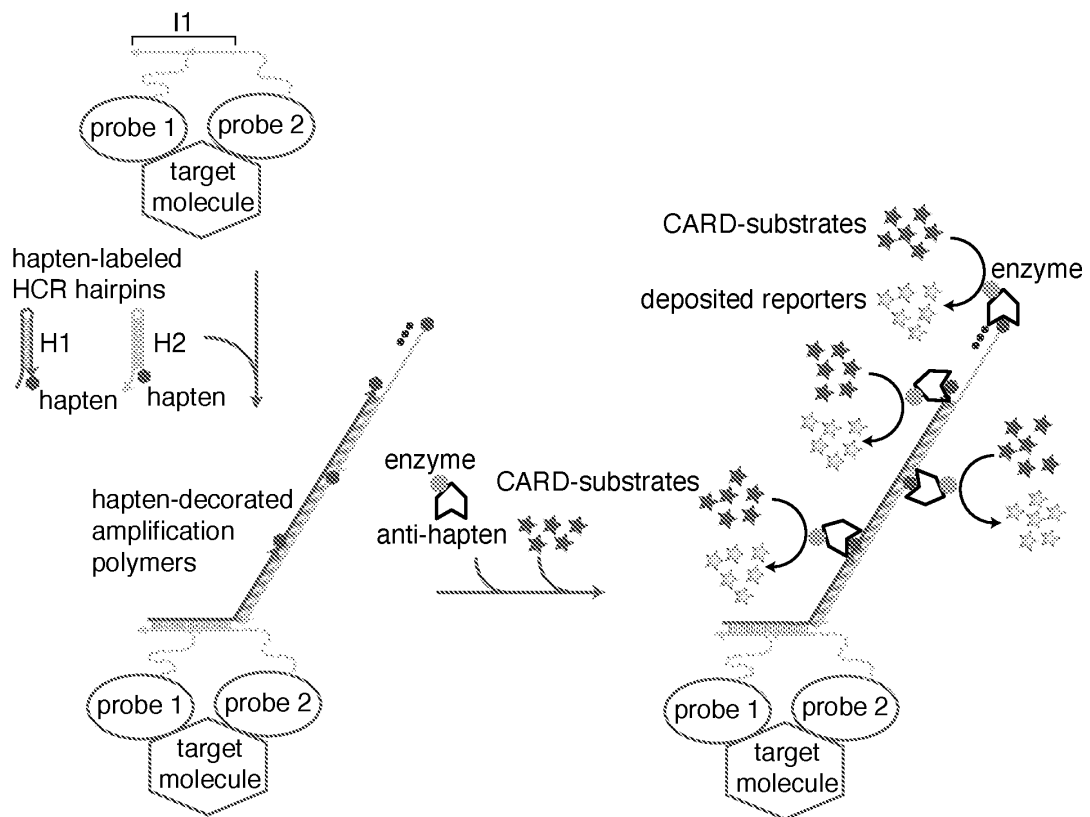


FIG. 34B

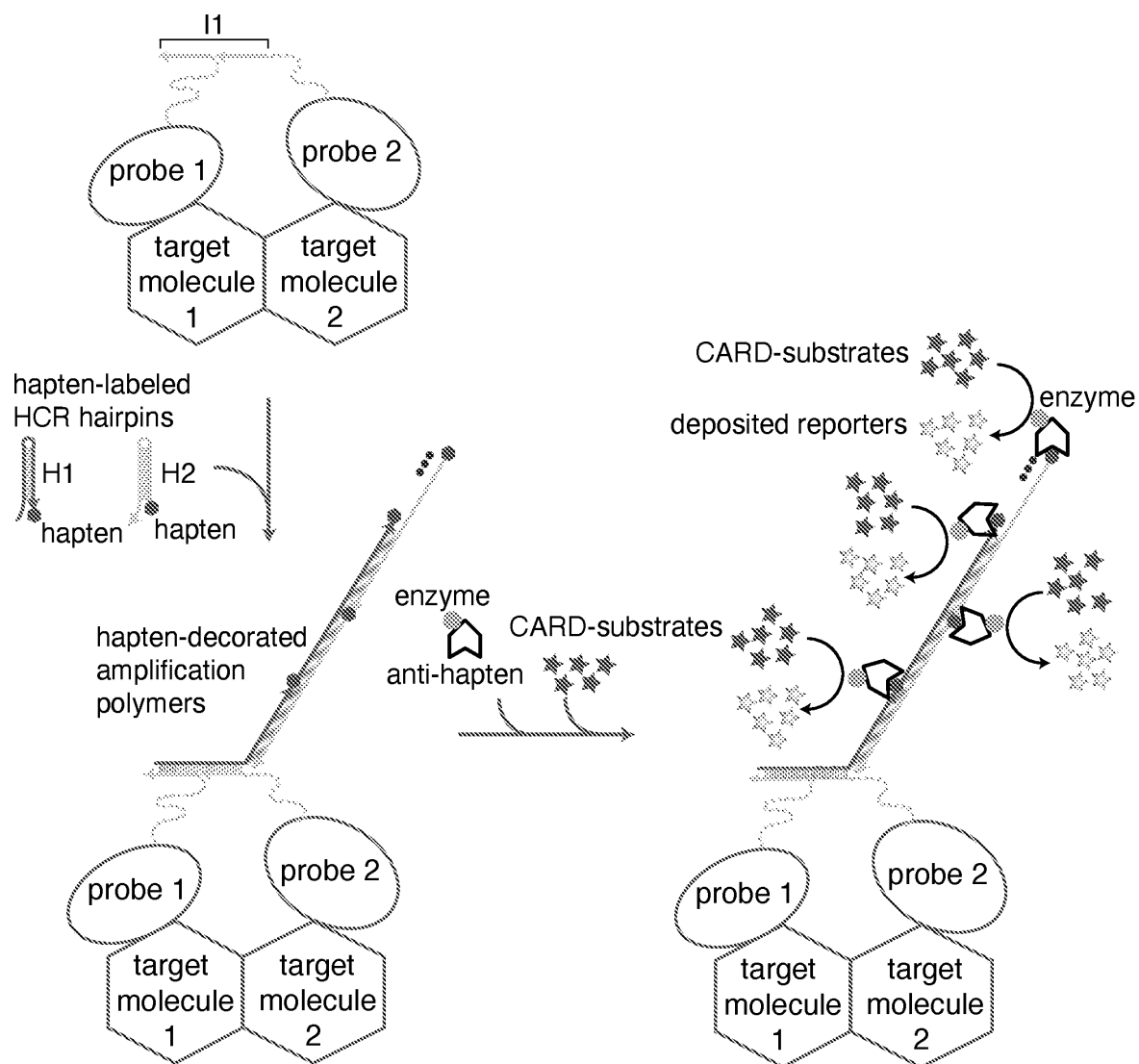


FIG. 34C

Hapten-labeled HCR hairpins

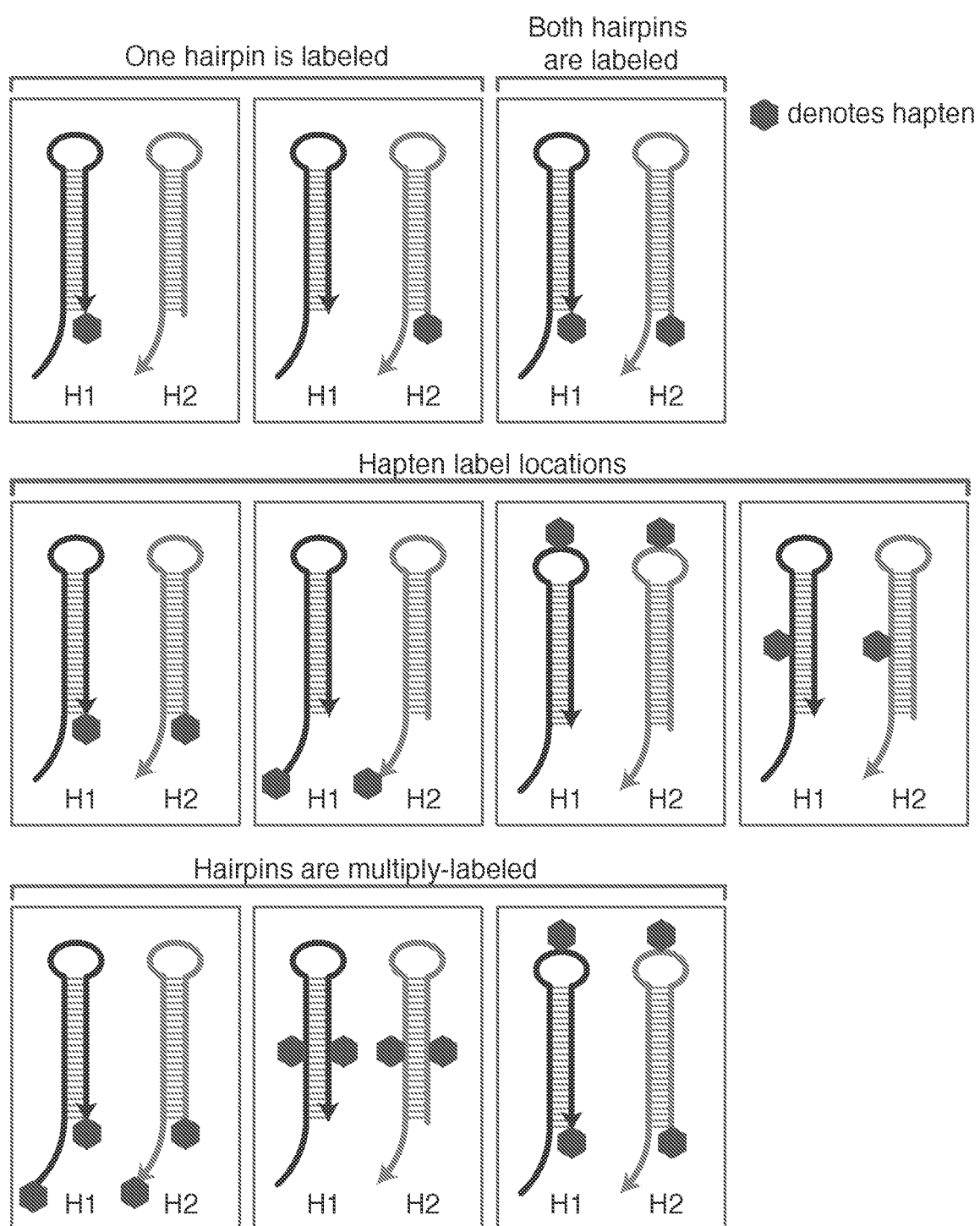


FIG. 35

Hapten-labeled HCR hairpins

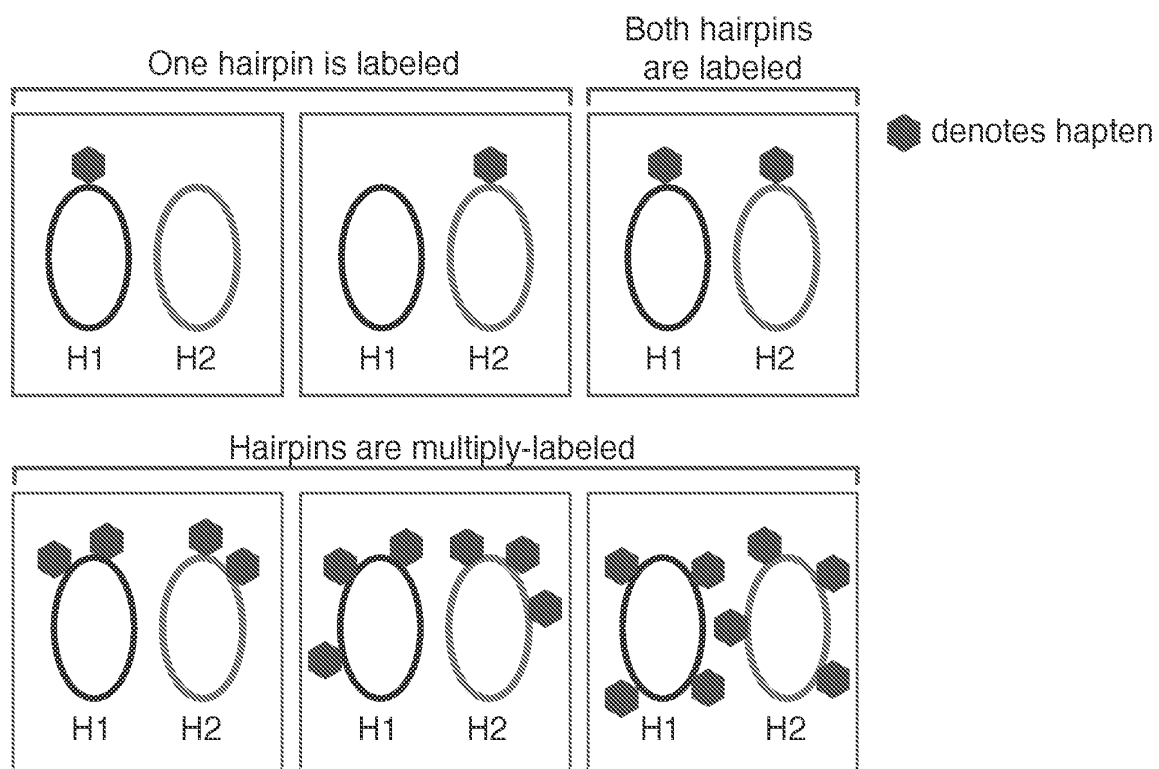
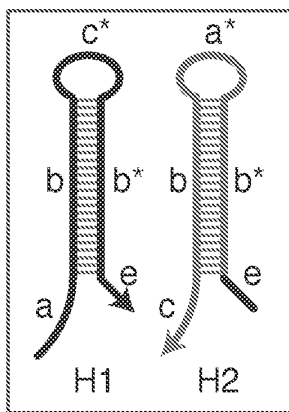
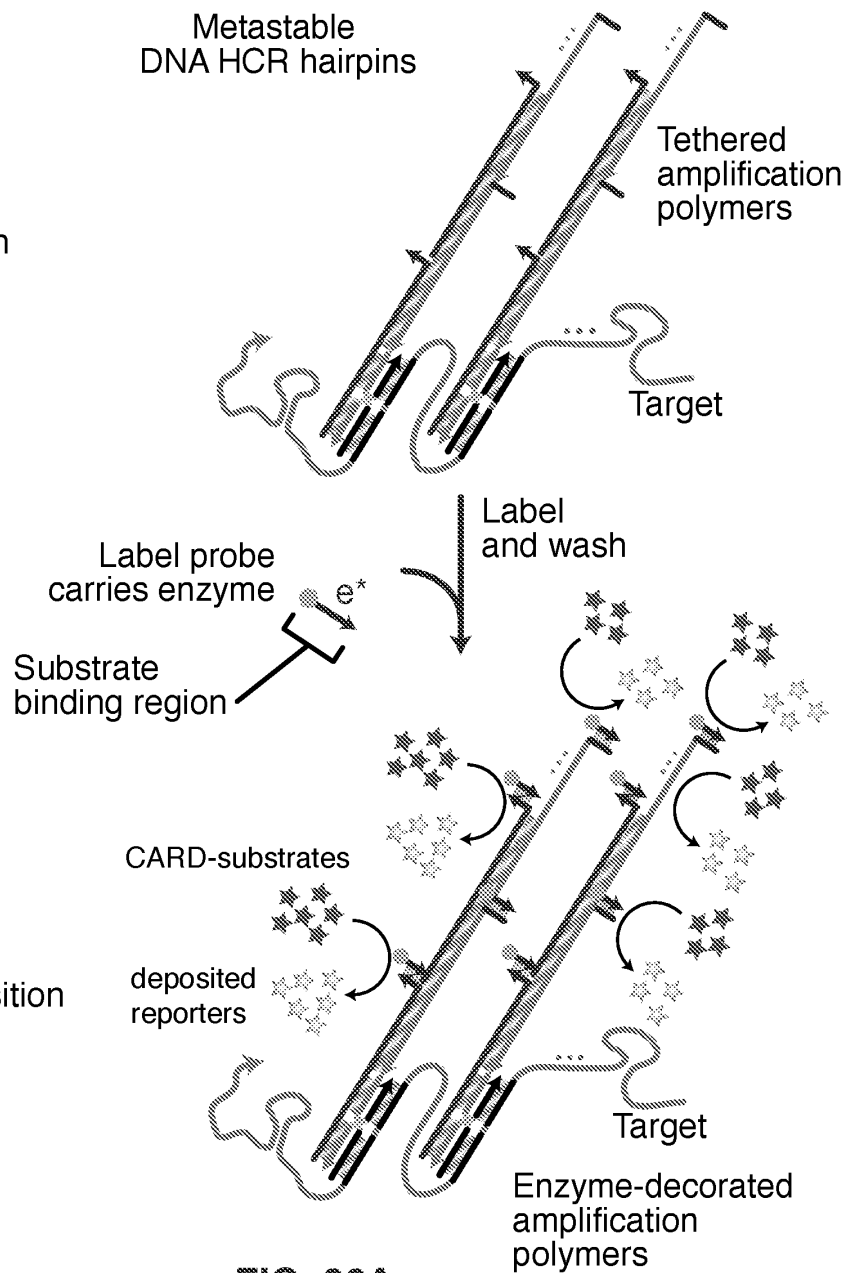
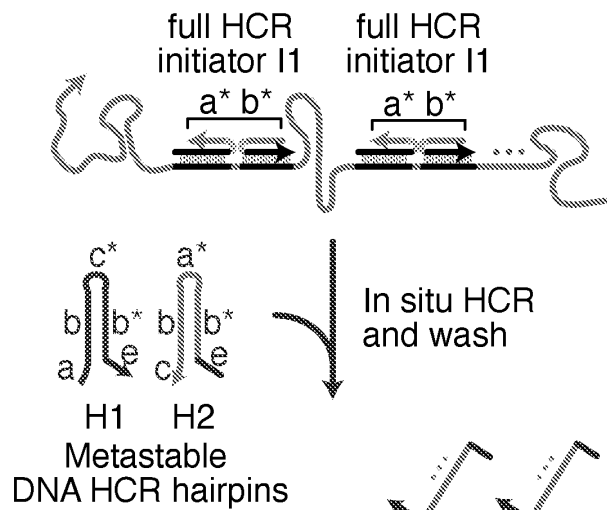


FIG. 35 (CONT.)



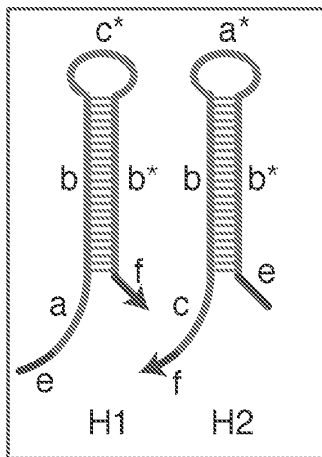
segments "e" are
substrates

Amplification



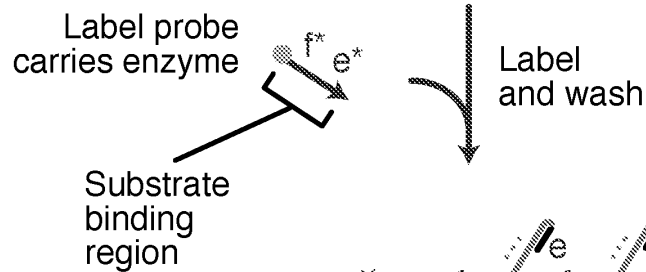
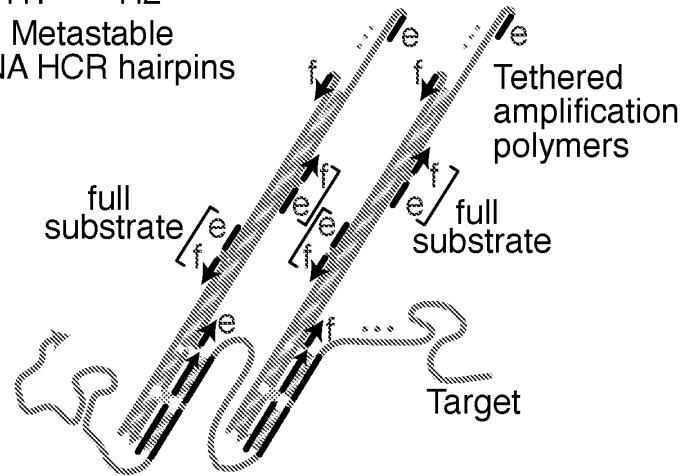
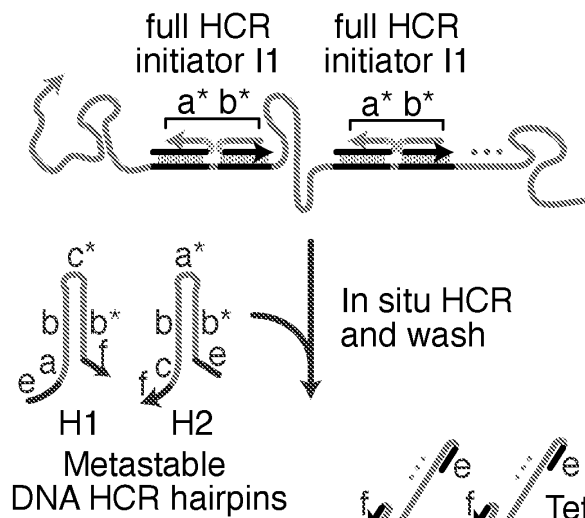
Labeling
&
Reporter Deposition

FIG. 36A



segments "e" and "f" are fractional substrates

Amplification



Labeling & Reporter Deposition

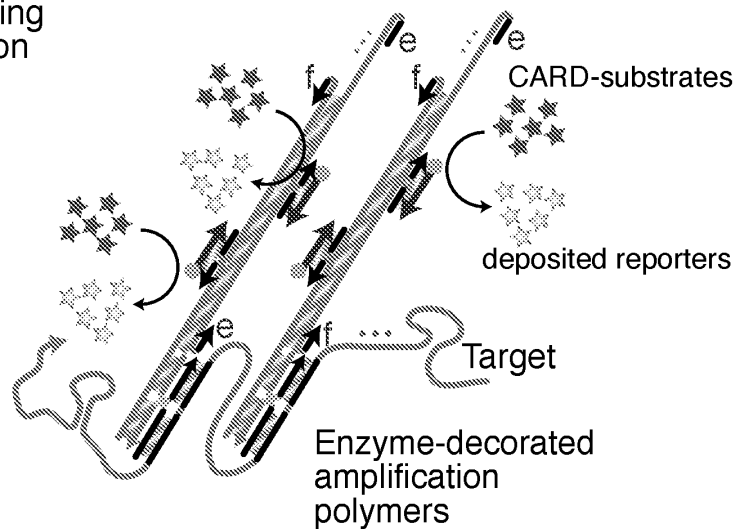


FIG. 36B

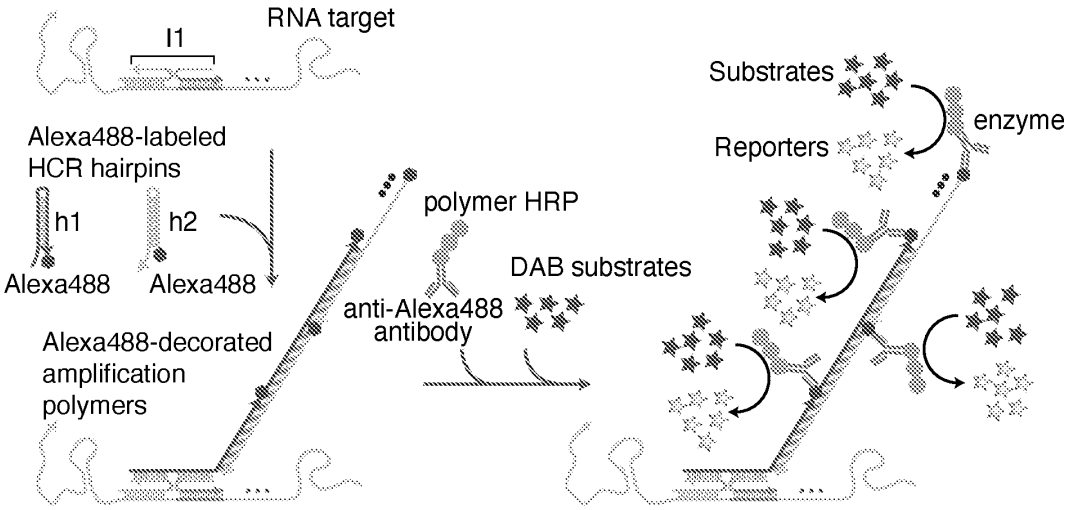


FIG. 37A

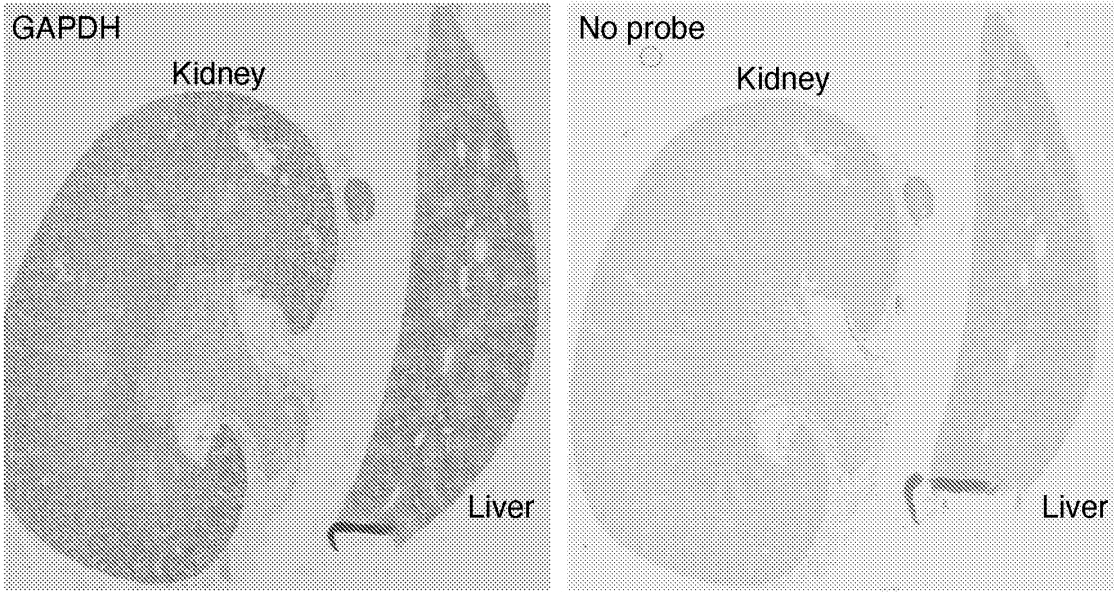


FIG. 37B

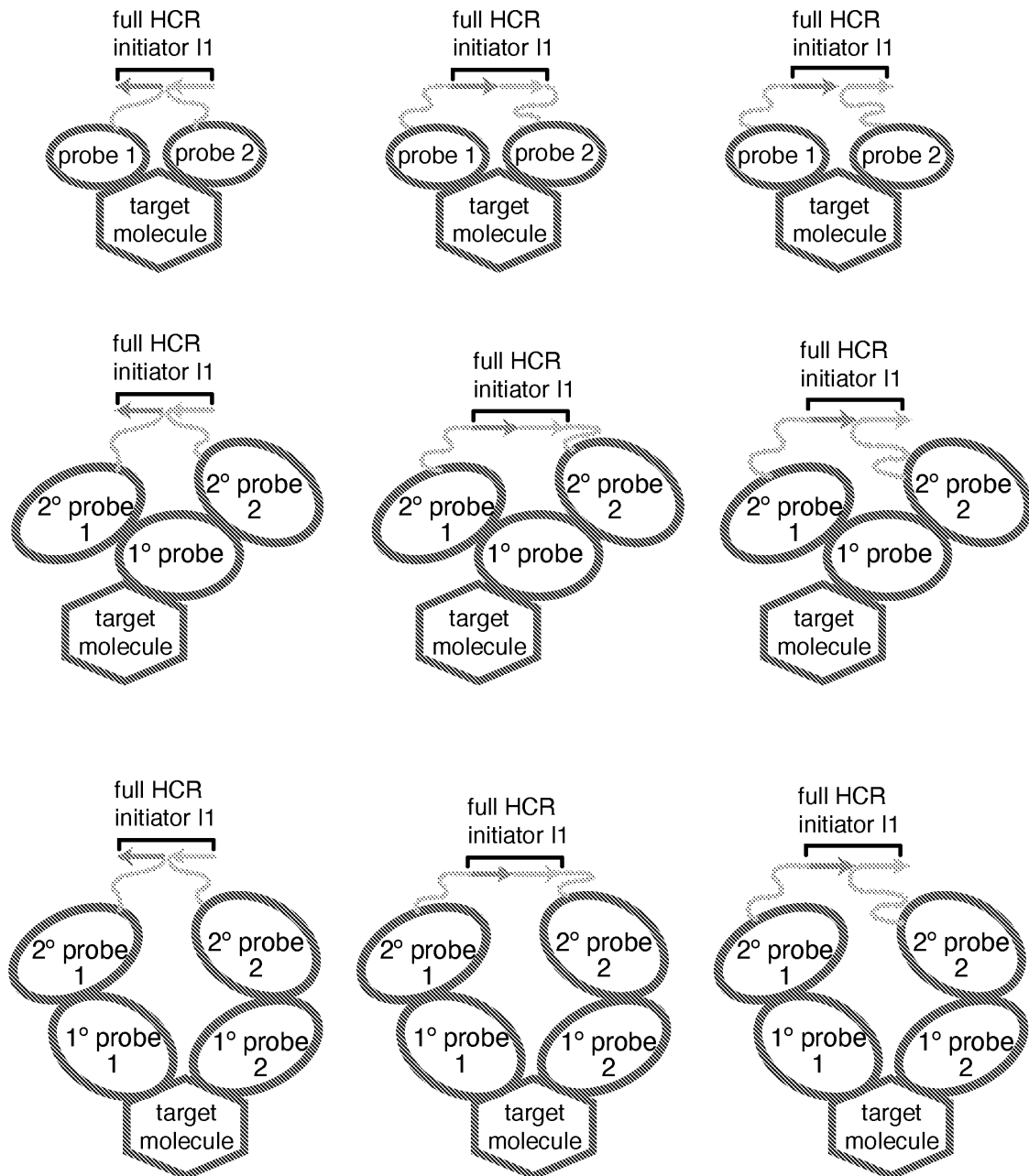


FIG. 38

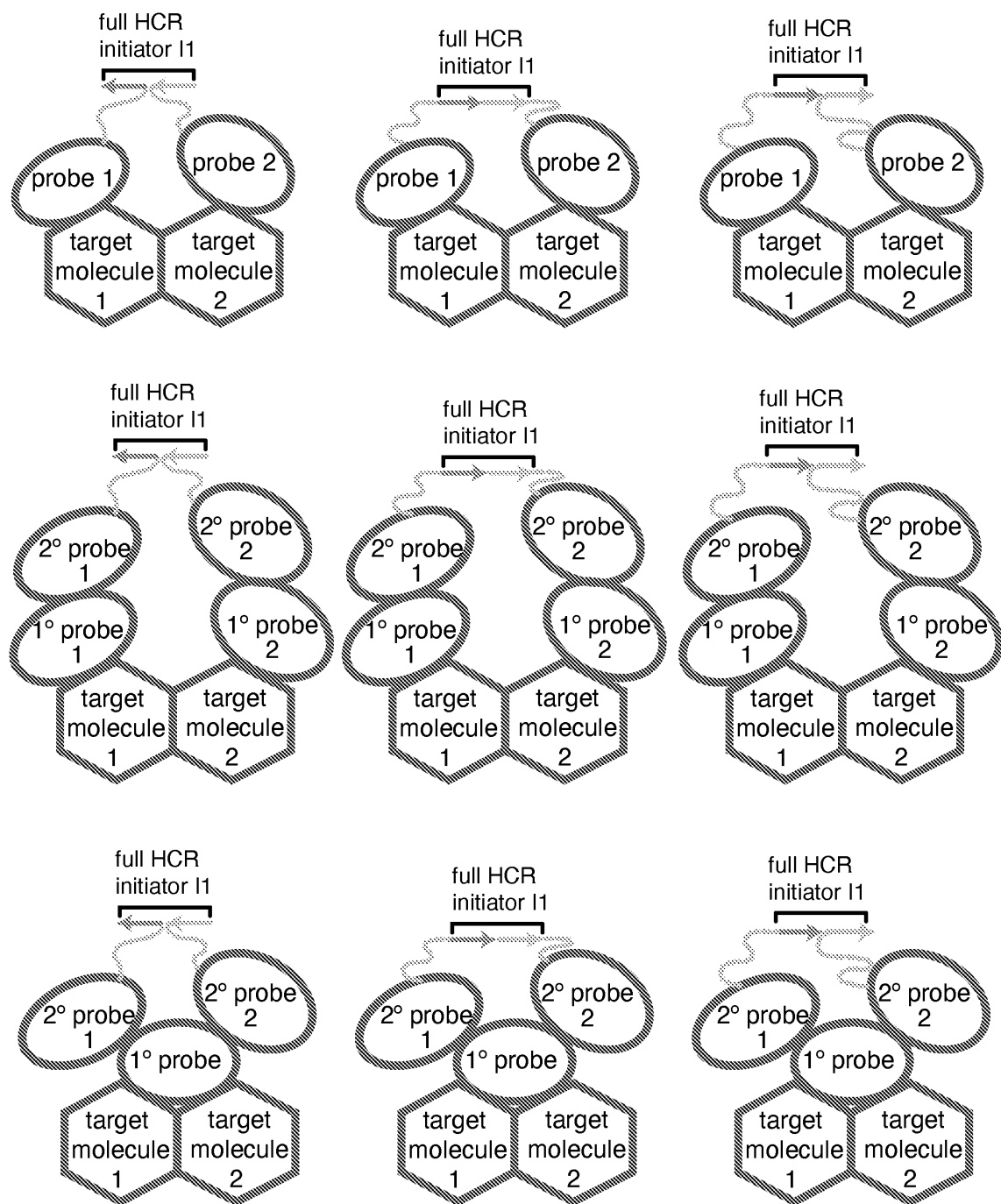


FIG. 38 (CONT.)

Initiator-labeled Probe
Single Initiator

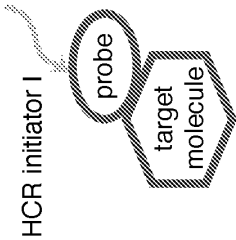


FIG. 39A

Initiator-labeled Probe
Multiple Initiators

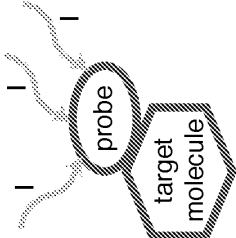


FIG. 39B

Secondary Initiator-labeled Probe
Single Initiator

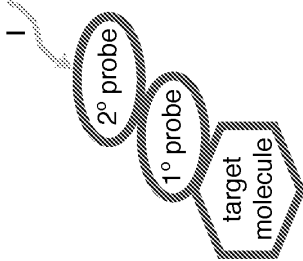


FIG. 39C

Secondary Initiator-labeled Probe
Multiple Initiators

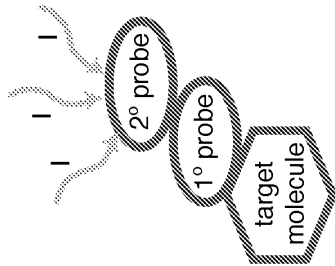


FIG. 39D

Initiator-labeled Probe
for Target Complex
Single Initiator

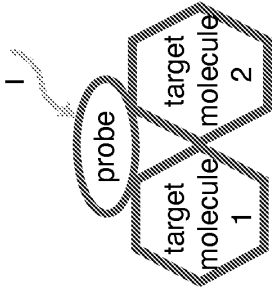


FIG. 39E

Initiator-labeled Probe
for Target Complex
Multiple Initiators

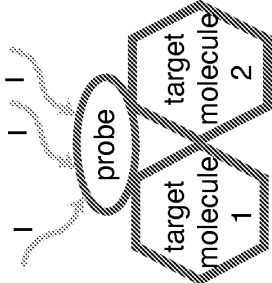


FIG. 39F

Nucleic Acid Probe
Single Initiator

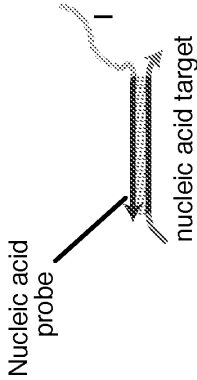


FIG. 39G

Nucleic Acid Probe
Multiple Initiators

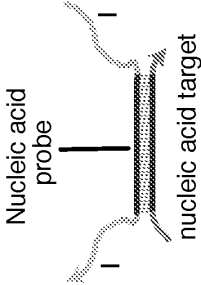


FIG. 39H

Primary-Antibody Probe
Single Initiator

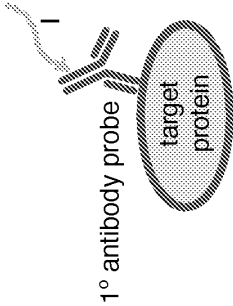


FIG. 39I

Primary-Antibody Probe
Multiple Initiators

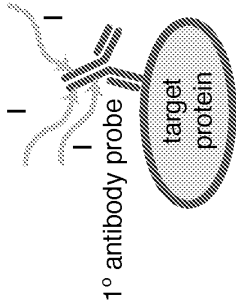


FIG. 39J

Secondary-Antibody Probe
Single Initiator

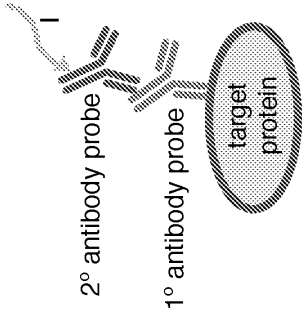


FIG. 39K

Secondary-Antibody Probe
Multiple Initiators

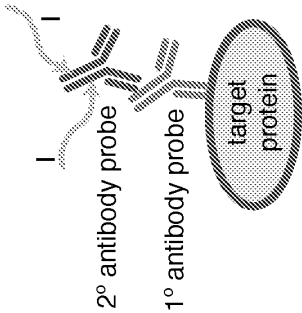


FIG. 39L

Nanobody Probe
Single Initiator

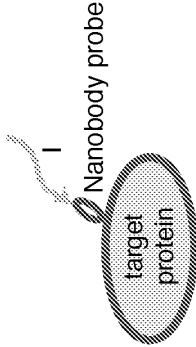


FIG. 39M

Nanobody Probe
Multiple Initiators

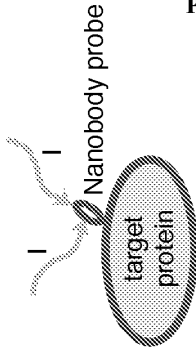


FIG. 39N

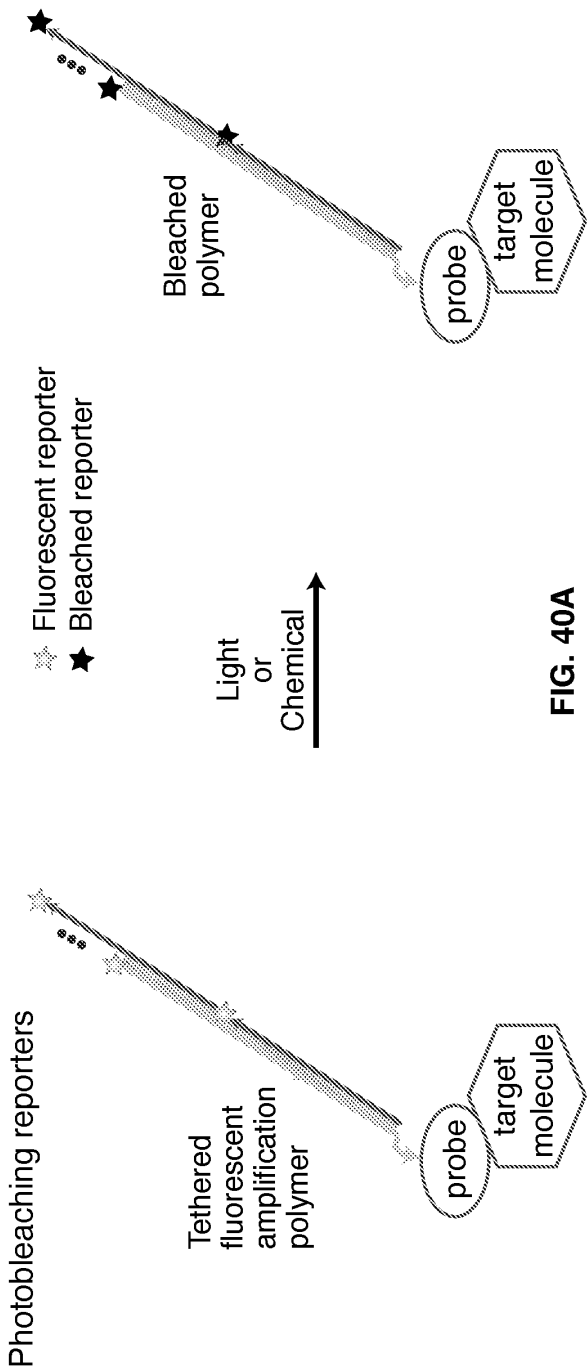


FIG. 40A

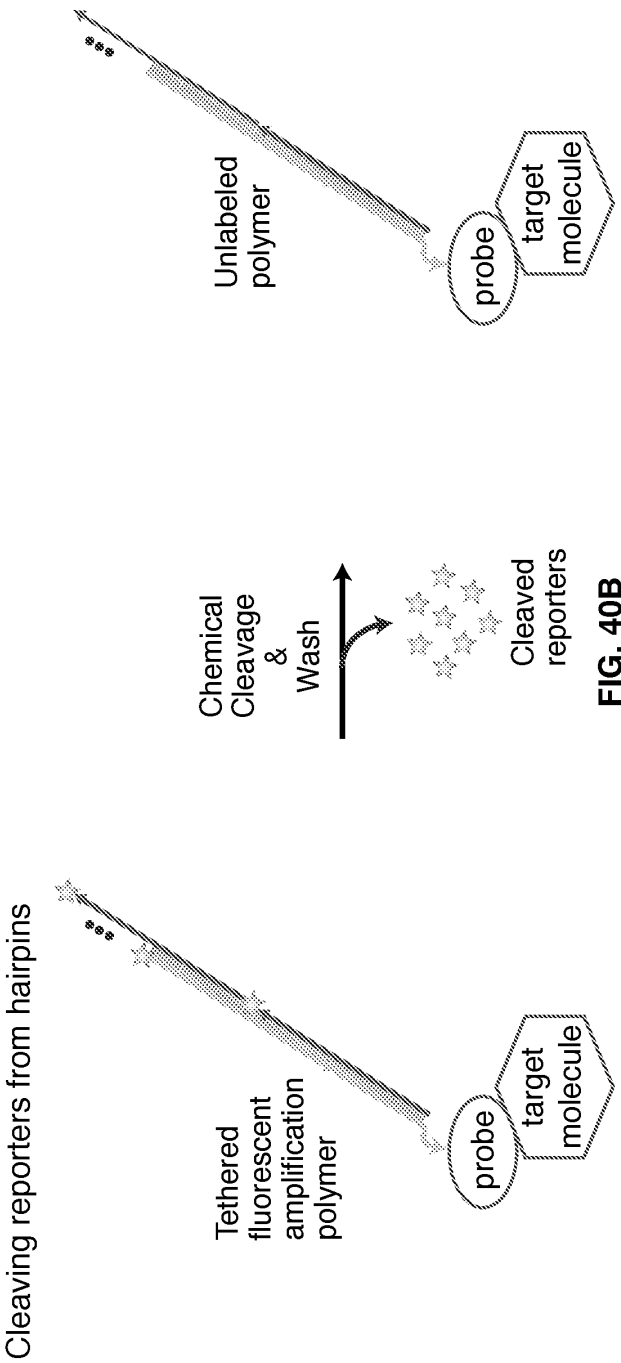


FIG. 40B

Cleaving reporters from label probes

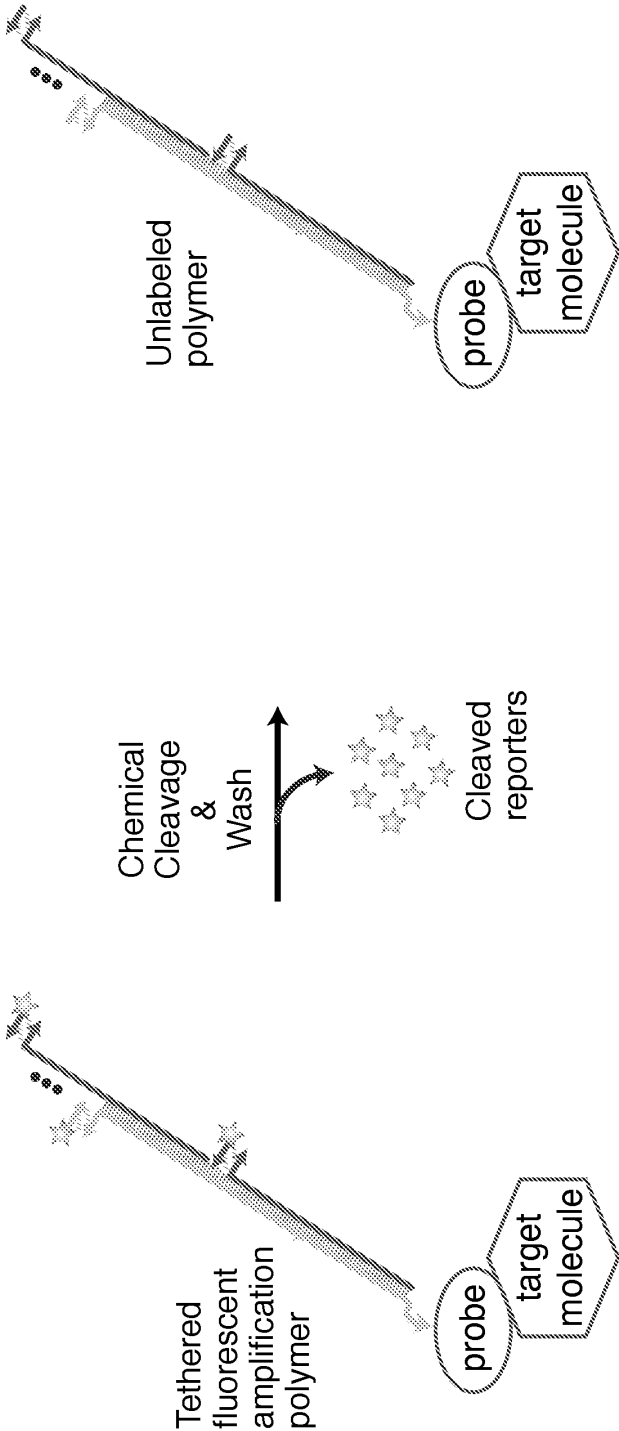


FIG. 40C

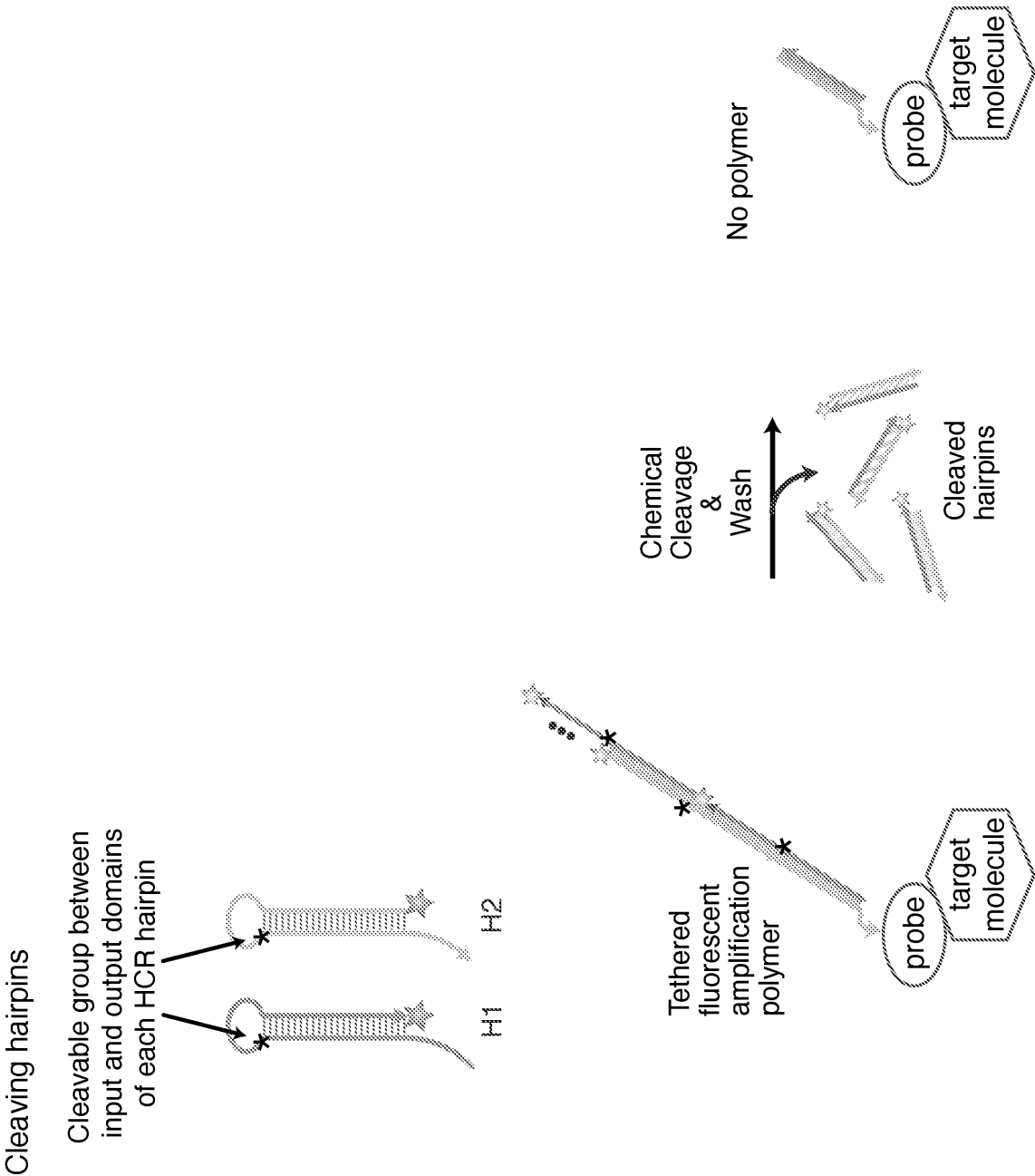


FIG. 40D

Cleaving probe

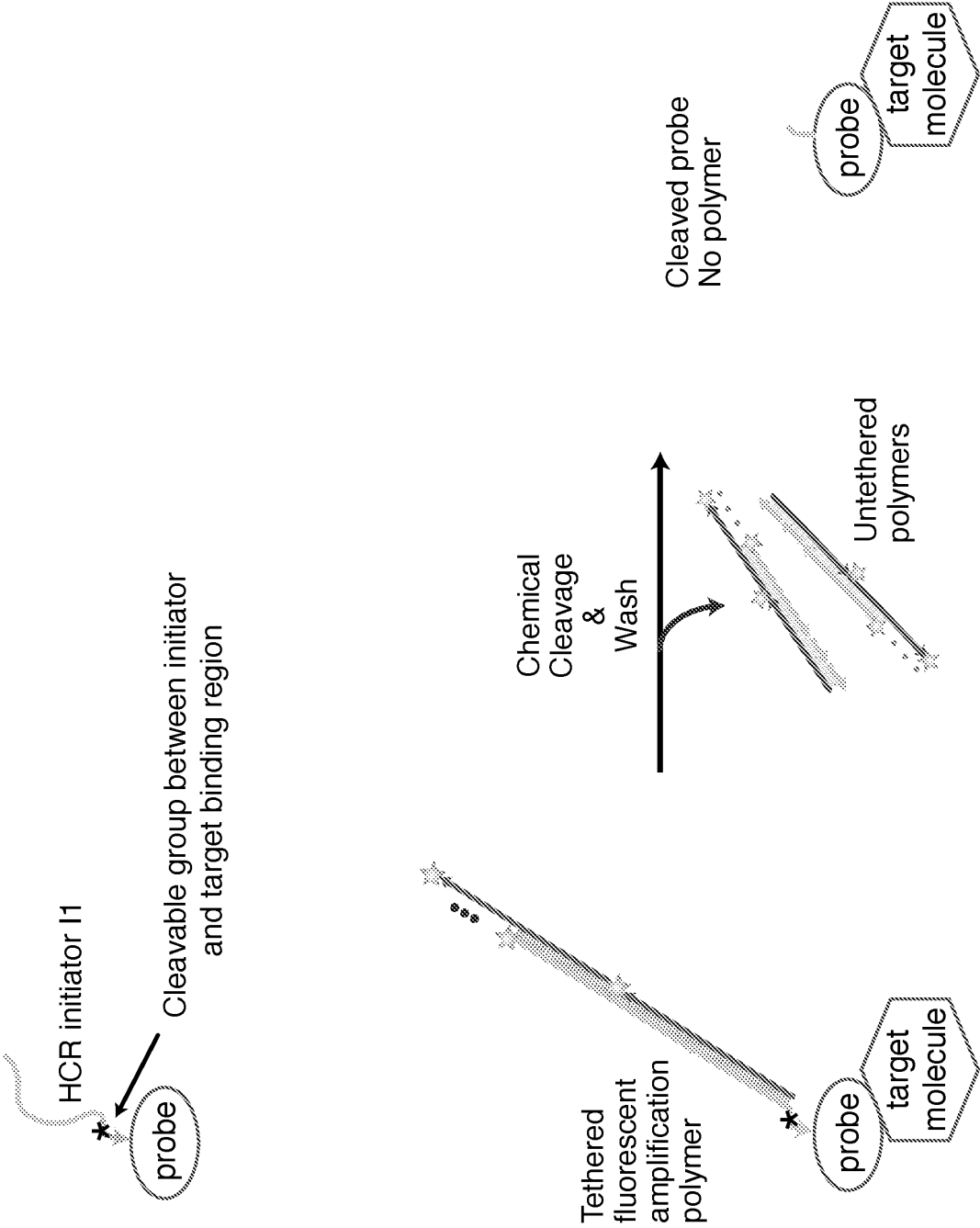


FIG. 40E

Dehybridizing hairpins with auxiliary strands

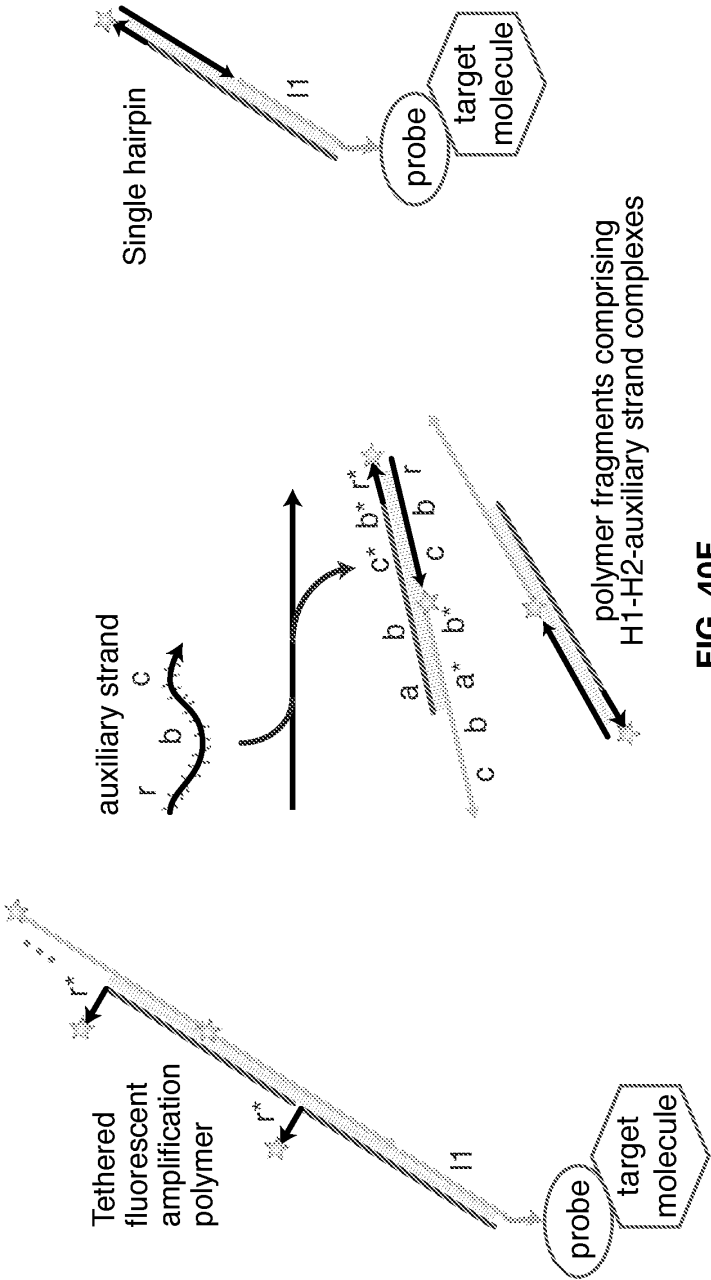
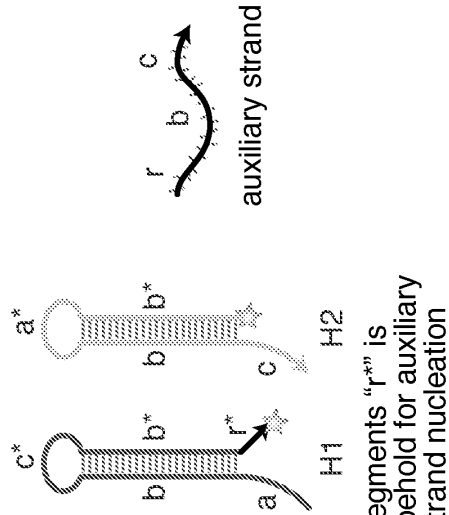


FIG. 40F

Dehybridizing label probes with auxiliary strands

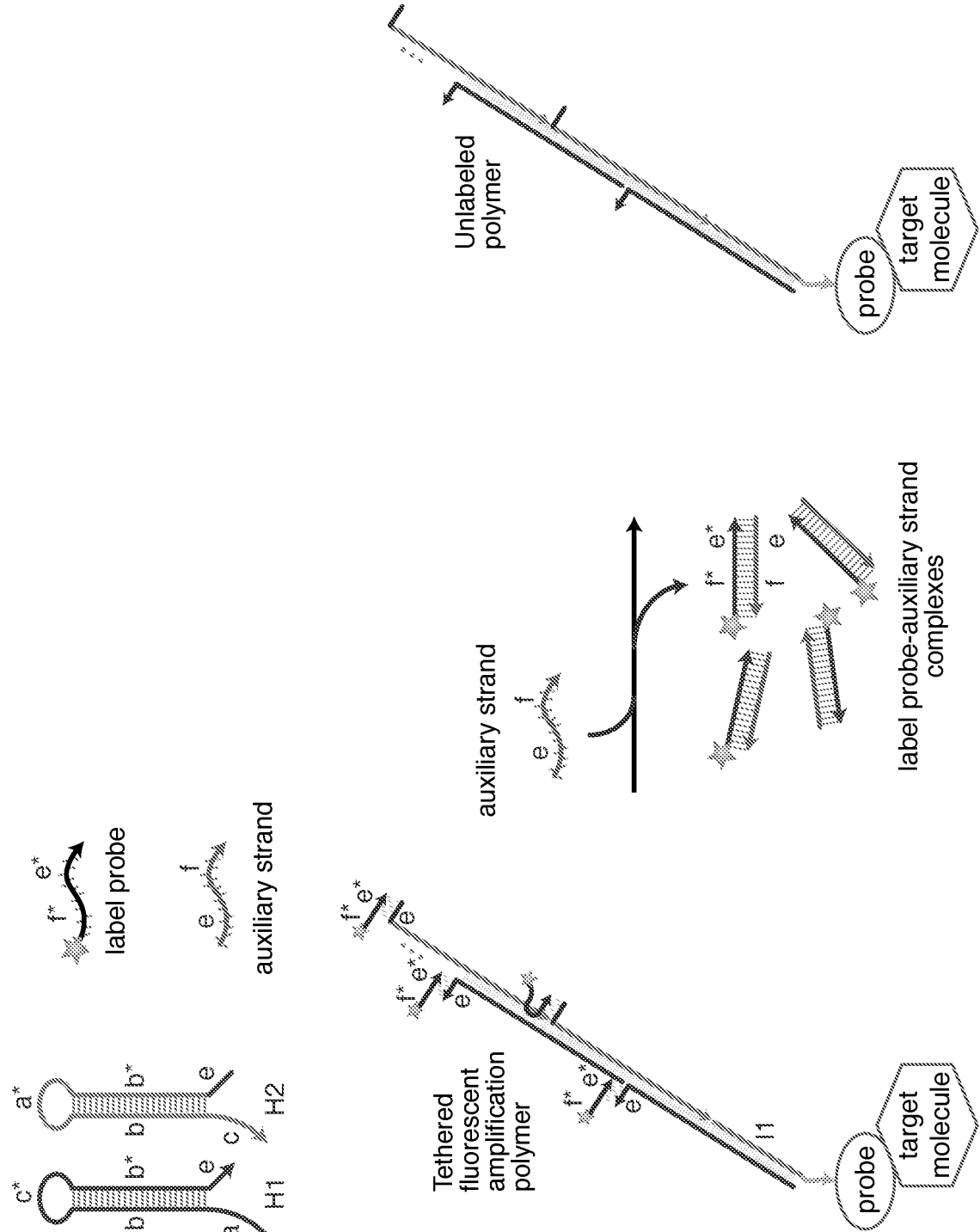


FIG. 40G

Destabilizing hairpins from polymers with chemical or elevated temperature

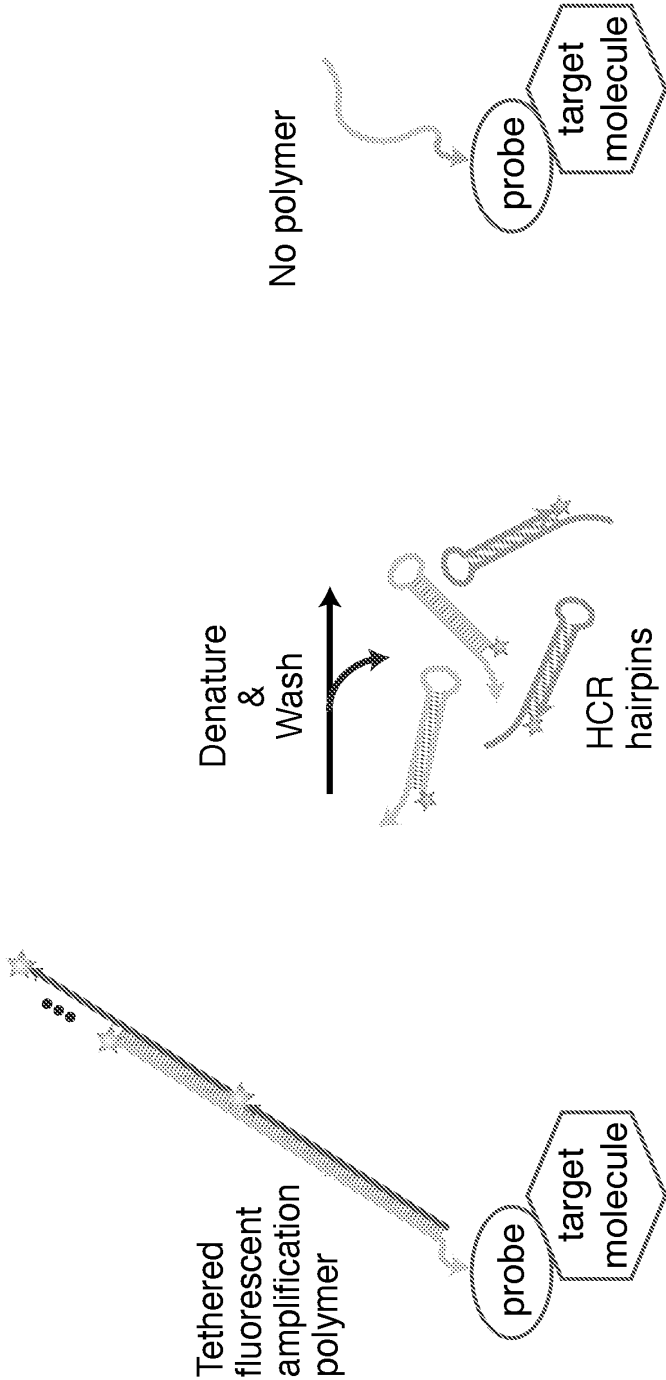


FIG. 40H

Destabilizing probes from target with chemical or elevated temperature

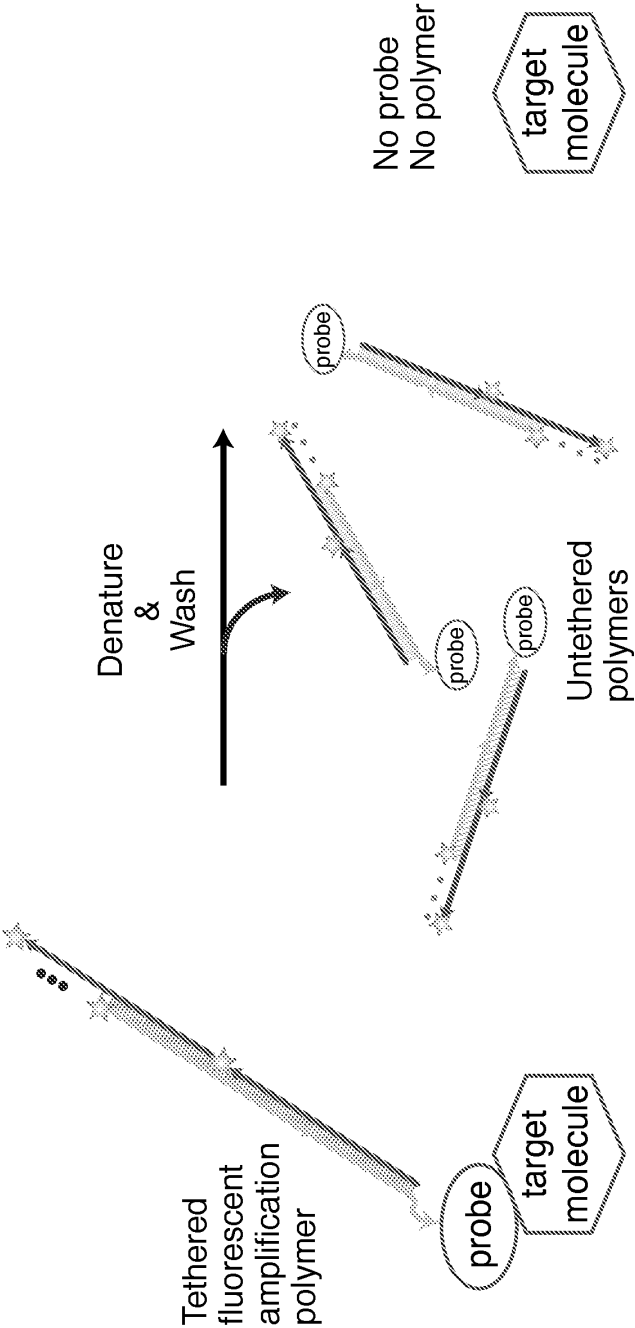


FIG. 40I

Destabilizing label probes from substrates with chemical or elevated temperature

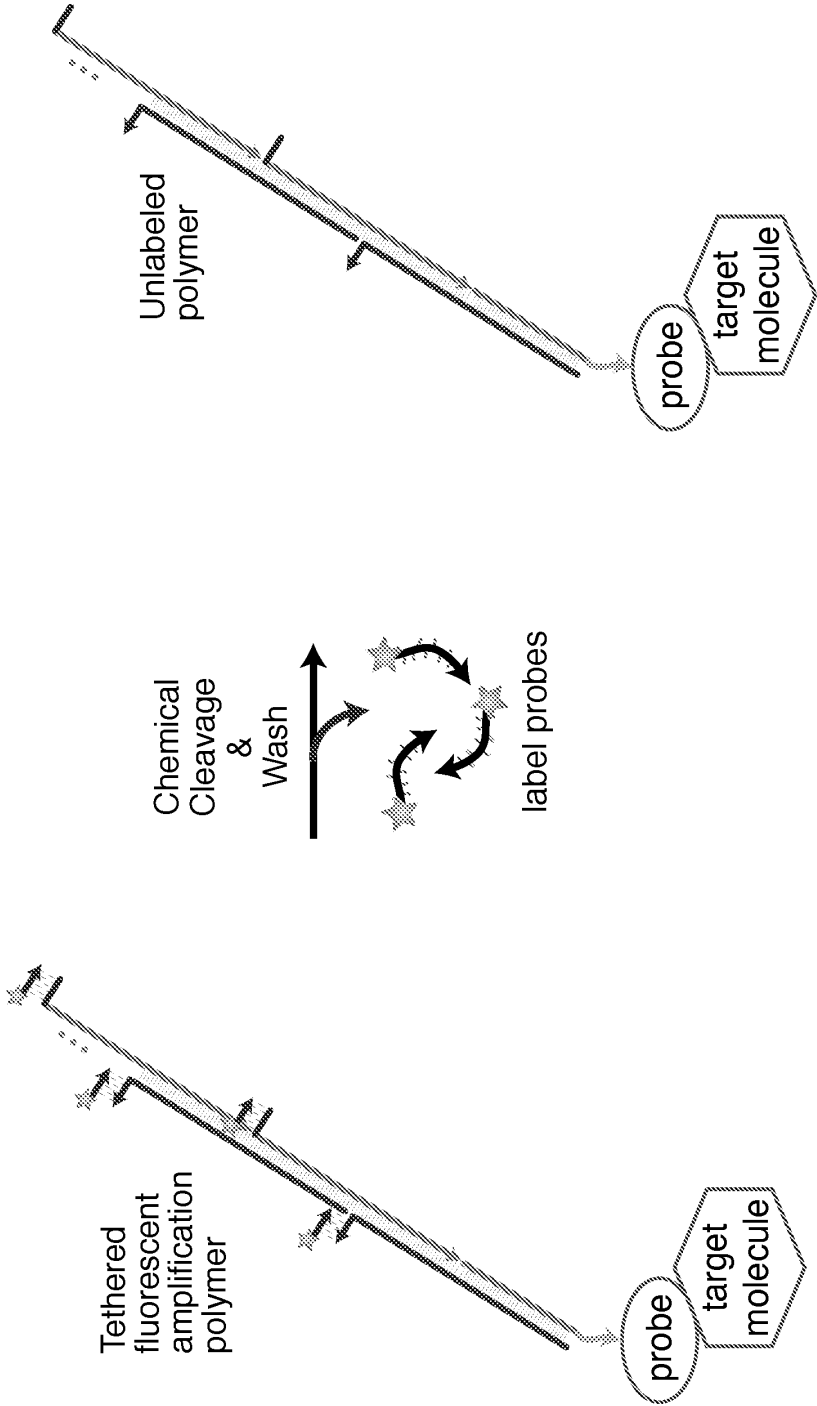


FIG. 40J

Degrading HCR probes and polymers with enzymes (e.g., DNase, RNase, protease)

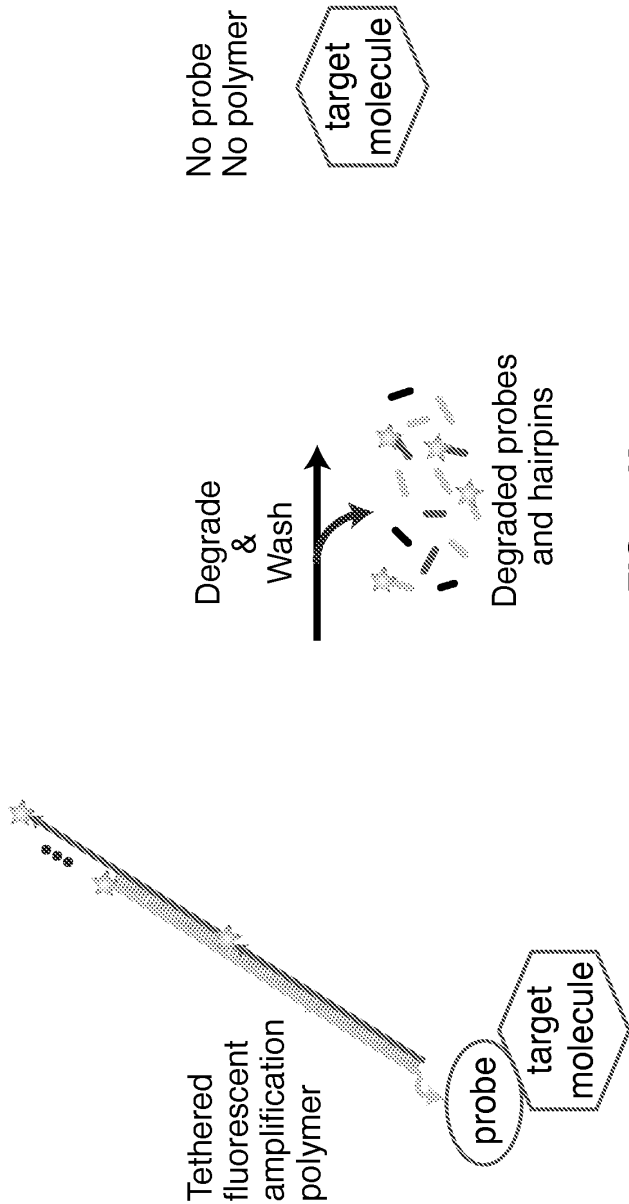


FIG. 40K

Degrading targets with enzymes (e.g. DNase, RNase, protease)

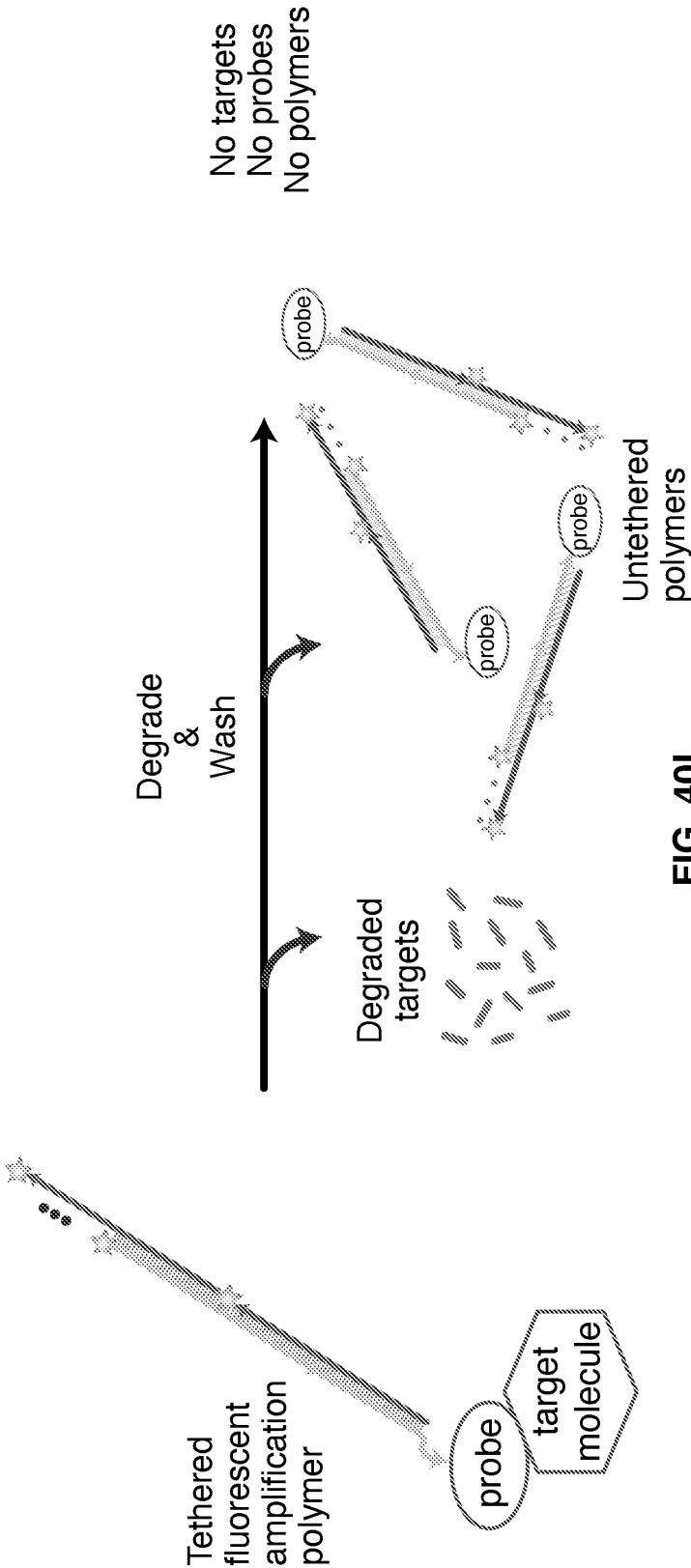


FIG. 40L

Degrading targets and HCR probes and hairpins with enzymes (e.g. DNase, RNase, protease)

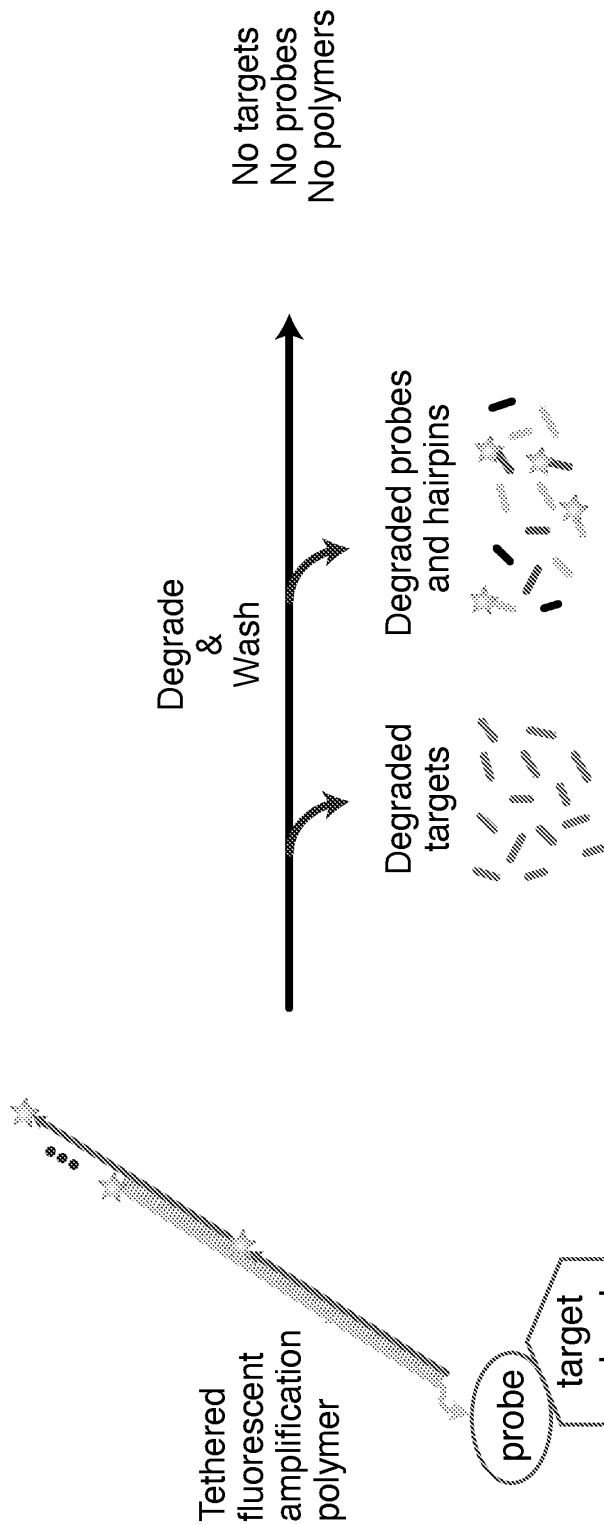


FIG. 40M

Dehybridizing hairpins with auxiliary strands

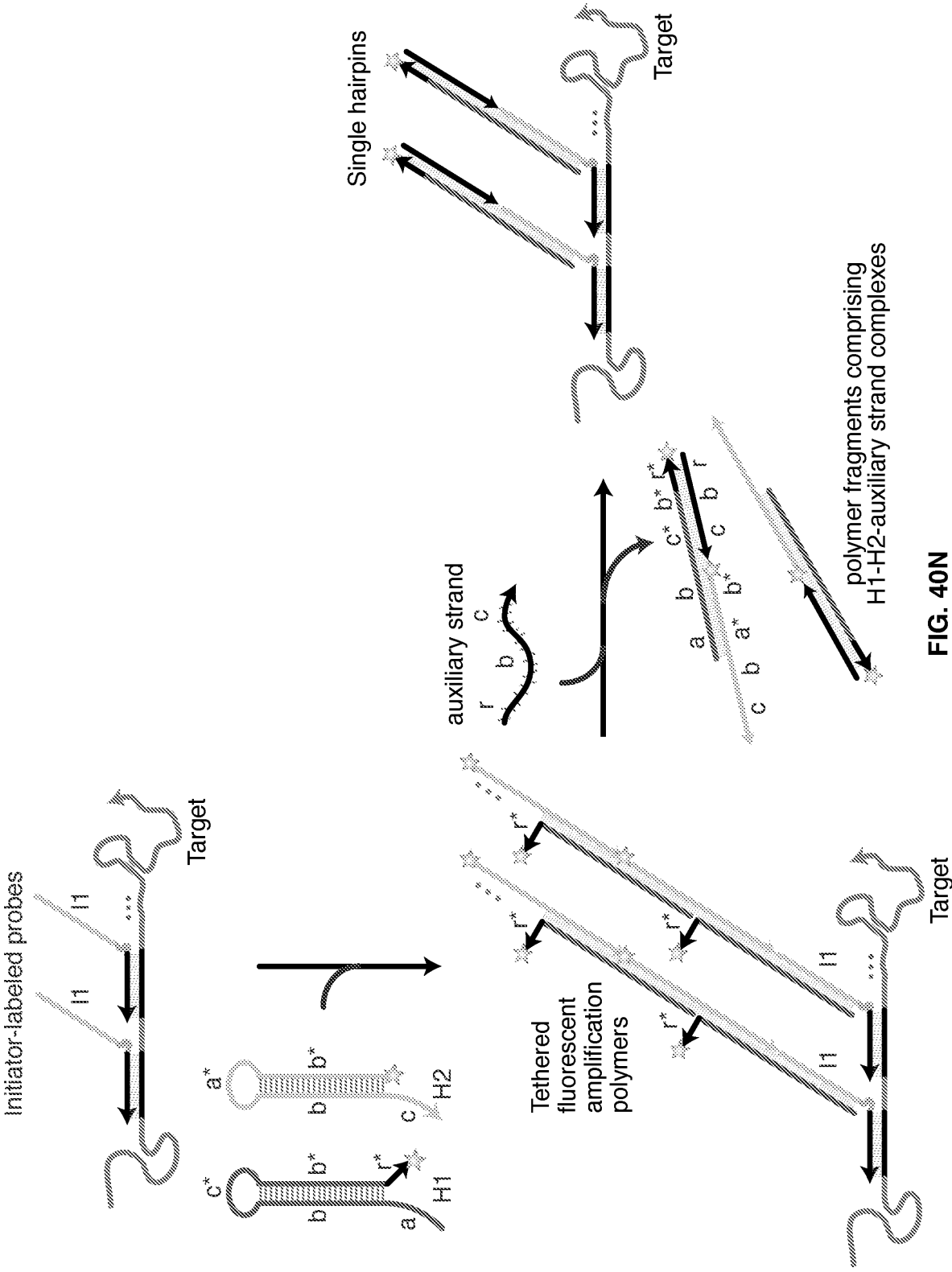


FIG. 40N

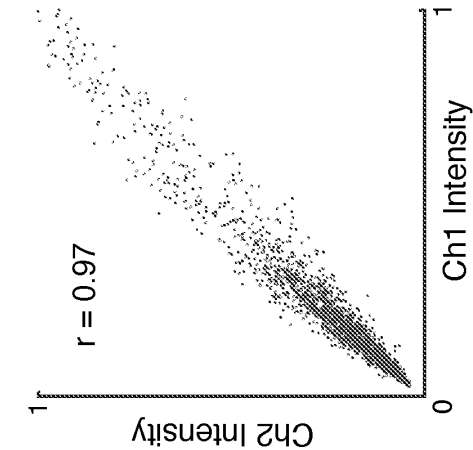


FIG. 41C

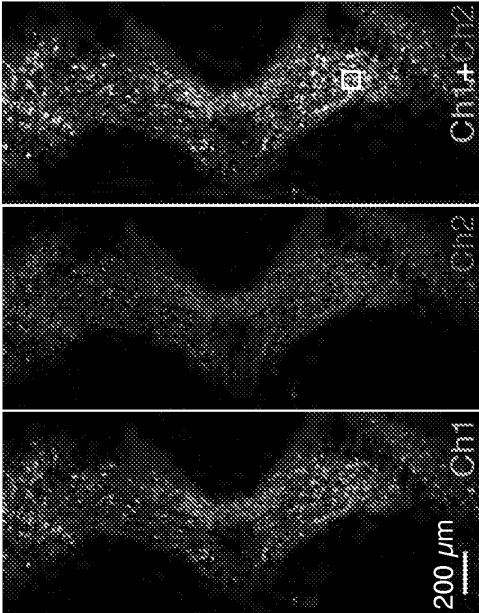


FIG. 41B

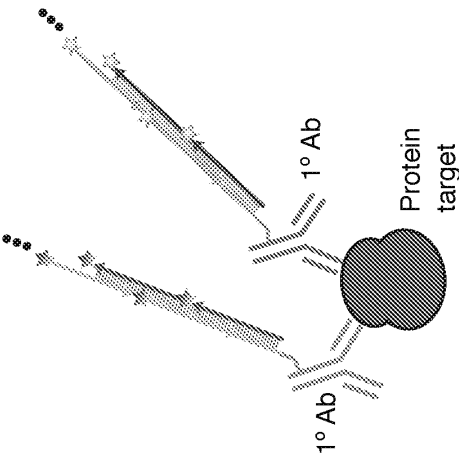


FIG. 41A

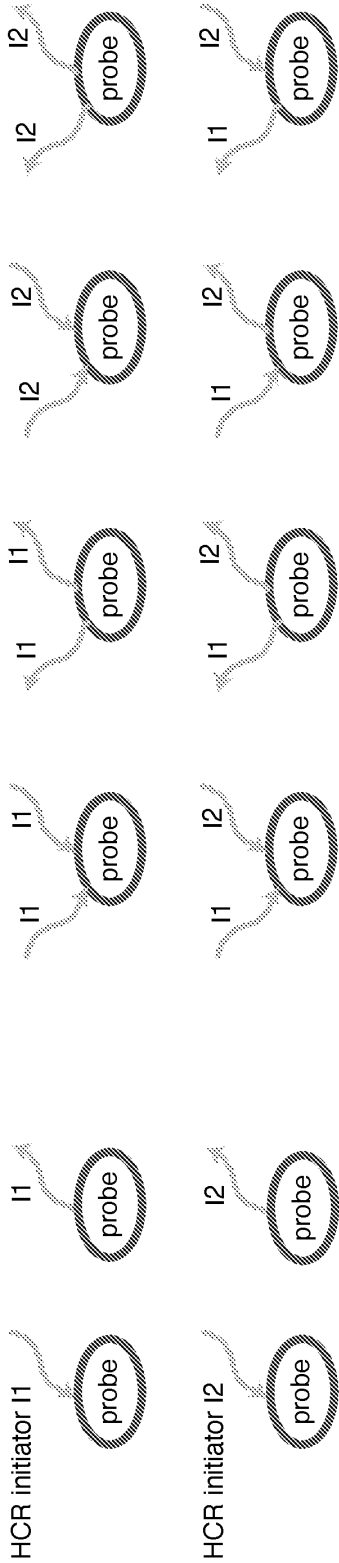


FIG. 42A

FIG. 42B

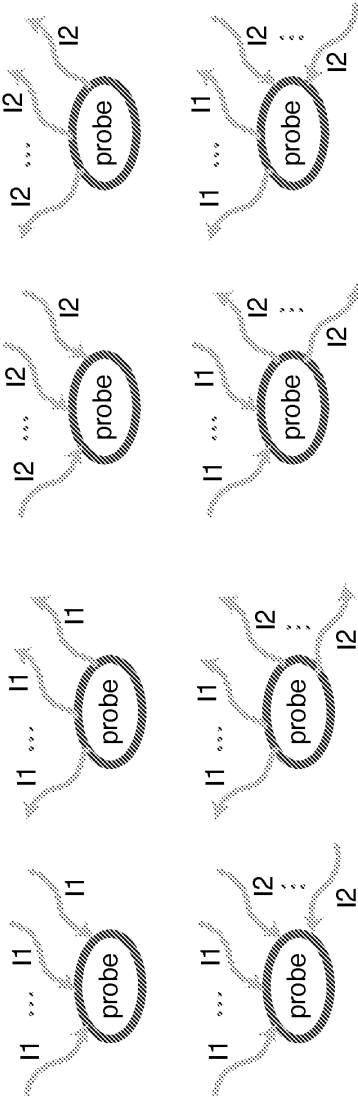


FIG. 42C

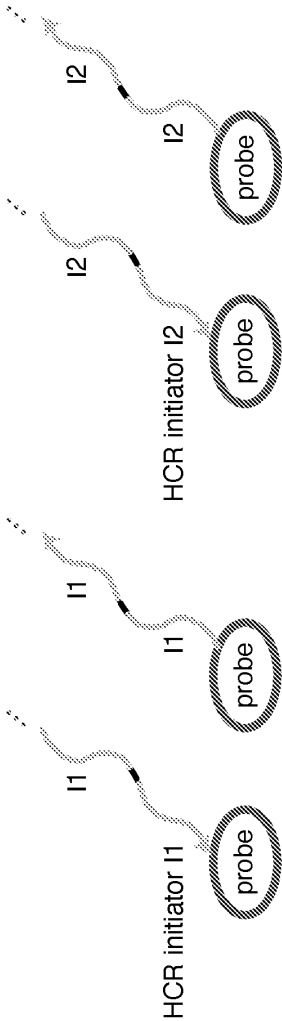


FIG. 42D

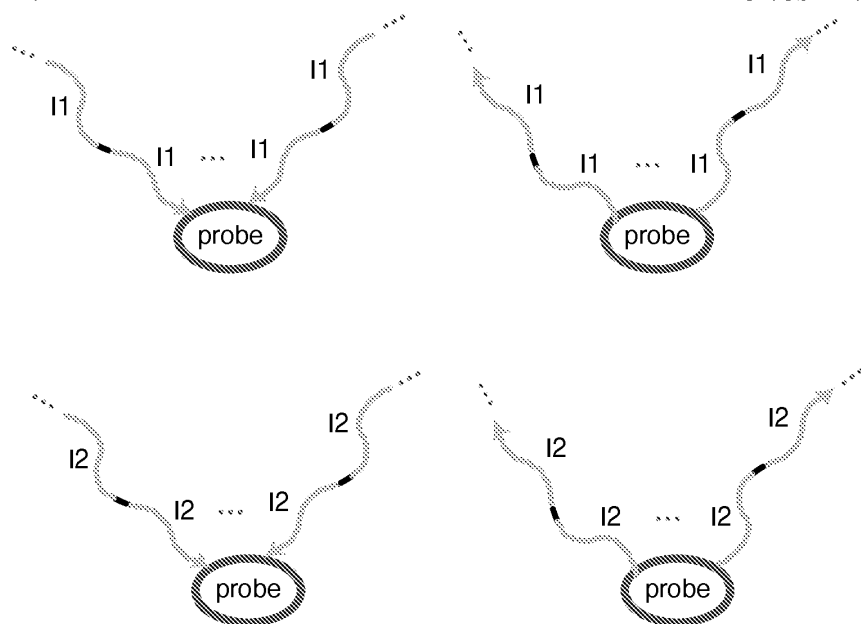


FIG. 42E

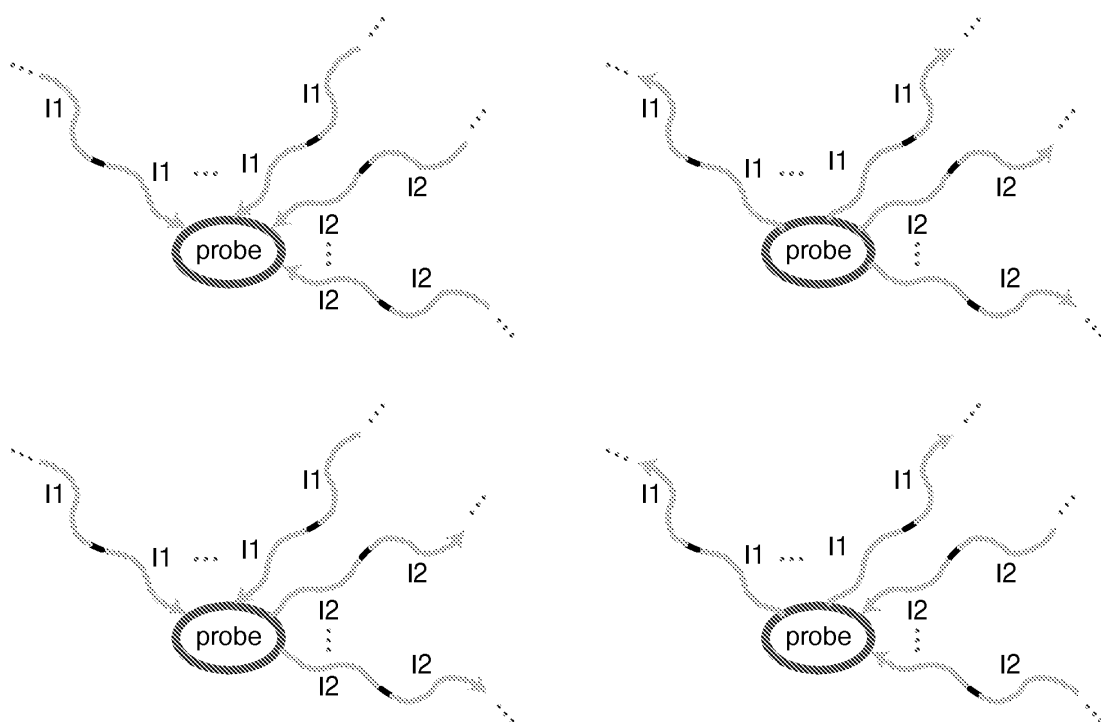


FIG. 42F

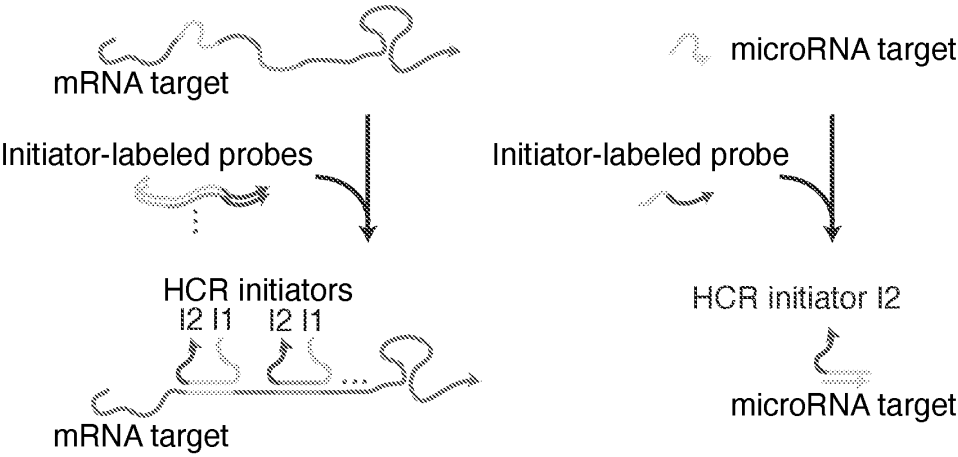


FIG. 43A

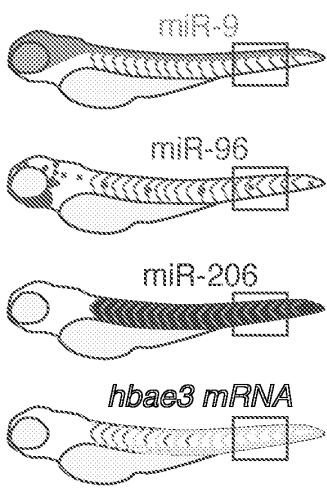


FIG. 43B

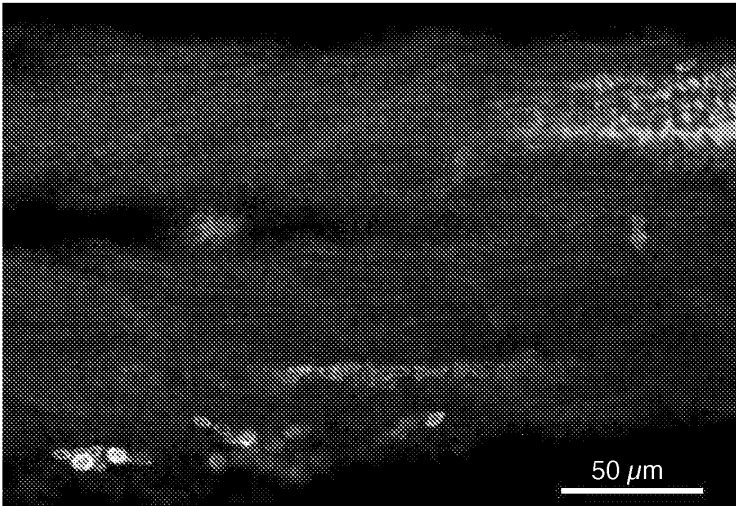


FIG. 43C

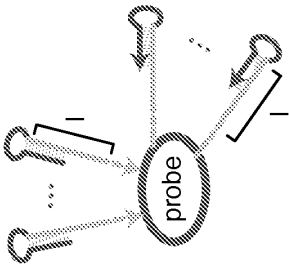


FIG. 44A

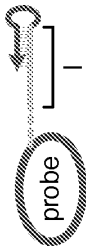


FIG. 44B

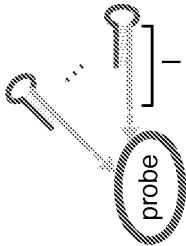


FIG. 44C

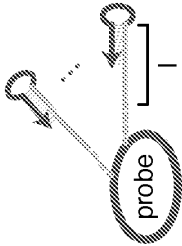


FIG. 44D

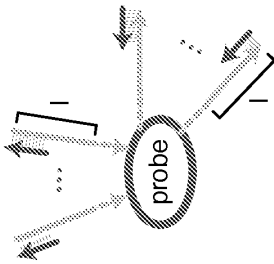


FIG. 44E



FIG. 44F

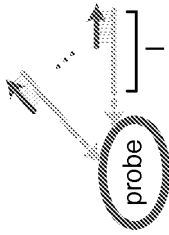


FIG. 44G

FIG. 44H

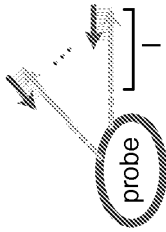


FIG. 44I

FIG. 44J

Nucleic Acid Probe
Multiple Initiators

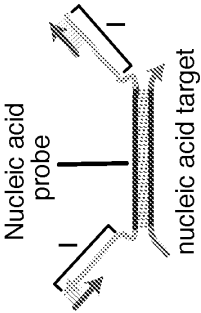


FIG. 44N

Nucleic Acid Probe
Single Initiator

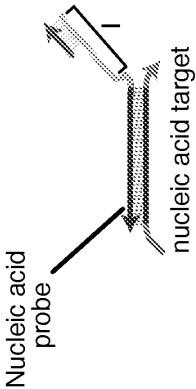


FIG. 44M

Nucleic Acid Probe
Multiple Initiators

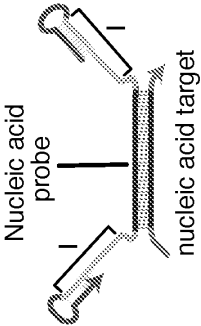


FIG. 44L

Nucleic Acid Probe
Single Initiator

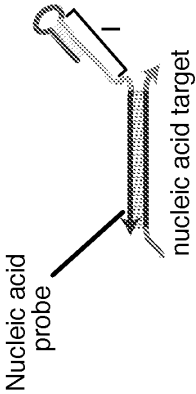


FIG. 44K

Primary-Antibody Probe
Multiple Initiators

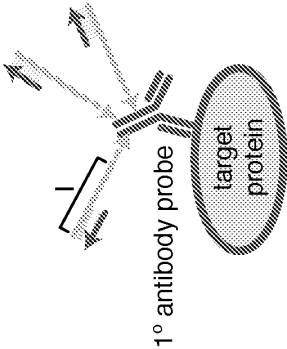


FIG. 44R

Primary-Antibody Probe
Single Initiator

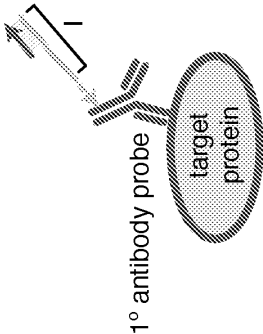


FIG. 44Q

Primary-Antibody Probe
Multiple Initiators

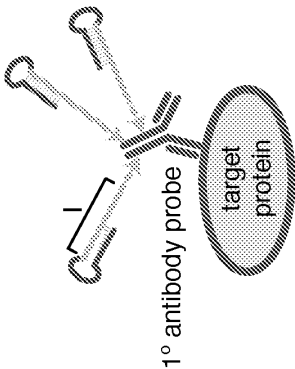


FIG. 44P

Primary-Antibody Probe
Single Initiator

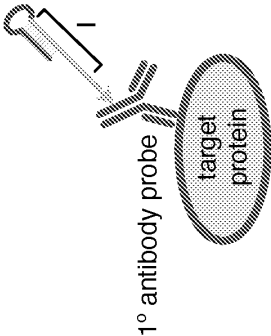


FIG. 44O

Secondary-Antibody Probe
Multiple Initiators

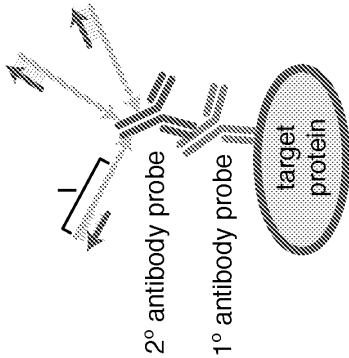


FIG. 44V

Secondary-Antibody Probe
Single Initiator

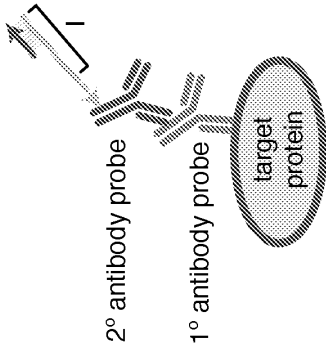


FIG. 44U

Secondary-Antibody Probe
Multiple Initiators

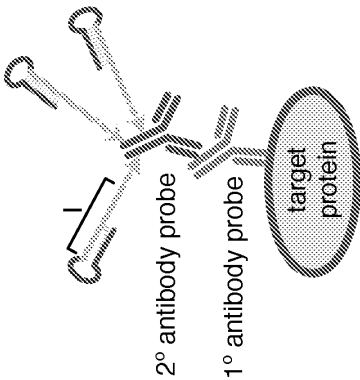


FIG. 44T

Secondary-Antibody Probe
Single Initiator

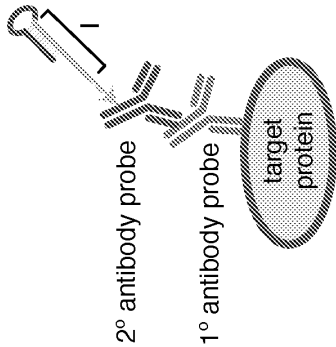


FIG. 44S

Nanobody Probe
Multiple Initiators

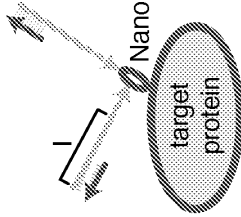


FIG. 44Z

Nanobody Probe
Single Initiator

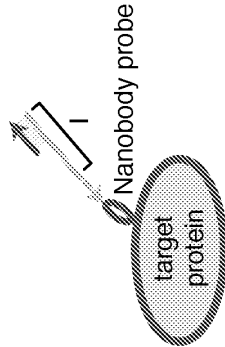


FIG. 44Y

Nanobody Probe
Multiple Initiators

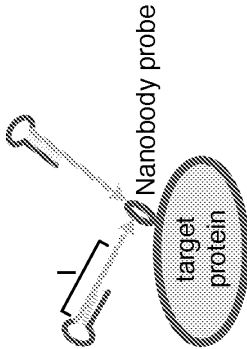


FIG. 44X

Nanobody Probe
Single Initiator

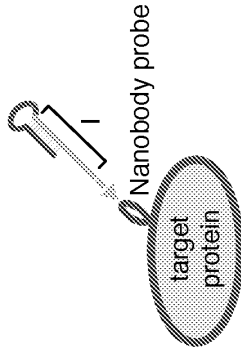


FIG. 44W

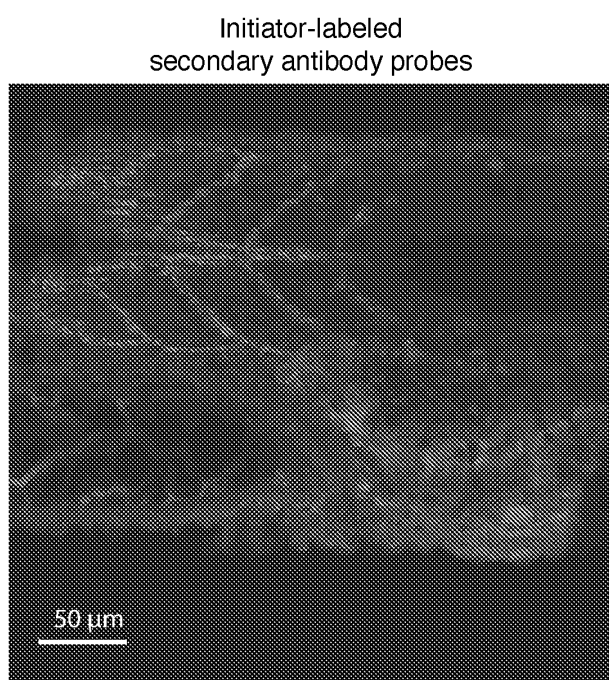


FIG. 45A

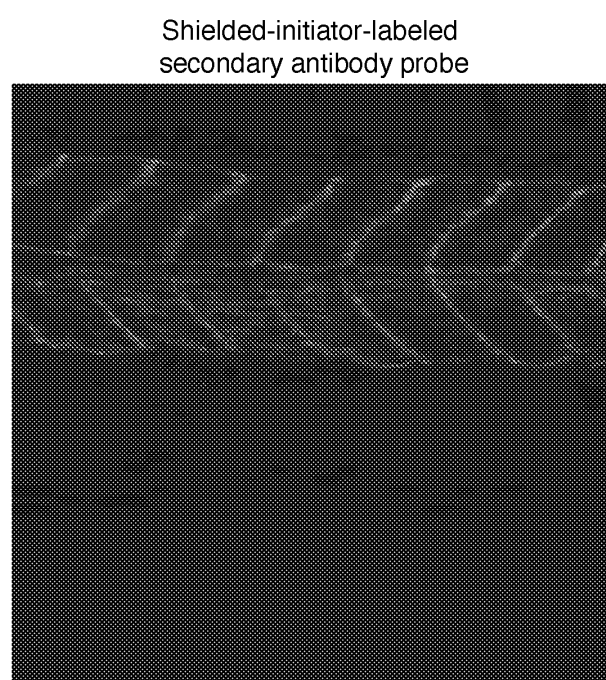


FIG. 45B

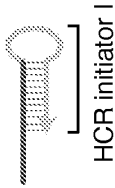


FIG. 46A



FIG. 46B

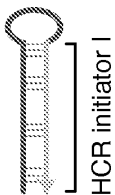


FIG. 46C

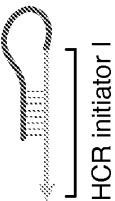


FIG. 46D

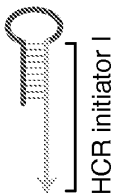


FIG. 46E



FIG. 46F



FIG. 46G



FIG. 46H

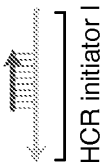


FIG. 46I



FIG. 46J



FIG. 46K



FIG. 46L

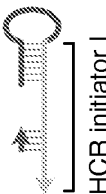


FIG. 46M

Hairpin H1 including toehold for auxiliary strand nucleation	5'- CGGGTTAAAGTTGAGTGGAGATATAGAGGCAGGGACA AAGTCTAATCCGTCCTGCCTCTATATCTCCACTCTATC AT-3' (SEQ ID NO: 1)
Hairpin H2	5'- GTCCTGCCTCTATATCTCCACTCAACTTTAACCCGGA GTGGAGATATAGAGGCAGGGACGGATTAGACTTT-3' (SEQ ID NO: 2)
Initiator I1	5'-GTCCCTGCCTCTATATCTCCACTCAACTTTAACCCG- 3' (SEQ ID NO: 3)
Auxiliary strand	5'- ATGATAGAGTGGAGATATAGAGGCAGGGACGGATTAG ACTTT-3' (SEQ ID NO: 4)

FIG. 47