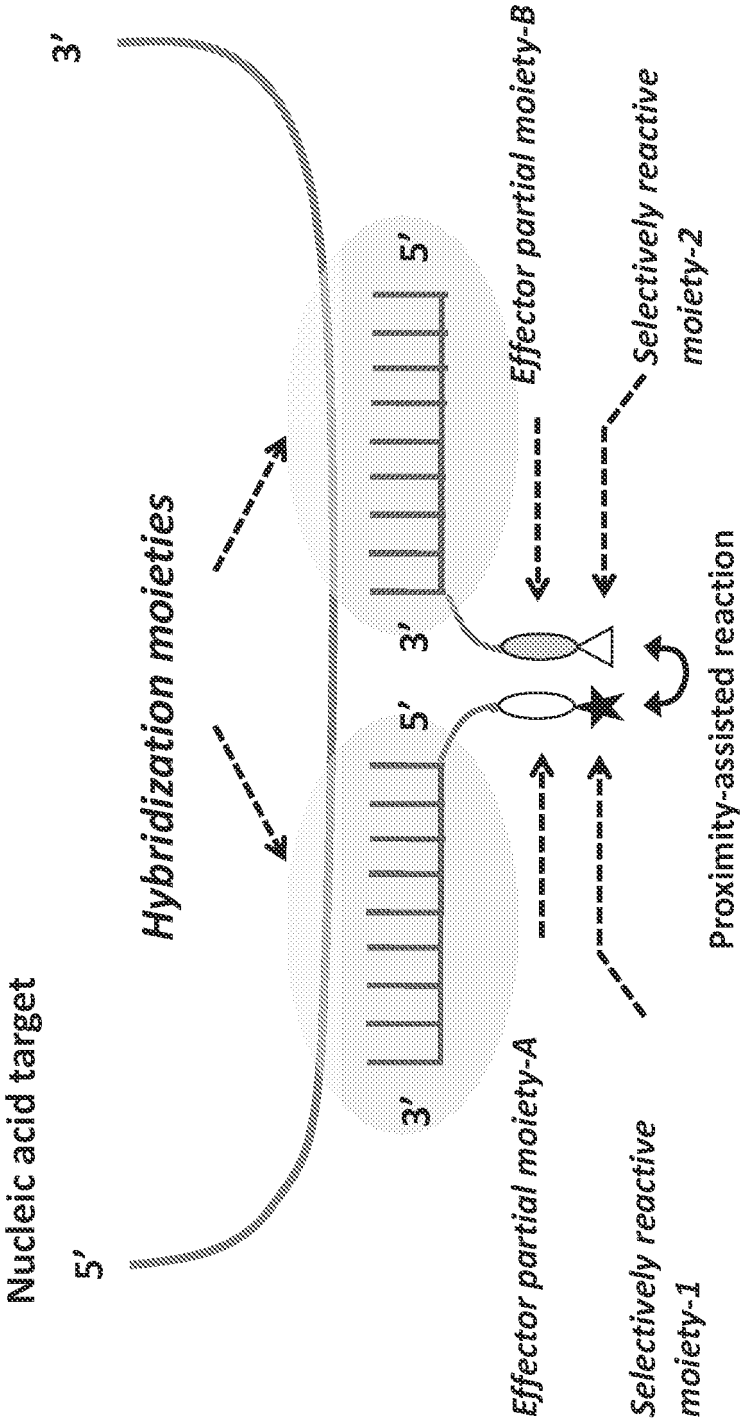

Nucleic acid target
5'
3'
Hybridization moieties
3'
5'
Effector partial moiety-A
Selectively reactive moiety-1
5'
3'
Effector partial moiety-B
Selectively reactive moiety-2
Proximity-assisted reaction

Figure 1A

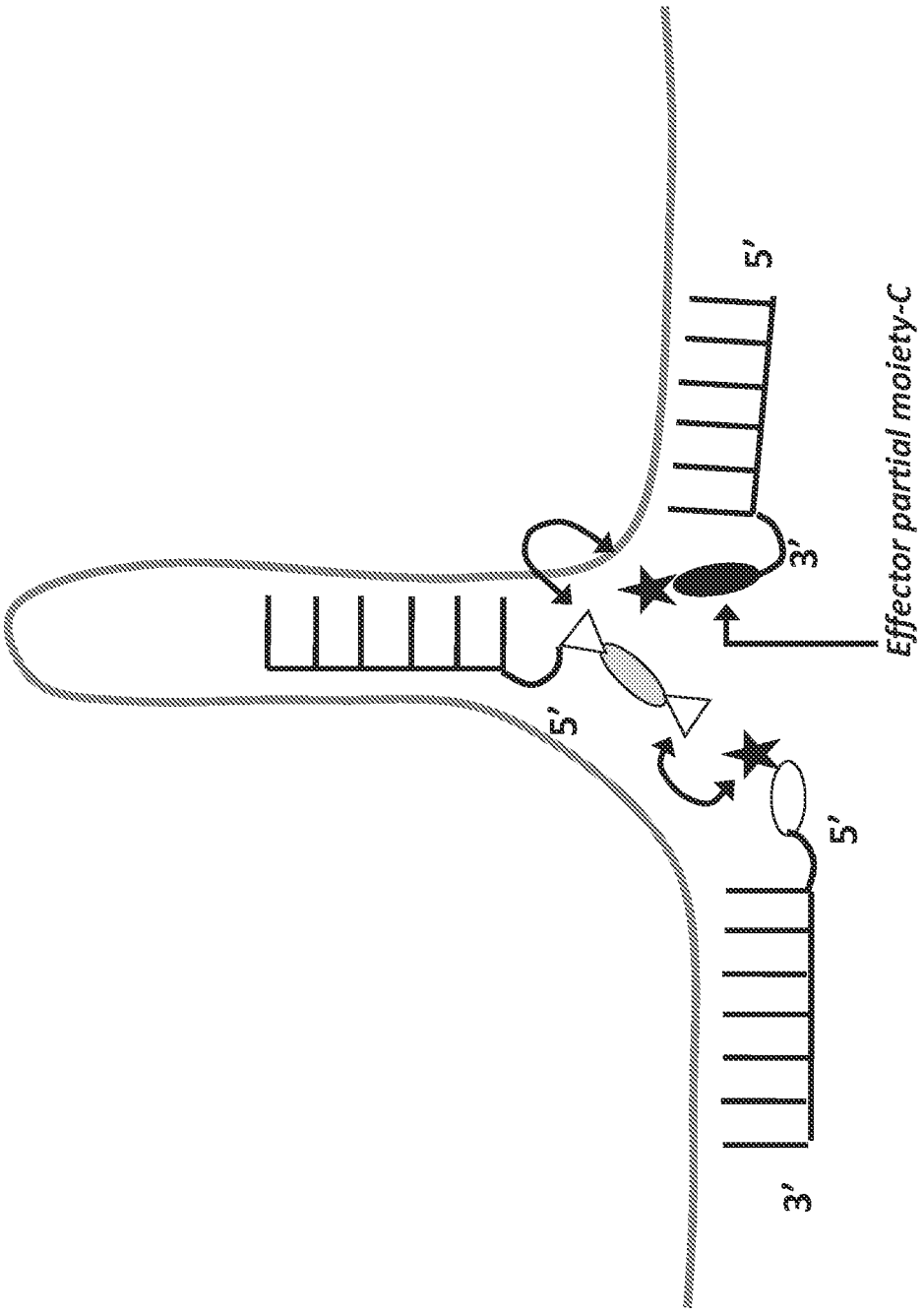


Figure 1B

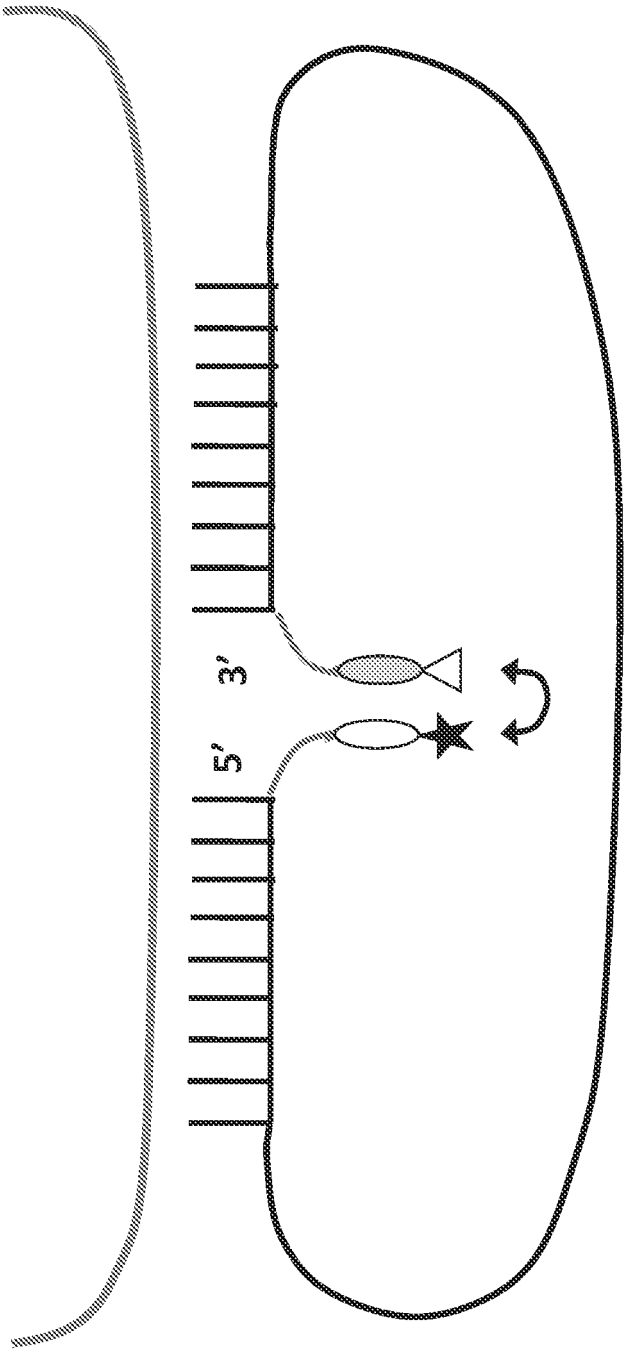


Figure 1C

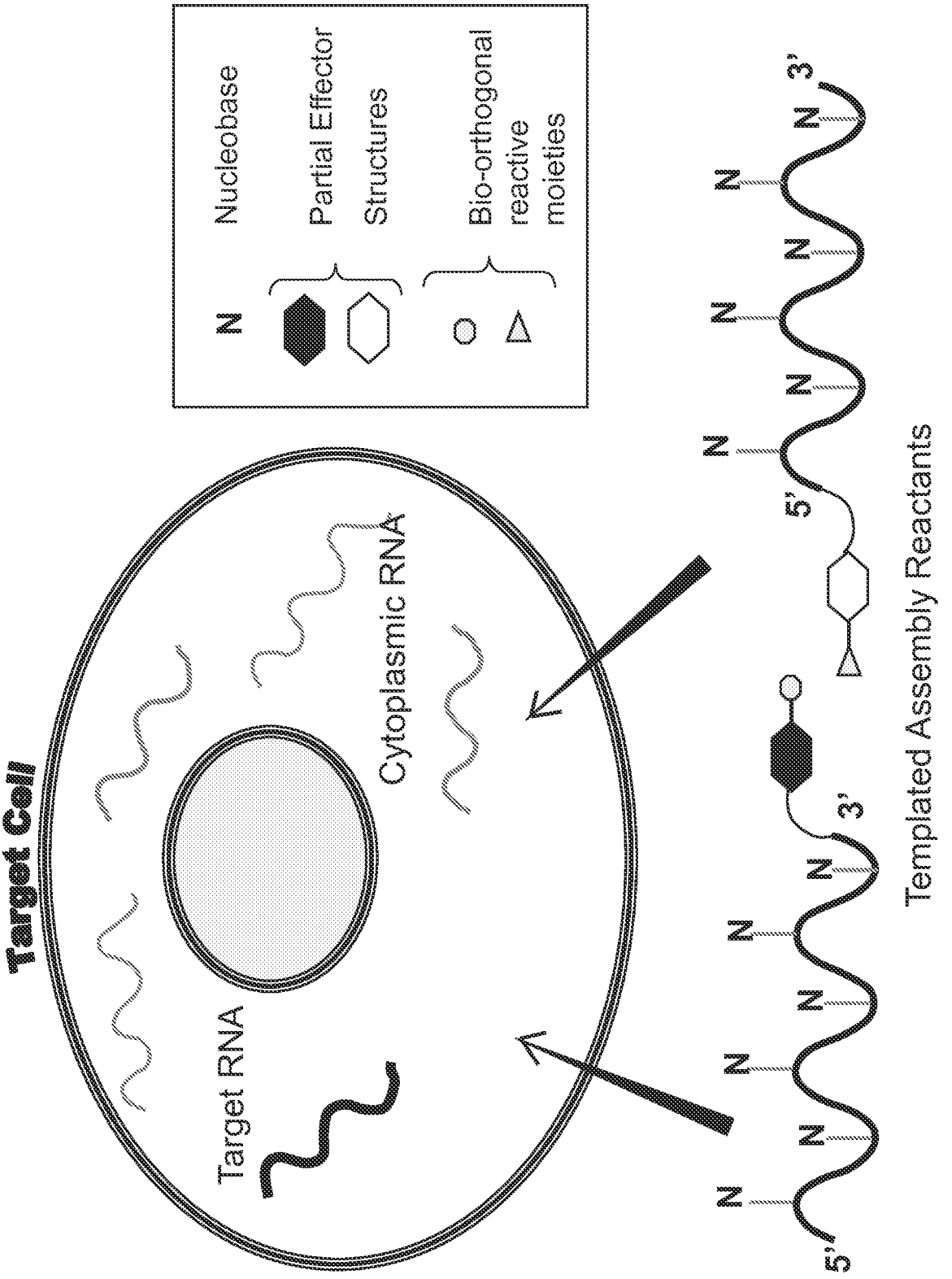

Nucleobase
N
Partial Effector
Structures
Bio-orthogonal
reactive
moieties
Target Cell
Target RNA
Cytoplasmic RNA
5'
3'
Templated Assembly Reactants

Figure 2A

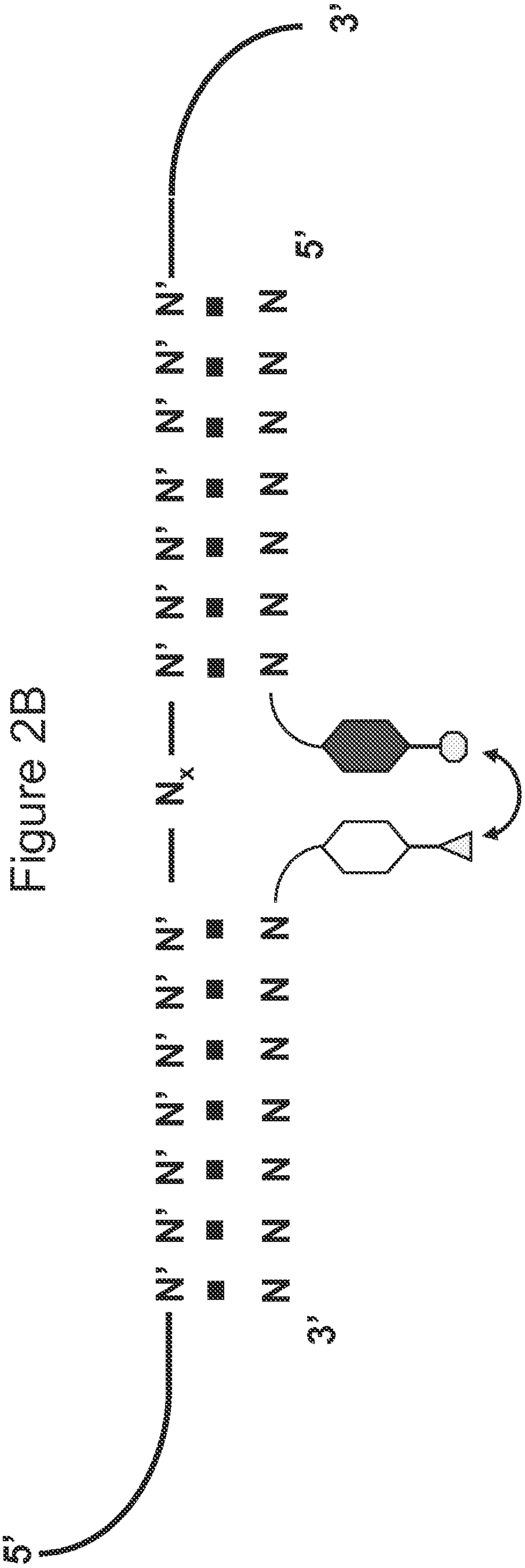


Figure 2B

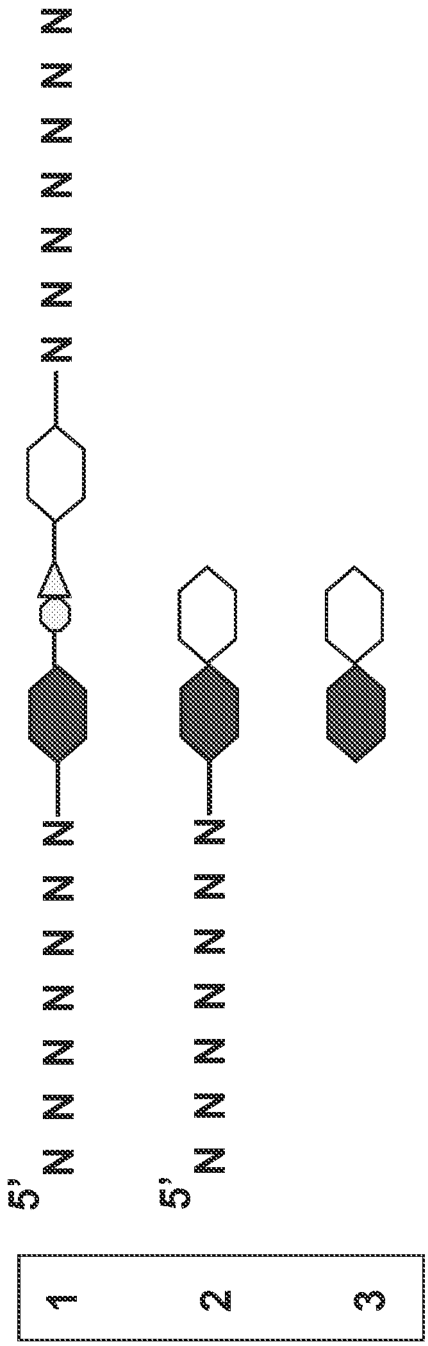


Figure 2C

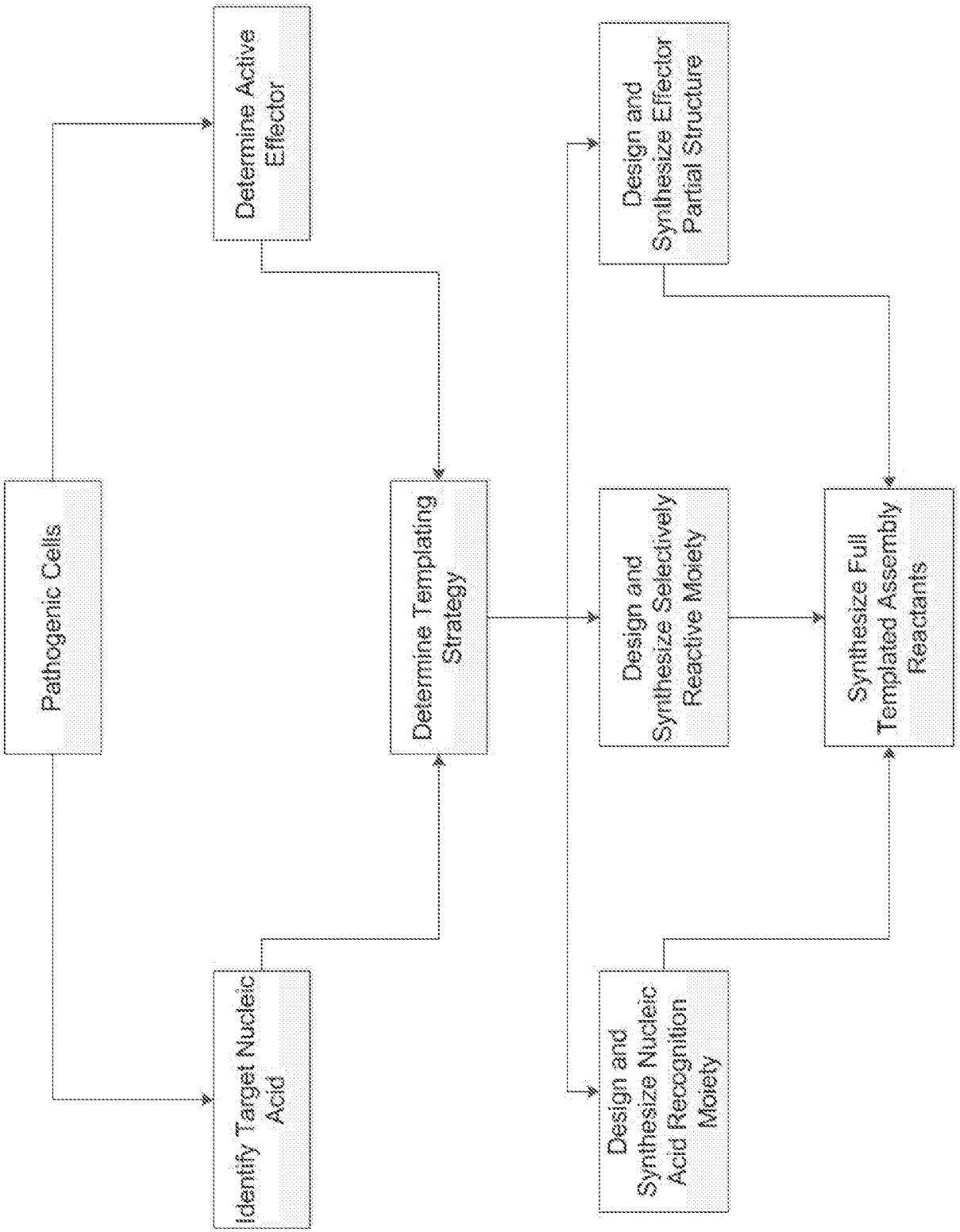

Pathogenic Cells
Determine Active Effector
Identify Target Nucleic Acid
Determine Templating Strategy
Design and Synthesize Effector Partial Structure
Design and Synthesize Selectively Reactive Moiety
Design and Synthesize Nucleic Acid Recognition Moiety
Synthesize Full Templated Assembly Reactants

Figure 3

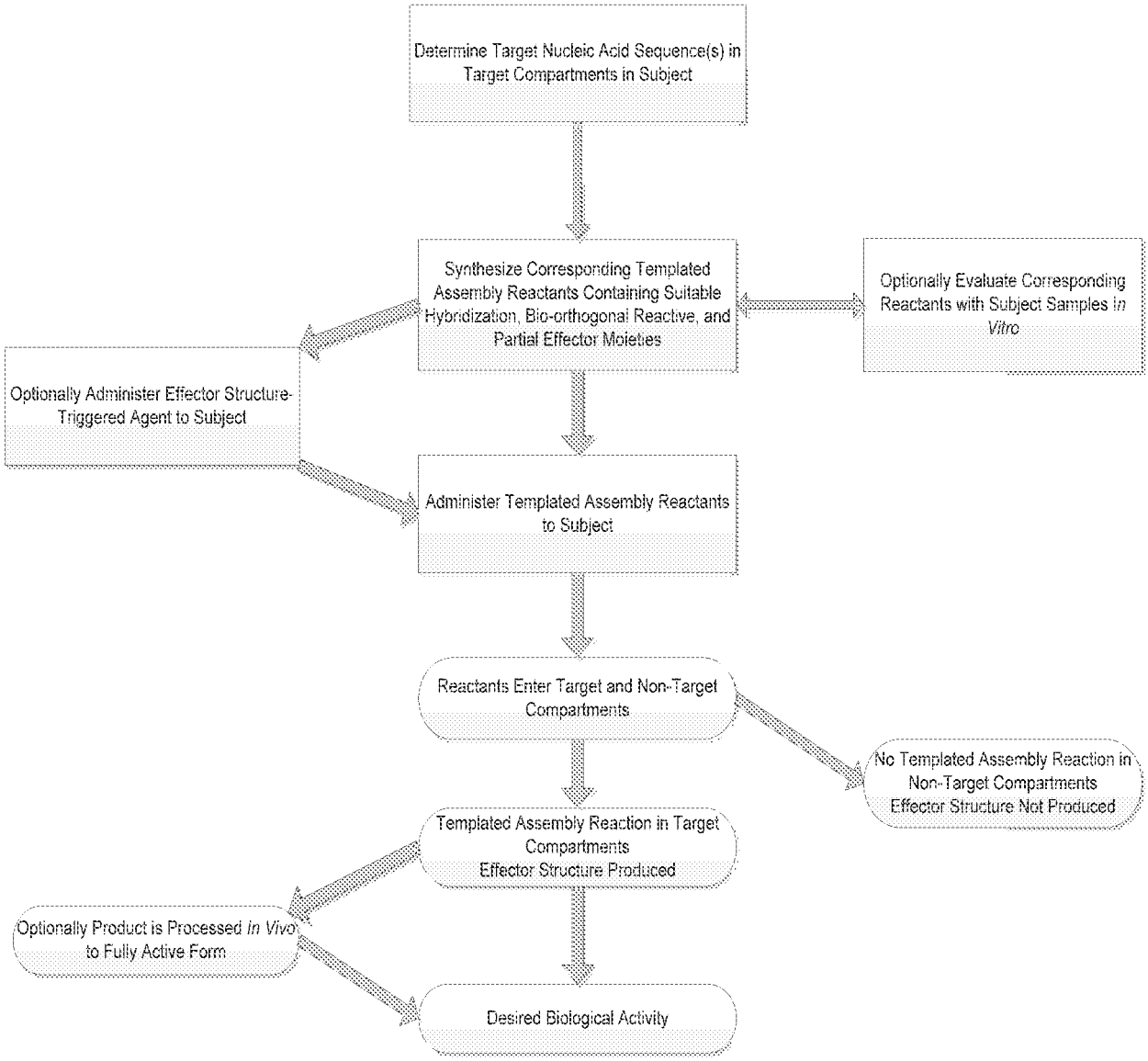
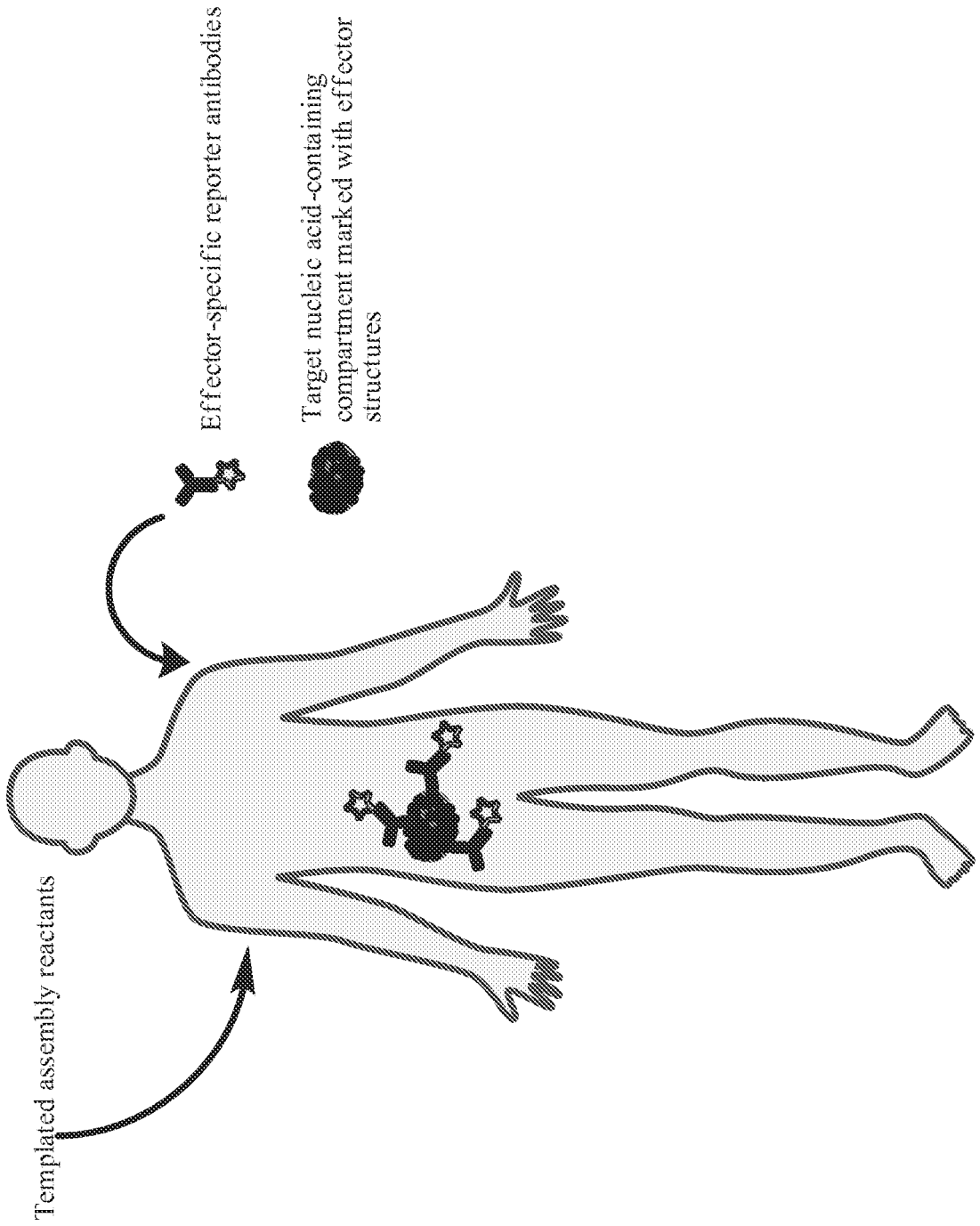

Determine Target Nucleic Acid Sequence(s) in Target Compartments in Subject
Synthesize Corresponding Templated Assembly Reactants Containing Suitable Hybridization, Bio-orthogonal Reactive, and Partial Effector Moieties
Optionally Evaluate Corresponding Reactants with Subject Samples *in Vitro*
Optionally Administer Effector Structure-Triggered Agent to Subject
Administer Templated Assembly Reactants to Subject
Reactants Enter Target and Non-Target Compartments
No Templated Assembly Reaction in Non-Target Compartments
Effector Structure Not Produced
Templated Assembly Reaction in Target Compartments
Effector Structure Produced
Optionally Product is Processed *In Vivo* to Fully Active Form
Desired Biological Activity

Figure 4



Localization of target compartments in a subject

Figure 5

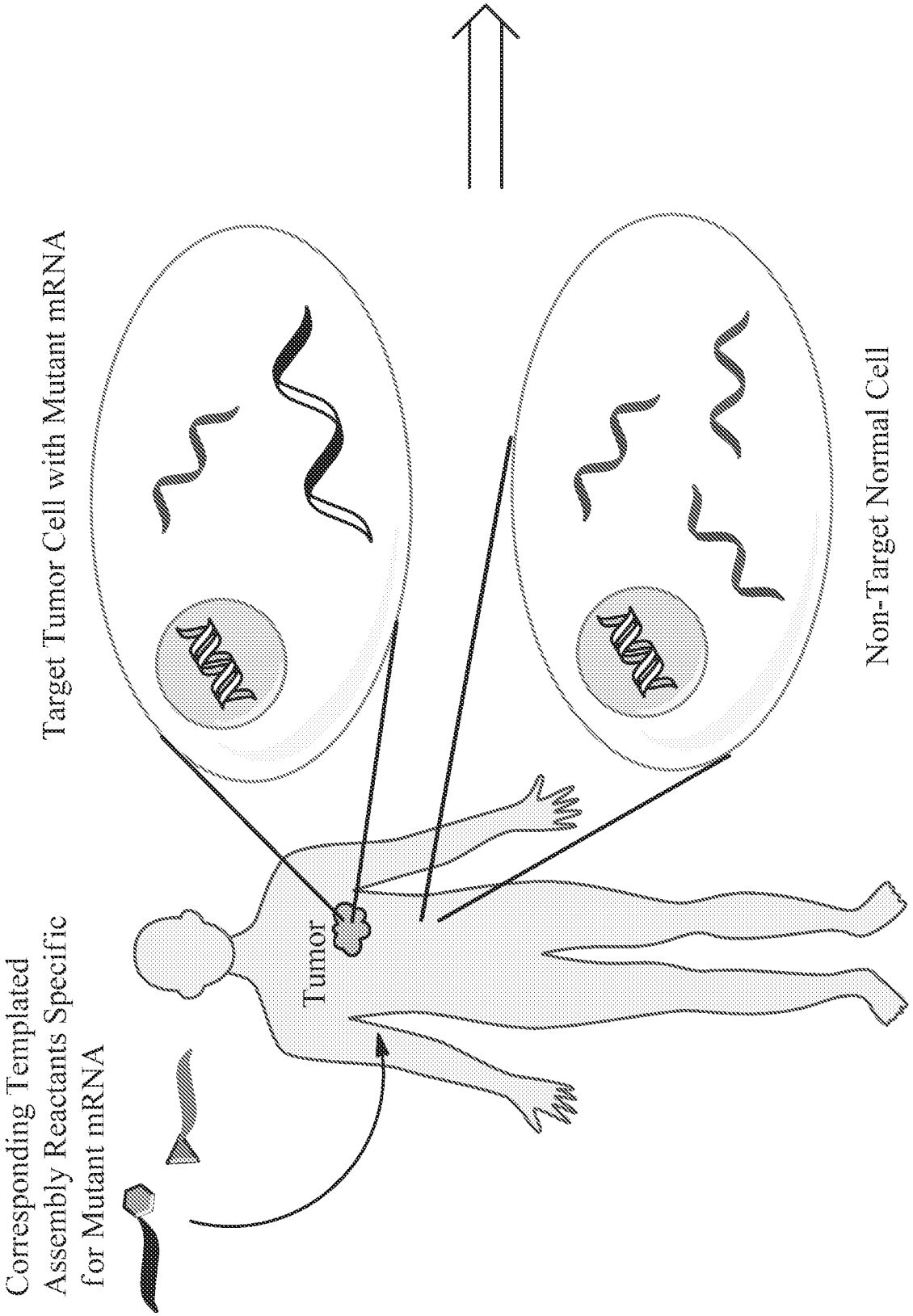

Corresponding Templated Assembly Reactants Specific for Mutant mRNA
Target Tumor Cell with Mutant mRNA
Tumor
Non-Target Normal Cell

Figure 6

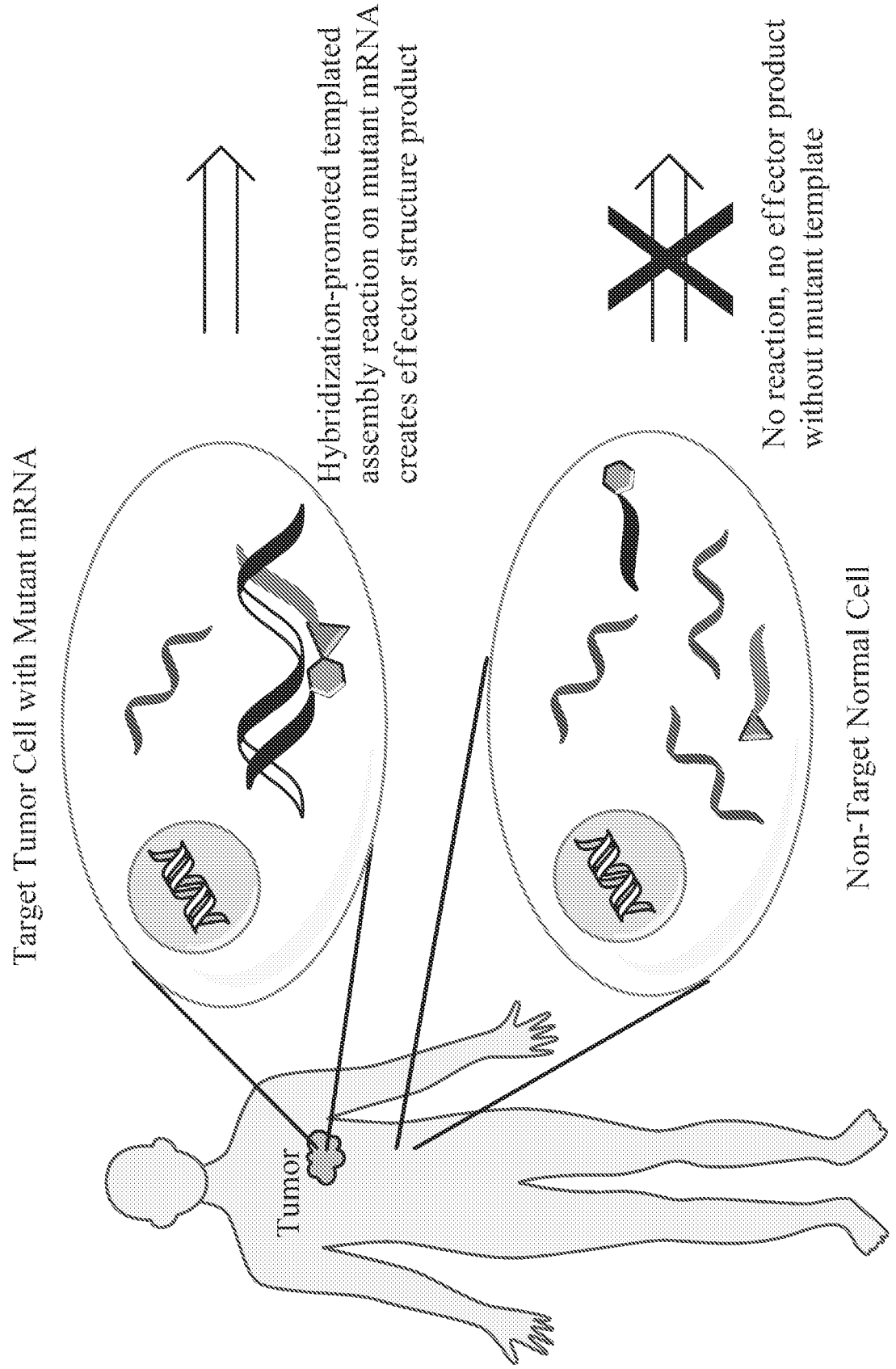

Target Tumor Cell with Mutant mRNA
Hybridization-promoted templated assembly reaction on mutant mRNA creates effector structure product
No reaction, no effector product without mutant template
Non-Target Normal Cell
Tumor

Figure 7

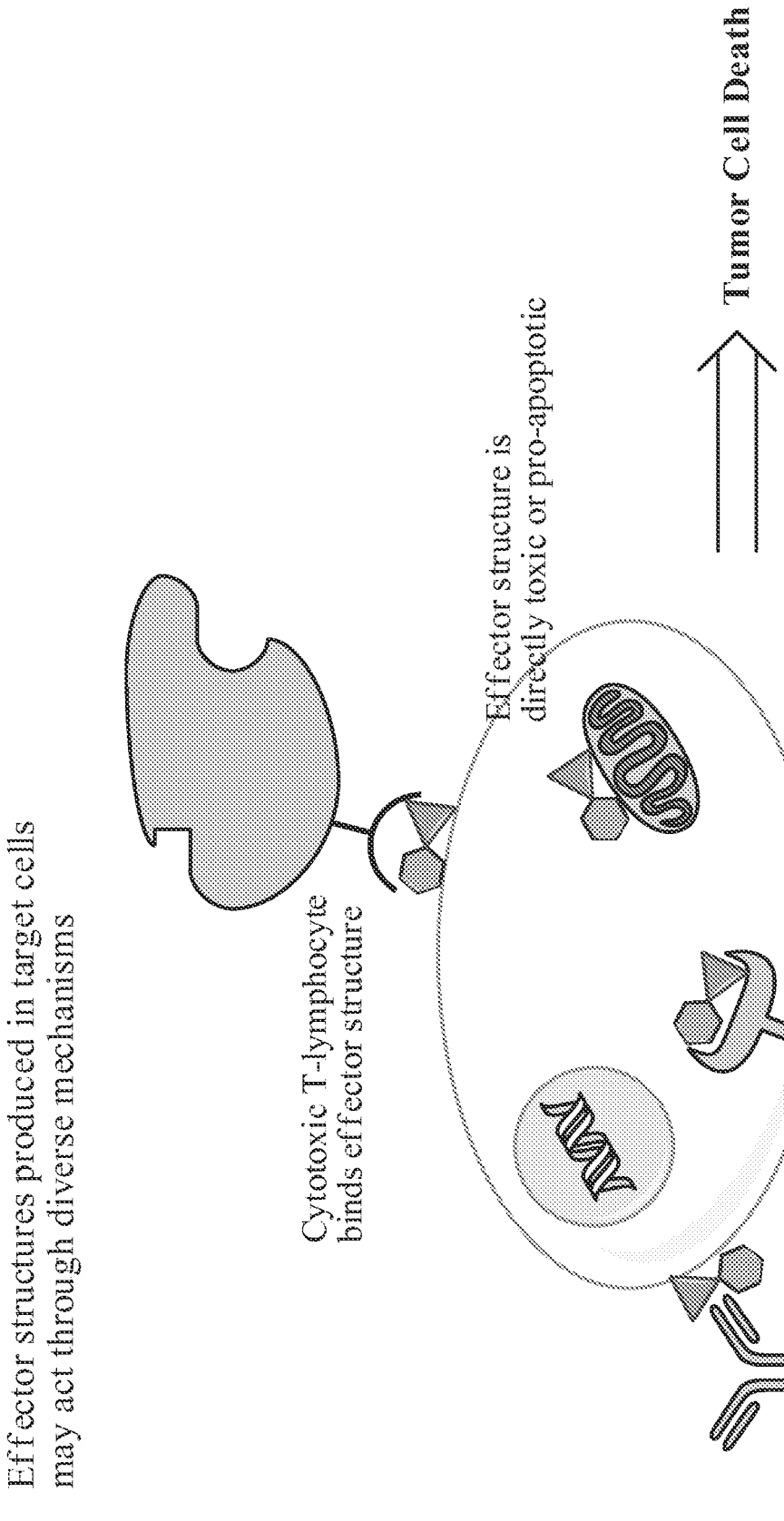

Effector structures produced in target cells
may act through diverse mechanisms
Cytotoxic T-lymphocyte
binds effector structure
Effector structure is
directly toxic or pro-apoptotic
Tumor Cell Death
Intracellular receptor binds
effector structure
Therapeutic antibody
binds effector structure

Figure 8

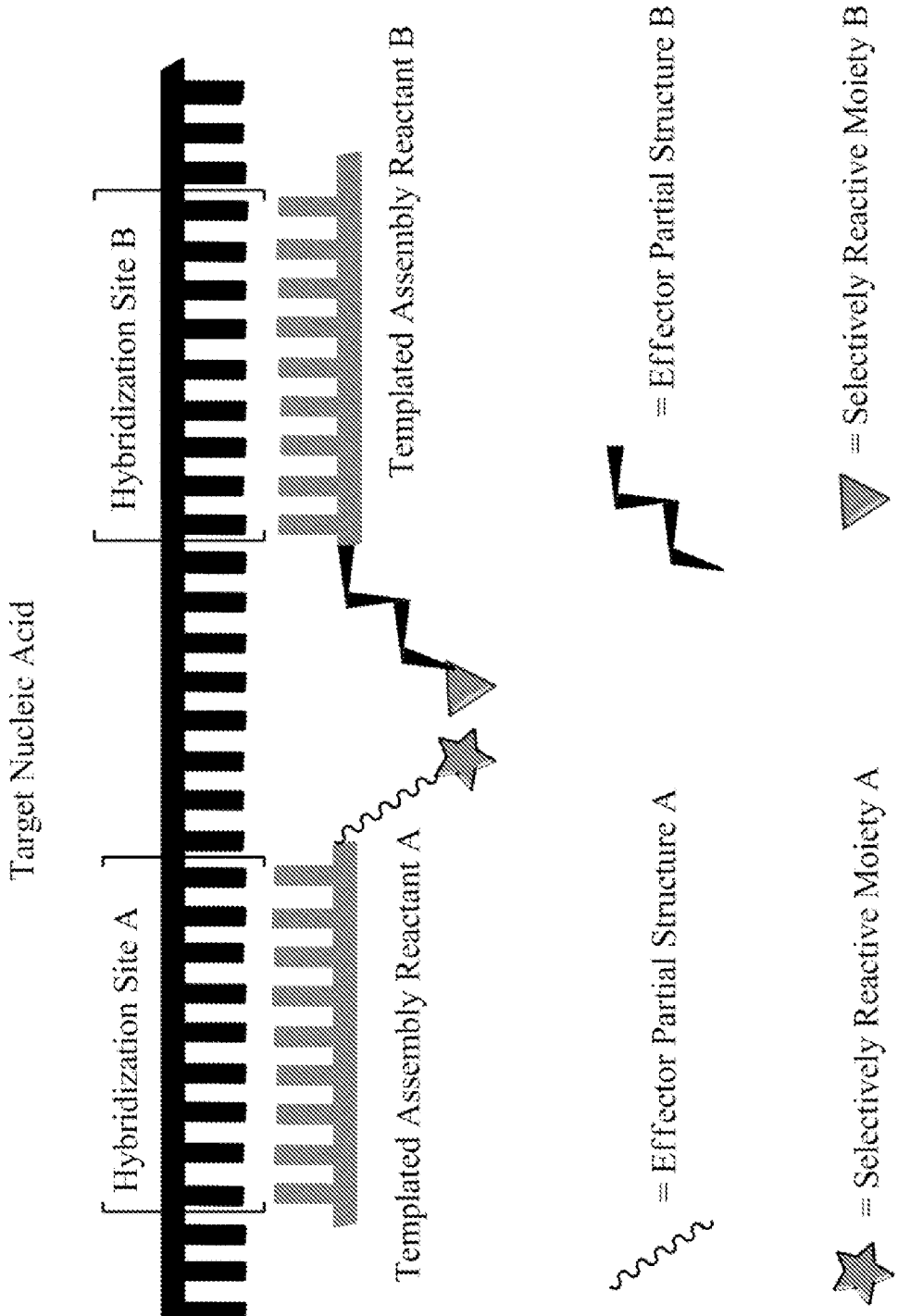

Target Nucleic Acid
Hybridization Site A
Hybridization Site B
Templated Assembly Reactant A
Templated Assembly Reactant B
= Effector Partial Structure A
= Effector Partial Structure B
= Selectively Reactive Moiety A
= Selectively Reactive Moiety B

Figure 9

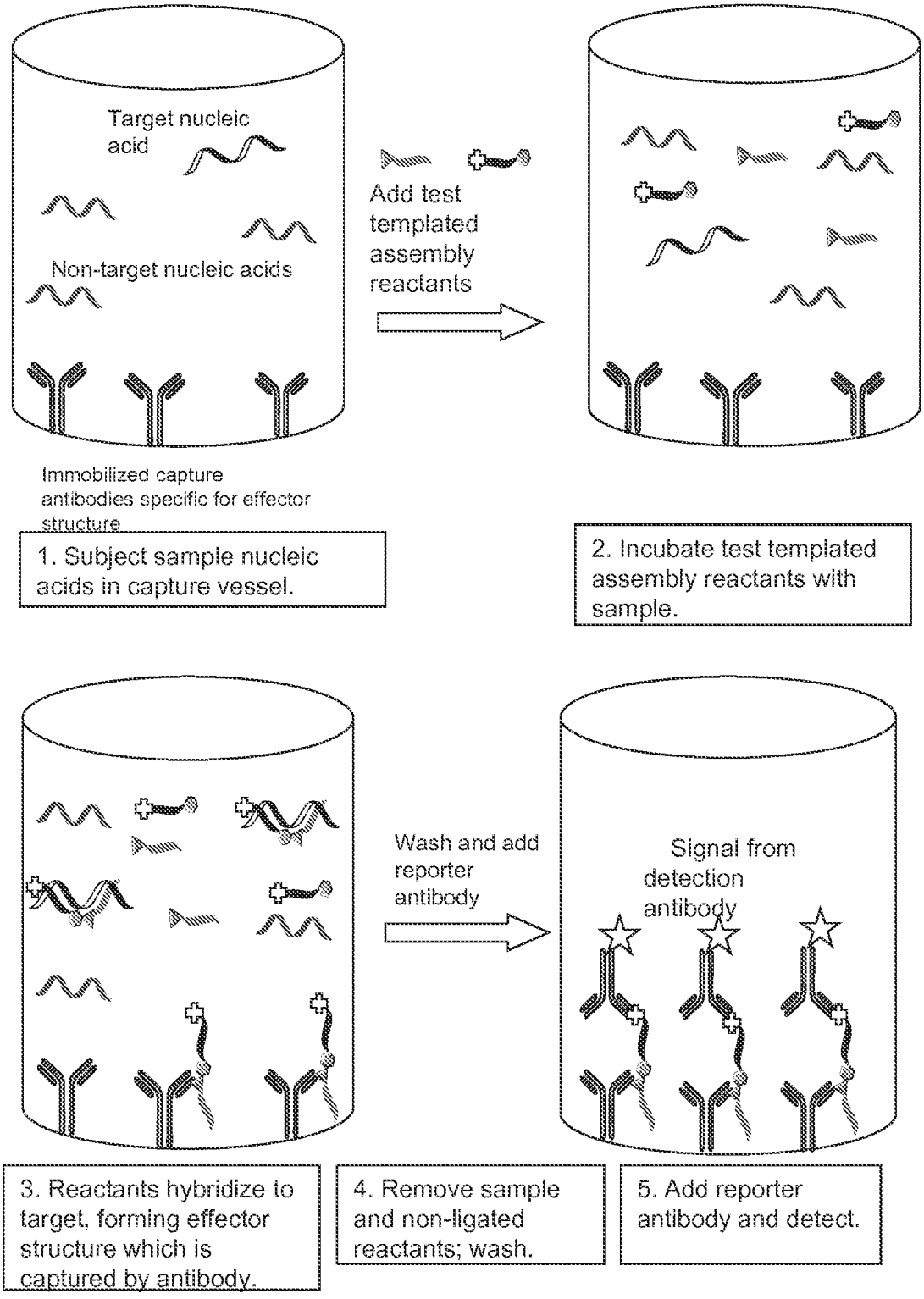

Target nucleic acid
Non-target nucleic acids
Immobilized capture antibodies specific for effector structure
1. Subject sample nucleic acids in capture vessel.
Add test templated assembly reactants
2. Incubate test templated assembly reactants with sample.
3. Reactants hybridize to target, forming effector structure which is captured by antibody.
Wash and add reporter antibody
4. Remove sample and non-ligated reactants; wash.
Signal from detection antibody
5. Add reporter antibody and detect.

Figure 10

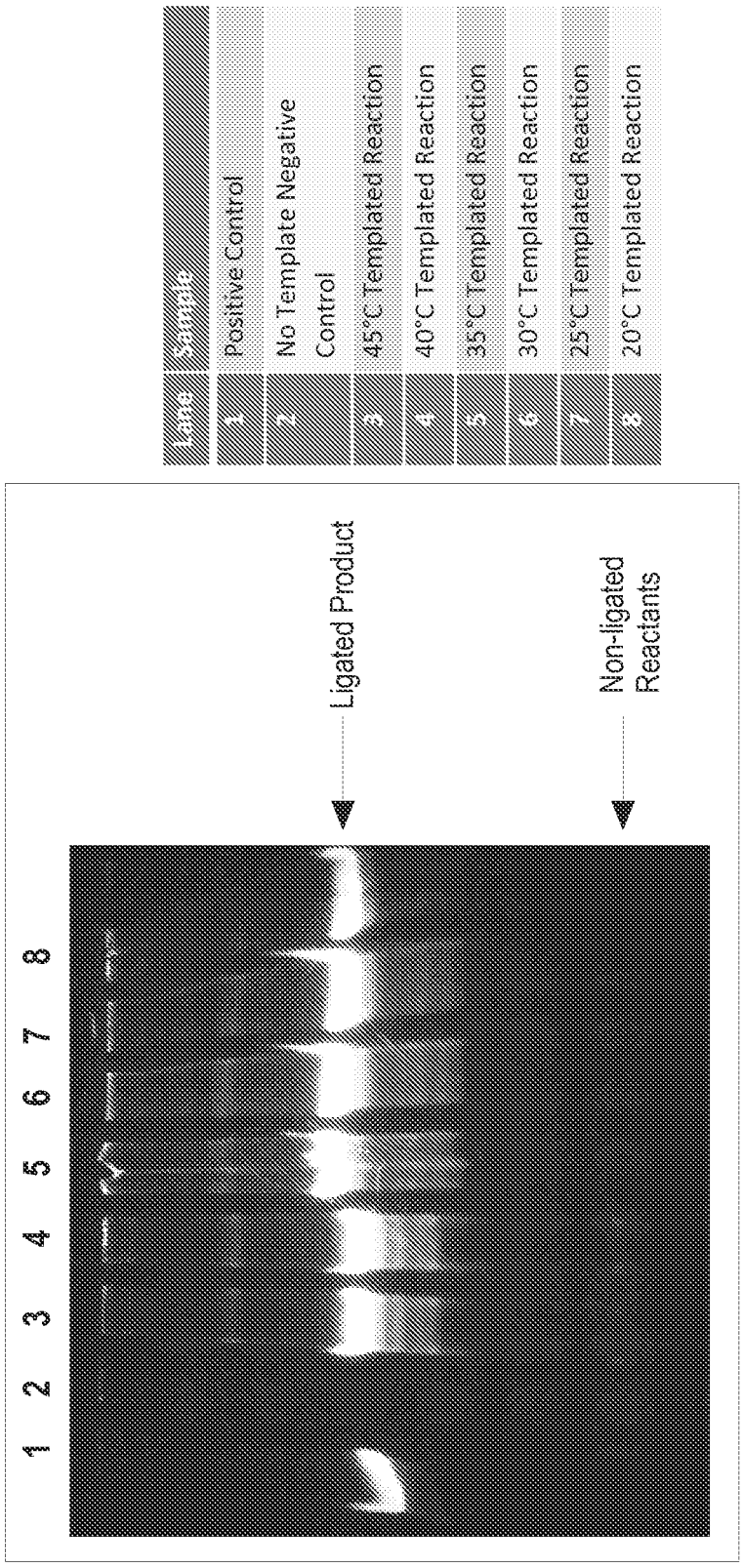

Lane
Sample
1
Positive Control
2
No Template Negative Control
3
45°C Templated Reaction
4
40°C Templated Reaction
5
35°C Templated Reaction
6
30°C Templated Reaction
7
25°C Templated Reaction
8
20°C Templated Reaction
1 2 3 4 5 6 7 8
Ligated Product
Non-ligated Reactants

Figure 11

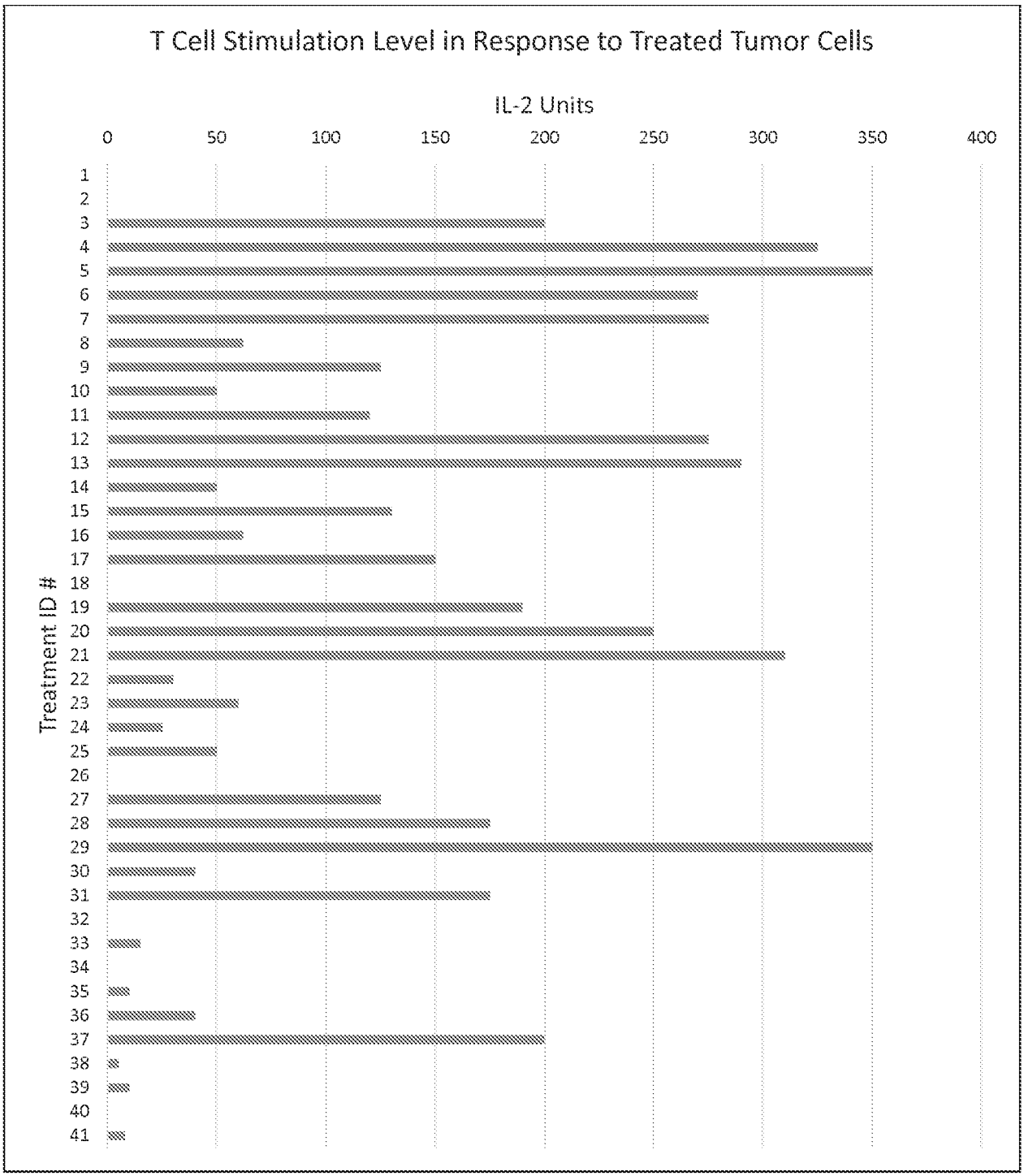

T Cell Stimulation Level in Response to Treated Tumor Cells
IL-2 Units
0
50
100
150
200
250
300
350
400
Treatment ID #
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

Figure 12