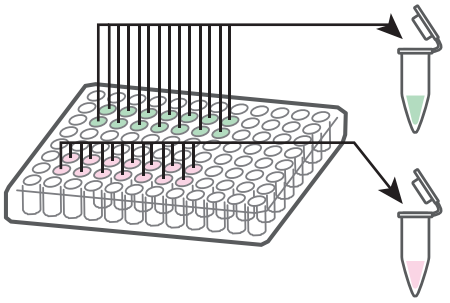
ISHpalette™ Quick Guide

Sep. 2025

Preparation of Target Probe Mix

ssDNA oligos synthesized by an oligo manufacturer

Mix equal amounts of the 36-base P1 probes and equal amounts of the 39-base P2 probes separately.



P1 Probe Mixture

P2 Probe Mixture

Add TE to adjust each ssDNA conc. to 1–10 μM.

↓

Frozen storage.

Day 1

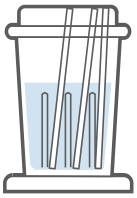
Preparation / Pre-treatment

(1 ～ 14 : For details, see pages 6–10 of the ISHpalette protocol.)

0 Hybridization buffer

Return to room temp.

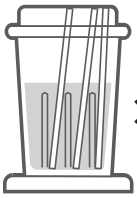
1 Sample slides



× 1 or more times

PBS 5 min.

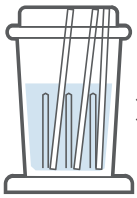
2



× 1 time

Methanol 10 min.

3

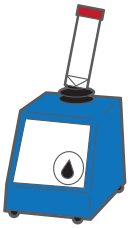


× 2 times

PBST 5 min.

Pre-hybridization

4 Hybridization buffer

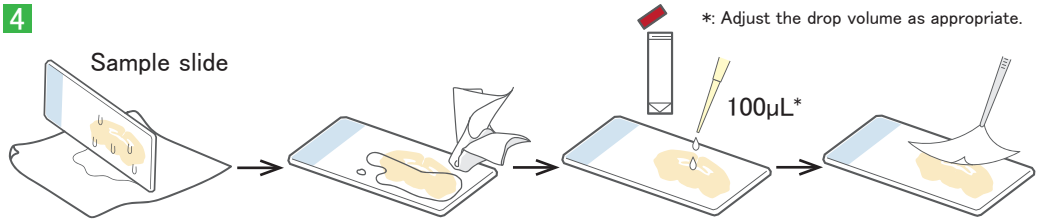


3 sec. × 3 times

Mix thoroughly.

4 Sample slide

*: Adjust the drop volume as appropriate.

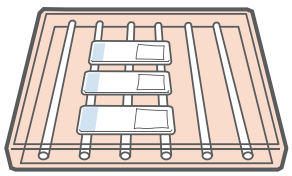


Wipe off liquid outside the sample using Kimwipes.

Drop Hybridization buffer.

Cover with Parafilm.

4



In a humid chamber, incubate at room temp. for 5 min.

Hybridization

5 Hybridization buffer

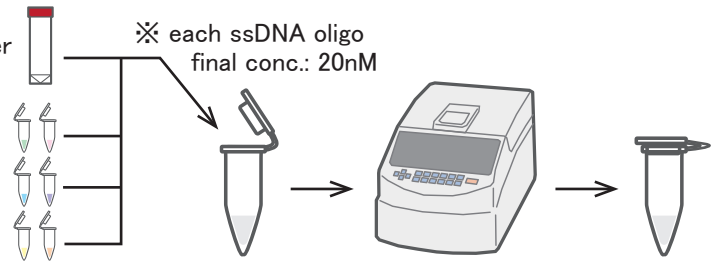
Probe Mixture

Gene A P1, P2

Gene B P1, P2

Gene C P1, P2

※ each ssDNA oligo final conc.: 20nM



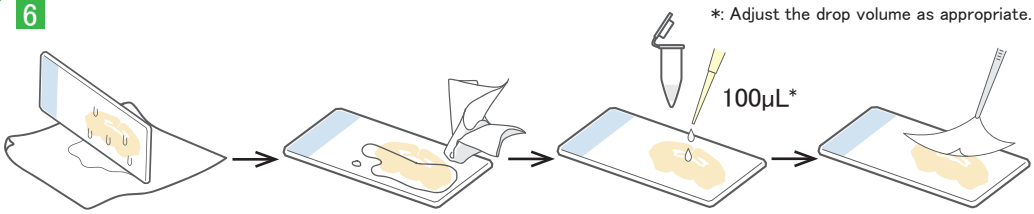
Preparation of probe Hybridization solution

95℃ 3 min.

Room temp.

6

*: Adjust the drop volume as appropriate.

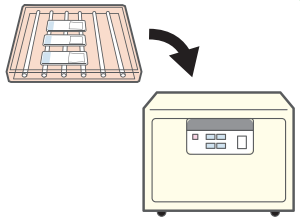


Wipe off liquid outside the sample using Kimwipes.

Drop probe Hybridization solution.

Cover with Parafilm.

6



In a humid chamber, incubate at 37℃ overnight.

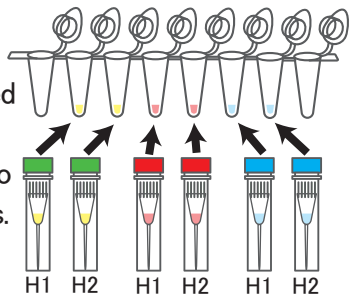
Day 2

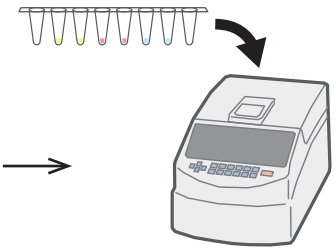
Heat treatment of hairpin DNA

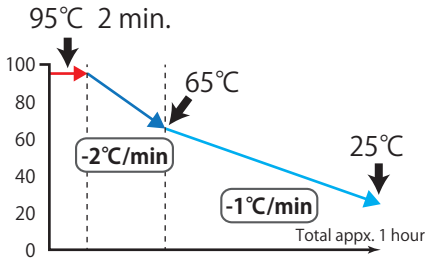
(1 ～ 14 : For details, see pages 6–10 of the ISHpalette protocol.)

7

Dispense the required amount of Short hairpin amplifier into separate PCR tubes.







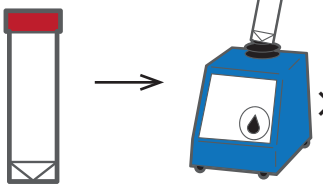
Perform heating → cooling using a thermal cycler.

While performing the heating and cooling, complete steps 8 & 9 below.

Washing slides

8

Amplification buffer

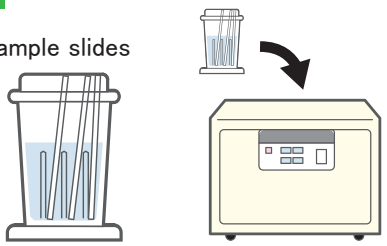


3 sec.
× 3 times

Return to room temp. Mix thoroughly.

9

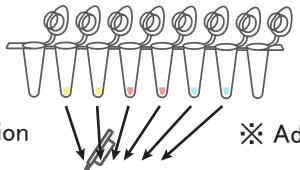
Sample slides



× 3 times

0.5 × SSCT 37℃ 10 min.

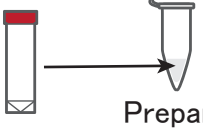
11



Cooled
Short hairpin amplifier

Amplification buffer

※ Add using separate pipette tips.



Final conc. of each hairpin DNA: 60 nM
+ Nuclear staining reagent (e.g. Hoechst)

3 sec.
× 3 times

Preparation of HCR reaction solution

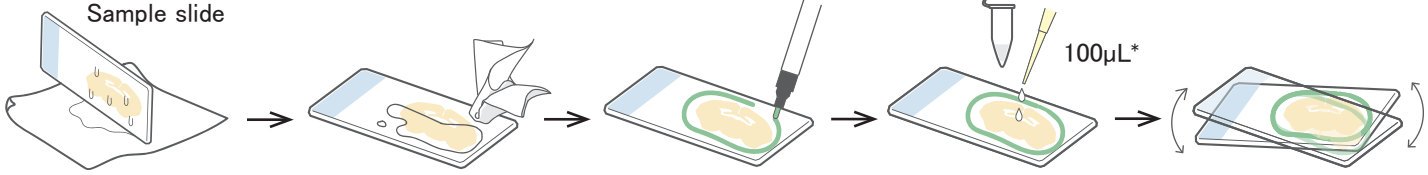
Mix throughly.

HCR

12

*: Adjust the drop volume as appropriate.

Sample slide



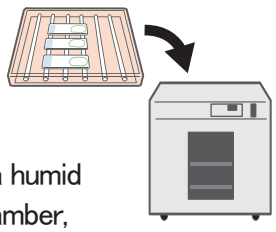
Wipe off liquid outside the sample using Kimwipes.

Draw a hydrophobic barrier line around the tissue using a PAP pen.

Drop HCR reaction solution.

Tilt slide several times to spread evenly.

12

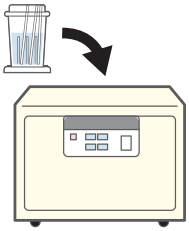


In a humid chamber, incubate at 25 ℃ or room temp. for 2 hours.

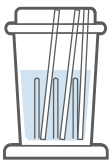
Washing slides

13

PBST



× 3 times

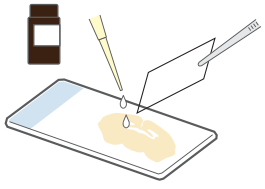


× 1 time

37℃ 10 min. PBS at room temp. >5 min.

14

Anti-fade mounting medium



Mounting