Design of Target Probes *(see figure on page 2)*

Each target probe set consists of two DNA probes:

- **Probe 1 (36 bases):** 25-base mRNA binding region, 2-base spacer, and a 9-base 5′ half of the initiator sequence.
- **Probe 2 (39 bases):** 25-base mRNA binding region, 2-base spacer, and a 12-base 3′ half of the initiator sequence.

Design guidelines:

- Select a 52-base region with 40–60% GC content (ideally 45–55%). Design two 25-base probes covering the 5′ and 3′ sides of this region. Avoid repetitive sequences.
- Use NCBI BLAST to minimize off-target complementarity. Cross-hybridization does not occur if sequence homology is ≤50%.
- Typically design 20 probe pairs (36- and 39-base) per target mRNA. If GC content or sequence homology limits probe design, a smaller number of probes can be used.
- Prefer CDS regions over UTRs as binding sites.

Note: *We also provide a custom design service for target probes. Based on the provided sequence information, users will need to order single-stranded oligos from an oligo synthesis company.*

Preparation of Target Probes

Combine all designed probes for each target mRNA, mix thoroughly, and dilute in TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). Store at −20 °C.
For use, prepare probe mixtures at ≥1 μM concentration.

Example 1: *10 probe sets per target mRNA*

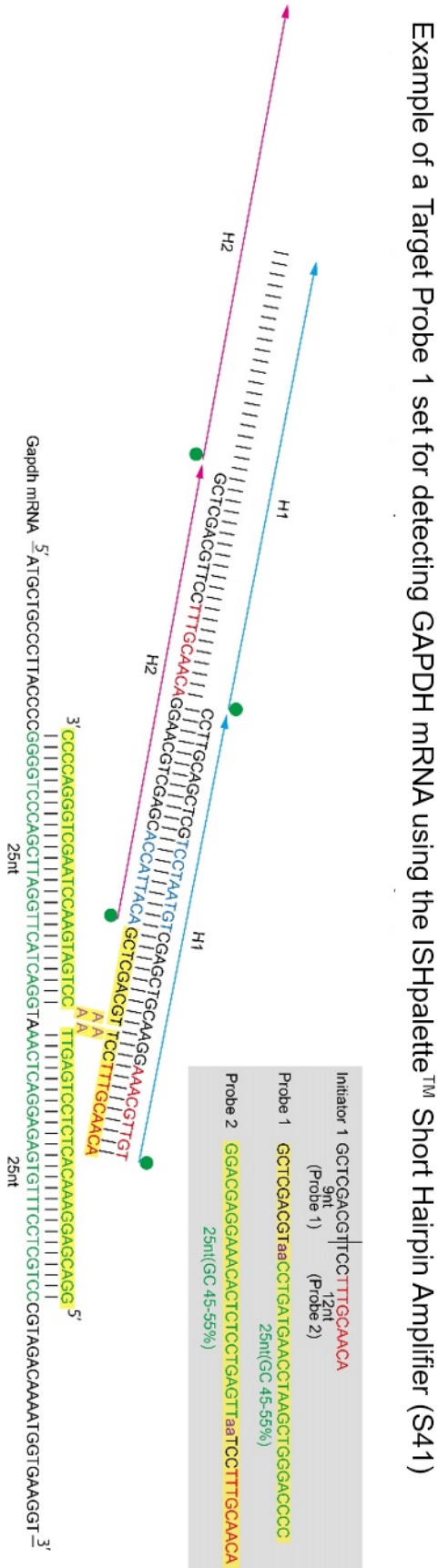
- Starting from 100 μM delivered stock, mix equal amounts of the 10 Probe 1 oligos → prepare a 10 μM Probe 1 stock; store at −20 °C.
- Do the same for Probe 2.
- For use, mix equal amounts of Probe 1 and Probe 2 to 1 μM in TE (10 μM Probe 1 mix 1 μL + 10 μM Probe 2 mix 1 μL + TE 8 μL = 10 μL).
- Add 2 μL of this mixture to 100 μL of hybridization buffer (final 20 nM).

Example 2: *20 probe sets per target mRNA*

- Starting from 100 μM delivered stock, mix equal amounts of the 20 Probe 1 oligos → prepare a 5 μM Probe 1 stock; store at −20 °C.
- Do the same for Probe 2.
- For use, mix equal amounts of Probe 1 and Probe 2 to 1 μM in TE (5 μM Probe 1 mix 2 μL + 5 μM Probe 2 mix 2 μL + TE 6 μL = 10 μL).
- Add 2 μL of this mixture to 100 μL of hybridization buffer (final 20 nM).

Note: *Hybridization with only one probe of a pair does not yield detectable signals. Single-probe mixtures may be used as negative controls.*

Reference: Tsuneoka & Funato, 2020. Front. Mol. Neurosci.



- This development product has been co-developed and commercialized in collaboration with Toho University, utilizing the technology (Patent No. 7482506) developed by Associate Professor Yousuke Tsuneoka, Department of Anatomy (Division of Microscopic and Morphological Anatomy), Faculty of Medicine, Toho University.