Protein Domain Microarray Compound Screening

1. Incubate the slide in blocking buffer for 1 h at room temperature (or overnight at 4°C).
2. Prebind the compound probe as follows:

Biotinylated Compound	100 nM
Cy3 Streptavidin (15.15 µM)	10 µL
1xPBS	to 20 µL
Total Volume	20 µL

3. Incubate on ice for 30 min.
4. To remove the unbounded FluoroLink™, it is necessary to incubate the compound probe with biotin agarose beads. During the prebinding step, prepare the beads by taking 60 µL of biotin agarose beads and wash with 1xPBST, then separate into three clean microcentrifuge tubes. Briefly spin down the tubes and discard the supernatant. Place the three tubes with the bead pellet on ice for the following step.
5. After incubation, add the labeled compound mix to one of the previously prepared tubes containing biotin beads and rock for 10 min at room temperature.
6. Add 230 µL of 1xPBST and rock 10 minutes at room temperature.
7. Centrifuge at 10,000g for 30 s with a "soft" stop at room temperature.
8. Transfer the supernatant to a clean tube from **step 4**. Repeat **steps 5 and 7** two times to remove all unbound FluoroLink™.
9. Save 230 µL of supernatant from the third tube and mix with 450 µL of blocking buffer.
10. Take out the slide from the blocking buffer (**step 1**). Do not allow the slide to dry, and place in a hybridization chamber. Add the compound probe and incubate at room temperature for 1 h in the dark.
11. Wash the probed slide three times by placing it in a glass slide-staining jar with 1xPBST. Shake for 30 min at 60 rpm at room temperature, covered from light. Change the buffer every 10 min.
12. Air-dry the slide.
13. Scan the slide with GenePix Scanner (Axon Instruments).