NxSeq™ HybCap Kit, Hybridisation-based, targeted enrichment quick protocol

1. For each enrichment reaction, build the following mixes; overfill for pipetting errors is included:

Hybridisation mix	
Component	**Volume**
Hyb Reagent 1	9.25 µL
Hyb Reagent 2	0.5 µL
Hyb Reagent 3	3.5 µL
Hyb Reagent 4	1.25 µL
HybCap™ Oligo Probes	5.5 µL
TOTAL	**20 µL**

Blocker mix	
Component	**Volume**
Blocker A	0.5 µL
Blocker B	2.5 µL
Blocker C	2.5 µL
TOTAL	**5.5 µL**

2. After pre-warming the hybridisation mix for 10 minutes at 60 °C, for each reaction, aliquot 18.5 µL of hybridisation mix to their own tubes – now known as "HMXs".
3. Combine 5 µL of blocker mix with 7 µL of each library in a tube – now known as "LBMXs".
4. Incubate the LBMXs in the thermal cycler for 5 minutes at 95 °C and then drop to the hybridisation temperature (e.g. normally 65 °C). Be sure to use a heated lid.
5. Put the HMXs in the thermal cycler and warm to the hybridisation temperature for 5 minutes.
6. Transfer 18 µL of each HMX to each LBMX, mix by pipetting, and incubate for 16-24 hours.
7. 1.5 hours before step 9, prepare Wash Solution F by combining 400 µL Hyb Reagent 2, 39.6 mL nuclease-free water and 10 mL Wash Solution in a 50 mL conical tube. Vortex thoroughly and warm to the hybridisation temperature for at least 45 minutes.
8. Prepare 30 µL of Magnetic Capture Beads per reaction by washing three times in 200 µL Binding Solution. Re-suspend the washed bead aliquots in 70 µL Binding Solution and warm the suspensions to the hybridisation temperature for at least 2 minutes.
9. Combine the warmed beads with the hybridisation reactions and incubate for 5 minutes at the hybridisation temperature, mixing after 2.5 minutes to keep beads suspended.
10. Pellet the beads and remove the supernatant. If using microcentrifuge tubes for clean-up, wash the beads three times with 375 µL warmed Wash Solution F, incubating 5 minutes at the hybridisation temperature. If using a 96-well magnetic rack and/or 0.2 mL strips/tubes, wash four times with 180 µL washes.
11. **Option 1:** If using the KAPA® HiFi HotStart ReadyMix for the amplification reaction, re-suspend the beads in 30 µL of Resuspension Solution and then use 15 µL of this suspension directly in a 50 µL PCR reaction with universal library primers. Following amplification, pellet the beads and purify the enriched library from the bead supernatant.
    Option 2: If using a different PCR master mix, elute the library from the beads by incubating the re-suspended beads for 5 minutes at 95 °C. Pellet the beads and then use 15 µL of the supernatant in a 50 µL PCR reaction with universal library primers. Following amplification, pellet the beads and purify the enriched library from the bead supernatant.

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