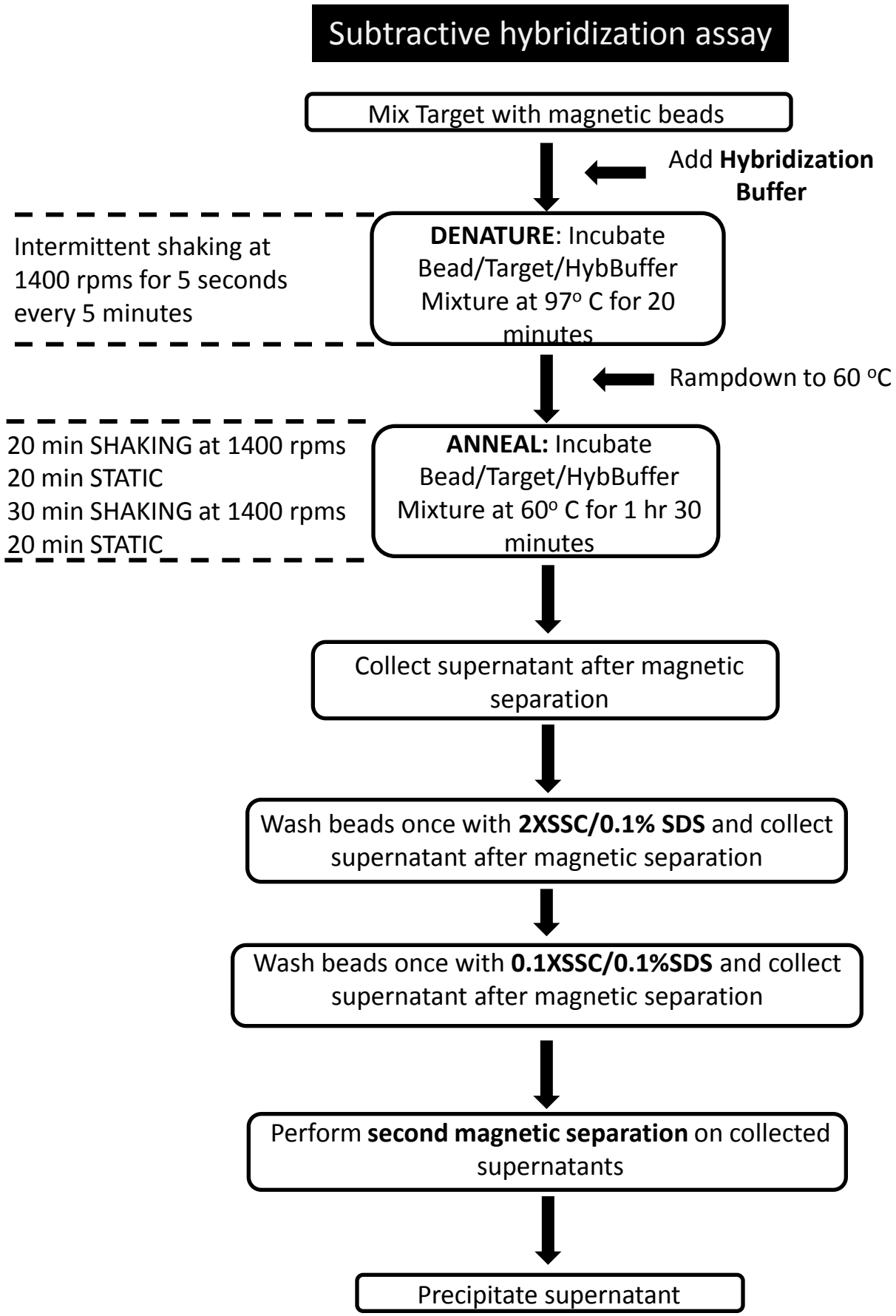
Subtractive hybridization assay
Mix Target with magnetic beads
Add **Hybridization Buffer**
Intermittent shaking at 1400 rpms for 5 seconds every 5 minutes
DENATURE: Incubate Bead/Target/HybBuffer Mixture at 97° C for 20 minutes
Rampdown to 60 °C
20 min SHAKING at 1400 rpms
20 min STATIC
30 min SHAKING at 1400 rpms
20 min STATIC
ANNEAL: Incubate Bead/Target/HybBuffer Mixture at 60° C for 1 hr 30 minutes
Collect supernatant after magnetic separation
Wash beads once with **2XSSC/0.1% SDS** and collect supernatant after magnetic separation
Wash beads once with **0.1XSSC/0.1%SDS** and collect supernatant after magnetic separation
Perform **second magnetic separation** on collected supernatants
Precipitate supernatant