ForenSeq Kintelligence Kit Checklist

VEROGEN

Amplify and Tag Targets

- ☐ 1. Using nuclease-free water, dilute 1 ng gDNA to 40 pg/µl.
- ☐ 2. In the Master Mix tube, combine the following volumes per sample:
  - kPCR1 (18.5 µl)
  - KPM (5 µl)
  - FEM (1.5 µl)
- ☐ 3. Pipette and centrifuge briefly.
- ☐ 4. Add 25 µl Master Mix to the ForenSeq Sample Plate.
- ☐ 5. Dilute 10 ng/µl NA24385 stock:
  - ☐ a. In the Control DNA Dilution 1 tube, combine:
    - 10 ng/µl NA24385 (2 µl)
    - Nuclease-free water (48 µl)
  - ☐ b. Gently pipette and centrifuge briefly.
  - ☐ c. In the Control DNA Dilution 2 tube, combine:
    - 400 pg/µl NA24385 (10 µl)
    - Nuclease-free water (90 µl)
  - ☐ d. Gently pipette and centrifuge briefly.
- ☐ 6. Add 25 µl 40 pg/µl gDNA to the ForenSeq Sample Plate. Pipette to mix.
- ☐ 7. Add 25 µl 40 pg/µl NA24385. Pipette to mix.
- ☐ 8. Add 25 µl nuclease-free water. Pipette to mix.
- ☐ 9. Centrifuge at 1000 × g for 30 seconds.
- ☐ 10. Place on the thermal cycler and run kPCR1.

SAFE STOPPING POINT

If you are stopping, store the plate at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Purify Targets

- ☐ 1. Add 75 µl ProK/SPB2 to Purification Bead Plate 1.
- ☐ 2. Transfer 45 µl reaction from the ForenSeq Sample Plate to Purification Bead Plate 1.
- ☐ 3. Shake at 1800 rpm for 2 minutes.
- ☐ 4. Incubate at room temperature for 10 minutes.
- ☐ 5. Place on the magnetic stand until clear.
- ☐ 6. Centrifuge at 1000 × g for 30 seconds.
- ☐ 7. Place on the magnetic stand until clear.
- ☐ 8. Remove and discard all supernatant.
- ☐ 9. Wash as follows.
  - ☐ a. Add 200 µl fresh 80% EtOH.
  - ☐ b. Incubate for 30 seconds.
  - ☐ c. Remove and discard all supernatant.
- ☐ 10. Wash a **second** time.
- ☐ 11. Centrifuge at 1000 × g for 30 seconds.
- ☐ 12. Place on the magnetic stand until clear.
- ☐ 13. Remove residual EtOH.
- ☐ 14. Remove from the magnetic stand.
- ☐ 15. Add 30 µl RSB.
- ☐ 16. Shake at 1800 rpm for 2 minutes.
- ☐ 17. If necessary, pipette or reshake.
- ☐ 18. Incubate at room temperature for 2 minutes.
- ☐ 19. Place on the magnetic stand until clear.
- ☐ 20. Transfer 28 µl supernatant from each Purification Bead Plate 1 well to a fresh well in the same plate.
- ☐ 21. Add 45 µl ProK/SPB2.
- ☐ 22. Shake at 1800 rpm for 2 minutes.
- ☐ 23. Incubate at room temperature for 5 minutes.
- ☐ 24. Place on the magnetic stand until clear.
- ☐ 25. Centrifuge at 1000 × g for 30 seconds.
- ☐ 26. Place on the magnetic stand until clear.
- ☐ 27. Remove and discard all supernatant.
- ☐ 28. Wash as follows.
  - ☐ a. Add 200 µl fresh 80% EtOH.
  - ☐ b. Incubate for 30 seconds.
  - ☐ c. Remove and discard all supernatant.
- ☐ 29. Wash a **second** time.
- ☐ 30. Centrifuge at 1000 × g for 30 seconds.
- ☐ 31. Place on the magnetic stand until clear.
- ☐ 32. Remove residual EtOH.
- ☐ 33. Remove from the magnetic stand.
- ☐ 34. Add 27 µl RSB.
- ☐ 35. Shake at 1800 rpm for 2 minutes.
- ☐ 36. If necessary, pipette or reshake.
- ☐ 37. Incubate at room temperature for 2 minutes.
- ☐ 38. Place on the magnetic stand until clear.
- ☐ 39. Transfer 25 µl supernatant from Purification Bead Plate 1 to the Purified Targets Plate.

SAFE STOPPING POINT

If you are stopping, store the plate at -25°C to -15°C overnight.

Enrich Targets

- ☐ 1. Centrifuge the Purified Targets Plate at 1000 × g for 30 seconds.
- ☐ 2. Add 5 µl UDI adapter.
- ☐ 3. Briefly centrifuge kPCR2 and pipette to mix.
- ☐ 4. Add 20 µl kPCR2.
- ☐ 5. Pipette to mix.
- ☐ 6. Centrifuge at 1000 × g for 30 seconds.
- ☐ 7. Place on the thermal cycler and run kPCR2.

SAFE STOPPING POINT

If you are stopping, store the plate at 2°C to 8°C for up to 7 days. Alternatively, leave on the thermal cycler overnight.

ForenSeq Kintelligence Kit Checklist

VEROGEN

Purify Libraries

- ☐ 1. Add 45 µl ProK/SPB2 to Purification Bead Plate 2.
- ☐ 2. Transfer 45 µl reaction from the Purified Targets Plate to Purification Bead Plate 2.
- ☐ 3. Shake at 1800 rpm for 2 minutes.
- ☐ 4. Incubate at room temperature for 5 minutes.
- ☐ 5. Place on the magnetic stand until clear.
- ☐ 6. Remove and discard all supernatant.
- ☐ 7. Wash as follows.
  - ☐ a. Add 200 µl fresh 80% EtOH.
  - ☐ b. Incubate for 30 seconds.
  - ☐ c. Remove and discard all supernatant.
- ☐ 8. Wash a **second** time.
- ☐ 9. Centrifuge at 1000 × g for 30 seconds.
- ☐ 10. Place on the magnetic stand until clear.
- ☐ 11. Remove residual EtOH.
- ☐ 12. Remove from the magnetic stand.
- ☐ 13. Add 52.5 µl RSB.
- ☐ 14. Shake at 1800 rpm for 2 minutes.
- ☐ 15. If necessary, pipette or reshake.
- ☐ 16. Incubate at room temperature for 2 minutes.
- ☐ 17. Place on the magnetic stand until clear.
- ☐ 18. Transfer 50 µl supernatant from Purification Bead Plate 2 to the Purified Library Plate.
- ☐ 19. Centrifuge at 1000 × g for 30 seconds.

SAFE STOPPING POINT

If you are stopping, store the plate at -25°C to -15°C for up to 1 year.

Normalize Libraries

- ☐ 1. Place on the magnetic stand.
- ☐ 2. Quantify using a fluorometric method.
- ☐ 3. If concentration is > 0.75 ng/µl, prepare RSB to dilute each library to 0.75 ng/µl:
  - ☐ a. Use the formula $C_1V_1/C_2=V_2$ to calculate the value for V_2.
  - ☐ b. Calculate the volume of RSB (V_2 - 8 µl).
  - ☐ c. Add the calculated volume to the Normalized Library Plate or tube.
- ☐ 4. Transfer 8 µl each library to the Normalized Library Plate or tube.

SAFE STOPPING POINT

If you are stopping, store the plate at -25°C to -15°C for up to 6 months.

Pool Libraries

- ☐ 1. Transfer 5 µl each library to the Pooled Libraries tube.
- ☐ 2. Store the Normalized Library Plate or tube at -25°C to -15°C for ≤ 30 days.
- ☐ 3. Vortex and centrifuge briefly.

SAFE STOPPING POINT

If you are stopping, store the tube at -25°C to -15°C for up to 6 months.

Denature and Dilute Libraries

- ☐ 1. In the 0.2 N NaOH tube, combine:
  - Nuclease-free water (90 µl)
  - HP3 (10 µl)
- ☐ 2. Invert the tube several times.
- ☐ 3. In the Denatured HSC tube, combine:
  - HSC (2 µl)
  - 0.2 N NaOH (2 µl)
- ☐ 4. Vortex and centrifuge briefly.
- ☐ 5. Incubate at room temperature for 5 minutes.
- ☐ 6. Add 36 µl HT1.
- ☐ 7. In the 20 pM Denatured Library tube, combine:
  - 0.75 ng/µl library pool (5 µl)
  - 0.2 N NaOH (5 µl)
- ☐ 8. Vortex briefly and centrifuge briefly.
- ☐ 9. Incubate at room temperature for 5 minutes.
- ☐ 10. Add 990 µl HT1 to the 20 pM Denatured Library tube.
- ☐ 11. In the 12 pM Denatured Library tube, combine:
  - 20 pM library pool (360 µl)
  - HT1 (238 µl)
  - Denatured HSC (2 µl)
- ☐ 12. Vortex and centrifuge briefly.
- ☐ 13. Transfer entire volume to the reagent cartridge.

Acronyms

Acronym	Definition
EtOH	Ethanol
FEM	ForenSeq Enzyme Mix
HP3	2 N NaOH
HSC	Human Sequencing Control
HT1	Hybridization Buffer
kPCR1	Kintelligence PCR1 Reaction Mix
kPCR2	Kintelligence PCR2 Reaction Mix
KPM	Kintelligence Primer Mix
NA24385	NA24385 Positive Amplification Control DNA
NaOH	Sodium hydroxide
ProK	Proteinase K

ForenSeq Kintelligence Kit Checklist

Acronym	Definition
RSB	Resuspension Buffer
SPB2	Sample Purification Beads 2
UDI	Unique Dual Index