

Genome-Wide Human SNP Nsp/Sty Assay 6.0

Improvements to step 7 of the SNP Assay 6.0, PCR cleanup, using an isopropanol precipitation method

This user bulletin includes updated instructions for performing step 7 of the Genome-Wide Human SNP Nsp/Sty Assay 6.0. Please note: this is intended for customers who are using the original SNP Assay 6.0 Kit. You may use this bulletin in conjunction with the quick reference cards (QRCs) to view relevant diagrams associated with this protocol.

About this stage

During this stage, you will:

- Pool the Sty and Nsp PCR reactions into a single deep-well pooling plate
- Add EDTA into each well and incubate
- Add isopropanol to each well and incubate
- Spin at 4°C to precipitate DNA into a pellet
- Discard the supernatant
- Ethanol wash the sample and spin DNA into a pellet
- Discard supernatant as necessary
- Elute the DNA

Location and duration

Location: Main lab

Duration: 130 minutes, 45 minutes hands-on time

Table 1: Steps, duration, and hands-on time.

Steps in process	Step duration (minutes)	Hands-on time (minutes)
PCR pooling	10-15	10-15
EDTA	10-15	5
Precipitation (RT)	30	<10
Spin (4°C)	30	<2
Discard supernatant and gently dry	5	<2
Ethanol wash and spin (4°C)	5	5
Discard supernatant and gently dry	5	<2
Elution time	30	5
Total time	130+	<45

Input required from previous stage

The input required is:

- Three plates Sty PCR product
- Four plates Nsp PCR product

Equipment and consumables required

Table 2: Equipment required to perform this stage.

Qty	Item
1	Plate centrifuge equivalent to Eppendorf Centrifuge 5804 R, capable of 4°C temperature
1	Boekel Scientific Jitterbug Microplate Thermoshaker or shaker
1	ABgene 2.2 mL Deep Well Plate (ThermoFisher Scientific cat#AB-0932)
1	Plate, elution catch, 96-well V-bottom
1	12-channel P20 pipette
1	12-channel P200 pipette
1	12-channel P1200 pipette
As needed	Pipettes and tips for pipettes listed above
As needed	KimWipes

Table 3: Chemicals required to perform this stage.

Qty	Item	Recommended supplier
1	0.5 M EDTA, pH 8.0	Ambion, cat#9260G or Affymetrix, cat#15694
1	Ammonium acetate solution for molecular biology, 7.5M	Sigma-Aldrich, cat#A2706-1L or Affymetrix, cat#11251
1	Isopropanol (2-propanol) for molecular biology, ≥99%	Sigma-Aldrich, cat#I9516-500ML
1	Ethanol absolute, 200 proof, >99.5%	Sigma-Aldrich, cat#459844-1L (ACS grade)
1	Buffer EB	Qiagen, cat#19086
1	Water molecular biology grade	Affymetrix, cat#71786



Important information about this stage

To help ensure the best results, carefully read the information below before you begin this stage of the protocol.

- Use all solutions at room temperature to minimize co-precipitation of salt.
- Centrifuge should be carried out at 4°C to prevent overheating of the samples.
- Only use the ABgene Deep Well Plate (AB-0932) for this purification, because pellets are not attached well with other types of deep-well plates.
- Care should be taken when removing the supernatant and 75% ethanol as pellets from 2-propanol precipitation are more loosely attached to the plate.
- **Do not over-dry the pellet; otherwise it is difficult to re-suspend.**

Procedure

1. Pool 700 μ L of PCR product into the deep-well plate (3 Sty + 4 Nsp), row by row.
2. Pipette 0.5 M EDTA into a reservoir with enough to add 12 μ L for each well. Add 12 μ L to each sample by multi-channel pipette and change tips for the next row. After adding EDTA to all samples, use 200 μ L tips to mix samples 5-10 times the same way as adding EDTA.
3. Cover the plate and incubate for 10 minutes at room temperature. Inspect the plate and make sure the samples have turned clear from a cloudy state. If not, incubate for another 5 minutes.
4. Prepare master mix, 200 μ L of NH₄OAC (7.5 M) and 700 μ L of 2-propanol, for a single sample. Transfer 900 μ L master mix to each pooled PCR product and mix 5 times with the same tips. Change tips and move to the next row until all samples are done.
5. Cover the plate with a loose-fitting lid and leave at room temperature/bench top for 30 minutes. Note: turn on the centrifuge and cool to 4°C.
6. Seal the plate with clear adhesive seal. Centrifuge the plate at 2,250 relative centrifugal force (RCF) for 30 minutes at 4°C. Note: RCF is not the same as RPM.
7. Remove the plate seal and carefully decant the supernatant by slowly inverting the plate upside down without disturbing the pellets. Discard supernatant to waste container.
8. Place the plate upside down on lab tissue for 2 minutes. Do not tap the plate!
9. Wash the pellets by adding 1.6 mL of 75% ethanol (room temperature). Pipet directly onto the pellets to help dislodge them from the bottom of the plate. The pellets will be disturbed and dislodged from the plate. Leave the plate at room temperature/bench top for 2 minutes.
10. Seal the plate with clear adhesive seal. Spin the samples at 2,250 RCF for 5 minutes at 4°C.
11. Remove the plate seal and carefully pour off/discard the ethanol wash by slowly inverting the plate upside down without disturbing the pellets.
12. Place the plate upside down on lab tissue for 2 minutes. Do NOT tap the plate on KimWipes **immediately!** Change the KimWipes if too wet. After 2 minutes, tap the plate gently on the KimWipes until **no wet spots** are observed on the KimWipes. Then flip the plate upright and let dry another 2 minutes on the bench.
13. Dissolve the pellets by adding 55 μ L of Buffer EB. Check that all of the pellets are immersed in the EB. If not, use a pipette tip to push the pellet down or rinse it with the Buffer EB.
14. **Seal the plate.** Shake gently for 30 minutes on a Jitterbug (setting 5) or a shaker. Extend the time if necessary until none of the pellets are visible. Gently vortex the sealed plate on vortex until all pellets are dissolved; then quickly spin the plate in a centrifuge.
15. With a multi-channel pipette set at 47 μ L, mix the samples a few times and transfer them to a fragmentation plate.
16. Quantify results and check DNA purity by adding 2 μ L of DNA to 198 μ L of H₂O and reading the absorbance at 260 nm.

Affymetrix, Inc. +1-888-362-2447 ■ Affymetrix UK Ltd. +44-(0)1628-552550 ■ Affymetrix Japan K.K. +81-(0)3-6430-4020
Panomics Products +1-877-PANOMICS www.panomics.com ■ USB Products +1-800-321-9322 www.usb.affymetrix.com

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