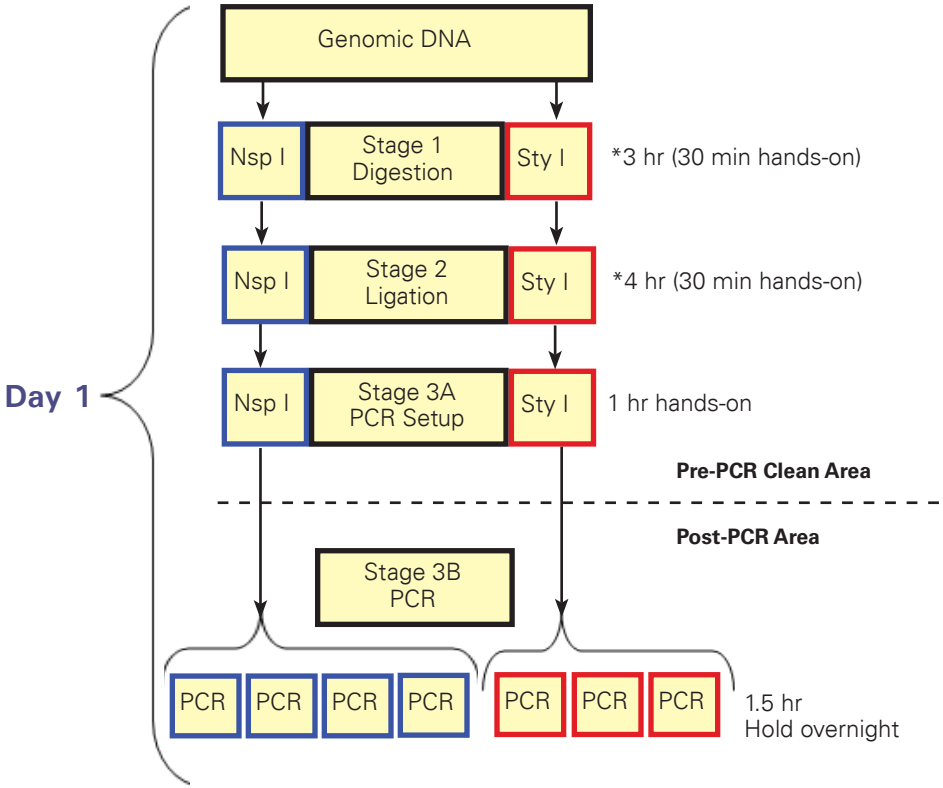
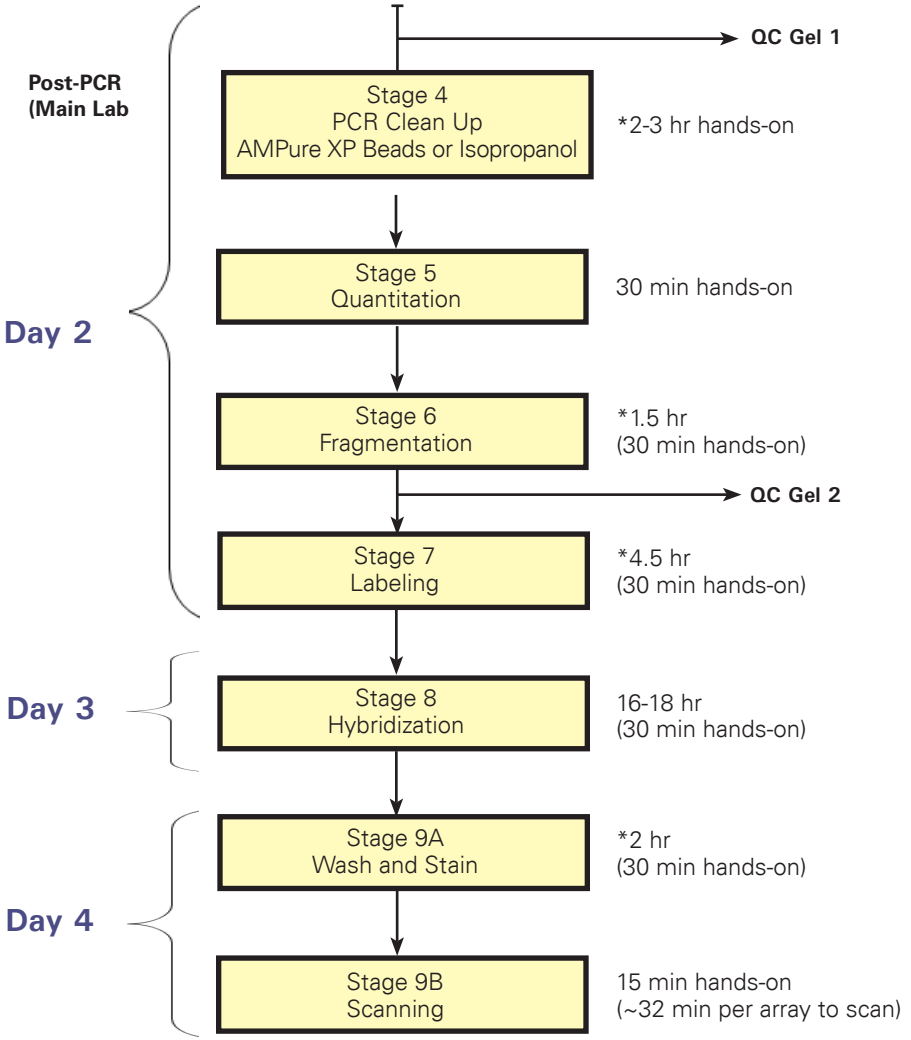
Quick Reference Card

SNP 6.0 Cytogenetics Copy Number Assay
Core Reagent 100 reaction Kit, 3x30 Sample
Workflow Overview





*Time includes both incubation and hands-on time.

Note: All Master mix calculations for '30 samples' in this QRC include sufficient volume to process 30 samples plus one positive and one negative control.

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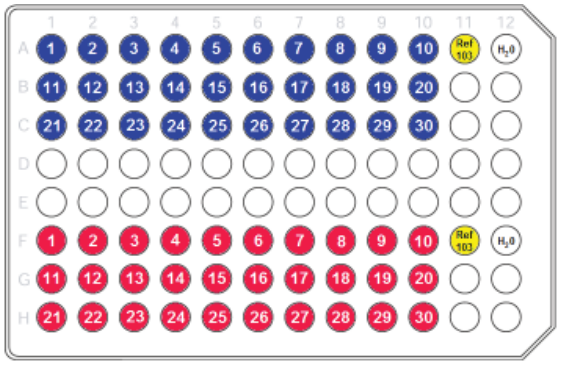
SNP 6.0 Cytogenetics Copy Number Assay
Core Reagent 100 reaction Kit, 3x30 Sample
Stage 1 – Digestion

DIGESTION MASTER MIX

Reagent	Per Sample	30 Samples Nsp	✓	30 Samples Sty	✓	Lot Number
Water, molecular biology grade	11.55 µL	423.5 µL		423.5 µL		
Nsp Buffer	2.00 µL	73.3 µL		—		
Sty Buffer	2.00 µL	—		73.3 µL		
BSA (100X; 10 mg/mL)	0.20 µL	7.3 µL		7.3 µL		
Nsp I (10 U/ µL)	1.00 µL	36.7 µL		—		
Sty I (10 U/ µL)	1.00 µL	—		36.7 µL		
Total Volume	**14.75 µL**	**540.8 µL**	—	**540.8 µL**	—	—

Suggested Digestion and Ligation Plate Map



Nsp Samples	Volume
gDNA (50 ng/ µL)	5.00 µL (250 ng)
Nsp Master Mix	14.75 µL
Total Volume	**19.75 µL**

Sty Samples	Volume
gDNA (50 ng/ µL)	5.00 µL (250 ng)
Sty Master Mix	14.75 µL
Total Volume	**19.75 µL**

1. Seal plate with adhesive film.
2. Vortex plate at high speed for 3 sec.
3. Spin down at 2000 rpm for 30 sec.
4. Ensure lid of thermal cycler is preheated.
5. Load plate onto thermal cycler and run the *Cyto Digest* program.

Proceed to Ligation

Cyto Digest	
Temp	**Time**
37 °C	2 hr
65 °C	20 min
4 °C	Hold

Important Points

- Aliquot genomic DNA (gDNA) to opposite ends of the plate to lessen the chance of pipetting errors.
- Add gDNA to all sample wells.
- Two digestion master mixes are prepared (Nsp and Sty).
  - Be sure to use the correct enzyme for each master mix (Nsp or Sty)
  - Leave Nsp and Sty enzymes at –20 °C until ready to use.
- Add 5 µL Ref103 DNA as positive control to wells marked "Ref 103".
- Add 5 µL water (molecular biology grade) as negative control to wells marked "H_20".

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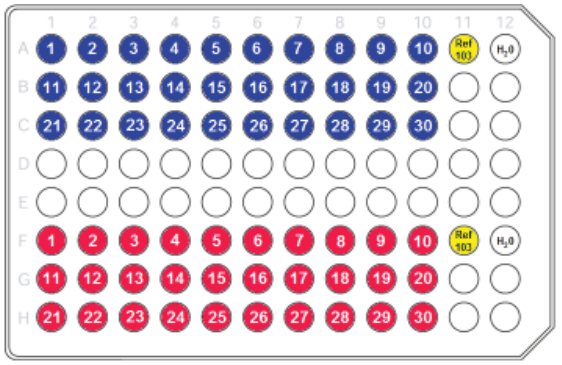
SNP 6.0 Cytogenetics Copy Number Assay
Core Reagent 100 reaction Kit, 3x30 Sample
Stage 2 – Ligation

LIGATION MASTER MIX

Reagent	Per Sample	30 Samples Nsp	✓	30 Samples Sty	✓	Lot Number
10X T4 DNA Ligase Buffer	2.50 µL	116.0 µL		116.0 µL		
Nsp Adaptor	0.75 µL	34.8 µL		—		
Sty Adaptor	0.75 µL	—		34.8 µL		
T4 DNA Ligase (400 U/µL)	2.00 µL	92.8 µL		92.8 µL		
Total Volume	**5.25 µL**	**243.6 µL**	—	**243.6 µL**	—	—

Suggested Digestion and Ligation Plate Map



Nsp Samples	Volume
Nsp Digested Sample	19.75 µL
Nsp Master Mix	5.25 µL
Total Volume	**25.00 µL**

Sty Samples	Volume
Sty Digested Sample	19.75 µL
Sty Master Mix	5.25 µL
Total Volume	**25.00 µL**

1. Seal plate with adhesive film.
2. Vortex plate at high speed for 3 sec.
3. Spin down at 2000 rpm for 30 sec.
4. Ensure lid of thermal cycler is preheated.
5. Load plate onto thermal cycler and run the *Cyto Ligate* program.

Proceed to PCR Setup

Cyto Ligate	
Temp	**Time**
16 °C	3 hr
70 °C	20 min
4 °C	Hold

Important Points

- Sample plate used for digestion and ligation.
- Two ligation master mixes are prepared (Nsp and Sty).
  - Be sure to use the correct adaptor for each master mix (Nsp or Sty)
  - Leave T4 DNA Ligase at –20 °C until ready to use.
  - Thaw T4 DNA Ligase Buffer on ice. Vortex to ensure any precipitate is resuspended and buffer is clear.

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Core Reagent 100 reaction Kit, 3x30 Sample
Stage 3a – PCR

1. Dilute ligated samples.

Nsp Samples	Volume Per Sample
Nsp Ligated Sample	25.0 µL
Water, molecular biology grade	75.0 µL
Total Volume	**100 µL**

Sty Samples	Volume Per Sample
Sty Ligated Sample	25.0 µL
Water, molecular biology grade	75.0 µL
Total Volume	**100 µL**

2. Seal plate with adhesive film.
3. Vortex at high speed for 3 sec; spin down at 2000 rpm for 30 sec.

4. Transfer four 10 µL aliquots of each Nsp sample to the PCR plate.
5. Transfer three 10 µL aliquots of each Sty sample to the PCR plate.

6. Prepare the PCR Master Mix.

PCR MASTER MIX				
Reagent	**Per Sample**	**30 Samples**	**✓**	**Lot Number**
Water, molecular biology grade	39.5 µL	9,730 µL		
TITANIUM™ *Taq* PCR Buffer (10X)	10.0 µL	2,468 µL		
GC-Melt/Beatine (5M)	20.0 µL	4,918 µL		
dNTPs (2.5 mM each)	14.0 µL	3,448 µL		
PCR Primer 002 (100 µM)	4.5 µL	1,103 µL		
TITANIUM™ *Taq* Polymerase	2.0 µL	490 µL		
Total Volume	**90.0 µL**	**22,157 µL**		

7. Add PCR Master Mix to samples.

Sample	Volume
Nsp or Sty Sample	10 µL
PCR Master Mix	90 µL
Total Volume	**100 µL**

8. Seal PCR plate, vortex at high speed for 3 sec, spin down at 2000 rpm for 30 sec.
9. Keep plate on ice and move to Post-PCR Room/Area.

Pre-PCR Area

Post-PCR Area

10. Ensure thermal cycler lid is preheated.
11. Load plate onto thermal cycler and run the *Cyto PCR* program.
12. Hold overnight.

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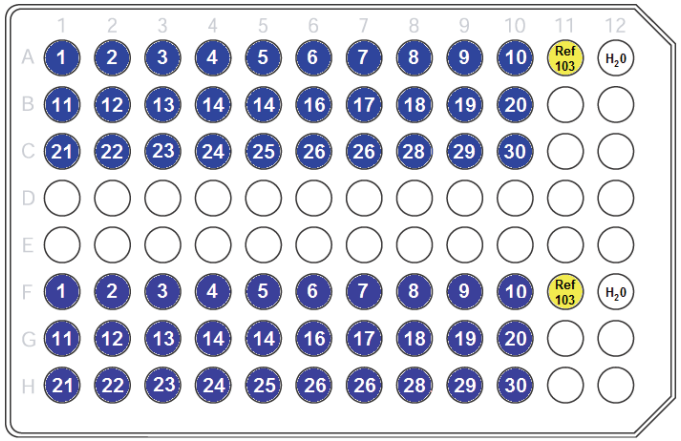
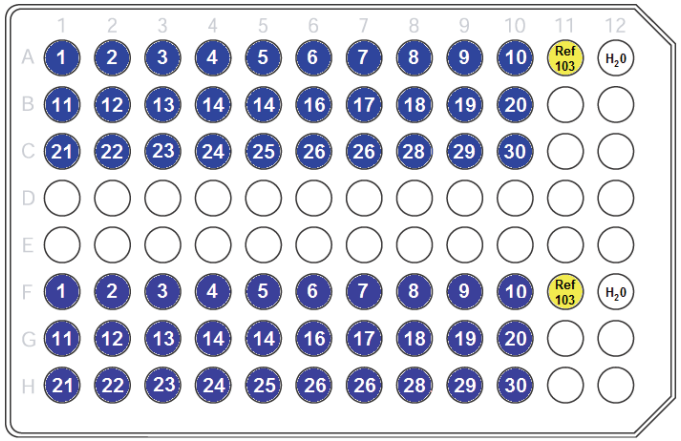
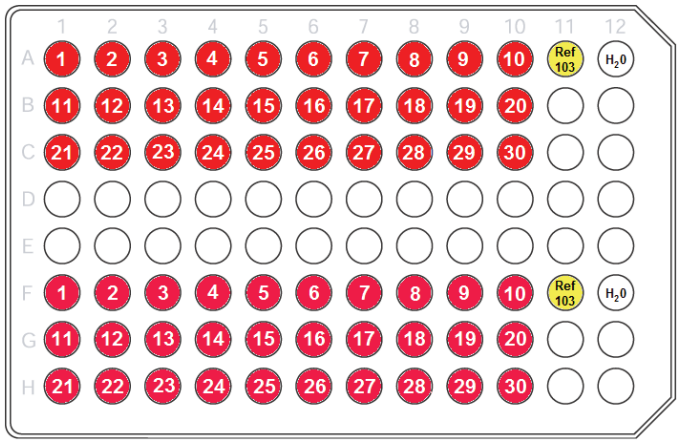
Core Reagent 100 reaction Kit, 3x30 Sample
Stage 3a – PCR Plate Maps and Thermocycler Programs

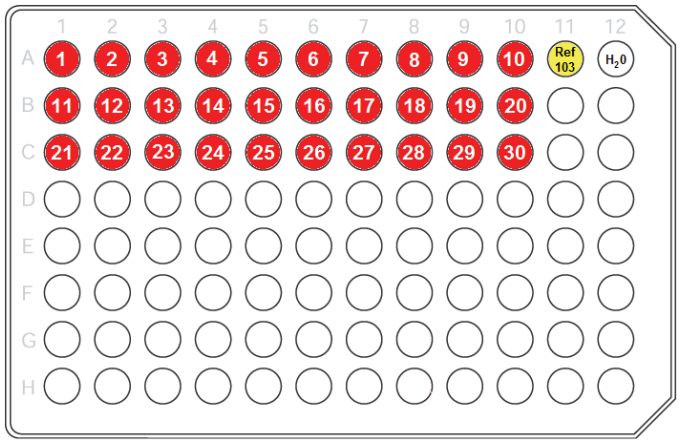
Suggested Nsp PCR Plate Map





Suggested Sty PCR Plate Map





Thermocycling Programs

Cyto PCR – ABI 9700		
Temp	**Time**	**Cycles**
94 °C	3 min	—
94 °C	30 sec	30
60 °C	45 sec	
68 °C	15 sec	
68 °C	7 min	—
4 °C	Hold	—

Cyto PCR – MJ Tetrad PTC-225		
Temp	**Time**	**Cycles**
94 °C	3 min	—
94 °C	30 sec	30
60 °C	30 sec	
68 °C	15 sec	
68 °C	7 min	—
4 °C	Hold	—

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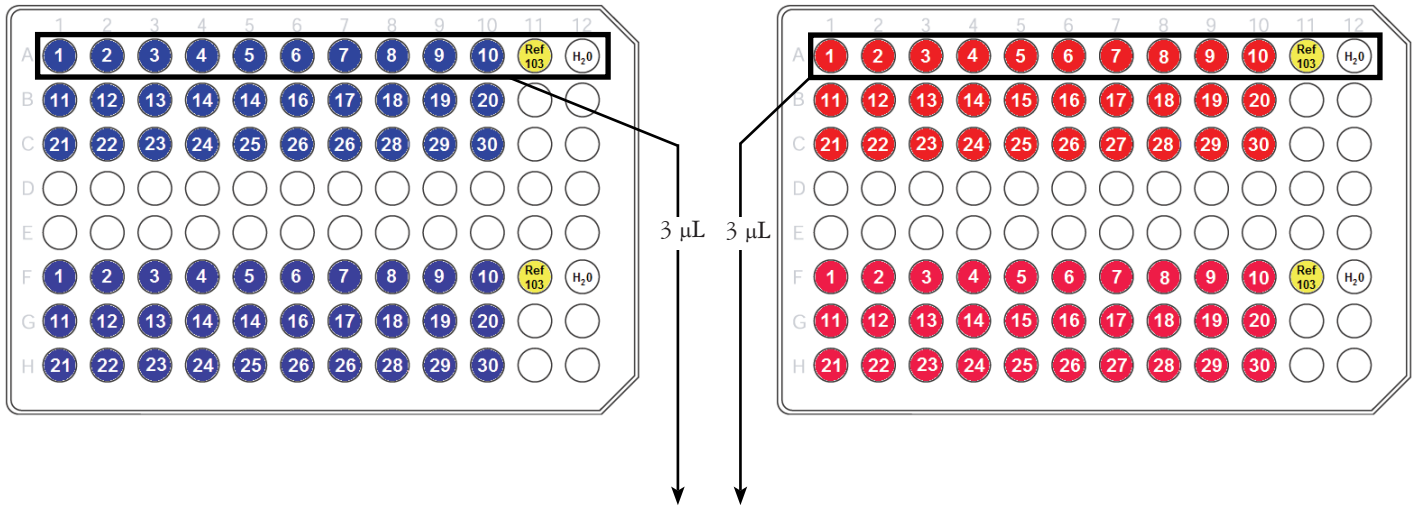
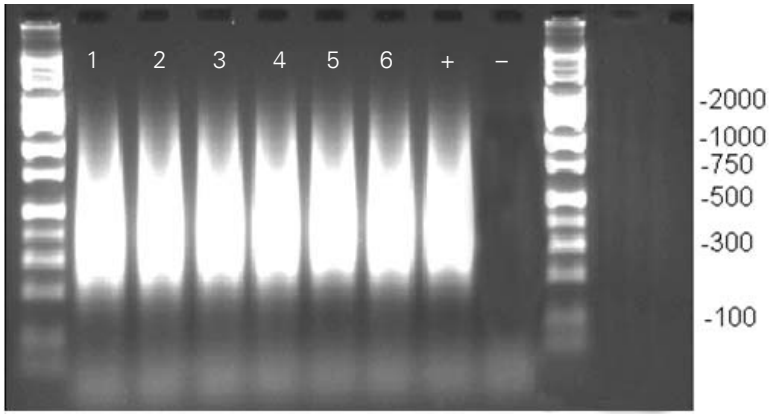
Suggested PCR Plate Maps



Transfer to corresponding wells of 96-well plate.
These wells should already contain 3 µL Gel Loading Dye.

1. Aliquot 3 µL of 2X Gel Loading Dye to a new 96-well plate labeled "PCR gel". Mark rows A-D as Nsp and rows F-H as Sty.
2. Transfer 3 µL of each representative Nsp PCR product to the PCR gel plate (rows A-D).
3. Transfer 3 µL of each representative Sty PCR product to the PCR gel plate (rows F-H).
   Be sure to cover reactions across thermal cyclers. Be sure to include the positive and negative control.
4. Seal the gel plate.
5. Vortex on high speed for 3 sec; spin down at 2000 rpm for 30 sec.
6. Load the total volume of each well from the PCR gel plate onto a 2% TBE gel with ladder.
7. Run the gel at 120V for one hour.
8. Confirm that the product distribution is between ~200 to 1100bp.
9. Save the image.
10. Continue to stage 4 – PCR purification.



Example of PCR products run on a 2% TBE gel at 120 volts for 1 hour.
Average product size is between 200 and 1100 bp.

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IMPORTANT: There are two methods that can be used to clean up the PCR product. See the tables below for method comparison.

Bead-Based Protocol
Stage 4A (page 8-10)

Stage 4A – DNA Clean Up Using AMPure XP Beads Duration ~180 min		
Steps in Process	**Step Duration (min)**	**Hands on Time (min)**
Sample/magnetic bead incubation	10	5
Initial vacuum step	40 - 60	5
First ethanol vacuum step (wash step)	10 - 20	5
Final ethanol vacuum step (dry step)	15	5
Elute DNA in Elution Buffer (EB)	30	< 2
Resuspend beads in Elution Buffer (EB)	30	5
Elution on vacuum manifold	5 - 15	< 2
Total Time	**140 - 180**	**< 45**

Isopropanol Method
Stage 4B (page 11)

Stage 4B – DNA Clean Up Using Isopropanol Duration ~130 min		
Step	**Step Duration (min)**	**Hands on Time (min)**
EDTA incubation	10 - 15	5
Isopropanol precipitation	30 - 40	< 10
Centrifugation	30	< 2
Pour off isopropanol	5	< 2
Ethanol wash	10	5
Pour off ethanol and dry	5	5
Resuspend pellet in Elution Buffer (EB)	30 - 50	5
Total Time	**90 - 155**	**< 35**

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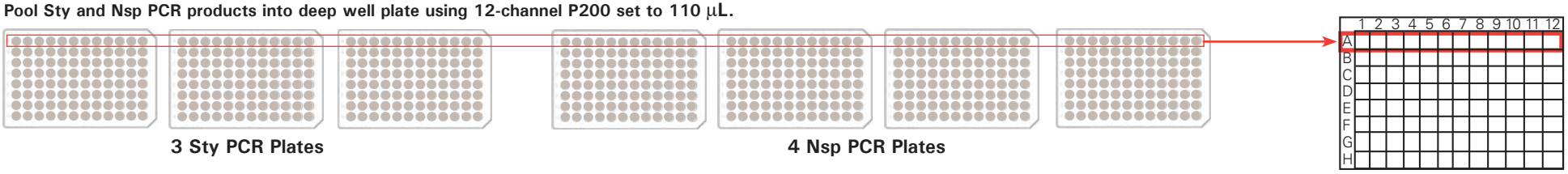
Core Reagent 100 reaction Kit, 3x30 Sample
Stage 4a – Post PCR Clean-up Using AMPure XP Beads

1. Pool Sty and Nsp PCR products into deep well plate using 12-channel P200 set to 110 µL.



3 Sty PCR Plates

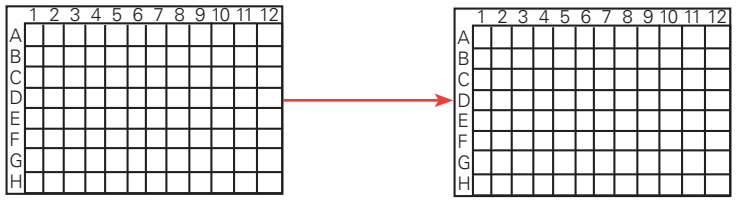
4 Nsp PCR Plates

Deep Well Pooling Plate
Total 700 µL each well
(3 Sty + 4 Nsp)

2. Add magnetic beads, mix and incubate.

A. Shake bottle of magnetic beads vigorously until all beads are resuspended.
B. Add 1.0 mL magnetic beads to each pooled sample.
C. Mix well 5X by pipetting up and down using 12-channel P1200.
D. *Visually confirm that each well is mixed.*
E. Cover plate and incubate for 10 min at room temperature.

3. Place Millipore filter plate on vacuum manifold; then transfer incubated samples to filter plate (2 x 900 µL). Seal the empty wells.



Deep Well Pooling Plate

Millipore Filter Plate
(P/N MDRLN0410)

Note:

- Take care not to mix up samples while pooling. Work uninterrupted.
- Do not touch filters with pipet tips.
- Preheat hybridization oven for 30 min at 50 °C

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4. **Apply vacuum until all of the liquid has been pulled through the filter (~40 to 60 min).**
   Visually check that all wells are dry, they should appear dull (matte).

   Ensure all liquid is removed from the filter plate.
   A. With the vacuum on, tap the top of the plate twice with the palm of your hand.
   B. Turn the vacuum off and remove the plate.
   C. Firmly blot the plate on lint-free tissue until no wet spots are observed.
   D. Place the plate back on the manifold.
   E. Turn on the vacuum for 3 min.
   F. With the vacuum on, tap the top of the plate twice with the palm of your hand. (Repeat step 4B - 4D, 1x.)

5. **Wash each sample with 1.8 ml 75% EtOH (room temperature) using a P1200 as follows:**
   A. With the vacuum on, add 900 µl to each sample.
   B. After ~2 min under vacuum, add another 900 µl to each sample.
   C. Continue to apply vacuum until all of the liquid has been pulled through the filter (~10-15 min).
   Visually check that all wells are dry. Maximum time 20 min.

6. **Ensure all EtOH is removed from the filter plate**
   A. With the vacuum on, tap the top of the plate twice with the palm of your hand.
   B. Turn the vacuum off and remove the plate.
   C. FIrmly blot the plate on lint-free tissue until no wet spots are observed.
   D. Return the plate to the manifold and apply vacuum for 2 - 5 min.
   E. Turn the vacuum off and remove the plate.
   F. Firmly blot the plate on lint-free tissue until no wet spots are observed.
   G. Let sit at room temperature for 10 min.

7. **Elute DNA from beads at 50C for 30 min.**
   A. Attach elution catch plate using lab tape.
   Seal on two sides only – do not seal completely.
   B. Add 60 µl buffer EB to each sample. Buffer EB at 4 °C.
   Tap plate stack to move all buffer EB onto the filters at the bottom of each well.
   C. Loosely cover with plate lid or clear lid from pipet tip box.
   D. Place the covered plate stack in a 50 °C hyb oven for 30 min
   If using an Affymetrix hybridization oven, place plate on the right front side (away from the air vent).
   E. Remove the plate cover and seal all wells tightly with adhesive film.
   F. Place on Jitterbug for 30 min at setting 7.
   Inspect each well to ensure all beads are resuspended.
   G. Remove the seal and place plate stack INSIDE the vacuum manifold.
   Ensure empty wells are sealed.
   H. Apply vacuum until all liquid has been pulled through (~5 to 15 min).
   Visually check that all wells are dry. If any wells are still wet or shiny, continue to apply vacuum for 15 min.
   I. Turn off vacuum, seal entire plate and centrifuge the plate stack for 5 min at 1400 RCF.
   Centrifuge at room temperature.
   J. Remove the catch plate containing the eluate and seal.
   There should be ~ 50 µl in each well.

NOTE:

- Do not extend vacuum steps beyond the maximum time listed.
  UNDER DRYING CAN RESULT IN INCOMPLETE ELUTION.
- Once samples are applied to the filter plate, keep at room temperature. If stored at 4 °C, yields will be reduced and array results compromised.

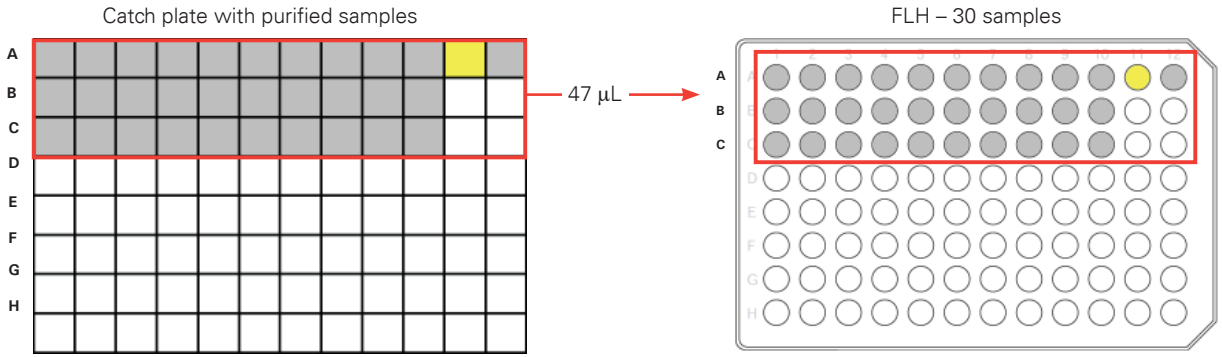
8. Prepare fragmentation/label/hyb plate (FLH).

A. Mix eluted DNA before transfer for fragmentation and quantification on the Jitterbug for 5 min at setting 7.

B. Spin briefly, then remove seal.

C. Transfer 47 µl to a new PCR plate using a 12-channel P200 (this is the fragmentation plate).

NOTE: To avoid mixing up your samples, re-label top 4 rows of plate FLH (Frag, Label, Hyb plate).

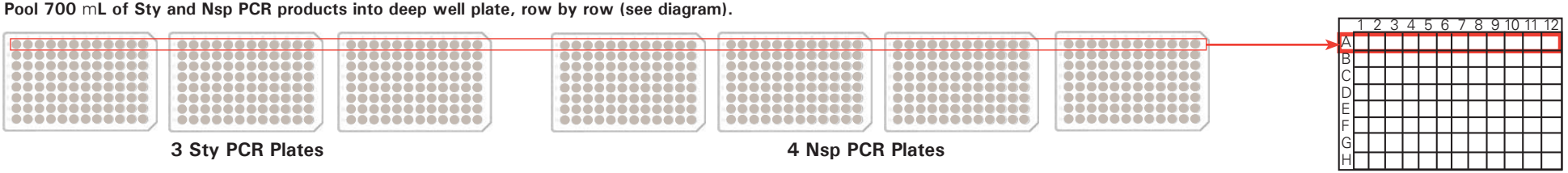


OD is measured in Stage 5. Proceed to page 12 for instructions.

Quick Reference Card

SNP 6.0 Cytogenetics Copy Number Assay
Core Reagent 100 reaction Kit, 3x30 Sample
Stage 4b – Post PCR Clean up Using Isopropanol

1. Pool 700 mL of Sty and Nsp PCR products into deep well plate, row by row (see diagram).



3 Sty PCR Plates **4 Nsp PCR Plates**

Deep Well Pooling Plate
Total 700 µL each well
(3 Sty + 4 Nsp)

2. Add EDTA and mix well.

A. Pipette 1 mL of 0.5 M EDTA into a reservoir.
B. From this reservoir, pipette 12 µL of 0.5 M EDTA.into each well using a multi-channel pipette.
C. Mix the sample thoroughly by aspirating up and down 5 - 10 times using a P200 multi-channel pipette set at 200 µL. Change tips between rows.

3. Cover the plate incubate for 10 min at room temperature.
Inspect the plate and make sure the samples have turned clear.

4. Prepare Master Mix: 200 µL of NH4OAC (7.5M) and 700 µL of isopropanol for each sample.
Transfer 900 µl of the Master Mix to each pooled PCR product.
Change tips and move to the next row until all the samples are done.

5. Cover the plate with a loose fitting lid and leave at room temperature for 30 min.
Note: Turn on the centrifuge and cool to 4 °C.

6. Seal the plate with clear adhesive seal. Centrifuge the plate at 2,250 RCF for 30 minutes at 4 °C.
Note: RCF is NOT the same as RPM. Use RCF (relative centrigual force)

7. Remove the plate seal and carefully decant the supernatant by slowly inverting the plate upside down without disturbing the pellets. Discard supernatant in a waste container.

8. Place the plate upside down on lab tissue for 2 min on a bed of Kimwipes® tissues. Do not tap the plate!

9. Wash the pellets by adding 1.6 mL of 75% ethanol. The ethanol MUST be at room temperature.
Pipet directly onto the pellets to help dislodge them from the bottom of the plate.
Once the pellets are dislodged, leave the plate at room temperature on the bench top for 2 min.

10. Seal the plate with clear adhesive seal.
Spin the samples again at 2,250 RCF for 5 min at 4 °C.

11. Remove the plate seal and carefully pour off the ethanol wash by slowly inverting the plate upside down without disturbing the pellets.

12. Place the plate upside down on Kimwipes® tissue for 2 min. Do NOT immediately tap the plate on the Kimwipes tissues! Change the tissues if too wet. After the 2 min time is up, tap the plate gently on the Kimwipes until no wet spots are observed on the tissues. Then flip the plate upright and tap on the bench to bring the pellets to the bottom of each well. Let dry another 2 min on the bench.

13. Add 55 µL EB buffer to each sample. Check that pellets are immersed.

14. Seal the plate. Shake gently for 30 min (Jitterbug setting 5).
Vortex until pellets are dissolved.

15. Using a multi-channel pipette, transfer 47 µL to a new PCR plate.

Proceed to Stage 5 - Quantitation on page 12.

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Plate Spectrophotometer

1. Dilute purified PCR 1:100.
   - — Pipette 2 µL from the Fragmentation plate into 198 µL water.
   - — Thoroughly mix the samples and water using one of these methods:

     Seal the plate, vortex, and spin down OR pipet up and down 5 times.
2. Measure the OD of each PCR product at 260, 280 and 320 nm.
3. Determine the OD260 measurement for the water blank and average.
4. Calculate one OD reading for every sample: OD = (sample OD) – (average water blank OD)
5. Calculate the undiluted concentration for each sample in µg/µL: OD X 0.05 µg/uL X 100.

NanoDrop

1. Dilute purified PCR 1:10.
   - — Pipette 2 µL from the Fragmentation plate into 18 µL water.
   - — Thoroughly mix the samples and water using one of these methods:

     Seal the plate, vortex, and spin down OR pipet up and down 5 times.
2. Blank the NanoDrop using water.
3. Take 2 µL of 1:10 diluted sample and measure the OD of each PCR product at 260, 280 and 320 nm.
4. Calculate the undiluted concentration of each sample in µg/µL: OD reading X 0.05 µg/uL X 10.

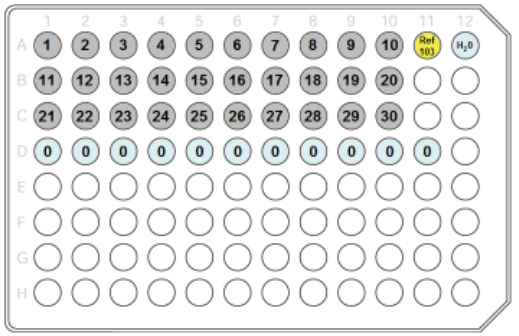
Assess OD Readings

- An acceptable OD should fall within the range of 0.9 to 1.4.
- DNA yield equivalent = 4.5 to 7.0 µg/µL.
- The OD260/OD280 ratio should be between 1.8 and 2.0.
- The OD320 measurement should be very close to zero (< 0.1).
- If metrics fall outside of these ranges, refer to the *Affymetrix® Cytogenetics Copy Number Assay User Guide* for more information.

Example showing one row of samples

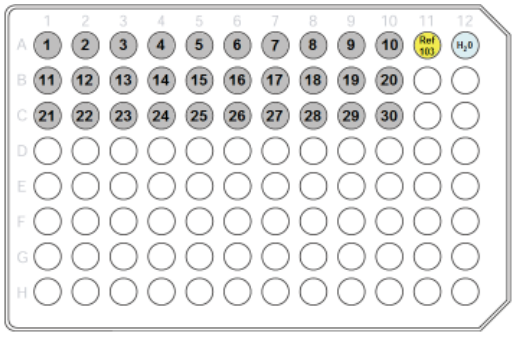
Plate Spectrophotometer

Row A: 198 µl water + 2 µl sample
Row B: 200 µl water



NanoDrop

Row A: 18 µl water + 2 µl sample



NOTE: Optimal PCR yield for downstream steps is above 5.5 µg/µL.

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Quick Reference Card

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Core Reagent 100 reaction Kit, 3x30 Sample
Stage 6 – Fragmentation

FRAGMENTATION MASTER MIX					
Reagent	**2 U/µL**	**2.5 U/µL**	**3.0 U/ µL**	**✓**	**Lot Number**
Water, molecular biology grade	170 µL	215 µL	260 µL		
10X Fragmentation Buffer	20 µL	25 µL	30 µL		
Fragmentation Reagent	10 µL	10 µL	10 µL		
Total Volume	**200 µL**	**250 µL**	**300 µL**		

NOTE: Please refer to manual if the U/µL of this reagent is different from the table above or if processing lessthan 30 samples.

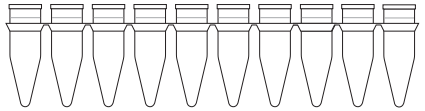
1. Add 5 µL of 10X Fragmentation Buffer to each sample.

Purified Sample	45 µL
10X Fragmentation Buffer	5 µL
Total Volume	**50 µL**

2. Prepare the Fragmentation Master Mix from table above.
3. Aliquot the master mix equally to one set of strip tubes.
4. Using a multi-channel pipet, add 5 µL of Fragmentation Master Mix to each sample.
5. Seal the plate with adhesive film.
6. Vortex at high speed for 3 sec.
7. Spin down at 2000 rpm for 30 sec.
8. Start the Cyto Frag program and pause when it reaches temperature.
9. Load plate onto thermal cycler and un-pause to run program.

Diluted sample	50 µL
Fragmentation Master Mix	5 µL
Total Volume	**55 µL**

Aliquot Fragmentation Master Mix equally to strip tubes. Use a multi-channel pipet to add to samples.



Cyto Fragment	
Temp	**Time**
37 °C	35 min
95 °C	15 min
4 °C	Hold

Proceed immediately to Labeling.

Important Points – Fragmentation Master Mix Preparation

- Check concentration of Fragmentation Reagent (enzyme; varies between 2 and 3 U/µL).
- Leave Fragmentation Reagent (enzyme) at –20 °C until ready to use.
- Thaw 10X Fragmentation Buffer on ice.
- Keep all reagents, including water, on ice. Perform all additions on ice.
- Preheat thermal cycler block to 37 °C.

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Quick Reference Card

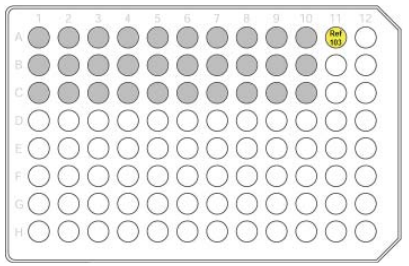
SNP 6.0 Cytogenetics Copy Number Assay
Core Reagent 100 reaction Kit, 3x30 Sample
Stage 7 – Labeling and QC #3, Fragmentation QC Gel

Labeling

LABELING MASTER MIX Reagent	Per Sample 2 U/µL	30 Samples	✓	Lot Number
5x TdT Buffer	14.0 µL	537 µL		
30 mM DNA Labeling Reagent	2.0 µL	77 µL		
30U/ µL TdT Enzyme (30 U/µL)	3.5 µL	134 µL		
Total Volume	**19.5 µL**	**748 µL**		

1. Transfer 2 µL of each fragmented sample to the corresponding well of a fresh 96-well plate (the Fragmentation QC Gel Plate).
2. Prepare the Labeling Master Mix.
3. Add 19.5 µL of master mix to each sample.
4. Tightly seal the plate, and vortex at high speed for 3 sec.
5. Spin down at 2000 rpm for 30 sec.
6. Load plate onto thermal cycler and run the *Cyto Label* program.

2 µL

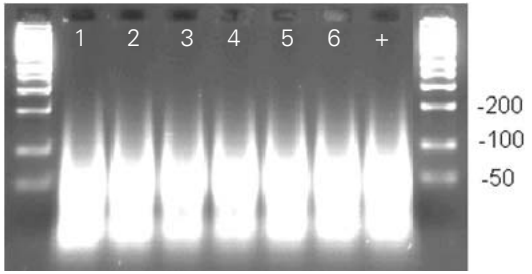


Fragmentation QC Gel Plate

Cyto Label	
Temp	Time
37 °C	4 hr
95 °C	15 min
4 °C	Hold

If possible, store the Label plate overnight at –20 °C. Otherwise, OK to hold at 4 °C overnight.

Fragmentation QC Gel



Example of fragmented samples run on a 4% TBE gel at 120 volts for 1 hr. Average fragment size is < 180 bp.

1. While the *Cyto Label* program is running, finish preparing the gel plate by adding 4 µL of Gel Loading Dye to each sample.
2. Seal the plate, vortex, and spin down.
3. Onto a 4% TBE gel, load 10 µL of BioNexus All Purpose Hi-Lo Ladder to the first and last lanes.
4. Load the samples and run the gel.
5. Inspect the gel and compare against the figure shown here.

Important Points

- Leave the TdT enzyme at –20 °C until ready to use.
- Thaw the 5X TdT Buffer and DNA Labeling Reagent on ice.
- Ensure the plate is tightly sealed to avoid evaporation while on the thermal cycler.

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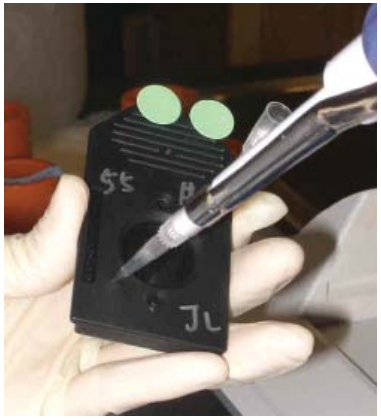
HYBRIDIZATION MASTER MIX				
Reagent	**Per Sample**	**30 Samples**	**✓**	**Lot Number**
Hyb Buffer Part 1	165 µL	6,325 µL		
Hyb Buffer Part 2	15 µL	575 µL		
Hyb Buffer Part 3	7 µL	268.3 µL		
Hyb Buffer Part 4	1 µL	38.3 µL		
OCR	2 µL	76.7		
Total Volume	**190 µL**	**7,283.3 µL**		



Use a P200 pipet to load arrays.

1. Unpackage the arrays and allow to equalibrate to room temperature prior to use.
2. Preheat the hybridization ovens for at least 1 hr at 50 °C with the rotation turned on.
3. Prepare the Hybridization Master Mix.
4. Add 190 µL of master mix to each sample.
5. Tightly seal the plate, vortex at high speed for 30 sec, and spin down at 2000 rpm for 30 sec.
6. Load plate onto thermal cycler and run the *Cyto Hyb* program.

Cyto Hyb	
Temp	**Time**
95 °C	10 min
49 °C	Hold

7. Leaving the samples on the thermal cycler, load 200 µL of sample onto each array using a single-channel P200 pipet.
8. Clean any excess fluid from around the septa.
9. Apply Tough-Spots to the septa and press firmly.
10. Load arrays into the hybridization oven four at a time.

Hyb arrays
16 to 18 hr at 50 °C

Important Points

- Samples must remain on the thermal cycler while loading the arrays.
- To avoid damaging the septa, use a single-channel P200 pipet to load the arrays.
- Shake arrays a few times to ensure bubbles are not visible through the window.
- When 4 arrays are loaded, immediately place them into the hybridization oven.

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All buffers are prepared for you. You will need to aliquot the following from the bottles:

Stain Cocktail 1
600 µL/sample

Stain Cocktail 2
600 µL/sample

Array Holding Buffer
1000 µL/sample

Washing and Staining Arrays

1. Remove the hybridization solution from each array.
2. Fill the arrays with 270 µL **1X Array Holding Buffer**.
3. Load arrays onto the Fluidics Station.
4. Using AGCC, run the *GenomeWideSNP6_450* protocol.

Before Scanning

1. Ensure no bubbles are visible through the window.
2. Cover the septa with Tough-Spots; then load onto the scanner.

Important Points

- The hybridization solution removed from the arrays can be stored long term at –80 °C.
- Stain Cocktail 1 and the Array Holding Buffer are light sensitive and must be stored at 4 °C.
- If necessary, the array can be stored in Array Holding Buffer at 4 °C for up to 3 hr before washing and staining.