

Quick Reference Card

Cytogenetics Assay

Stage 1a – Whole Genome Amplification

1. Preparation

- Genomic DNA (gDNA) sample preparation:
 - 3 μL of each gDNA sample at a concentration of 33 ng/ μL in 1X TE Buffer. Refer to the *Cytogenetics Assay User Manual* for more information.
 - Sample plate must be at room temperature.
 - Chilled aluminum block on ice.
 - Optional: Prepare a spreadsheet with sample information in AGCC.
- Thermal cycler: preheat the lid for 10 min.
- Prepare the Cyto Amplif Rxn Buffer — **IMPORTANT** This reagent must be thoroughly mixed before use.
 - Thaw at room temperature.
 - Vigorously vortex at maximum speed for 10 sec.
 - Immediately place on ice.

- Prepare the Cyto 10X Denat and Neutral Solns
 - Thaw at room temperature.
 - Vortex and spin.
 - Leave at room temperature.

2. Dilute the Cyto 10X Denat and Neutral Solns to 1X

- Dilute only the volume required for your current experiment. Do not dilute the entire volume provided.
- Dilute each buffer in a 1.5 mL Eppendorf tube.
- Vortex and spin.
- 1X Denat Soln – leave at room temperature.
- 1X Neutral Soln – place on ice.

Cyto 10X Denat Soln (DS) Dilution Table

Nbr of Samples	Vol 10X Cyto Denat Soln (DS)		Water (from Kit)		Final Volume 1X Denat Soln (20% extra)
1 to 4	2 μL	+	18 μL	=	20 μL
5 to 8	4 μL	+	36 μL	=	40 μL
9 to 12	5 μL	+	45 μL	=	50 μL
13 to 24	10 μL	+	90 μL	=	100 μL
25 to 36	15 μL	+	135 μL	=	150 μL
37 to 48	20 μL	+	180 μL	=	200 μL
49 to 56	24 μL	+	216 μL	=	240 μL
57 to 64	27 μL	+	243 μL	=	270 μL
65 to 72	30 μL	+	270 μL	=	300 μL
73 to 84	35 μL	+	315 μL	=	350 μL
85 to 96	40 μL	+	360 μL	=	400 μL

Cyto 10X Neutral Soln (NS) Dilution Table

Nbr of Samples	Vol 10X Cyto Neutral Soln (NS)		Water (from Kit)		Final Volume 1X Neutral Soln (20% extra)
1 to 4	4 μL	+	36 μL	=	40 μL
5 to 8	8 μL	+	72 μL	=	80 μL
9 to 12	10 μL	+	90 μL	=	100 μL
13 to 24	20 μL	+	180 μL	=	200 μL
25 to 36	30 μL	+	270 μL	=	300 μL
37 to 48	40 μL	+	360 μL	=	400 μL
49 to 56	48 μL	+	432 μL	=	480 μL
57 to 64	54 μL	+	486 μL	=	540 μL
65 to 72	60 μL	+	540 μL	=	600 μL
73 to 84	70 μL	+	630 μL	=	700 μL
85 to 96	80 μL	+	720 μL	=	800 μL

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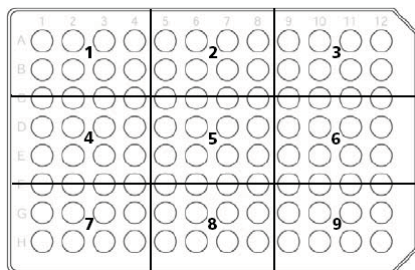
Stage 1b – Whole Genome Amplification

AMPLIFICATION MASTER MIX										Number of Samples Run	✓
Reagent	1	4 (+20%)	8 (+20%)	12 (+20%)	16 (+20%)	20 (+20%)	24 (+20%)	48 (+20%)	96 (+20%)	Lot Number	
Cyto Amplif Rxn Buffer	50 µL	240 µL	480 µL	720 µL	960 µL	1200 µL	1440 µL	2880 µL	5760 µL		
Cyto Amplif Enzyme Mix	2.5 µL	12 µL	24 µL	36 µL	48 µL	60 µL	72 µL	144 µL	288 µL		
Total Volume	52.5 µL	252 µL	504 µL	756 µL	1008 µL	1260 µL	1512 µL	3024 µL	6048 µL		

3. Add 1X Denat and Neutral Solns to Samples

- Add 3 µL 1X Denat Soln to each sample (total volume 6 µL/well).
— **IMPORTANT** Do NOT pipette up and down to mix. Pipette to wall of well.
- Seal, vortex and spin.
- Incubate in plate holder on bench top at room temperature for 3 min.
- Place plate in aluminum block on ice and *immediately* add 6 µL 1X Neutral Soln.
— **IMPORTANT** Do NOT pipette up and down to mix. Pipette to wall of well.
- Seal, vortex and spin.
- Place back in aluminum block on ice (no incubation).

Always vortex plates 1 to 2 sec each sector.
(total of 9 sectors as shown in figure below)



Cyto Amp	
Temp	Time
30 °C	16 hr
65 °C	3 min
4 °C	Hold

4. Prepare and Add Amplification Master Mix

- Prepare the Amplification Master Mix:
 - **IMPORTANT** Cyto Amplif Enzyme Mix: flick to mix, then quick spin.
 - **IMPORTANT** Amplification Master Mix:
 - » Gently vortex (50% maximum speed), then flick and invert tube 2X.
 - » Repeat vortex, flick and invert 1X.
 - » Quick spin and place on ice.
- If using a multi-channel pipet, *slowly* aliquot the master mix equally to strip tubes or a reagent reservoir and place on ice.
IMPORTANT Aliquot slowly to avoid introducing bubbles. Keep master mix and samples on ice throughout this procedure.
- If using strip tubes, quick spin to remove any bubbles.
- Slowly* add 52.5 µL of the master mix to each sample (total volume 64.5 µL/well).
- Seal, vortex and spin.
- Load plate onto thermal cycler and run the *Cyto Amp* program.
- When the program is finished, do one of the following:
 - Proceed directly to Stage 2a, Purification.
 - Store the samples at –20 °C.

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Cytogenetics Assay Stage 2a – Purification

1. Aliquot Reagents to Reservoirs (if using multi-channel pipets)

Cyto Magnetic Beads

1. Invert the Cyto Magnetic Bead bottle 10X or more to thoroughly mix (solution should appear homogenous - no bead clumps on bottom of bottle).
2. Vortex and spin down the plate.
3. Aliquot 90 μ L per sample plus 20% extra to a reservoir. If < 8 samples, aliquot directly to each sample.

Cyto Elution Buffer

- If < 24 samples, *aliquot to strip tubes* 50 μ L per sample plus 25% extra.
- If \geq 24 samples, *aliquot to a reservoir* 50 μ L per sample plus 25% extra.

2. Add Magnetic Beads and Incubate

1. Add 90 μ L of beads to each sample and *slowly* pipet up and down 10X to mix.
IMPORTANT Avoid introducing bubbles. Thorough mixing critical to ensure DNA binds to beads.
2. Cover plate and incubate at room temperature for 5 min.

3. Transfer Samples and Discard Supernatant

1. Transfer full volume in each well to Costar round bottom plate.
2. Place plate on magnetic-ring stand, cover, and allow to sit at room temperature for 5 min.
3. Set a P200 single or multi-channel pipet to 160 μ L.
4. Remove and discard supernatant.
IMPORTANT Place pipet tip in center of bead ring – do not disturb beads – pull straight up and out of wells after aspiration. OK if supernatant is a light brown color.

Preparing Purif Wash Buffer

- Add 99.9% ethanol to Cyto Purif Wash Buffer bottle:
 - 24 sample reagent kit: 10.5 mL
 - 96 sample reagent kit: 45 mL
- Cap bottle and invert 5 times to mix.
- Check the box on bottle to indicate the buffer has been prepared.
- Close cap tightly to store.

4. Add Cyto Purif Wash Buffer

IMPORTANT Ethanol must be added to the Purif Wash Buffer bottle.

1. Aliquot 400 μ L per sample plus 20% extra to a reservoir.
2. Leaving the Costar plate on the magnetic stand, wash the beads twice as follows:
 - A. Add 200 μ L of Purif Wash Buffer to each sample.
 - B. Incubate at room temperature for 1 min. Remove and discard the supernatant.
 - C. Add 200 μ L of Purif Wash Buffer to each sample.
 - D. Incubate at room temperature for 1 min.
 - E. Remove and discard the supernatant.
 - F. Examine the wells. If > 5 μ L in any well, remove with a P20 pipette and discard.

IMPORTANT Immediately elute the samples. Do not allow beads to dry. Drying can negatively effect yields.

5. Elute the Samples

1. Leaving the Costar plate on the magnetic ring-stand, add 50 μ L Cyto Elution Buffer to each sample (do not touch bead ring).
2. Tightly seal plate and vortex in foam adaptor for 15 min at maximum setting.
IMPORTANT Ensure the foam adaptor is securely attached to the vortex. Secure the plate in the foam adaptor with lab tape.
We also recommend placing an anti-vibration pad under the vortex to prevent its movement across the bench top (*e.g.*, Richter Pad, ISC BioExpress, P/N S-7350-25).
3. Spin the plate and remove the seal.
4. Place plate on magnetic-ring stand, cover and allow to sit at room temperature for 30 min.
5. *Slowly* aspirate and transfer 45 μ L of eluate from each sample to a fresh plate.
6. Seal, vortex, spin, and proceed to quantitation.

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Cytogenetics Assay Stage 2b – Quantitation

Plate Spectrophotometer Only – Prepare a Dilution Plate

1. Dilute 2 μL of each sample in 198 μL molecular biology-grade water in an OD plate.
2. Include at least one well or row of water only as a blank.
3. Seal and invert the plate 3X to mix.

Dilution Plate for Plate Spectrophotometer

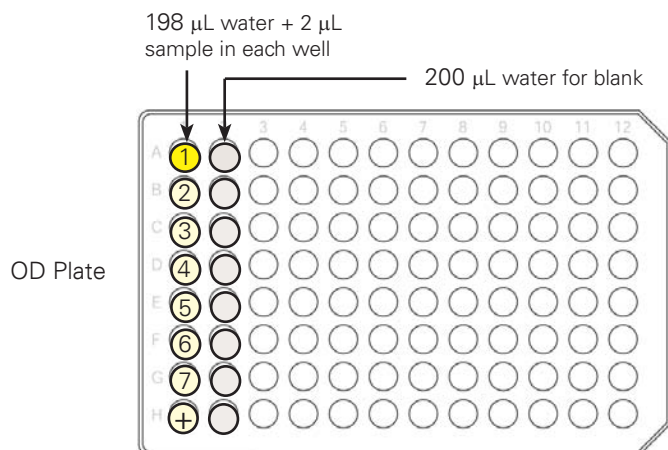


Plate Spectrophotometer

1. Measure the OD of each sample at 260, 280 and 320 nm.
2. Determine the OD₂₆₀ measurement for the water blank and average.
3. Calculate one OD reading for every sample:
$$\text{OD} = (\text{sample OD}) - (\text{average water blank OD})$$
4. Calculate the undiluted concentration for each sample in $\mu\text{g}/\mu\text{L}$:

$$\text{OD} \times 0.05 \text{ ug/uL} \times 100 = \text{undiluted sample concentration}$$

NanoDrop or Infinite 200 NanoQuant

1. Blank the instrument using water.
2. Take 2 μL of sample and measure the OD at 260, 280 and 320 nm.

Assess OD Readings

- An acceptable OD should be greater than or equal to 0.55 $\mu\text{g}/\mu\text{L}$ (no upper limit).
Based on use of a conventional UV spectrophotometer plate reader; assumes a 1 cm path length.
- 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 Assay User Manual* for more information.

When finished with quantitation, do one of the following:

- Proceed directly to Stage 3a, Fragmentation and Labeling.
- Store the samples at -20°C .

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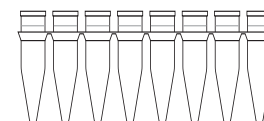
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Stage 3a – Fragmentation and Labeling

1. Preparation

1. Preheat the thermal cycler lid for 10 min.
2. Transfer 37 μL of each sample with an acceptable OD range to a fresh 96-well plate.
3. Thaw the Cyto Frag and Label Buffer at room temperature, then place on ice.
4. Place the purified, normalized samples in an aluminum block on ice.
5. Place a set of strip tubes and a 1.5 mL Eppendorf tube on ice.

Aliquot the master mix equally to strip tubes before adding to samples using a multi-channel pipet.



2. Prepare and Add Fragmentation and Labeling Master Mix

FRAGMENTATION and LABELING MASTER MIX										Number of Samples Run	✓	
Reagent	1	4 (+20%)	8 (+20%)	12 (+20%)	16 (+20%)	20 (+20%)	24 (+20%)	48 (+20%)	96 (+20%)	Lot Number		Volume Prepared
Cyto Frag and Label Buffer	10.00 μL	48 μL	96 μL	144 μL	192 μL	240 μL	288 μL	576 μL	1152 μL			
Cyto Frag and Labeling Enz Mix	3.00 μL	14.4 μL	30 μL	43 μL	57.6 μL	72 μL	86.5 μL	173 μL	346 μL			
Total Volume	13 μL	62.4 μL	126 μL	187 μL	249.6 μL	312 μL	374.5 μL	749 μL	1498 μL			

1. Prepare the Fragmentation and Labeling Master Mix on ice.
2. If using a multi-channel pipet, aliquot the master mix equally to one set of strip tubes on ice.
3. Immediately add 13 μL of the master mix to each sample (total volume per well 50 μL).
4. Seal, vortex and spin.
5. Load plate onto thermal cycler and run the *Cyto Frag-Label* program.
6. Optional: We recommend that you run a QC Gel to check the fragmentation reaction (see the *Fragmentation QC Gel* quick reference card).
7. When finished, do one of the following:
 - Proceed directly to Stage 4, Hybridization.
 - Store the samples at $-20\text{ }^{\circ}\text{C}$.

If desired, you can add Hybridization Buffer to the samples prior to storing at $-20\text{ }^{\circ}\text{C}$. See the quick reference card, *Stage 4 — Hybridization*, for more information.

Cyto Frag-Label	
Temp	Time
37 $^{\circ}\text{C}$	2 hr
95 $^{\circ}\text{C}$	10 min
4 $^{\circ}\text{C}$	Hold

Important Points – Fragmentation Master Mix Preparation

- Leave Cyto Frag and Label Enz at $-20\text{ }^{\circ}\text{C}$ until ready to use.
- Perform all additions on ice.

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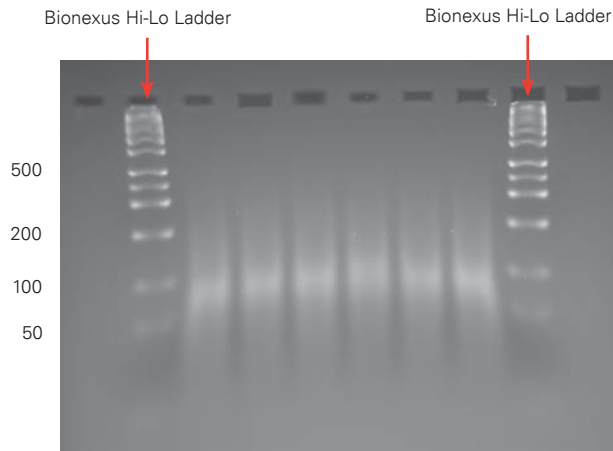
Stage 3b – Optional: Fragmentation QC Gel

1. Optional: Prepare and Run Fragmentation QC Gels

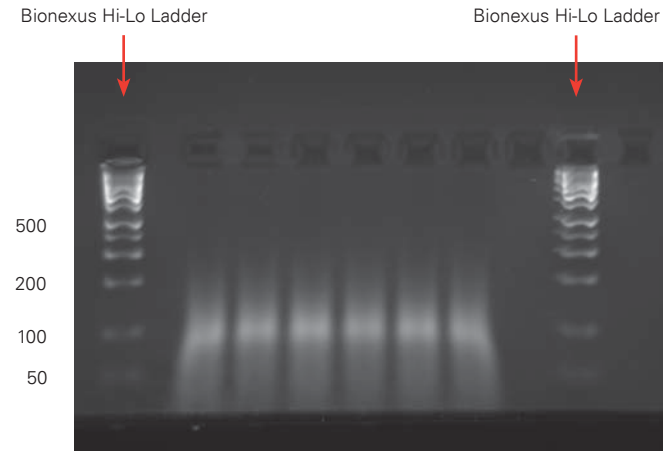
1. Remove the samples from the thermal cycler, spin, and place in an aluminum block on ice.
2. Remove 2.5 μ L of each sample and place in a fresh 96-well plate.
3. Add 2.5 μ L Gel Loading Buffer to each sample and mix.
4. Load 5 μ L Bionexus Hi-Lo Ladder.
5. Load the samples onto a precast 3% to 4% TBE agarose gel.
6. Run the gel at 5 V/cm.

2. Inspect the Gels

Inspect and compare the gels against the examples shown here.



Lonza 4% TBE agarose gel run for 60 min at 80V.



Bio-Rad 3% TBE agarose gel run for 60 min at 80V.

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Cytogenetics Assay Stage 4 – Hybridization

1. Preparation

- Arrays:
 - Unpackage and equilibrate to room temperature prior to use.
 - Number each array and insert a 200 μ L pipet tip in the upper right septum.
- Preheat each hybridization oven until the temperature is stabilized at 50 °C with the rotation on at 60 rpm.
- Place the Cyto Hyb Buffer on ice for 15 min.
- Samples:
 - If frozen, thaw at room temperature on bench top.
 - Vortex and spin, then place in an aluminum block on ice.



2. Batch Register Samples and Scan Array Barcodes

- If not already done, generate a Batch Registration file in AGCC.
- Enter your sample information into the spreadsheet.
- Scan the array barcodes and upload the file to AGCC.

3. Denature the Samples

- Slowly* invert the Cyto Hyb Buffer bottle 5X to mix.
- Add 100 μ L of Cyto Hyb Buffer to each sample.
Aspirate and dispense *slowly* to ensure accuracy.
- Seal, vortex, and spin.
- Load plate onto thermal cycler and run the *Cyto Denature* program.

Cyto Denature	
Temp	Time
95 °C	5 min
50 °C	15 min
50 °C	Hold



Use a P200 pipet to load arrays

4. Load Samples onto Arrays and Hyb

- Leave the samples on the thermal cycler.
- Working one row at a time, load each sample onto an array using a P200 pipet.
 - Cytogenetics 2.7M Array: 130 μ L
 - Cytogenetics 310K Array: 90 μ L
- Clean any excess fluid from around the septa.
- Apply Tough-Spots to the septa and press firmly.
- Load arrays into the hybridization oven 8 at a time.
- When all samples are loaded onto arrays and in oven, hybridize for 16 to 19 hr at 50 °C , rotation 60 rpm.

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.
- When 8 arrays are loaded, immediately place them into the hybridization oven.

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Stage 5 – Washing, Staining and Scanning

Cyto Stain Buffer 1

Invert vial 5X to mix.
500 μ L to Amber vial.
Position 1 on fluidics station.



Cyto Stain Buffer 2

Invert vial 5X to mix.
500 μ L to Blue vial.
Position 2 on fluidics station.



Cyto Holding Buffer

Invert vial 5X to mix.
800 μ L to Clear vial.
Position 3 on fluidics station.



1. Wash and Stain Arrays

1. Prime the fluidics stations (PRIME_450)
Be sure to use Cyto Wash Buffers A and B from the Cytogenetics Reagent Kit.
2. Aliquot Cyto Stain Buffer 1, Cyto Stain Buffer 2 and Cyto Holding Buffer to 1.5 mL vials.
3. Place stain buffers and holding buffer onto the fluidics stations.
4. Remove up to 8 arrays from the hybridization oven at a time
5. Remove the Tough-Spots and load onto the fluidics station.
6. Scan the array barcodes.
7. Using AGCC, run the *Cytogenetics_Array_450* or *Cytogenetics310K_Array_450* protocol.

2. Scan Arrays

1. Turn the scanner on and warm at least 10 min.
2. For each array:
 - A. Cover the septa with Tough-Spots.
 - B. Ensure no bubbles are visible through the window.
 - C. Load onto the scanner.

If no autoloader, keep un-scanned arrays at 4 °C.

Important Points

- Leave arrays in the hybridization oven until ready to wash and stain.
Remove up to 8 arrays at a time only.
If necessary, remove one batch of arrays after 16 hr; the next batch after 17 hr 30 min; and the final batch after 19 hr.
Optional: Remove Hyb Buffer from arrays and replace with Cyto Holding Buffer. Store at 4 °C for up to 4 hr. Do not exceed 4 hr.
- Keep unused Cyto Stain Buffers and Cyto Holding Buffer at 4 °C.
- Always use the reagents in the Cytogenetics Reagent Kit for this assay.
Do not substitute reagents from other Affymetrix reagent kits.
- **Fluidics Station Important Points**
 - Run the standard bleaching protocol prior to processing Cytogenetics Arrays. Thereafter, bleach these instruments on a weekly basis.
 - Change the peristaltic tubing prior to processing Cytogenetics Arrays. Thereafter, change the tubing on a monthly basis.