PLANT

PLOIDY

Report

www.plantploidy.com

Plant ploidy testing.

Walker Pawpaws
ATTN. Woody Walker

182 Westview Dr.
Sparta, NC 28675-8327
Email Thepawpawman@gmail.com

Invoice No.: 1091

Lab No.: 1628-1630
Date: 09-27-2024

Cannabis samples

Controls- Diploid is Blue, Tetraploid is red. Triploid is green. Daylily is purple.

Internal standard

Hemerocallis *Daylily 'Battle Angel'* genome size 8.99 pg 2C DNA content

genus Hemerocallis /ˌhɛmɪroʊˈkælɪs/, a member of the family Asphodelaceae, subfamily Hemerocallidoideae.

Daylily genomes sizes are in the following link.

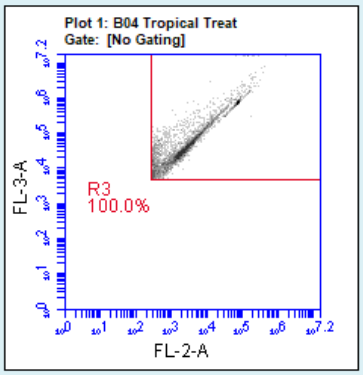
[Daylily Ploidy Genome Database (heavenlygardens.com)]

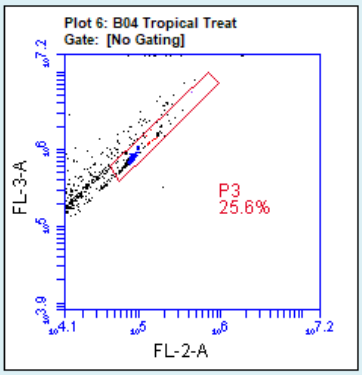
www.heavenlygardens.com/Daylily-Ploidy-database.htm

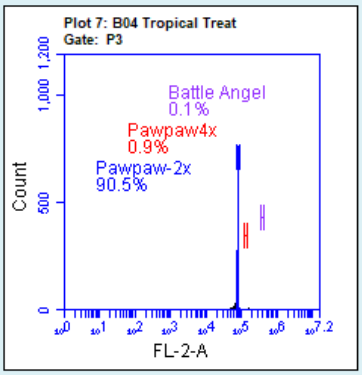
Sample Id is above peak profile.

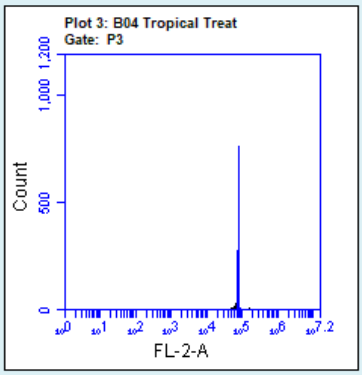
1075 Amity Road * Galloway, Ohio 43119 * (614) 419-1781 * (614) 851-1375 Fax

B4 - Tropical Treat ☑ Show Statistics









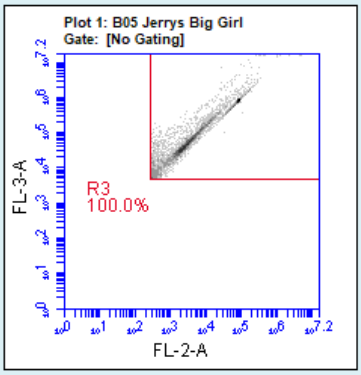
Plot 1: B04 Tropical Treat	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	4,605	171	100.00%	100.00%	26,723.22	320,432.21	206.24%	287.10%		
R3	4,605	171	100.00%	100.00%	26,723.22	320,432.21	206.24%	287.10%		

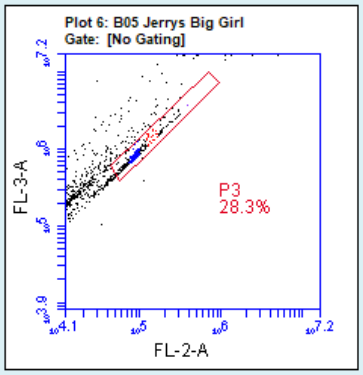
Plot 6: B04 Tropical Treat	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	4,605	171	100.00%	100.00%	26,723.22	320,432.21	206.24%	287.10%		
P3	1,181	44	25.65%	25.65%	83,188.36	791,936.29	24.61%	29.38%		

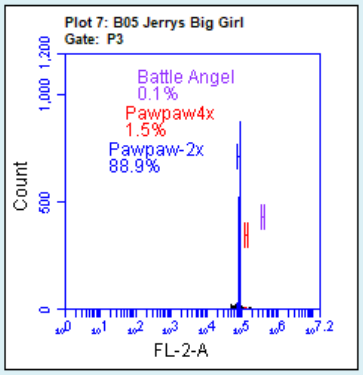
Plot 7: B04 Tropical Treat Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,181	44	100.00%	25.65%	83,188.36	24.61%	
Pawpaw-2x (72,358.0 / 97,...	1,069	40	90.52%	23.21%	81,376.52	3.53%	
Battle Angel (352,820.0 / 4...	1	0	0.08%	0.02%	429,147.00	0.00%	
Pawpaw4x (118,715.0 / 15...	11	0	0.93%	0.24%	139,047.00	8.70%	

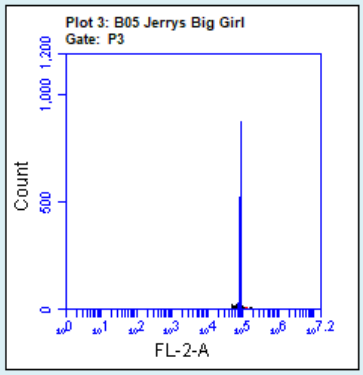
Plot 3: B04 Tropical Treat Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,181	44	100.00%	25.65%	83,188.36	24.61%	

B5 - Jerrys Big Girl ☑ Show Statistics









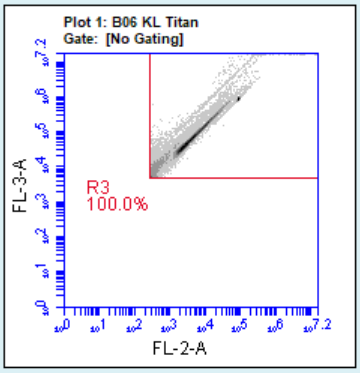
Plot 1: B05 Jerrys Big Girl	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	5,784	340	100.00%	100.00%	32,970.05	360,235.14	342.17%	250.84%		
R3	5,784	340	100.00%	100.00%	32,970.05	360,235.14	342.17%	250.84%		

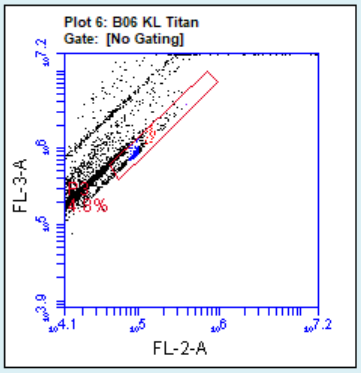
Plot 6: B05 Jerrys Big Girl	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	5,784	340	100.00%	100.00%	32,970.05	360,235.14	342.17%	250.84%		
P3	1,635	96	28.27%	28.27%	90,500.87	849,617.95	25.04%	28.07%		

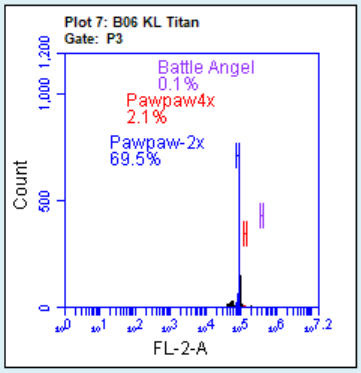
Plot 7: B05 Jerrys Big Girl Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,635	96	100.00%	28.27%	90,500.87	25.04%	
Pawpaw-2x (72,358.0 / 97,...	1,453	85	88.87%	25.12%	88,352.39	3.70%	
Battle Angel (352,820.0 / 47...	1	0	0.06%	0.02%	377,900.00	0.00%	
Pawpaw4x (118,715.0 / 159...	25	1	1.53%	0.43%	135,767.52	9.41%	

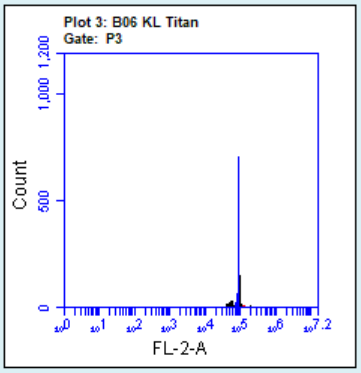
Plot 3: B05 Jerrys Big Girl Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,635	96	100.00%	28.27%	90,500.87	25.04%	

B6 - KL Titan ☑ Show Statistics









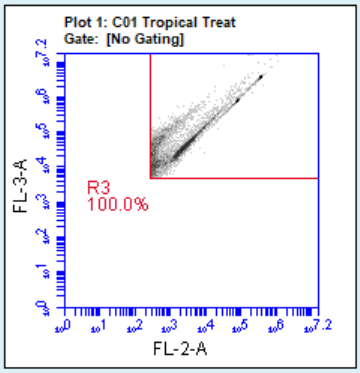
Plot 1: B06 KL Titan	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	24,232	1275	100.00%	100.00%	15,465.40	234,235.88	1,289.74%	455.68%		
R3	24,232	1275	100.00%	100.00%	15,465.40	234,235.88	1,289.74%	455.68%		

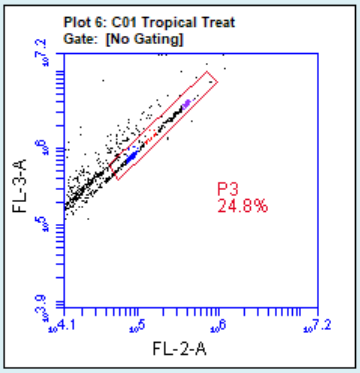
Plot 6: B06 KL Titan	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	24,232	1275	100.00%	100.00%	15,465.40	234,235.88	1,289.74%	455.68%		
P3	1,170	62	4.83%	4.83%	93,932.81	915,958.59	29.25%	32.58%		

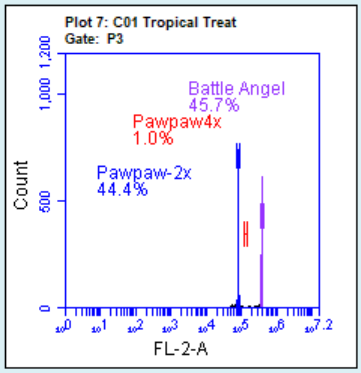
Plot 7: B06 KL Titan Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,170	62	100.00%	4.83%	93,932.81	29.25%	
Pawpaw-2x (72,358....	813	43	69.49%	3.36%	92,578.97	4.56%	
Battle Angel (352,82...	1	0	0.09%	0.00%	383,071.00	0.00%	
Pawpaw4x (118,715....	25	1	2.14%	0.10%	136,263.04	7.64%	

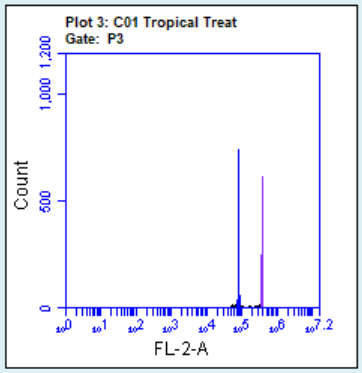
Plot 3: B06 KL Titan Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,170	62	100.00%	4.83%	93,932.81	29.25%	

C1 - Tropical Treat ☑ Show Statistics









Plot 1: C01 Tropical Treat	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	7,775	370	100.00%	100.00%	61,588.72	629,061.55	206.51%	199.88%		
R3	7,775	370	100.00%	100.00%	61,588.72	629,061.55	206.51%	199.88%		

Plot 6: C01 Tropical Treat	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	7,775	370	100.00%	100.00%	61,588.72	629,061.55	206.51%	199.88%		
P3	1,925	92	24.76%	24.76%	233,883.55	2,207,137.60	65.44%	65.25%		

Plot 7: C01 Tropical Treat Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,925	92	100.00%	24.76%	233,883.55	65.44%	
Pawpaw-2x (72,358.0 / 97,...	855	41	44.42%	11.00%	84,833.81	3.40%	
Battle Angel (352,820.0 / 4...	879	42	45.66%	11.31%	393,433.70	2.23%	
Pawpaw4x (118,715.0 / 15...	19	1	0.99%	0.24%	139,372.58	9.47%	

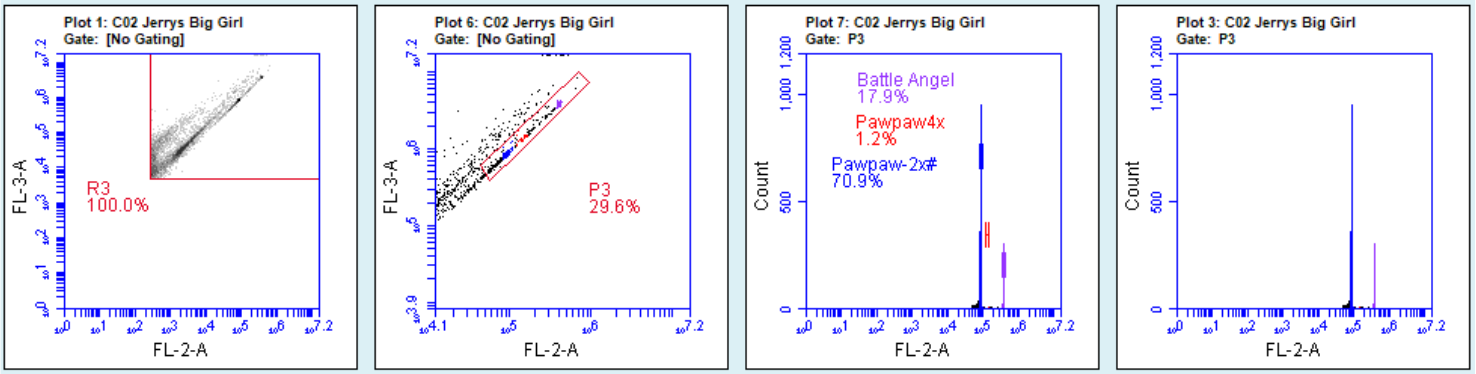
Plot 3: C01 Tropical Treat Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,925	92	100.00%	24.76%	233,883.55	65.44%	

Hemerocallis ***Daylily 'Battle Angel'*** **genome size 8.99 pg 2C DNA content**

Sample calculation = *Daylily 'Battle Angel'* genome size 8.99 pg 2C DNA content x (84,833.81 / 393,433.70) = 1.94 pg (2n DNA content)

Tropical Treat-wild genome size of 1.94 pg (2C DNA content).

C2 - Jerrys Big Girl ☑ Show Statistics



Plot 1: C02 Jerrys Big Girl	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	6,444	307	100.00%	100.00%	48,397.96	518,253.96	197.77%	216.26%		
R3	6,444	307	100.00%	100.00%	48,397.96	518,253.96	197.77%	216.26%		

Plot 6: C02 Jerrys Big Girl	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	6,444	307	100.00%	100.00%	48,397.96	518,253.96	197.77%	216.26%		
P3	1,909	91	29.62%	29.62%	149,288.28	1,412,033.01	82.12%	82.69%		

Plot 7: C02 Jerrys Big Girl Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,909	91	100.00%	29.62%	149,288.28	82.12%	
Pawpaw-2x# (79,889.0 / 10...	1,353	64	70.87%	21.00%	89,292.57	2.87%	
Battle Angel (352,820.0 / 47...	341	16	17.86%	5.29%	402,057.11	2.13%	
Pawpaw4x (118,715.0 / 159...	23	1	1.20%	0.36%	140,315.04	9.08%	

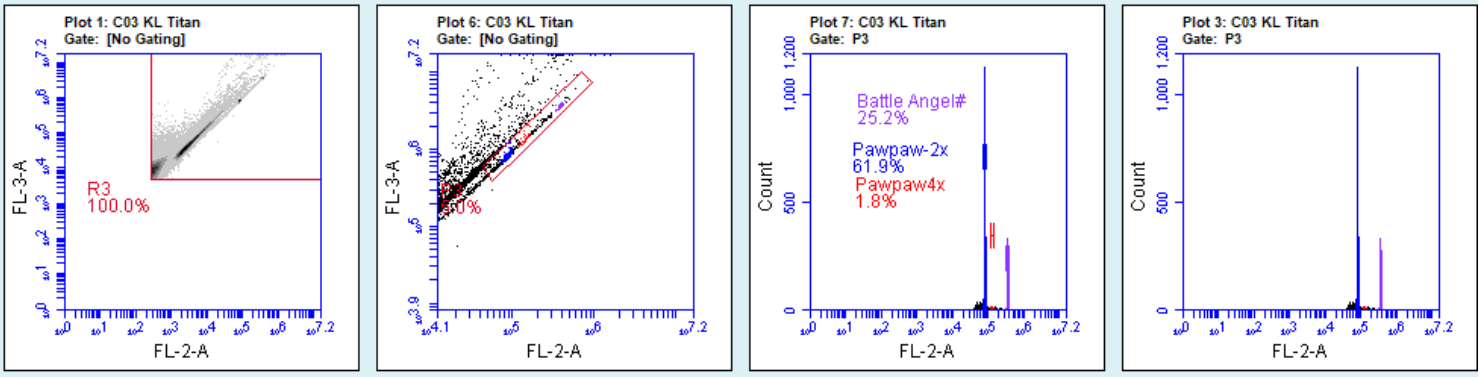
Plot 3: C02 Jerrys Big Girl Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	1,909	91	100.00%	29.62%	149,288.28	82.12%	

Hemerocallis *Daylily 'Battle Angel'* genome size 8.99 pg 2C DNA content

Sample calculation = *Daylily 'Battle Angel'* genome size 8.99 pg 2C DNA content x (89,292.57 / 402,057.11) = 2.00 pg (2n DNA content)

Jerry's Big Girl genome size of 2.00 pg (2C DNA content).

C3 - KL Titan ☑ Show Statistics



Plot 1: C03 KL Titan	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	50,023	2382	100.00%	100.00%	12,302.72	160,316.66	407.93%	449.19%		
R3	50,023	2382	100.00%	100.00%	12,302.72	160,316.66	407.93%	449.19%		

Plot 6: C03 KL Titan	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	Mean FL-3-A	CV FL-2-A	CV FL-3-A	Median FL-2-A	Median FL-3-A
All	50,023	2382	100.00%	100.00%	12,302.72	160,316.66	407.93%	449.19%		
P3	2,500	119	5.00%	5.00%	166,751.83	1,578,901.93	80.20%	79.57%		

Plot 7: C03 KL Titan Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	2,500	119	100.00%	5.00%	166,751.83	80.20%	
Pawpaw-2x (72,358....	1,548	74	61.92%	3.09%	86,497.21	3.38%	
Battle Angel# (319,5...	629	30	25.16%	1.26%	387,961.82	2.56%	
Pawpaw4x (118,715....	46	2	1.84%	0.09%	137,230.20	8.59%	

Plot 3: C03 KL Titan Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL-2-A	CV FL-2-A	Median FL-2-A
This Plot	2,500	119	100.00%	5.00%	166,751.83	80.20%	

Hemerocallis ***Daylily 'Battle Angel'*** **genome size 8.99 pg 2C DNA content**

Sample calculation = *Daylily 'Battle Angel'* genome size 8.99 pg 2C DNA content x (86,497.21 / 387,961.82) = 2.00 pg (2n DNA content)

KL Titan genome size of 2.00 pg (2C DNA content).

Results

2x genome size of 1.94 pg (2C DNA content). 2x
3x genome size of 2.91 pg (2C DNA content). 3x
4x genome size of 3.88 pg (2C DNA content). 4x

Tropical Treat-wild genome size of 1.94 pg (2C DNA content).
Jerry's Big Girl genome size of 2.00 pg (2C DNA content).
KL Titan genome size of 2.00 pg (2C DNA content).

I saw via internet Pawpaw trees as Asimina triloba has 18 chromosomes.
So
Tropical Treat-wild genome size of 1.94 pg (2C DNA content). = 18

So 1.94 / 18 = 0.1077 pg per chromosome diploid

Jerry's Big Girl genome size of 2.00 pg (2C DNA content). = 18.57
2.0 / 0.1077 = 18.57

KL Titan genome size of 2.00 pg (2C DNA content). = 18.57
2.0 / 0.1077 = 18.57

All samples are same as diploid control from client.
Possibility exists that two samples have an extra chromosome or due to added weight in DNA as calculated in genome size. Direct chromosome count for final confirmation would be required to answer if samples have an extra chromosome or due to added weight in DNA Samples are not triploid or tetraploid.

Notes 2C is given as first peak regardless of actual ploidy.
The highest peak area is in bold, in this case **2C.**

If needed, convert DNA mass in picograms to the numbers of base pairs or vice versa as follows 1 pg DNA = .978 x 10^9 Bp. (978000000 base pairs).
Sample amount in Gb /.978= pg (2C DNA content).

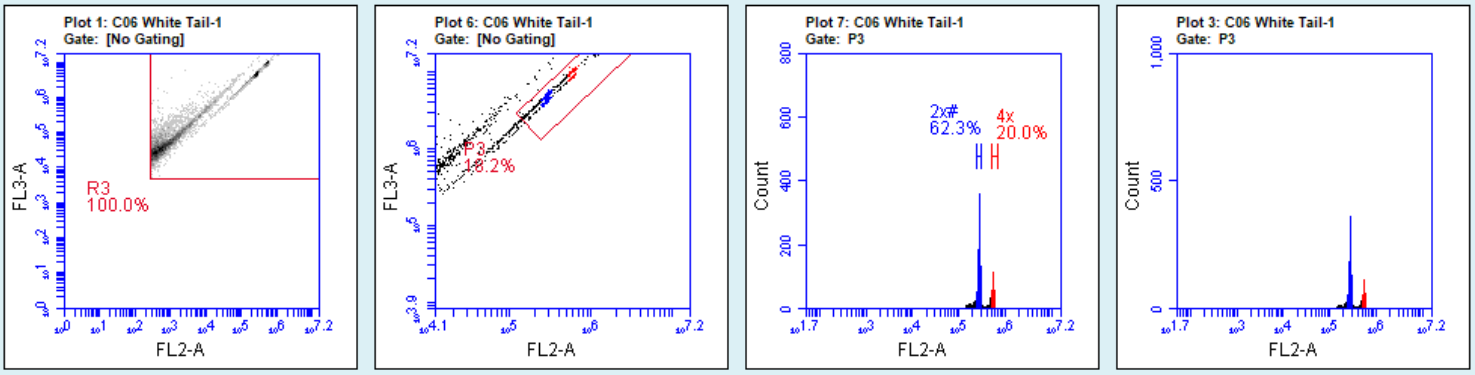
Notes

If you want to convert to actual % of peak. Use: 2x% / (2x% +4x%) x 100 = %2x actual

If you have less than 5% on 2nd peak, I'd go with 0% on that peak if plant going though growth phase.

Example

C6 - White Tail-1 ☑ Show Statistics



Plot 1: C06 White Tail-1	Count	Events / µL	% of This Plot	% of All	Mean FL2-A	Mean FL3-A	CV FL2-A	CV FL3-A	Median FL2-A	Median FL3-A
All	6,601	330	100.00%	100.00%	68,462.65	1,229,641.24	216.41%	201.37%		
R3	6,601	330	100.00%	100.00%	68,462.65	1,229,641.24	216.41%	201.37%		

Plot 6: C06 White Tail-1	Count	Events / µL	% of This Plot	% of All	Mean FL2-A	Mean FL3-A	CV FL2-A	CV FL3-A	Median FL2-A	Median FL3-A
All	6,601	330	100.00%	100.00%	68,462.65	1,229,641.24	216.41%	201.37%		
P3	1,200	60	18.18%	18.18%	352,896.79	5,879,836.90	40.21%	39.80%		

Plot 7: C06 White Tail-1 Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1,200	60	100.00%	18.18%	352,896.79	40.21%	
4x (514,894.0 / 751,925.0)	240	12	20.00%	3.64%	578,261.07	5.63%	
2x# (241,437.0 / 326,86...	747	37	62.25%	11.32%	287,028.61	5.87%	

Plot 3: C06 White Tail-1 Gated on P3	Count	Events / µL	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1,200	60	100.00%	18.18%	352,896.79	40.21%	

Use: 2x% / (2x% +4x%) x 100 = %2x actual

62.25% / (20% +62.25%) x 100 = 75.68% 2x

Use: 4x% / (4x% +2x%) x 100 = %4x actual

20% / (20% +62.25%) x 100 = 24.32% 4x

James Gossard, Laboratory Director

1075 Amity Road * Galloway, Ohio 43119 * (614)419-1781 * (614)851-1375 Fax

ID: Instrument QC

Report Date: SEP 25, 2024 12:18:52 EDT

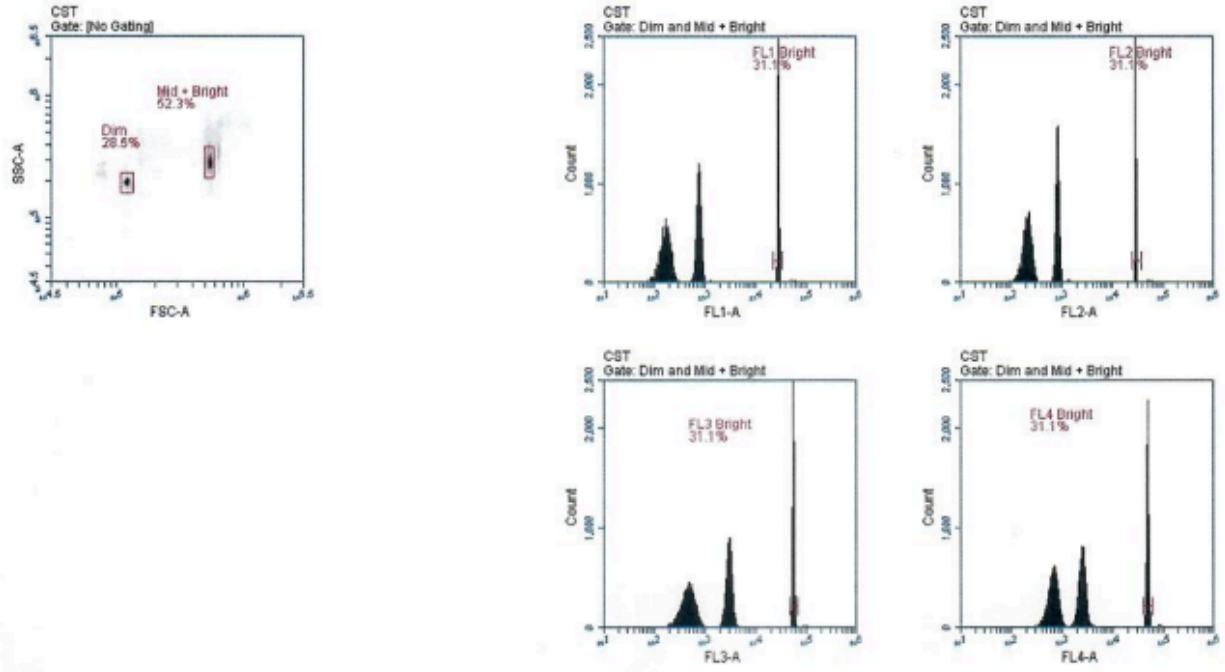
BD Accuri C6 Plus Serial #: R66051700347
BD CSampler Plus Software Version: 1.0.34.1

Result: Passed

BD CS&T Bead Lot	3077233	**Operator**	James Gossard
Expiration	FEB 28, 2025	**Lab Director**	James Gossard
Date Acquired	SEP 25, 2024 12:13:58 EDT		

Instrument QC Report File Name: C:\Cytometer Support Files\Instrument QC reports\InstrumentQC_3077233_20240925_121318.pdf

BD CS&T Beads



Parameter	Bright Bead Median	MFI Range		% Bright Bead rCV	Instrument Sensitivity	Sensitivity Spec.	Parameter Pass/Fail
FSC	546370	411710	764605	2.6%	273	30	Pass
SSC	288419	215730	400641	9.8%	70	50	Pass
FL1	29041	22157	41148	3.2%	270	80	Pass
FL2	29715	21674	40252	1.9%	749	200	Pass
FL3	57529	44845	83284	4.4%	128	40	Pass
FL4	50697	41652	77353	5.1%	110	70	Pass

QC Messages

Comments:

Method

Read this package insert carefully before use.

CyStain™ PI Absolute P **REF 05-5022**

INTENDED PURPOSE

For Research Use Only. Not for use in diagnostic procedures.

CyStain™ PI Absolute P is a reagent kit for nuclei extraction and DNA staining of nuclear DNA from different plant species and tissues in order to determine absolute or relative genome size and ploidy level. It is a staining protocol for the fluorescent staining of nuclear DNA of fixed and non-fixed cells from different origin. The kit was developed for the use in cells obtained from cell suspensions and solid tissues, respectively. The procedure may include fixation with 70 % ethanol. Prepared samples can be analysed on standard flow cytometers.

Intended to be used by professionals or trained personnel only.

KIT COMPONENTS

Packing contains reagents for 250 tests:

- 125 ml Nuclei Extraction Buffer
- 500 ml Staining Buffer
- 2 x 1.5 ml Propidium Iodide
- 1 x 5 mg RNase A

PRINCIPLE OF EXAMINATION METHOD

Reagent kit for nuclei extraction and DNA staining of nuclear DNA from plant tissues to determine absolute and relative genome size and ploidy level.

ADDITIONAL REQUIRED EQUIPMENT

- A calibrated pipette and pipette tips
- Sample tube(s) compliant to the flow cytometer
- Personal protective equipment

INSTRUMENT REQUIREMENTS

A flow cytometer with 488 nm or 532 nm laser light source and a fluorescence parameter of orange - red fluorescence emission (> 590 nm).

INSTRUCTIONS

For instrument alignment and quality control, please refer to the IFU of your flow cytometer.

Preparation of RNase A Stock Solution

1. Add 1.5 ml H_2O to the tube containing RNase A).
2. Mix thoroughly.

Store RNase A stock solution at -20 °C.

Preparation of Staining Solution

The following preparation will suffice for 10 samples.

1. Add 120 µl Propidium Iodide and 60 µl RNase A Stock Solution to 20 ml Staining Buffer.

Freshly prepared Staining Solution is stable for 24 hours, if stored at 2-8 °C and protected from light.

Preparation and staining of samples

1. Put approximately 0.5 cm^2 (or less) of leaf tissue or other plant material in a plastic petri dish (REF No. 04-2005).
2. Add 3 to 4 drops of 500 µl Nuclei Extraction Buffer onto the plant material.
3. Chop the sample by using a sharp razor blade for 30-60 seconds (Razor blades need to be exchanged after 5-10 samples).
4. Add the remaining Nuclei Extraction Buffer and incubate for 30-90 seconds.
5. Filter sample through 50 µm CellTrics™ filter (REF No. 04-0042-2317) into a Sample tube (REF No. 04-2000).
6. Add 2 ml Staining Solution.
7. Incubate for 30-60 minutes protected from light at room temperature.
8. Start analysis.

If samples do show oxidation or staining inhibition effects, e.g. samples showing brownish color, instable peak positions or peaks with a significant left shoulder we do recommend to use as alternative reagent CyStain™ PI OxProtect (REF No. 05-5027).

STORAGE AND STABILITY

Storage

2-8 °C in the dark

Shelf life

Please refer to the expiry date, labelled on the bottle(s).

Reagent solutions

RNase A stock solution has to be stored at -20 °C.

Prepared Staining Solution has to be stored at 2-8 °C in the dark and is stable for 24 hours only.

Rev.: 003
Rev. date: 09-09-2020
Doc. No.: 05-5022 PAI EN
CN 866

Equipment

Laser excitation	488 nm 640 nm
Laser profile	Blue laser beam: 9 x 94 µm Red laser beam: 11 x 104 µm
Laser power	488 nm solid-state blue laser: 20 mW 640 nm diode red laser: 12.5 mW

Item	Specification
Emission detection	4 colors, standard optical filters • FL1 533/30 nm • FL2 585/40 nm • FL3 >670 nm • FL4 675/25 nm
Optical alignment	Fixed alignment
Flow cell	200-µm ID quartz capillary
Minimum detectable particle size	0.5 µm
Minimum sample volume	50 µL (12 x 75-mm tubes) 150 µL (BD Trucount tubes) 100 µL (CSampler Plus, 12 x 75-mm tubes)
Flow rate	14–66 µL/min, depending on the test
Maximum events/sample	1 million events
Fluorescence sensitivity MESF*	FITC <75 PE <50
Fluorescence linearity	2 ±0.05% for chicken erythrocyte nuclei (CEN)
Fluorescence precision	≤3% CV for CEN
Data acquisition rate	10,000 events/s, maximum
Fluid bottle capacity	2 L sheath, 2 L waste 250 mL BD FACSClean 250 mL Detergent Solution

PLANT

PLOIDY

INVOICE

www.plantploidy.com

Invoice to:
Walker Pawpaws
ATTN. Woody Walker

182 Westview Dr.
Sparta, NC 28675-8327
Email Thepawpawman@gmail.com

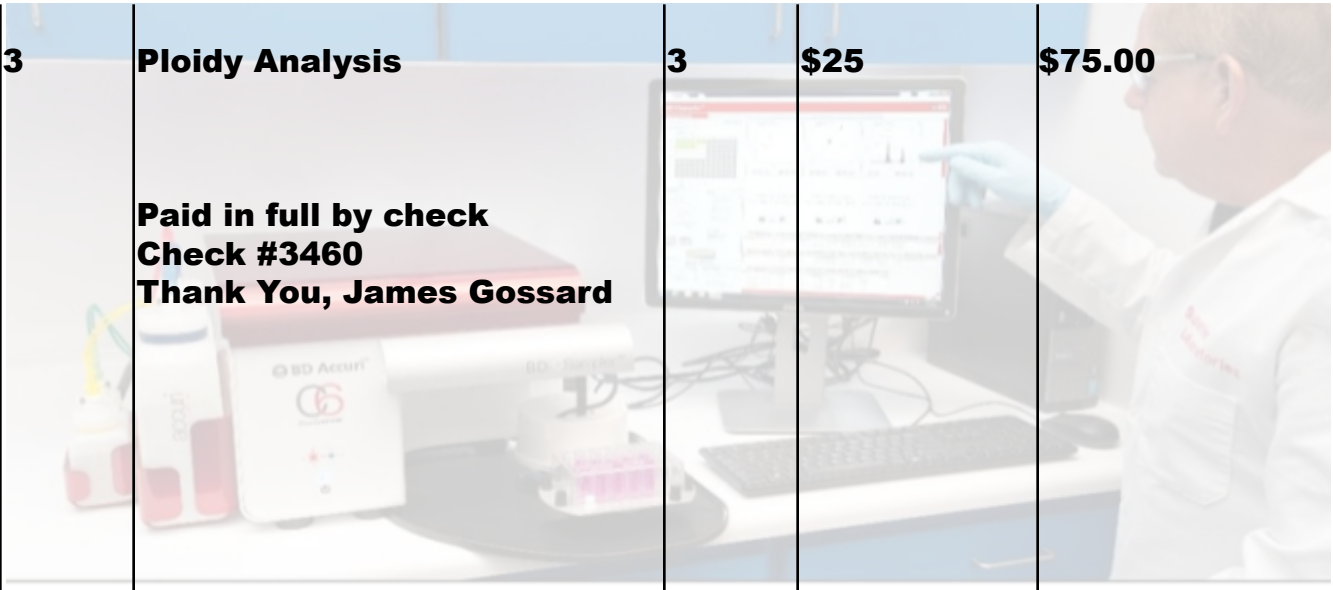
Invoice date: 9-27-2024.

Invoice No.: 1091

Lab No.: 1628-1630
Date: 09-27-2024

NO	DESCRIPTION	QTY	PRICE	SUBTOTAL

3	Ploidy Analysis	3	$25	$75.00
	Paid in full by check **Check #3460** **Thank You, James Gossard**			
			GRAND TOTAL	$000.00



Please include invoice number/copy with your remittance

Accounts 30 days past invoice date will be assessed 1.5% Finance Charge per month

Plant ploidy/ Heavenly Gardens/ Superior Laboratories

Payment Information: **Invoices are due upon receipt.**

PayPal to: Jgossard@aol.com

James Gossard

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