DNA contents of Texas bluegrass acquired from GRIN, collected in Texas and Oklahoma and interspecific hybrids with Kentucky bluegrass determined by flow cytometry

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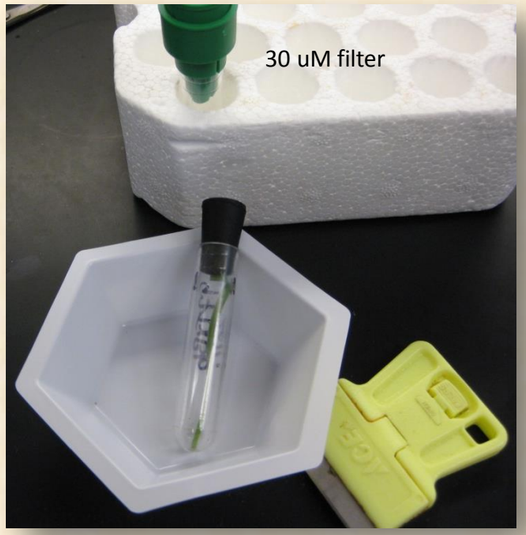
Texas Bluegrass (*Poa arachnifera*)

Texas bluegrass is a dioecious native cool season perennial grass that has withstood the region's heat, droughts and overgrazing for centuries. It produces nutritious and palatable forage during the late fall, winter and early spring when most rangeland forages are least preferred by livestock and lowest in nutrients. Interspecific hybrids with Kentucky bluegrass have the potential to produce turf-type material with greater heat tolerance than Kentucky bluegrass. Recently two different reports indicated that Texas bluegrass exits with a range of genome sizes based on flow cytometry measurements. In order to obtain a better idea of the distribution of genome sizes in Texas bluegrass and hybrids, ***the objectives were to use flow cytometry to estimate genome size in:***

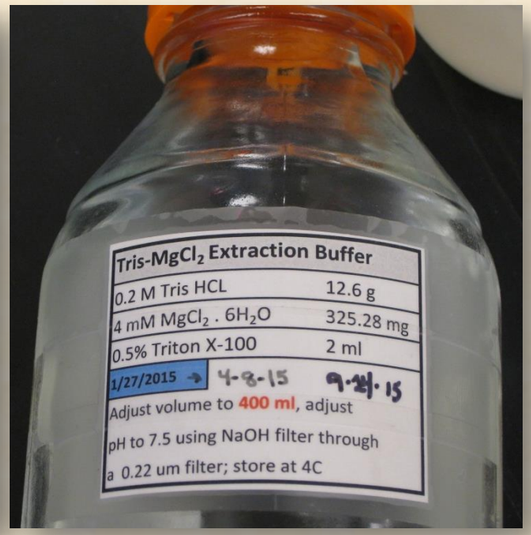
- Seeds acquired from GRIN (original submission and increased generation)
- Plants collected in Texas and Oklahoma
- Progeny from controlled crosses between male and female Texas bluegrass plants
- F1 hybrids obtained by controlled crosses between Texas and Kentucky bluegrass
- Advanced generation hybrids

Fresh leaves from plants in the greenhouse or field are placed in small capped tubes and stored in the fridge until processing.

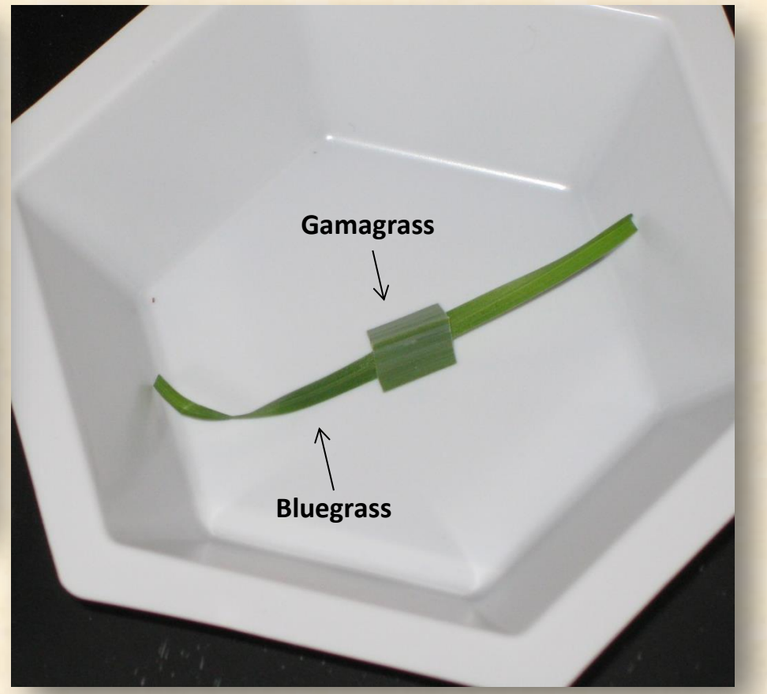
Flow Cytometry



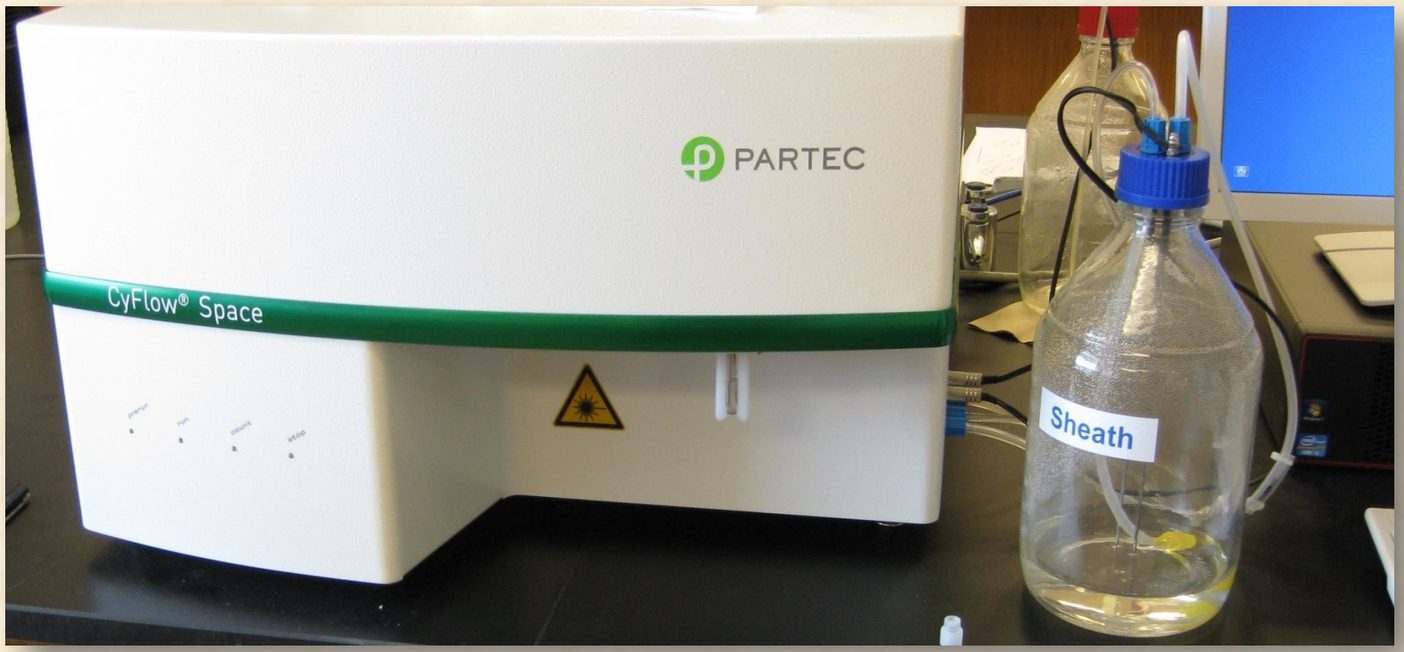
A sharp razor blade housed in a scraper is used to chop leaf tissue in a disposable weigh boat. The extract is filtered through a 30 uM Partec filter.



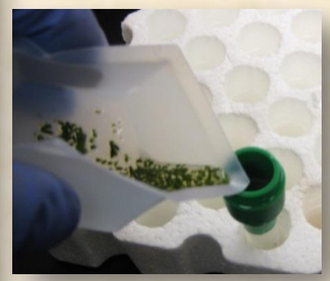
50 ug/mL propidium iodide(PI)
50 ug/mL Rnase
10 mg/mL PVP-40
Added to buffer before use
Buffer kept on ice in the dark



Leaf tissue before the addition of buffer and chopping

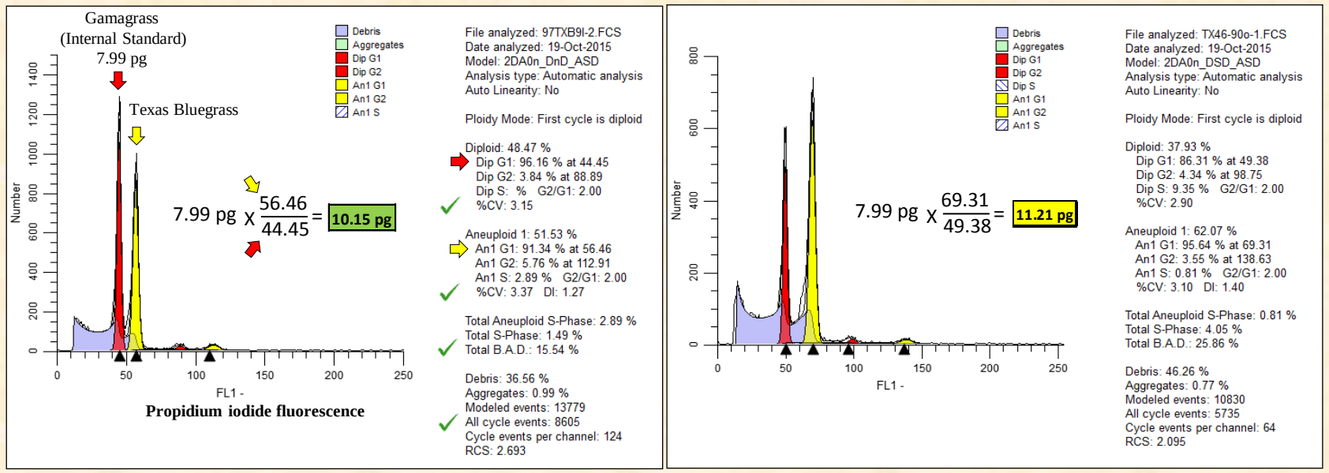


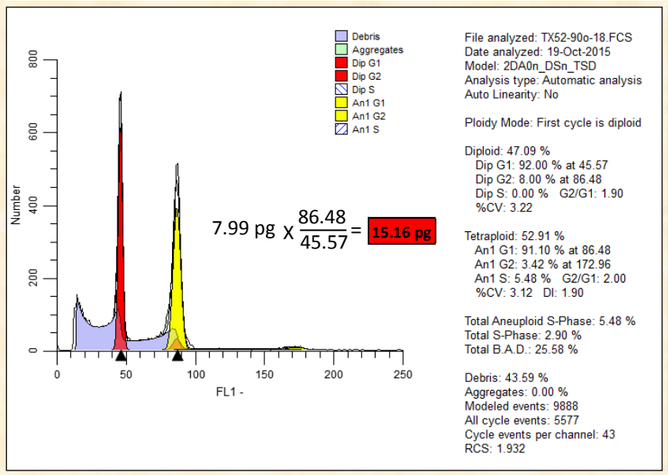
Samples were processed with a Partec CyFlow Space flow cytometer equipped with a 30 mW 532 nm green laser

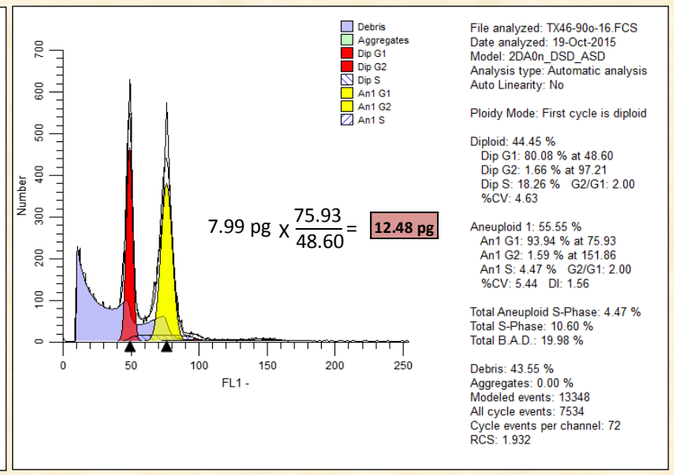


Leaf tissue after the addition of buffer and chopping. The weigh boat is then cupped, shaken, and poured through the green filter.

Histogram analysis to estimate DNA content



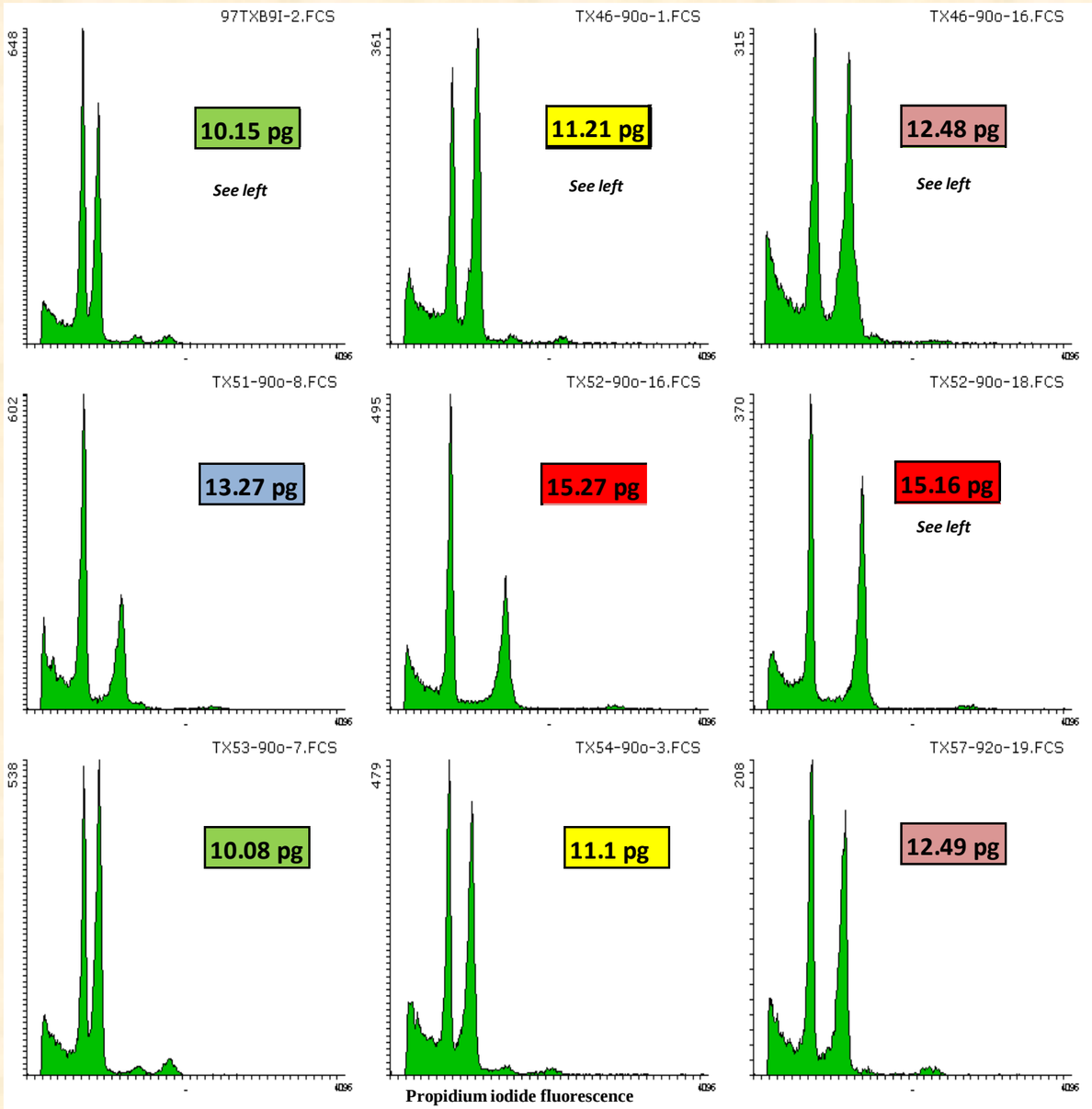




Examples of using Modfit LT 4.1.7 on raw *.fcs files to estimate the mean and ***coefficient of variation (CV)*** of the peaks of interest and the percentage of ***background aggregates and debris (BAD)***. The top left panel shows how peak means are used to calculate the DNA content of Texas bluegrass in picograms (pg) using eastern gamagrass leaf tissue (7.99 pg) as an internal standard.

For a precise DNA content estimate the histogram should have:

- ✓ High nuclei counts in peaks of interest
- ✓ Low coefficient of variation (CV) for peaks of interest
- ✓ A low background aggregates and debris (BAD) %



Examples of histograms produced using eastern gamagrass leaf as an internal standard with Texas bluegrass leaves. The image was created using the raw *.fcs files and the “image collector” function In Flowing Software 2.5.1.

Seed acquired from GRIN

Map-ID GRIN#	Source		Seedling Distribution					N
19-88	Original	J. Read	16		4			20
19-88	Increased		11	5	1			17
52-90	Original	J. Read			6	4	10	20
52-90	Increased				4	7	3	14
54-90	Original	J. Read	10	4	6			20
54-90	Increased		16	3	1			20
97TXB6	Original	A. Hopkins	15					15
97TXB6	Increased		19					19
97TXB9	Increased	A. Hopkins	20					20
51-90	Original	J. Read	16		3	1		20
53-90	Original	J. Read	16	1		1	2	20
53-90	Increased		1	1				2
47-90	Original	J. Read	17		3			20
47-90	Increased		18	9		2		29
4-88	Original	J. Read	10	2	6			18
4-88	Increased		2	2		1		5
49-90	Original	J. Read	17		3			20
49-90	Increased		1	3				4
46-90	Original	J. Read	12	3	4	1		20
46-90	Increased		6	7	2			15
55-90	Original	J. Read	16		3			19
56-90	Original	J. Read	10		6	4		20
39-88	Original	J. Read	7	2	11			20
39-88	Increased		3					3
57-92	Original	J. Read	18	1	1			20

Original = A sample of the original seed that either James Read or Andy Hopkins submitted to the GRIN system. **Increased** = Seed increased from the original seed by the GRIN system and is the class that is generally distributed upon request.

Plant Collections

RICE

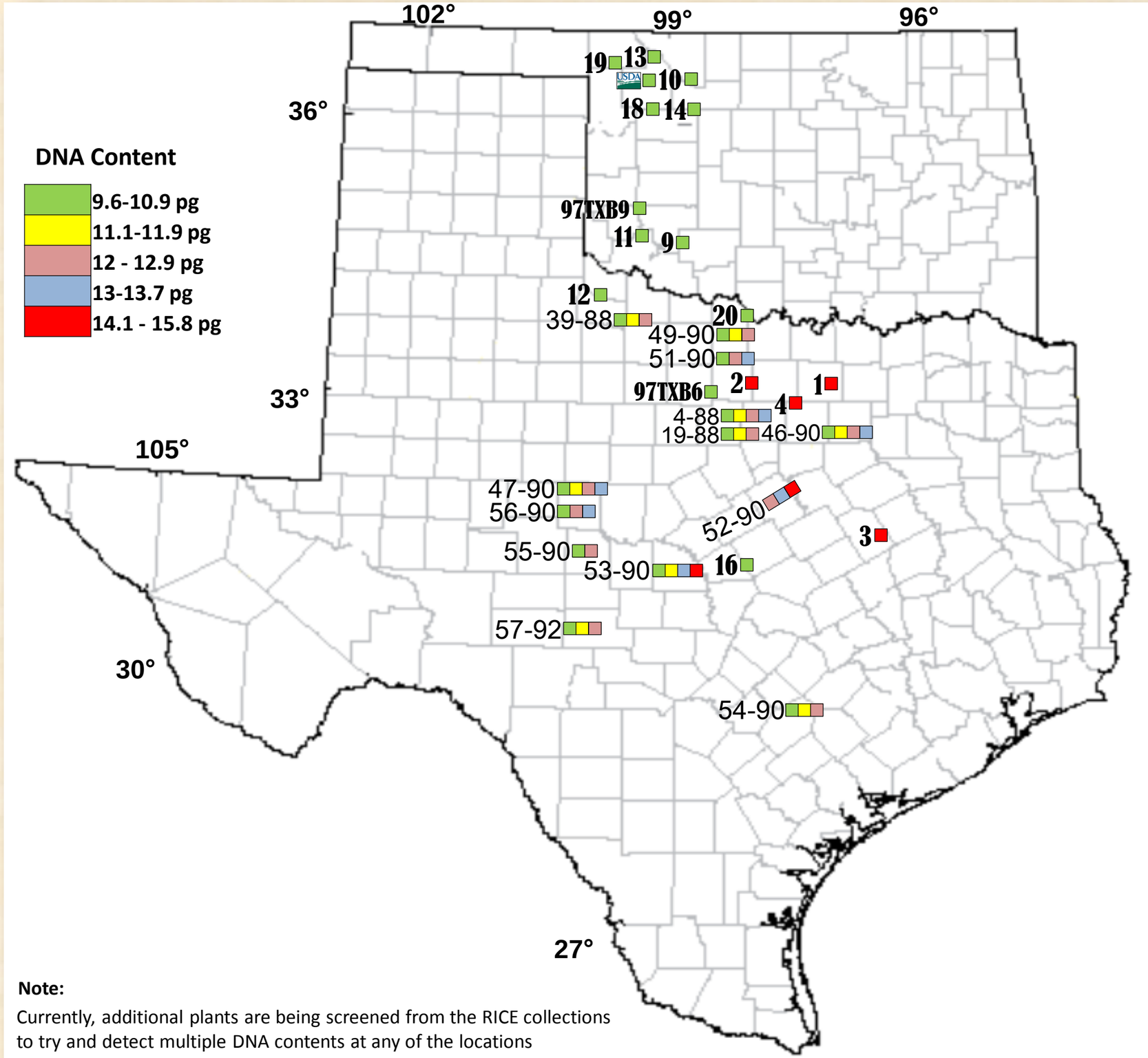
1,2,3,4		Rice U.					8	8
9-20		Rice U.	20					20

Leaves from a single male and female plant at each location were supplied by the Miller lab at Rice University for analysis.

USDA

USDA		J. Goldman	>20					>20

Plants collected within a 50 mile radius of the Woodward Oklahoma USDA – ARS field station.



Note:

Currently, additional plants are being screened from the RICE collections to try and detect multiple DNA contents at any of the locations

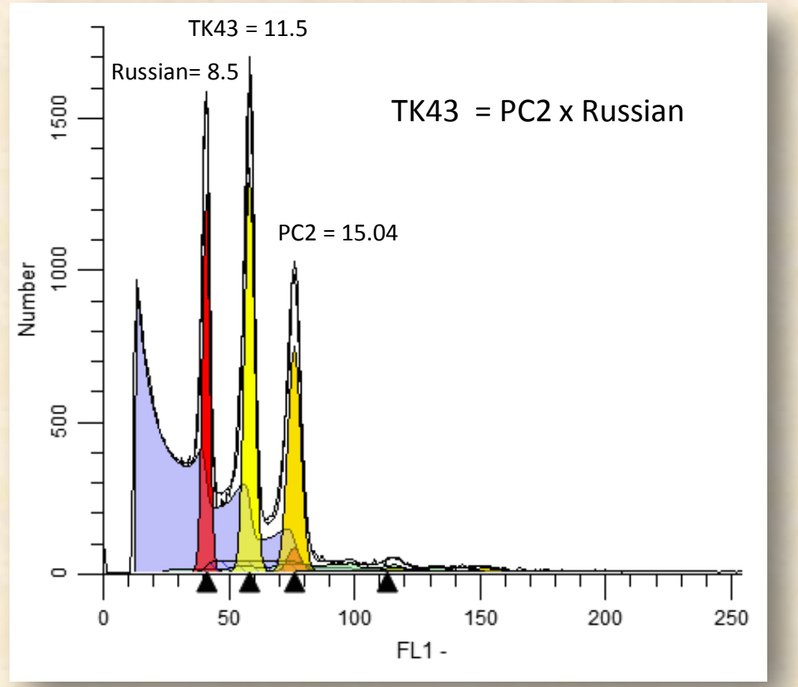
Texas X Texas Controlled Crosses

	Female	Male	Progeny				
D4 crosses			>50				
d4iso-2 x wliso-1 1-28	10.32	13.26		8	7		
wliso-7 x wliso-1 1-28	10.13	13.26		1	4		
d4iso-4 x wliso-1 2-4	10.3	13.26		11	12		
d4iso-7 x wliso-5 2-4	10.23	12.7		13	2		
d4iso-4 x wliso-5 2-4	10.3	12.7	1	7	6		
PC2 x JM2-2	15.3	10.04			5		

DNA content in progeny resulting from controlled crosses involving parents with different DNA contents

A female Texas bluegrass breeding parent. Each bagged inflorescence is a controlled cross pollination

Texas X Kentucky Crosses
F_1 and advanced generations



In some cases F1 hybrids contains a genome size intermediate between the parents as shown with TK43 above. In other cases F1 hybrids contain a genome size that is not intermediate and may be the result of an unreduced gamete or other genetic anomaly.

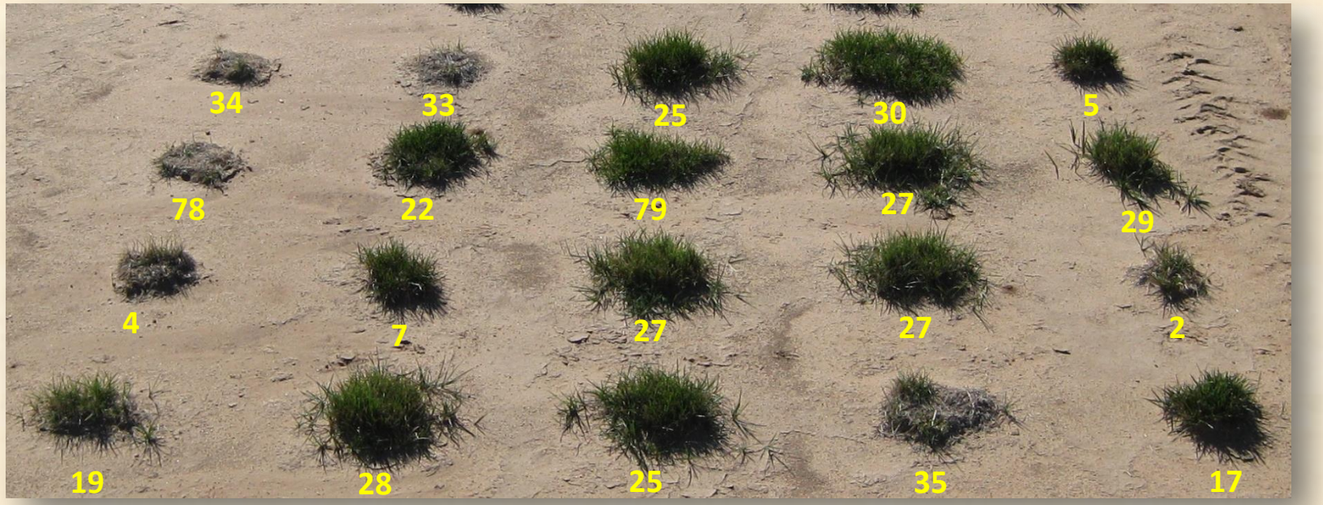
TK43 X Kenblue (KB)			
		KB (n)	KB (2n)
		3.205	6.41
TK43 (n)	5.675	8.88	12.085
TK43 (2n)	11.35	14.555	17.76

Progeny	N
9.5 pg	1
14-15 pg	31
17-17.4 pg	3

With (Texas x Kentucky) x Kentucky or (TK)K hybrids, in this case the majority of the progeny have a genome size larger than either parent and may be the result of an unreduced gamete from TK43.

Advanced generation hybrids derived from TK and (TK)K have been detected that range from 9 – 28 pg.

Seeded 9-25-15 **Photo 11-6-15**



A portion of a low-input turf trial containing advanced generation hybrids, pure Texas bluegrass, Kentucky bluegrass and commercial hybrid bluegrass checks.

17 - TK24 **13.9pg**
25 - (TK43 x Trenton) x Russian **16.38pg**
19 - TK#125 **9.1pg**
5,7 – TK24 x Huntsville – **14.2pg**
22 – TK24 x Huntsville – **15.6pg**

30 - SolarGreen **9.3pg**
27,28 D4 Pure Texas Population (**ca. 10pg**)
29 WL Pure Texas Population (**10-13pg**)
79 - Kentucky PI 206734 **6.83pg**
78 – Kentucky Tsunami

33 Bandera
34 Kentucky PI 539061 (AJC524) **8.2pg**
35 Absolute

Some further questions to try and answer:

Can multiple DNA contents be detected in Texas bluegrass from sources other than James Read? *Further sampling in progress*

Are there any detectable advantages / disadvantages in the performance of Texas bluegrass individual plants or populations that is related to DNA content?

Are there any advantages/disadvantages related to genome size when selecting female Texas bluegrass plants to be used for creating interspecific hybrids?

Is there a relationship between genome size and any positive or negative agronomic traits when evaluating hybrids derived from crosses between Texas and Kentucky bluegrass for low-input turf?

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I would also like to thank Vicki Bradley - USDA, ARS Washington State University Regional Plant Introduction Station for supplying the original and increased GRIN seed submissions .