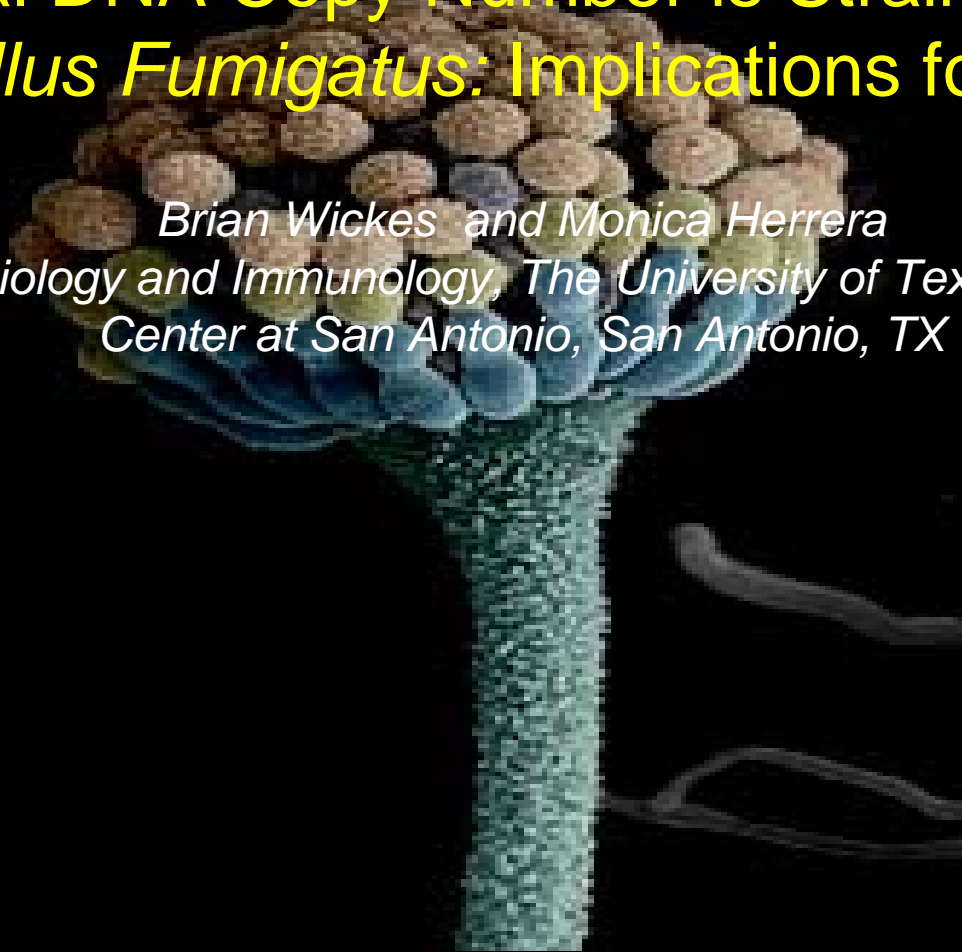


Ribosomal DNA Copy Number is Strain Dependent in *Aspergillus Fumigatus*: Implications for QRT-PCR

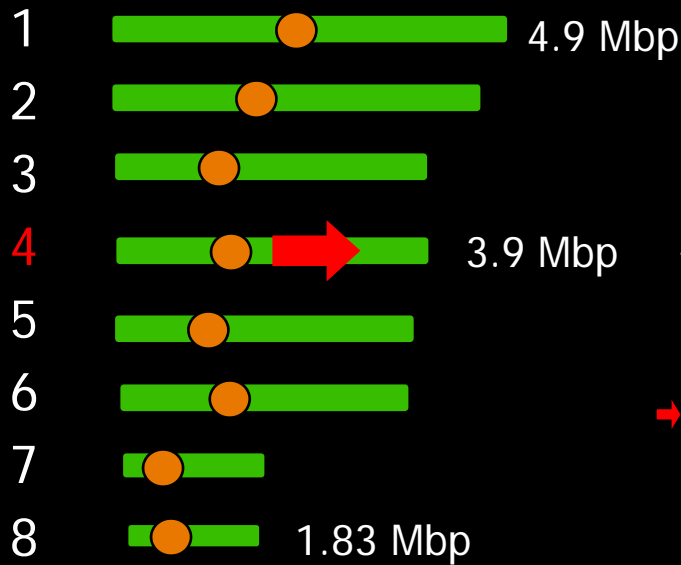
Brian Wickes and Monica Herrera

*Dept of Microbiology and Immunology, The University of Texas Health Science
Center at San Antonio, San Antonio, TX*



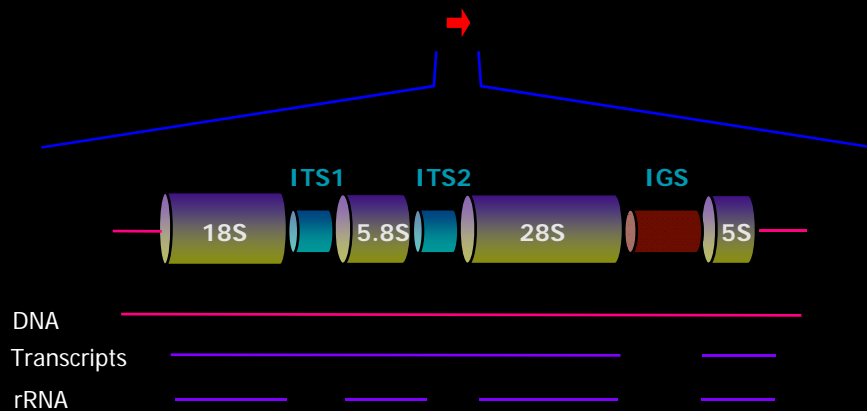
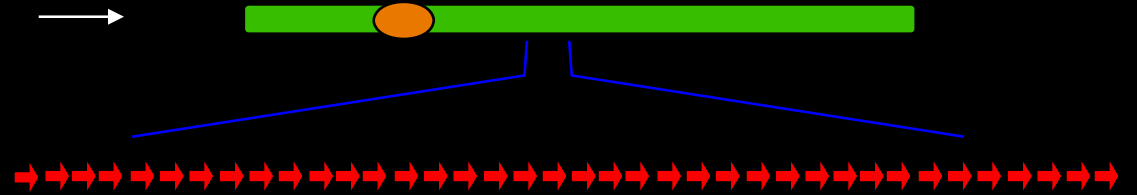
rDNA Organization of the *Aspergillus fumigatus* Genome

29.4 Megabases



→ = 1 repeat

AF293 has 35 repeats



Variation of rDNA Copy Number in Other Fungi

<i>Candida albicans</i>	Infect. Immun. 59:2480-4. 1991
<i>Kluyveromyces lactis</i>	Mol. Gen. Genet. 223:342-4. 1990
<i>Schizosaccharomyces pombe</i>	Mol. Gen. Genet. 236:448-52. 1993
<i>Saccharomyces cerevisiae</i>	J. Gen. Mic. 139:1409-15. 1993
<i>Leptosphaeria maculans</i>	Microbiol. Res. 152:261-7. 1997
<i>Yarrowia lipolytica</i>	Gene 42:273-82. 1986
<i>Cochliobolus heterostrophus</i>	Curr. Genet. 14:573-82. 1988
<i>Pythium insidiosum</i>	Genome 33:585-591. 1990

Does the rDNA variation observed in other fungi occur in *A. fumigatus*?

METHODS

Using known single copy genes as normalizers in qRT-PCR, rDNA copy number from individual strains were determined and compared using an Applied Biosystems 7900 instrument with Taqman probes

- *FKS1* confirmed as a single copy reference by comparison to *pyrG* and *ARG4* using relative quantitation

Beauvais et al., J. Bact 183:2273-2279 2001

Weidner et al., Curr. Genet. 33:378-385 1998

Nierman et al., Nature 438:1151-6 2005

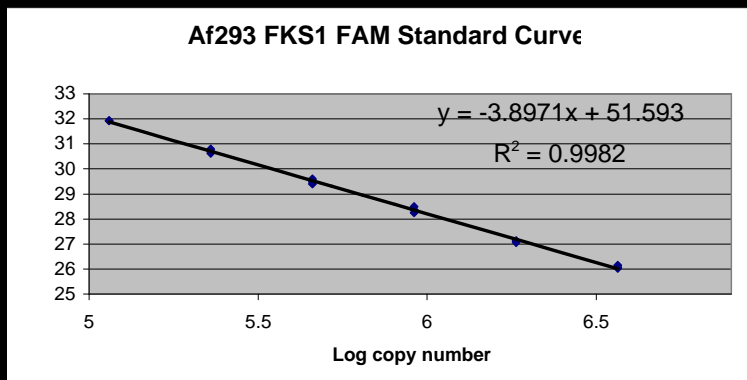
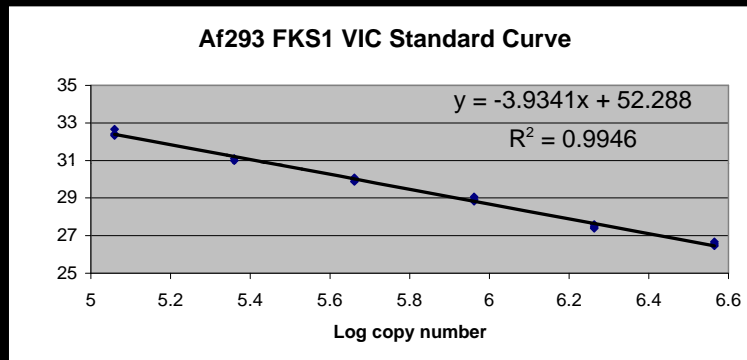
- *arsC* (arsenate reductase) is a duplicated gene and was used for strategy evaluation

Nierman et al., Nature 438:1151-6 2005

- 18s rDNA copy numbers were determined by absolute quantification from multiple strains

Comparison of *FKS1* across strains

Af293 standard curves, 2 dyes



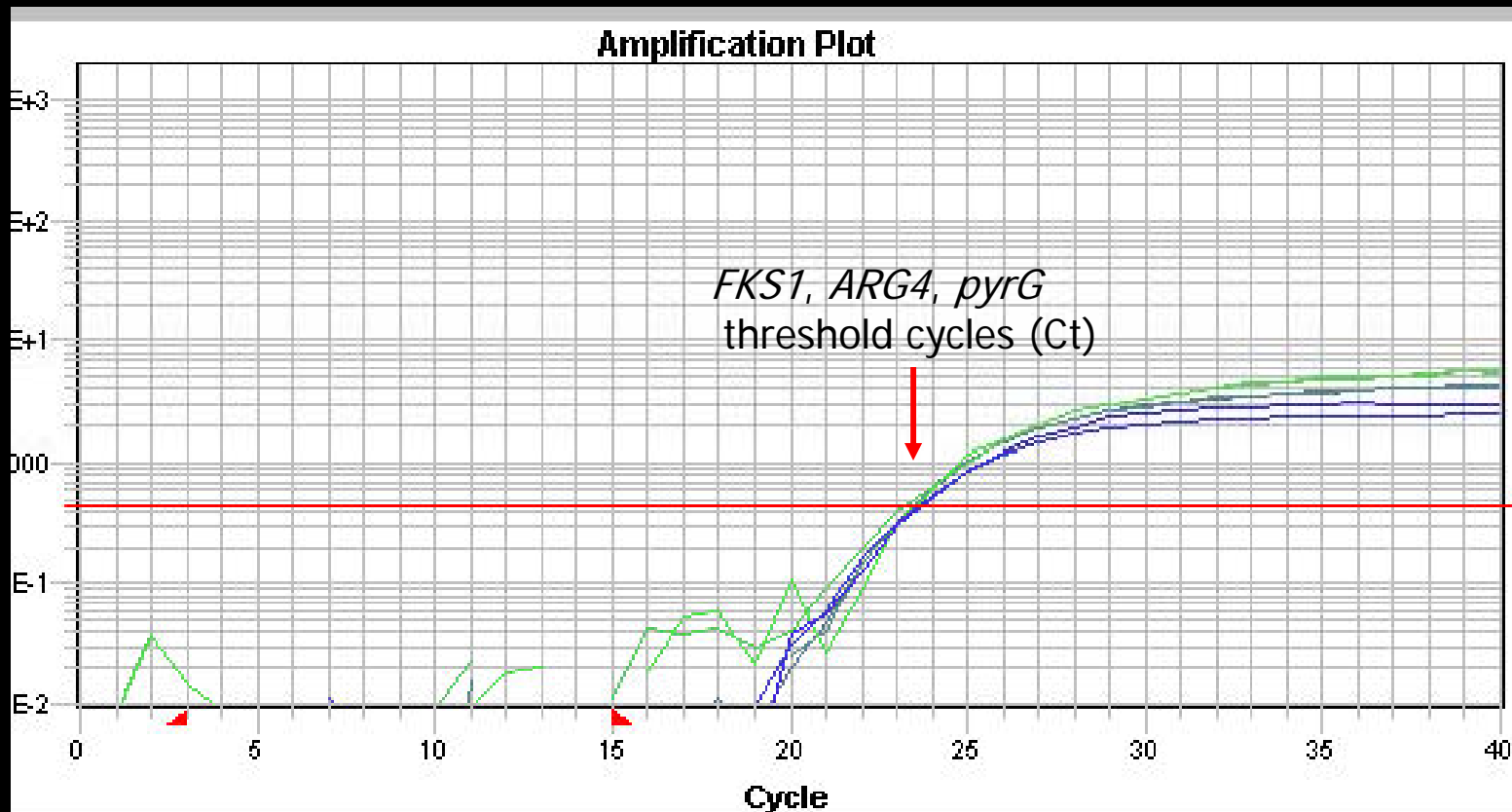
Copy# determination by absolute quantification

STRAIN	FKS1-FAM Ct	FKS1-VIC Ct	Copy#
Af293	26.01	26.03	1.05+/-0.02
WSA-450	28.05	28.10	0.97+/-0.13
WSA-172	24.02	24.11	0.93+/-0.14
WSA-445	25.87	25.66	0.98+/-0.11
WSA-621	22.13	22.23	0.94+/-0.10
WSA-419	24.15	24.09	1.10+/-0.26

N= 3 samples, done in duplicate

Comparison of *FKS1* to *ARG4* and *pyrG*

Af293 amplification plot



FKS1 vs. *ARG4* and *pyrG*

Confirmation of single copy genes by QRT-PCR

Comparison of *FKS1* vs *pyrG* and *ARG4*

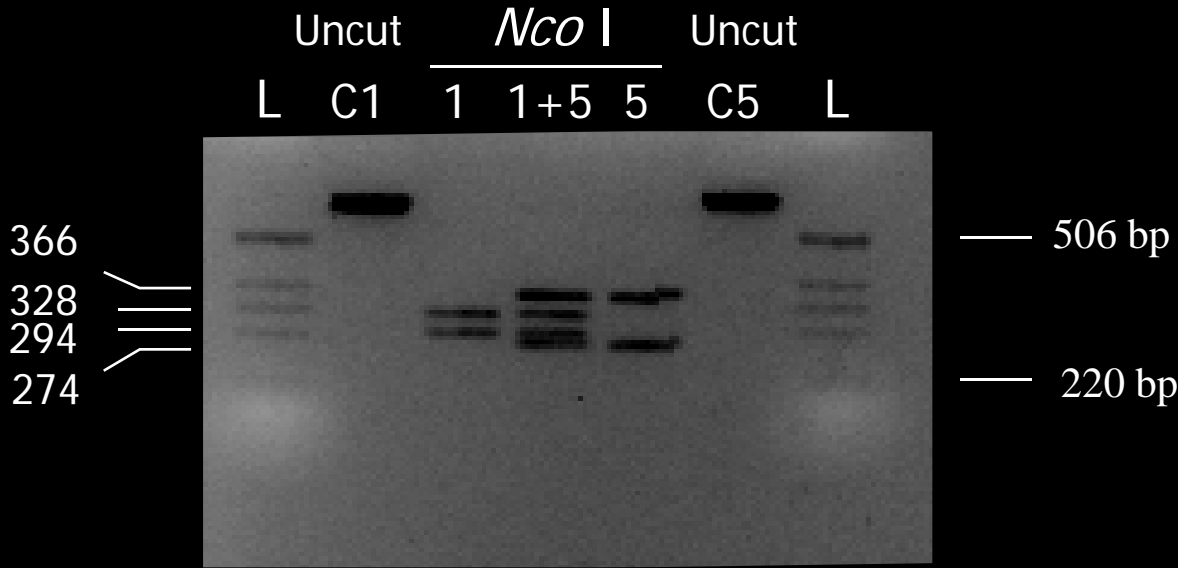
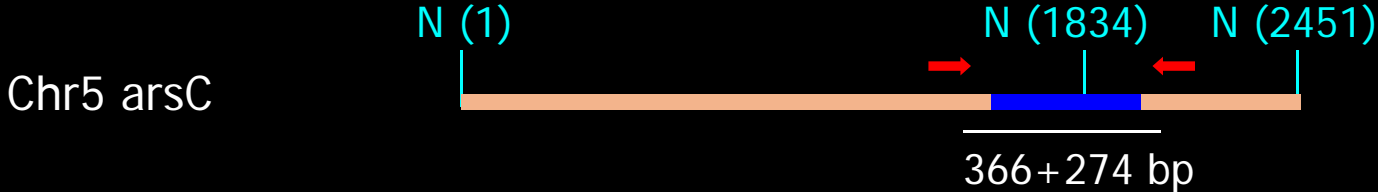
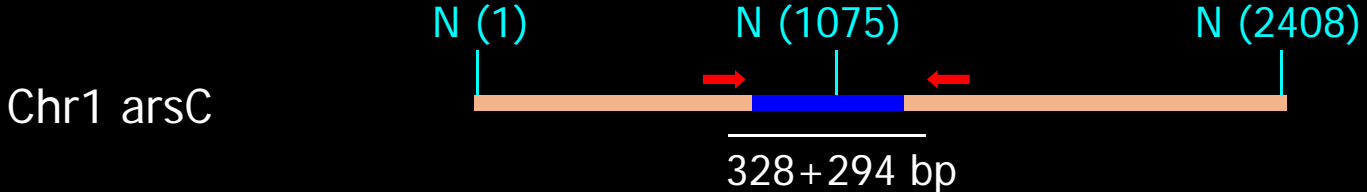
STRAIN	Avg Ct	FKS1 copy#	Avg Ct	pyrG copy#	Avg Ct	ARG4 copy#
Af293	19.16 +/- .012	1	19.33 +/- .005	1.13	19.06 +/- .008	1.07
WSA-450	19.92 +/- .099	1	20.00 +/- .005	1.06	20.30 +/- .006	0.77
WSA-172	21.39 +/- .012	1	21.34 +/- .001	0.97	21.23 +/- .007	1.11
WSA-445	22.26 +/- .049	1	22.55 +/- .003	1.22	22.31 +/- .004	0.97
WSA-621	24.34 +/- .014	1	23.52 +/- .004	1.29	24.54 +/- .007	1.10
WSA-419	21.40 +/- .050	1	21.82 +/- .003	1.34	21.40 +/- .005	1.00

N= 3 samples, run in duplicate

Copy # test gene (*pyrG* or *ARG4*) = $2^{-\Delta Ct}$

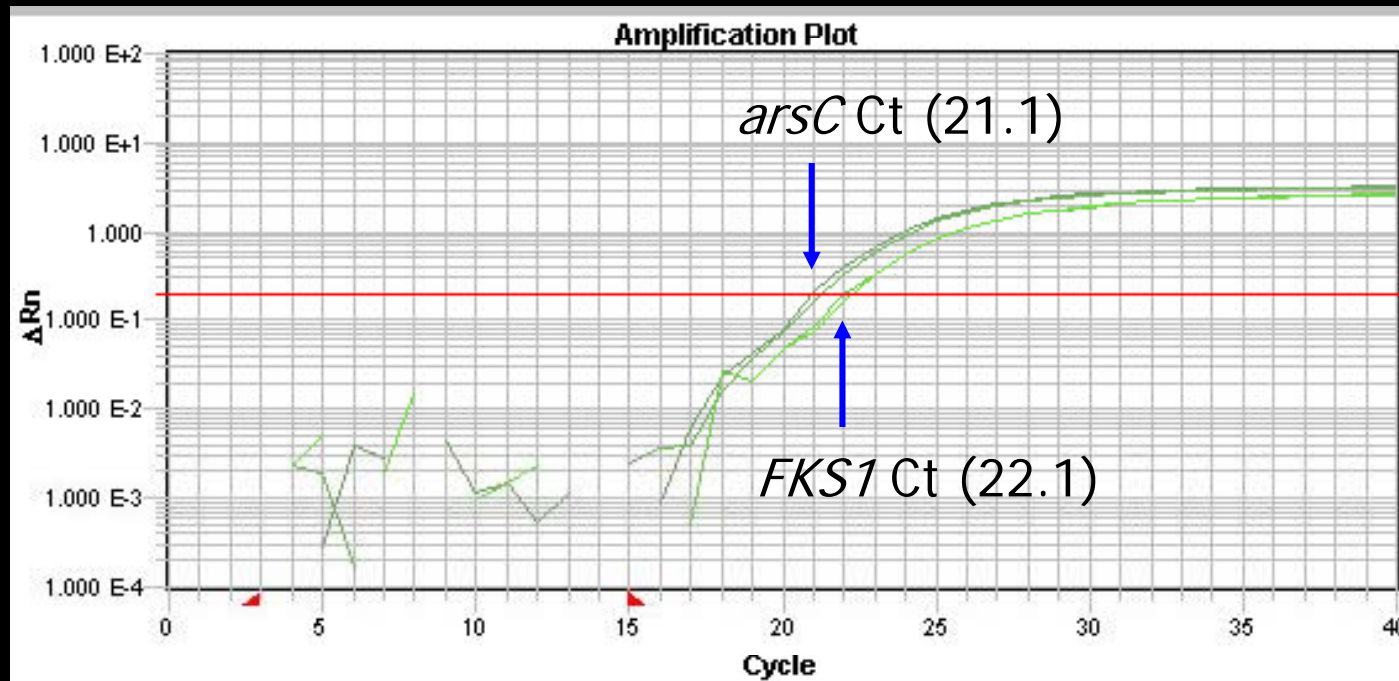
FKS1 selected as reference gene based on demonstration of 1 copy/genome

Confirmation of *arsC* Duplication



Confirmation of *arsC* copy number by QRT-PCR

Comparison of Af293 *FKS1* vs. *arsC*



Determination of *arsC* copy number by QRT-PCR

FKS1 vs *arsC*, all isolates

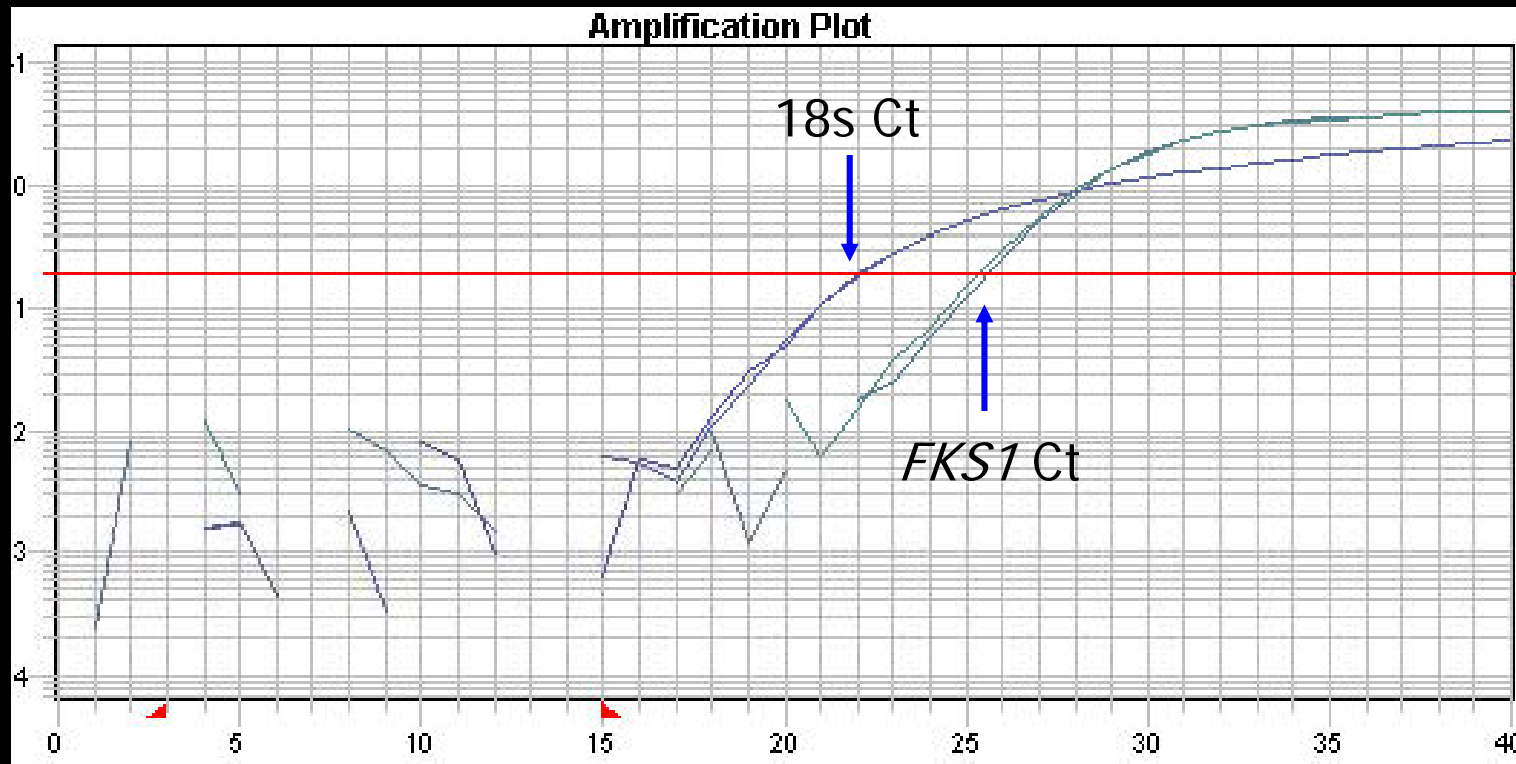
Strains	Avg C_t <i>FKS1</i>	<i>FKS1</i> copies	Avg C_t <i>arsC</i>	<i>arsC</i> copies
Af293	22.1 +/- .107	1	21.1 +/- .007	2 (2.00)
WSA-622	19.2 +/- .030	1	18.1 +/- .002	2 (1.71)
WSA-172	19.8 +/- .010	1	18.7 +/- .004	2 (2.14)
WSA-446	22.0 +/- .052	1	21.1 +/- .003	2 (1.89)
WSA-445	19.8 +/- .014	1	18.7 +/- .005	2 (2.16)
WSA-271	22.1 +/- .025	1	21.0 +/- .002	2 (2.07)
WSA-270	19.2 +/- .042	1	18.2 +/- .002	2 (2.00)
WSA-621	18.9 +/- .060	1	18.8 +/- .003	2 (2.01)
WSA-419	23.1 +/- .014	1	21.9 +/- .007	2 (2.42)

N= 3 samples, run in duplicate

arsC copy # = $2^{-\Delta Ct}$

Determination of 18s rDNA copy number by QRT-PCR

Af293 18s rDNA vs *FKS1*



A. fumigatus 18s rDNA copy number

Absolute quantitation method

Prepare Std curve of \log_{quantity} vs C_t

Obtain $C_t = m(\log \text{quantity}) + b$ from $y = mx + b$

Verify $r^2 > 0.980$

$$\text{Total copies} = 10^{\frac{(C_t - b)}{m}}$$

$$18s \text{ rDNA copy\#/genome} = \frac{\text{total copies } 18s}{\text{total copies } FKS1}$$

STRAIN	18s rDNA copy #
Af293	38 +/- 0.01
WSA-622	45 +/- 0.07
WSA-172	46 +/- 0.03
WSA-446	47 +/- 0.01
WSA-445	49 +/- 0.06
WSA-271	49 +/- 0.05
WSA-270	53 +/- 0.01
WSA-621	70 +/- 0.03
WSA-419	91 +/- 0.03

Range: 38-91, Avg: 54

Af293 genome=35, QRT-PCR=38

The Effect of Environmental factors on rDNA copy number

Morphology (5d growth)

Conidia
38.01 +/- 0.02

Hyphae
38.11 +/- 0.03

Culture age (RT, broth)

3d
38.03 +/- 0.04

5d
37.93 +/- 0.12

10d
38.32 +/- 0.03

25d
38.06 +/- 0.07

Growth Temperature (agar)

30°C
37.86 +/- 0.05

45°C
38.01 +/- 0.01

Tissue

Mouse
lung

kidney

Guinea Pig
lung

kidney

38.03 +/- 0.07

38.13 +/- 0.01

38.22 +/- 0.06

37.67 +/- 0.13

Summary and Conclusions

- Copy number varies with strain, 38-91 copies /haploid genome
- *rDNA should not be used as a reference for interstrain comparisons without additional controls*
- No evidence that copy number varies with morphology, temperature, age, tissue type

Acknowledgments

Wickes Lab, Dept of Microbiology, UTHSCSA

Monica Herrera
Maxinne Pineda
Jianmin Fu, PhD

Patterson Lab, Dept of Medicine, UTHSCSA

Tom Patterson, MD
Ana Vallor, PhD
Laura Najvar

NIH, TIGR, Sanger