



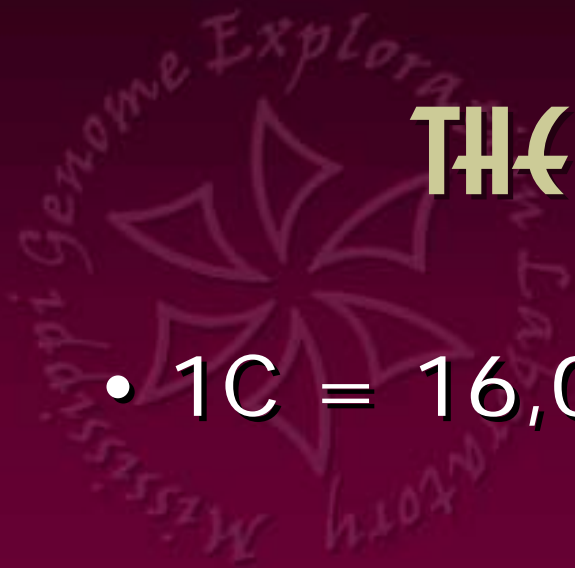
GENE-ENRICHMENT STRATEGIES FOR ANALYSIS OF THE WHEAT GENE SPACE

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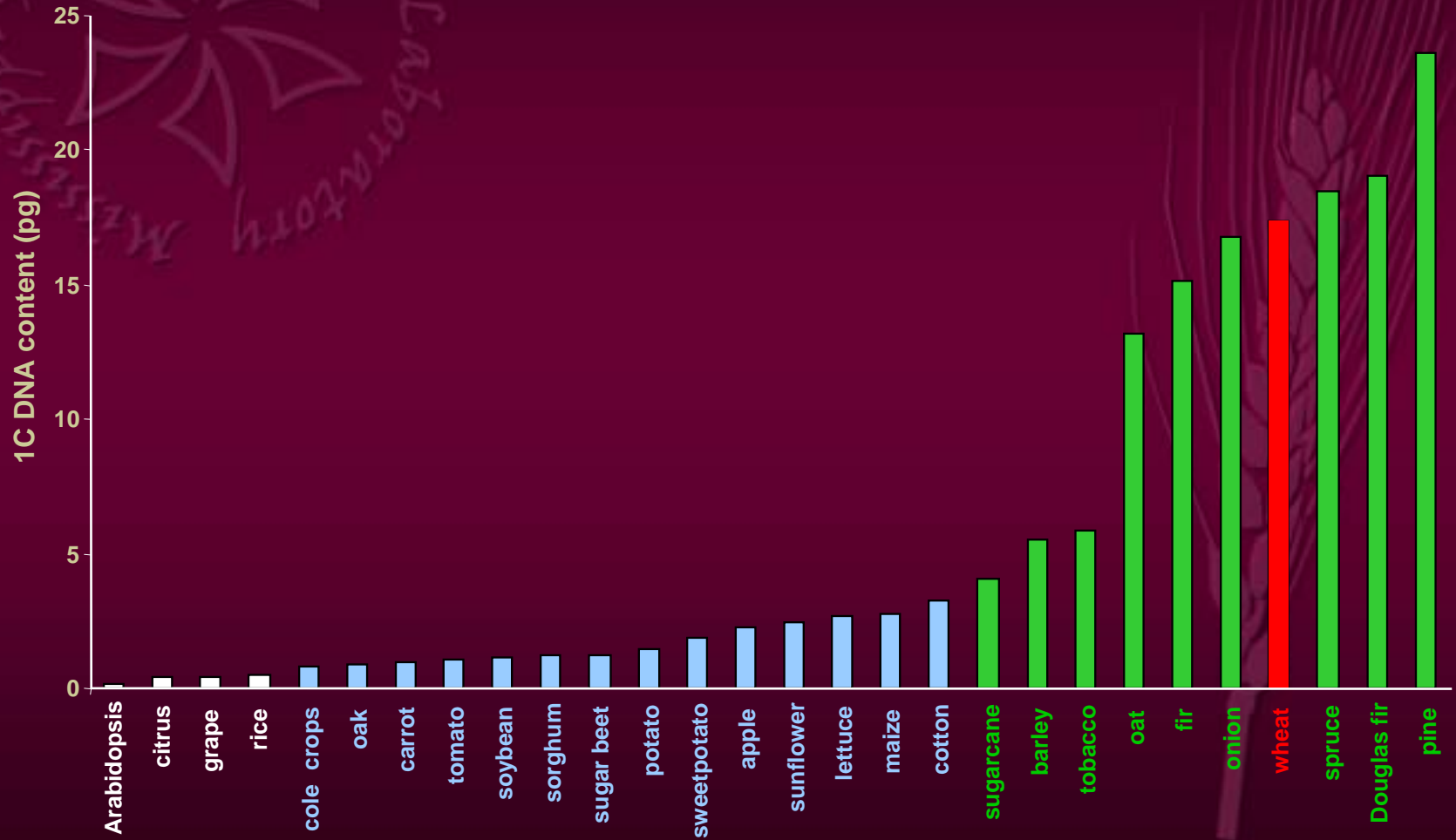
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THE WHEAT GENOME

- 1C = 16,000 Mb
- 5.1 times larger than human genome
- Hexaploid (AABBDD)



COMPARATIVE GENOME SIZES



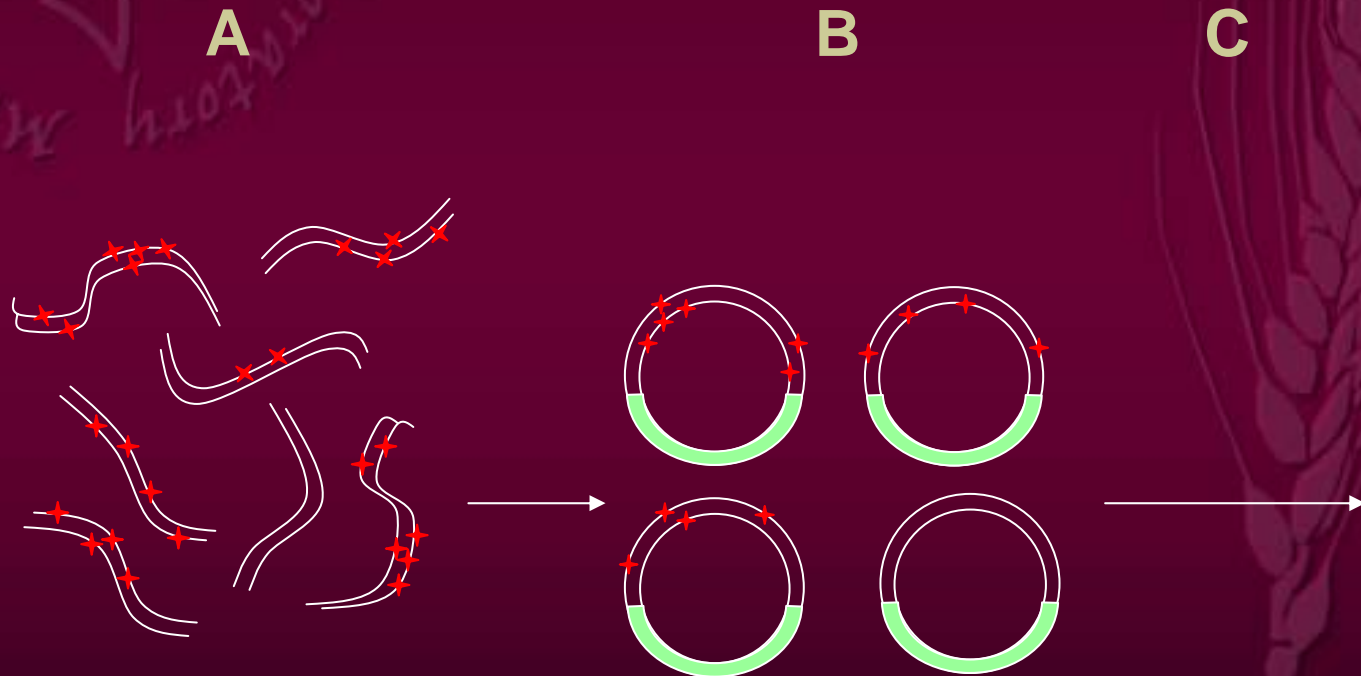
WHEAT GENES & REPETITIVE DNA

- Repeated sequences = at least 85% of wheat genome (Smith and Flavell 1975)
- Wheat retroelements – Some up to several kb in length; relatively well conserved from one copy to another
- Wheat genes are mostly grouped in *gene islands* scattered in a *sea* of repetitive elements (Gill *et al.* 1996; Faris *et al.* 2000)

GENE ENRICHMENT TECHNIQUES

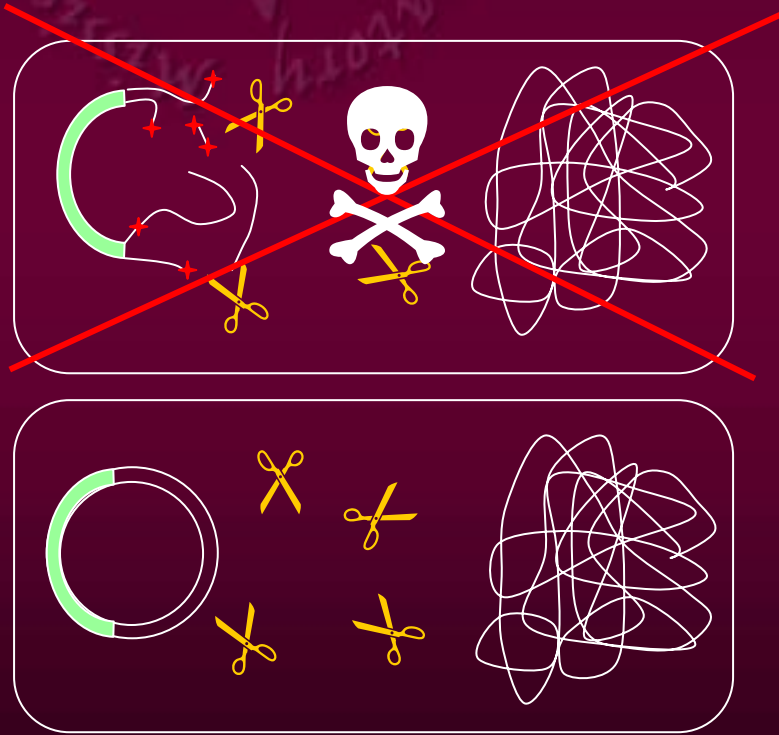
- EST sequencing
- **Methylation filtration (MF)** – Orion Genomics
 - Rabinowicz et al. (1999)
 - Methyl filtration, GeneThresher[®]
- **Cot filtration (CF)**
 - Peterson et al. (2001, 2002)
 - Cot-based cloning & sequencing (CBCS)
 - High Cot (HC) sequencing

METHYLATION FILTRATION (MF)

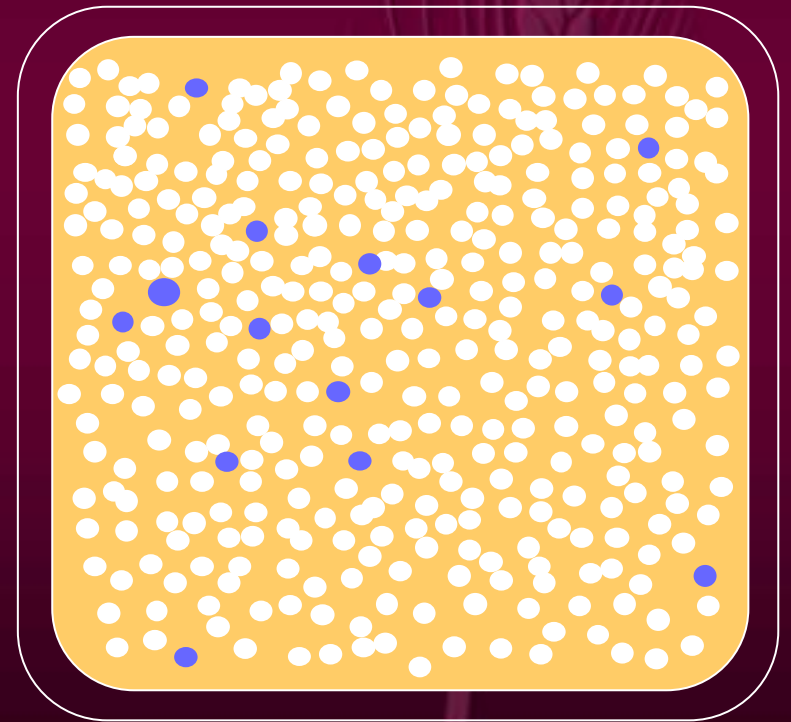


mf CONTINUED

D



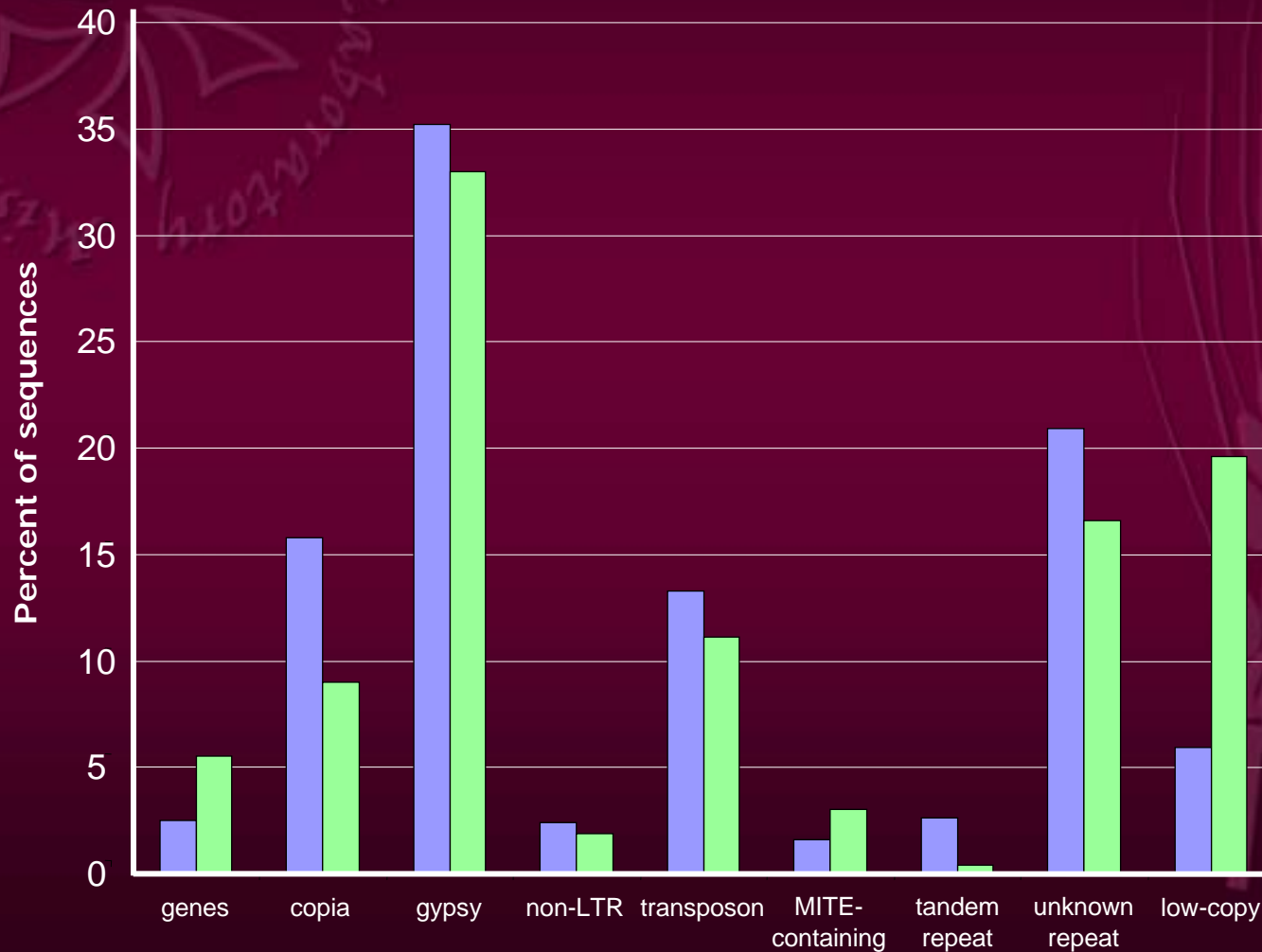
E



INITIAL WHEAT MF RESEARCH

- Li W, Zhang P, Fellers JP, Friebe B, Gill BS (2004) Plant J. 40: 511-515.
- Obtained sequences of 913 MF and 3,830 unfiltered (UF) *Ae. tauschii* clones
- Performed comparative analysis of MF and UF sequences

A. TAUSCHII - Uf VS. Mf



Ae. TAUSCHII MF RESULTS

- Most repeated sequences are largely methylated, but genes, low-copy sequences and MITEs are hypomethylated
- 2.2-fold enrichment for genes
- 3.3-fold enrichment for low-copy sequences
- 3.0-fold enrichment for both genes and low-copy DNA
- 1.2-fold reduction in repetitive DNA

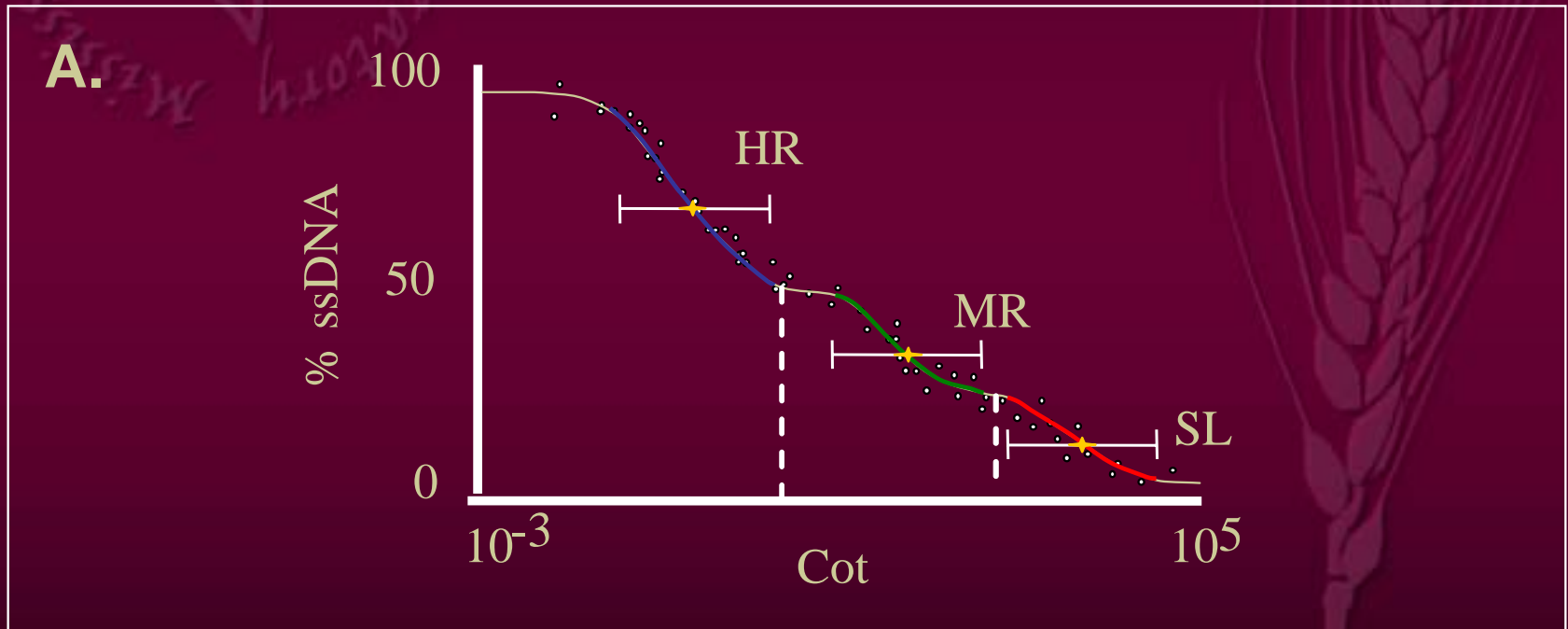
MF IN *T. AESTIVUM*

- Rabinowicz et al., in preparation
- Comparison of Chinese Spring MF and UF libraries revealed 4.7-fold gene enrichment by MF

(COT FILTRATION (C)

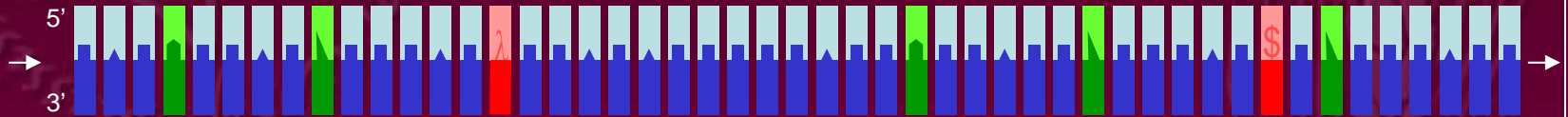
- Based on the principles of DNA reassociation kinetics
- That is, in a sample of denatured genomic DNA, a particular DNA sequence will renature at a rate proportional to its copy number in the genome.

COT ANALYSIS

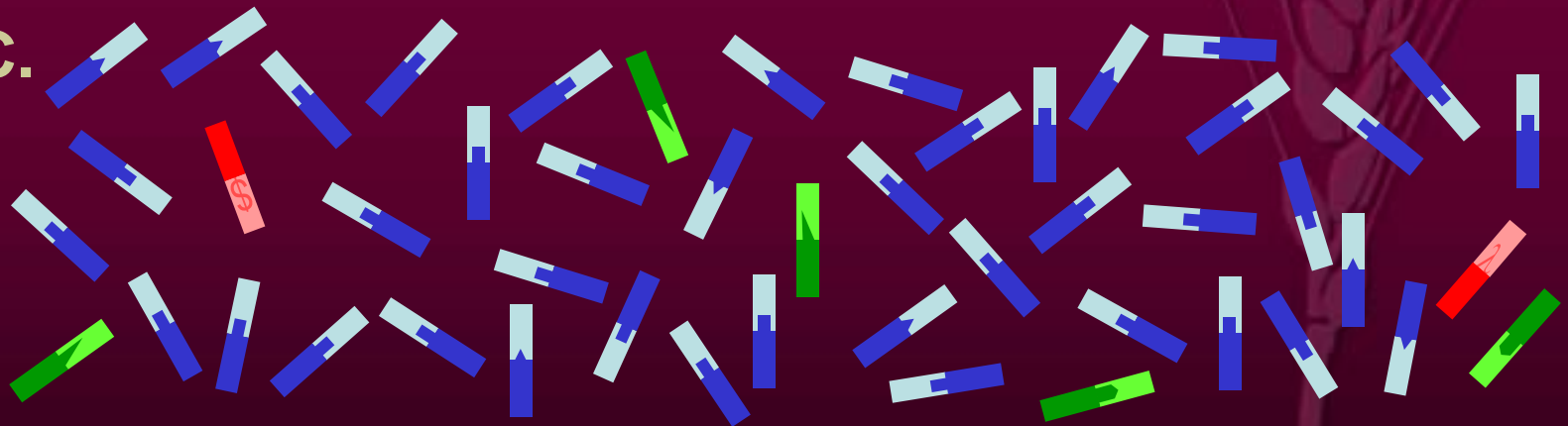


UNSHEARED DNA & SHEARING

B.

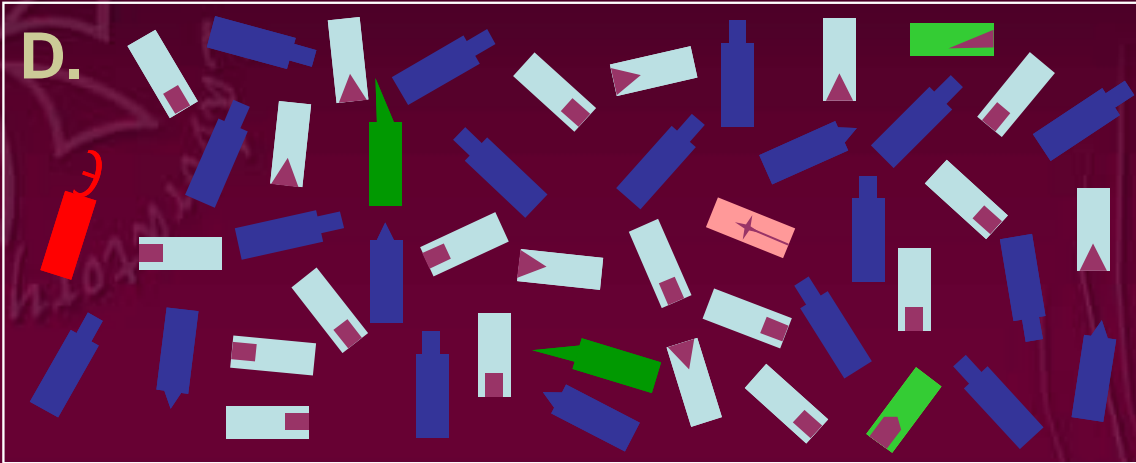


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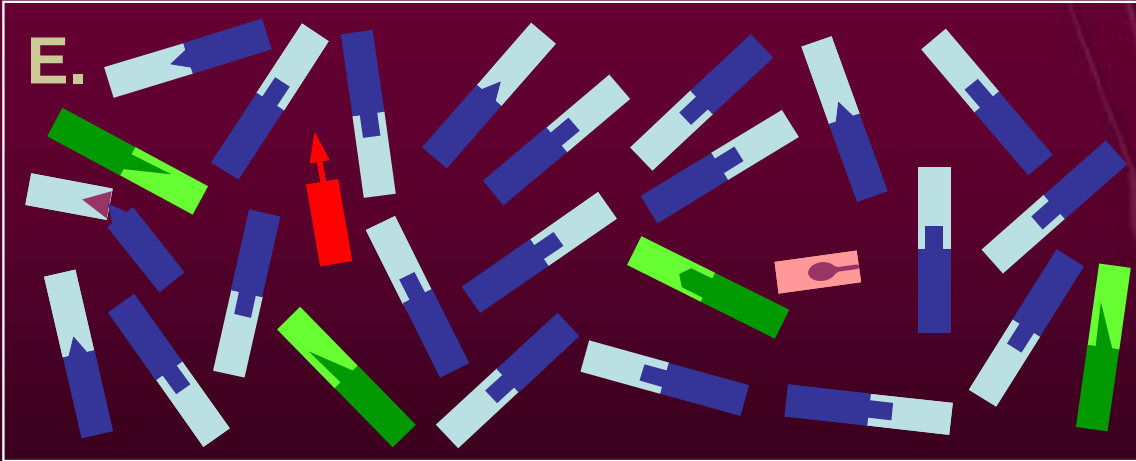


DENATURATION & REASSOCIATION

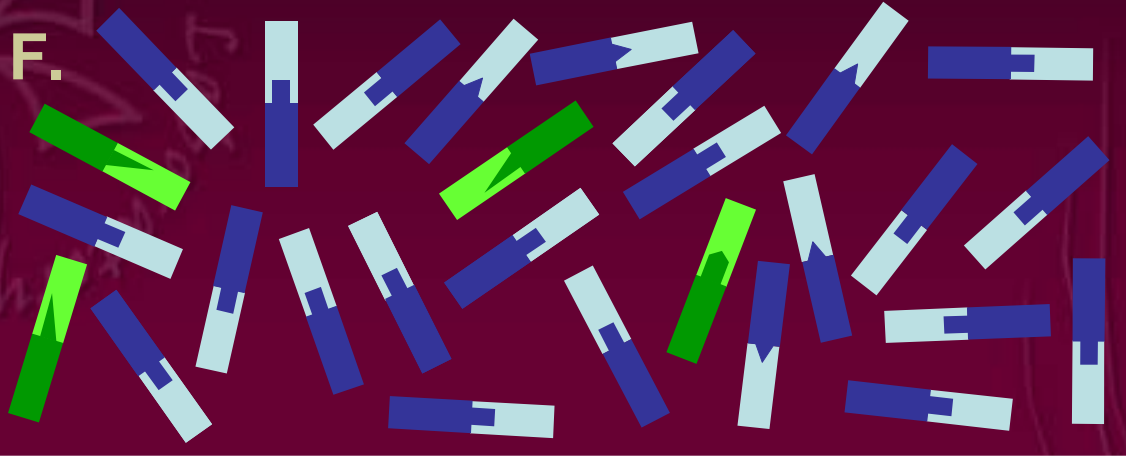
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HAP CHROMATOGRAPHY

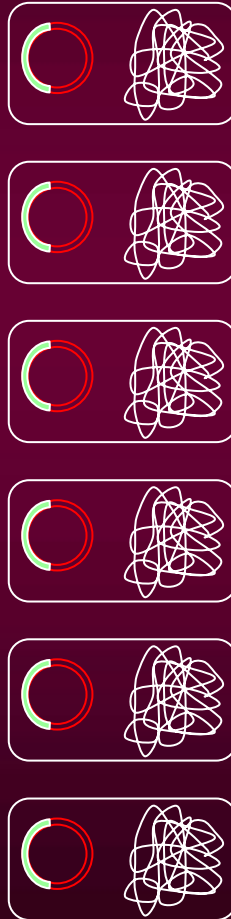


2ND-STRAND SYNTHESIS & CLONING

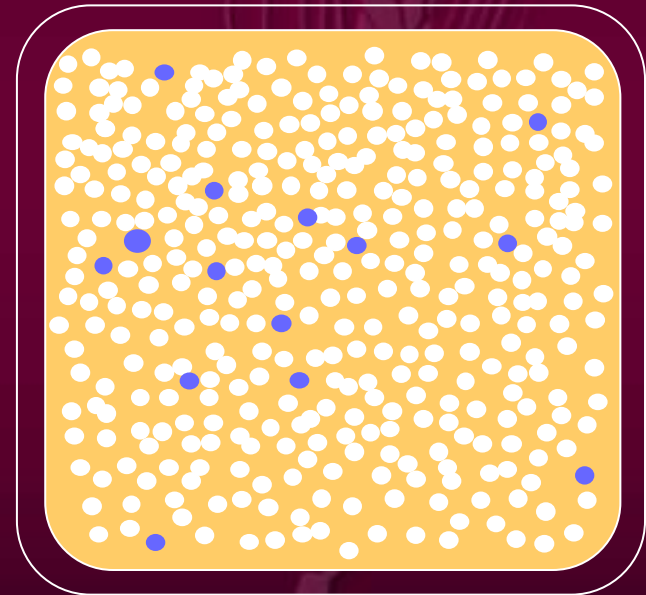
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I



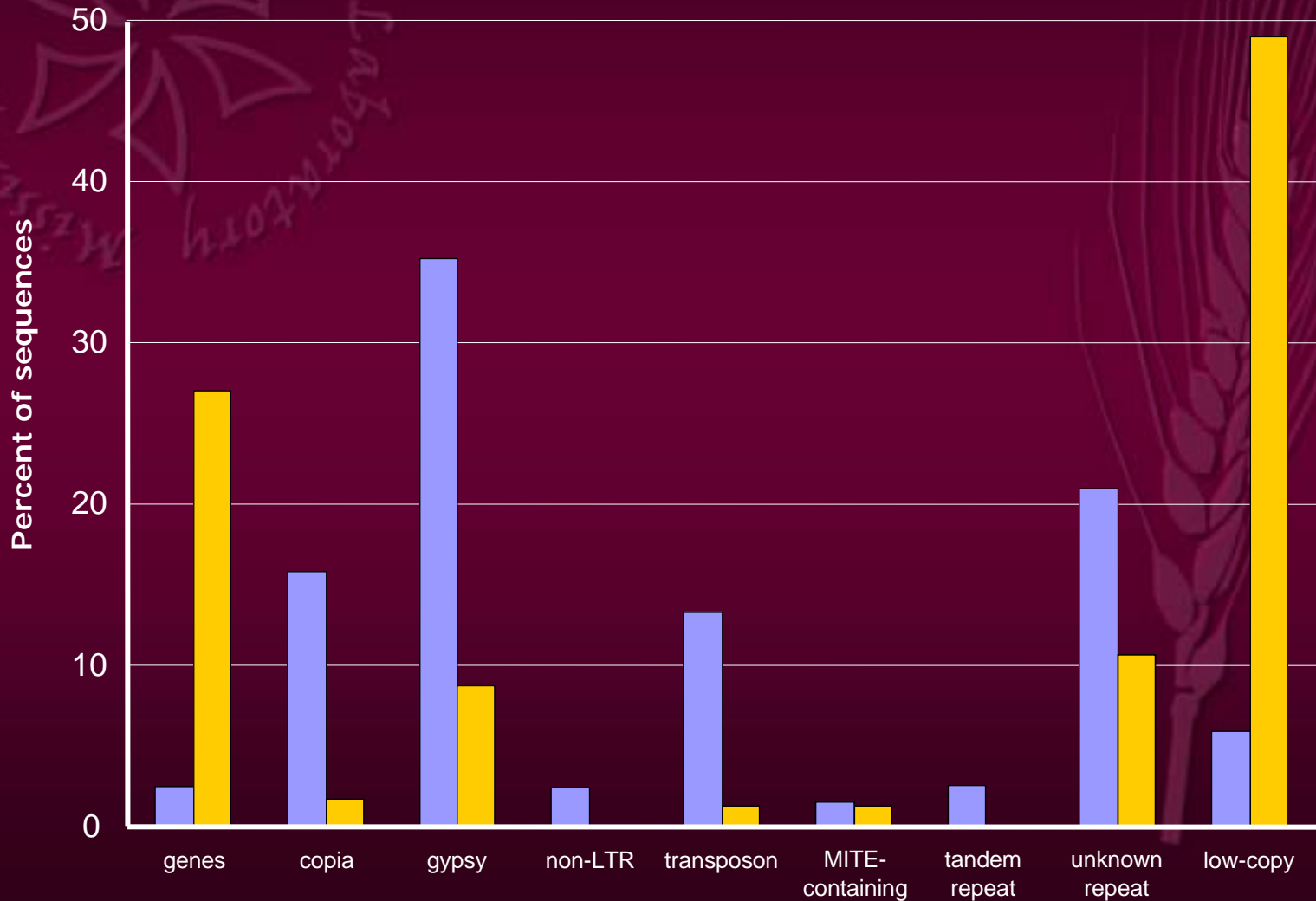
J



INITIAL WHEAT CF RESEARCH

- Lamoureaux, Peterson, Li, Fellers, & Gill
– unpublished results
- Sequenced 4,200 clones from a CF library (Cot > 1639) developed from Chinese Spring
- Performed comparison between CF and *Ae. tauschii* UF data

Uf (*A. TAUSCHII*) VS. Cf (*T. AESTIVUM*)



RESULTS

- 10.8-fold gene enrichment
- 9.3-fold enrichment for low-copy sequences
- 9.0-fold enrichment for genes & low-copy DNA
- 3.8-fold reduction in repetitive DNA

CONCLUSIONS

- Both MF and CF should be useful in sequencing the wheat *gene space*.
- In wheat, CF appears to be particularly good at reducing repeat content and capturing low-copy sequences not detected by other means.
- New MF results suggest MF will be of more use in sequencing the *T. aestivum* genome than it would be in sequencing the *Ae. tauschii* genome.

THANKS TO...

- Bikram Gill & the wheat *Group-3 Consortium*
- Andrew Paterson
- John, Paul, George, and Ringo