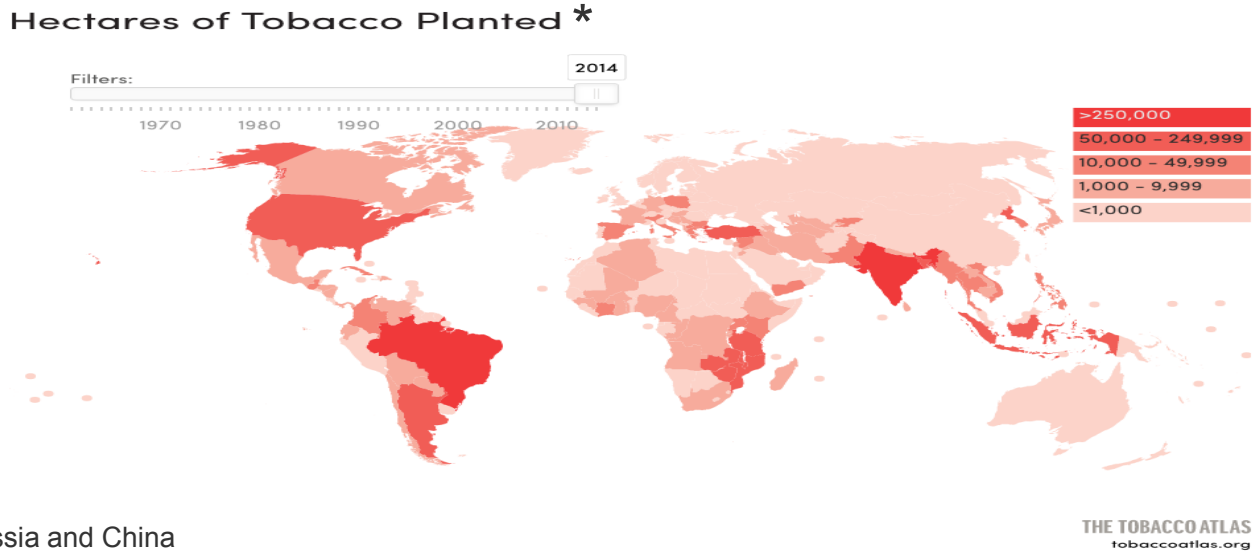
Recent Developments and Practical Applications of New Breeding Technologies (NBTs)

James A. Strickland, Ph.D.
Vice President – Altria Client Services
October 25, 2018

Altria
Altria Client Services

Tobacco Industry To Date

- **Previous challenges faced and met**
  - A global commodity
  - Relatively stable global supply



*Excluding Russia and China

Current Challenges in Tobacco Agriculture

Product Quality

Diseases

Regulation

Increased Regulations – United States

- **FDA Proposed Rule on NNN Levels in Smokeless Tobacco**
  - Published January 2017
  - Mean level of 1 ppm (dwb) through the end of shelf life
  - Technical achievability

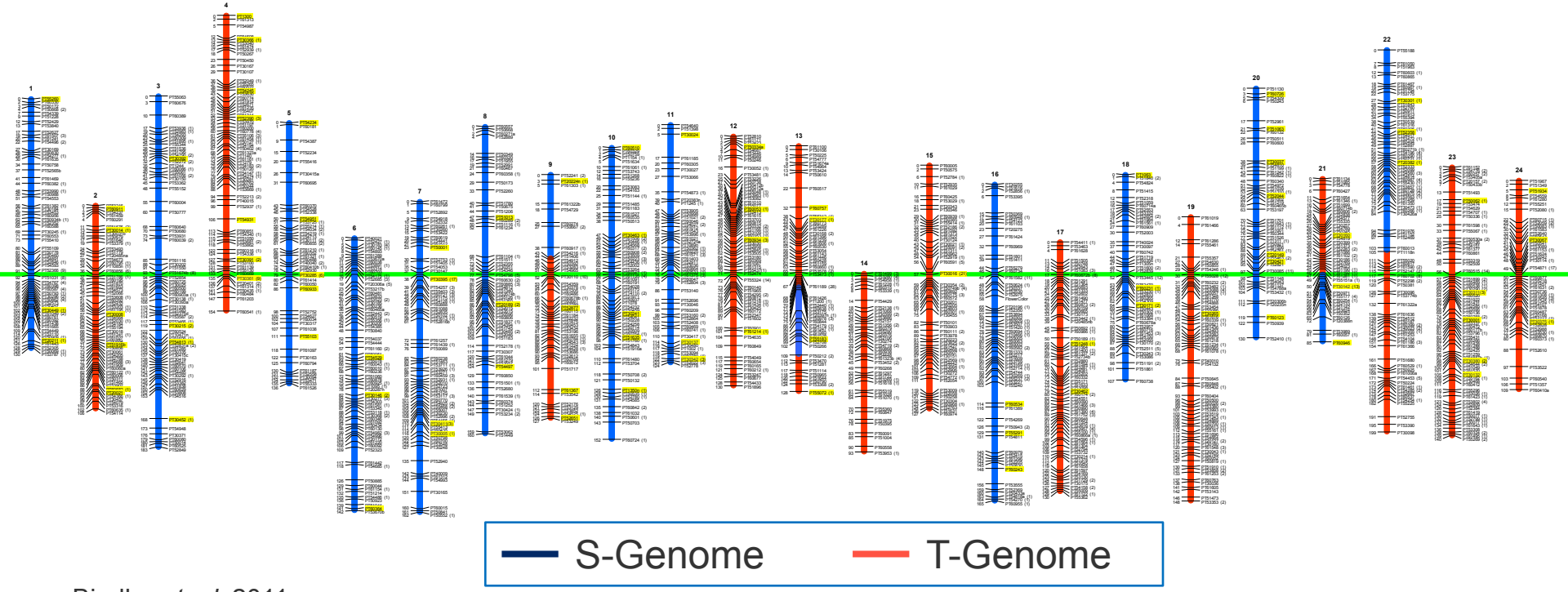
- **FDA ANPRM on Nicotine Levels In Combusted Cigarettes**
  - Published March 2018
  - Extremely low nicotine levels (0.3, 0.4, or 0.5 mg/g filler - dwb)
  - Technical achievability

- **Other Regulatory Pressures**
  - FDA ANPRM on Regulation of Flavors in Tobacco Products (March 2018)
  - Others

Nicotiana tabacum - Small Genetic Differences…Large Impacts

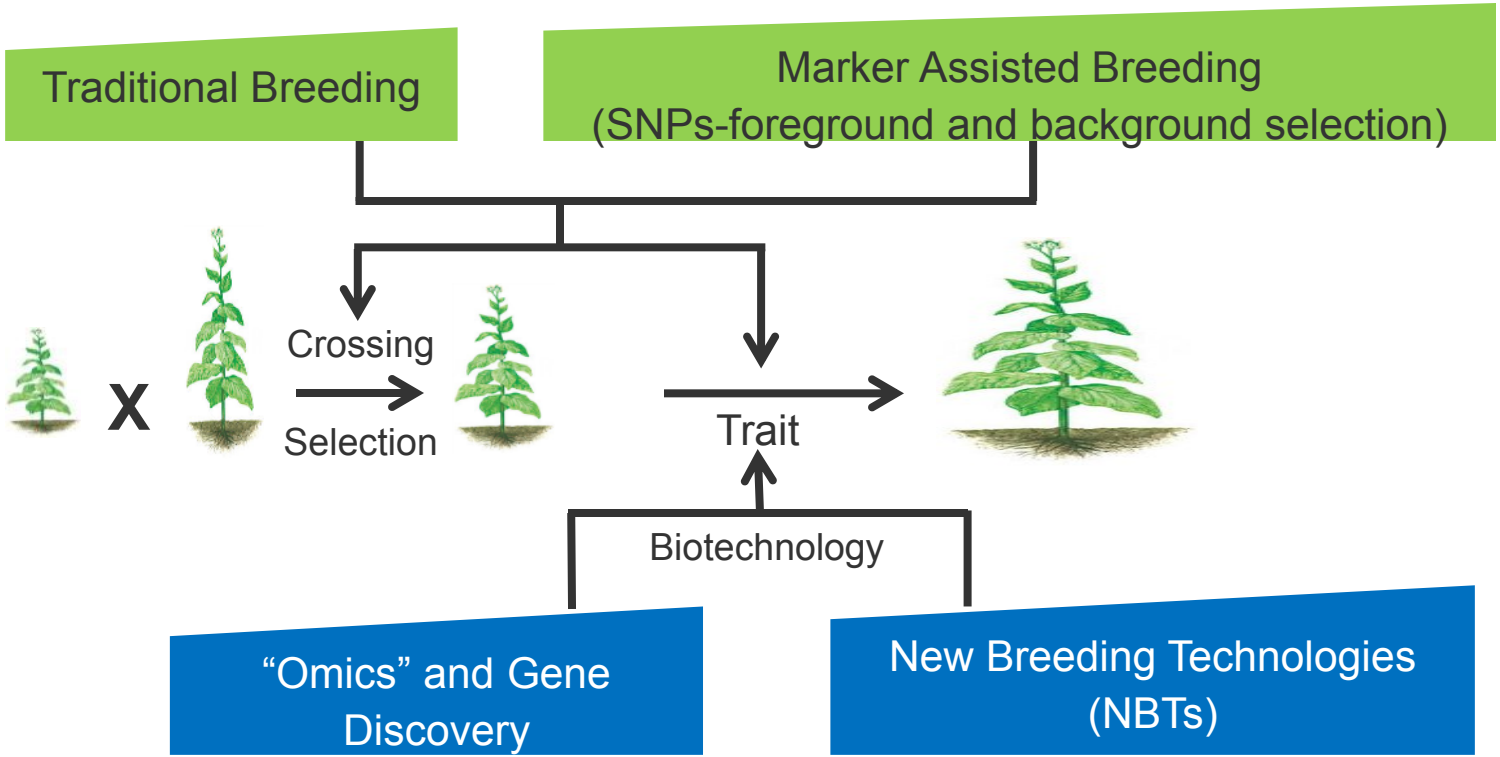
Variety	DNA Identity to TN90
Maryland 609	99.93373%
K326 – Flue Cured	99.91987%
NLM - Dark	99.9313%

Tobacco - An Allotetraploid Genome

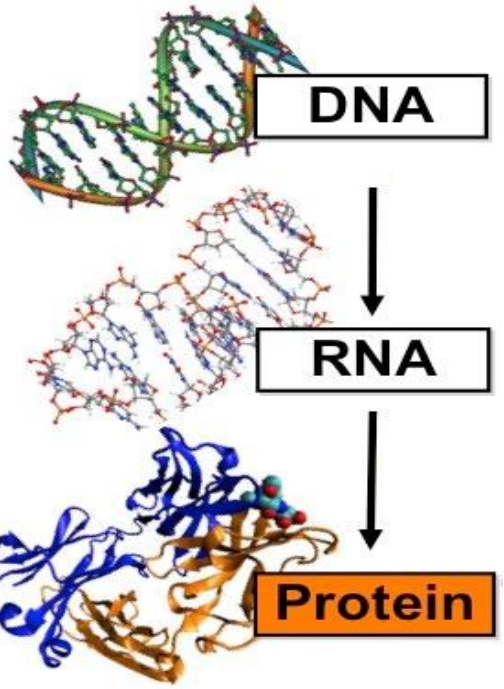


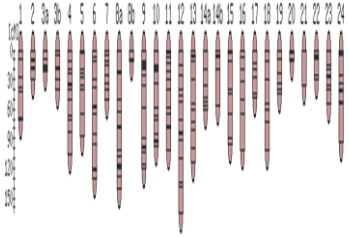
Bindler *et. al.* 2011

Technologies to Meet Regulatory Needs



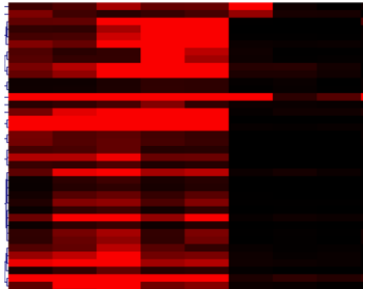
The Right Materials and the Right Tools





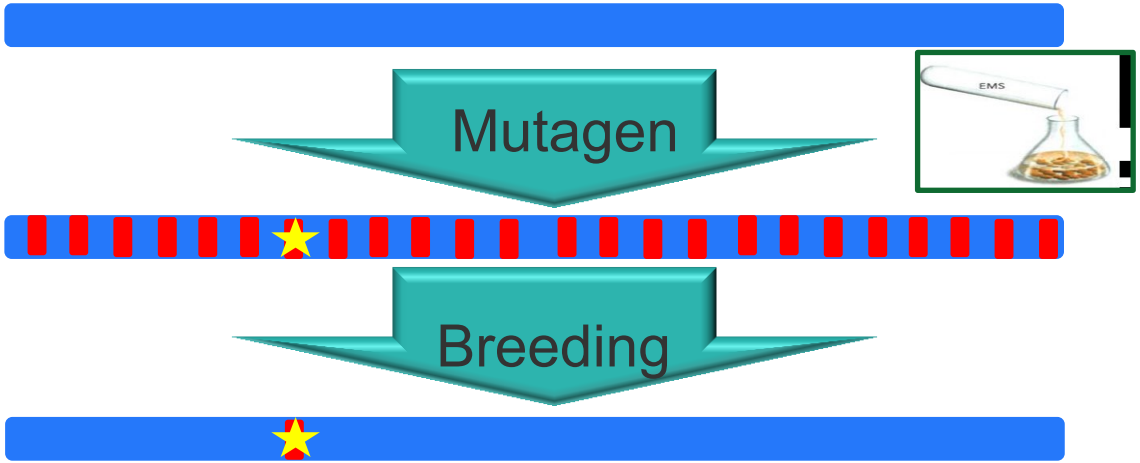
SNP Profile

Axiom® Chip

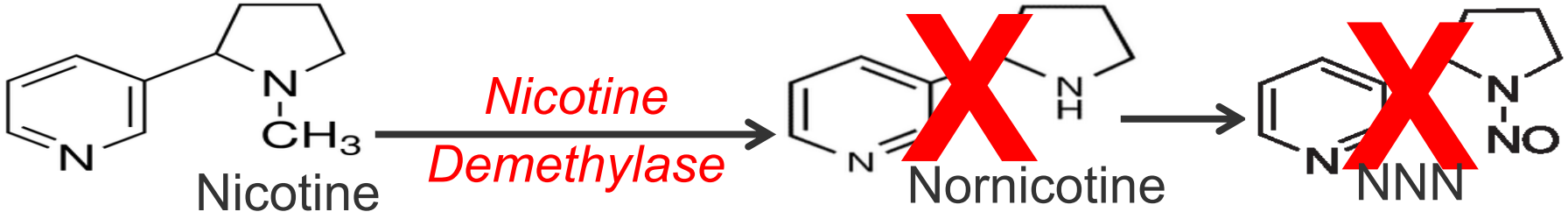


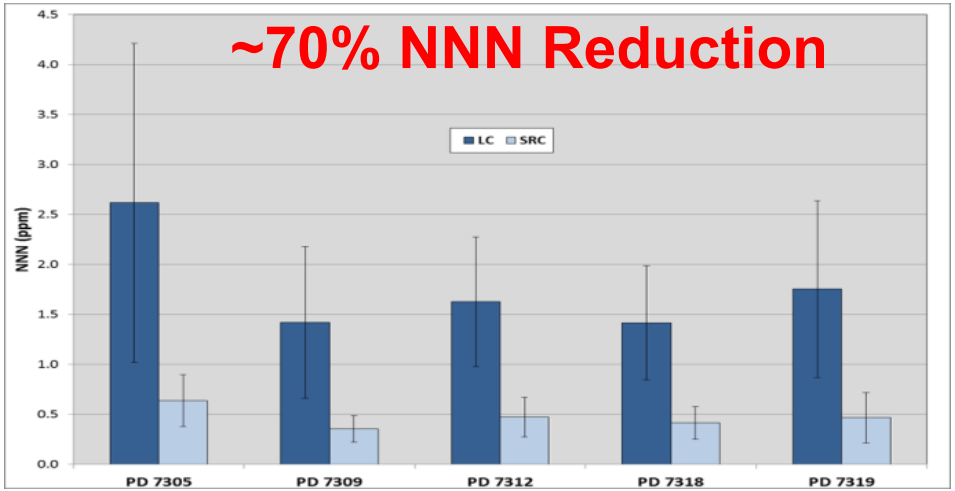
RNA Expression

Mutagenesis



Zyvert™ Development* -Low NNN Tobacco





*In collaboration with
North Carolina State University
and the University of Kentucky

Altria
Altria Client Services

New Breed Technologies – GMOs?

Cisgenic

Same Tobacco Promoter and Gene

Intragenic

Different Tobacco Promoter

Different Tobacco Gene

Transgenic

Non-tobacco Promoter

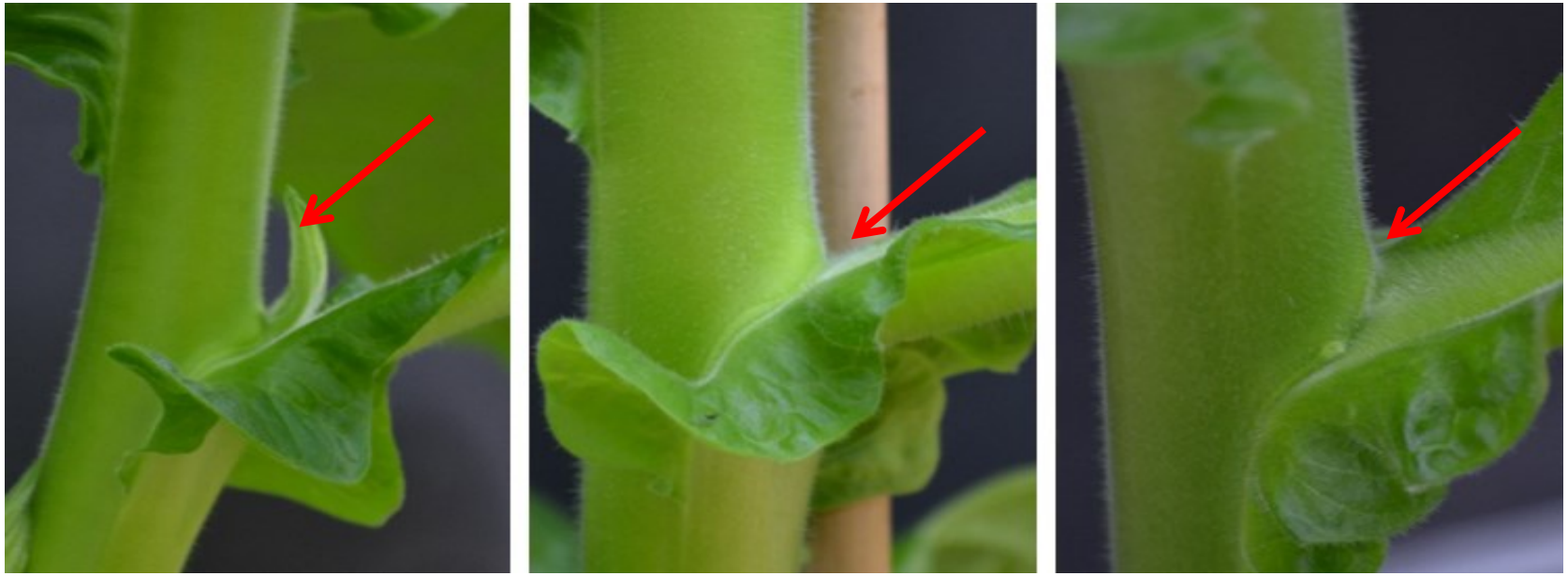
and/or

Non-tobacco Gene

The definition has now expanded

Transgenic Tobacco – Sucker Control

1 Week Post Topping



Wild Type

Tobacco Promoter::Non-tobacco Cell-Death Gene

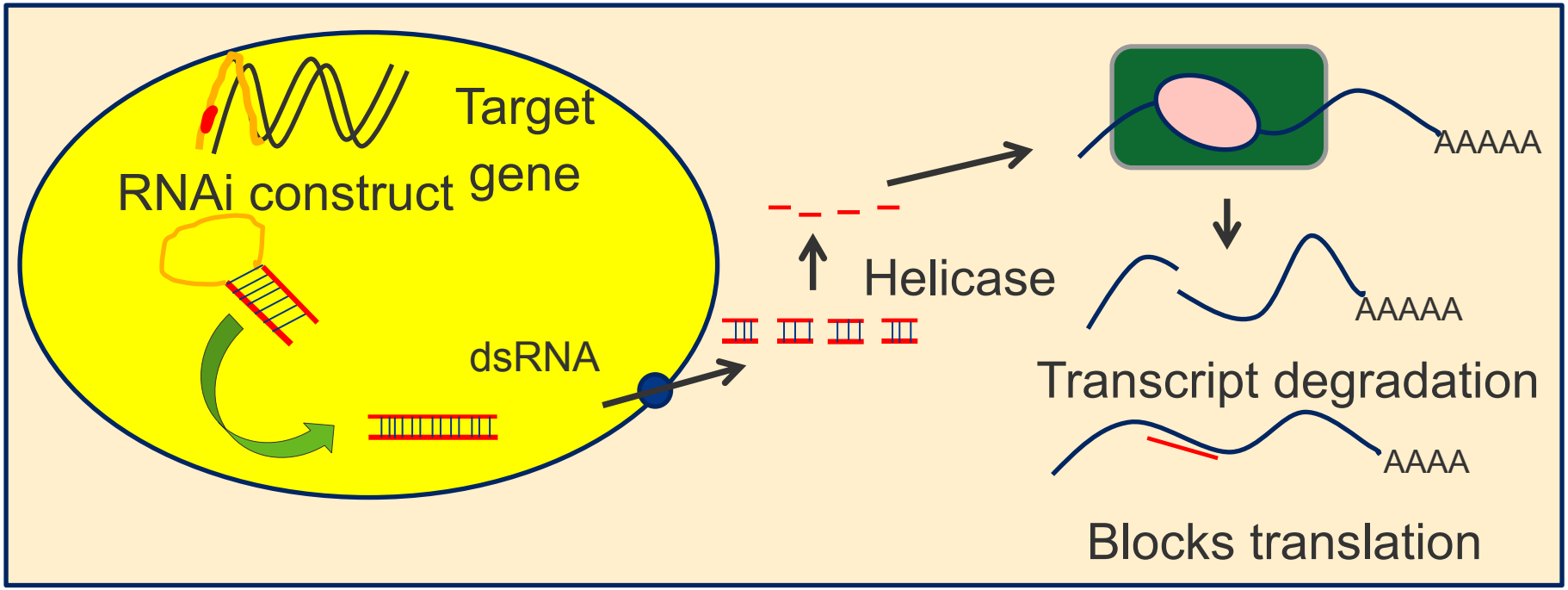
Very Low Nicotine Tobacco

Burley 21

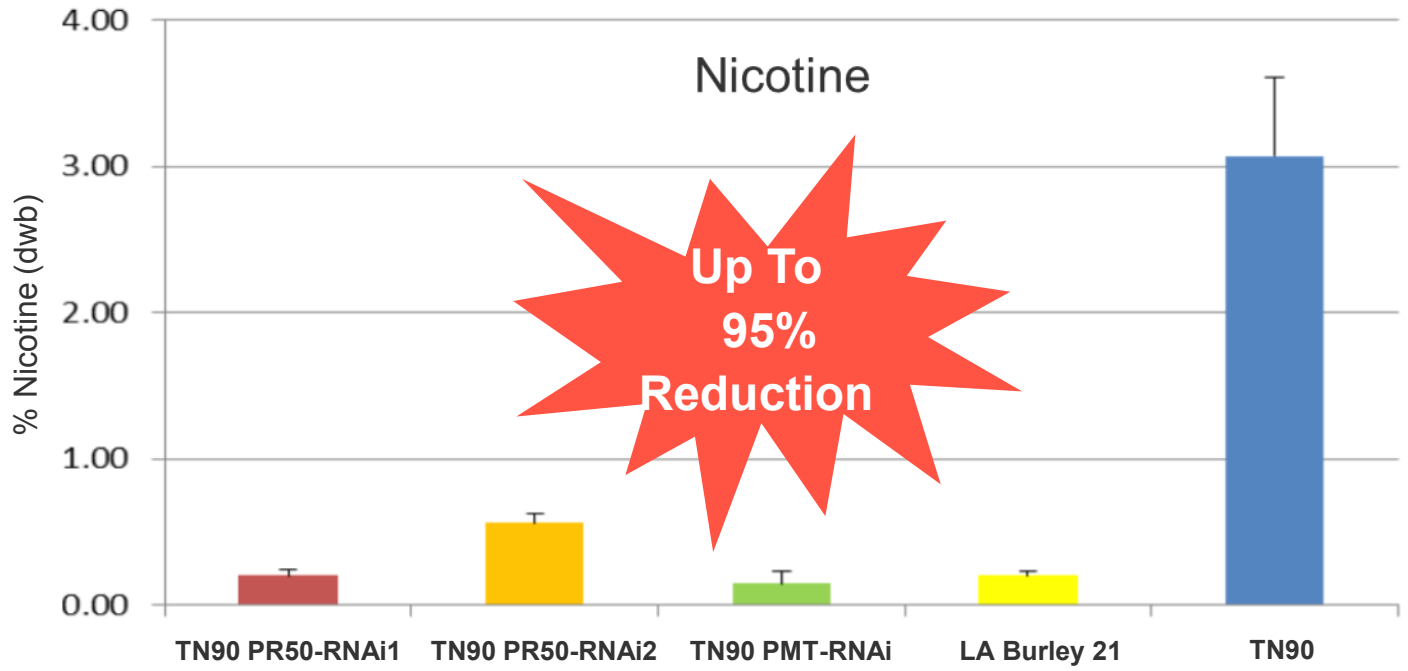
LA Burley 21

Altria
Altria Client Services

RNA Interference - RNAi



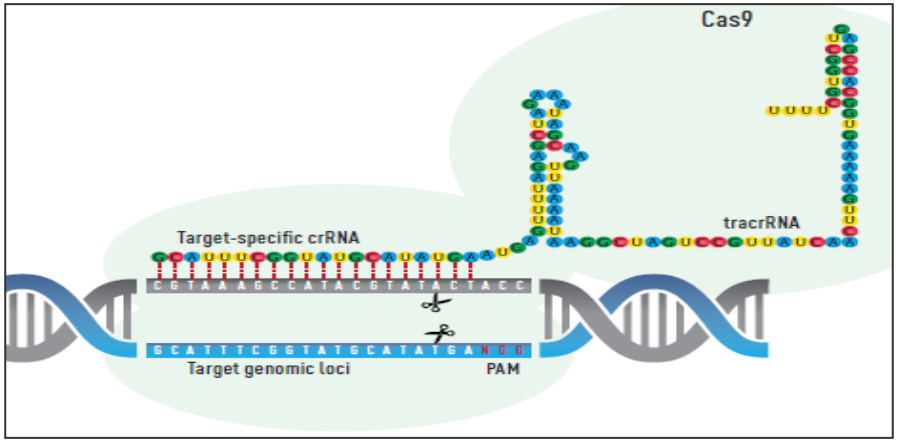
Nicotine Levels of RNAi Experimental Lines



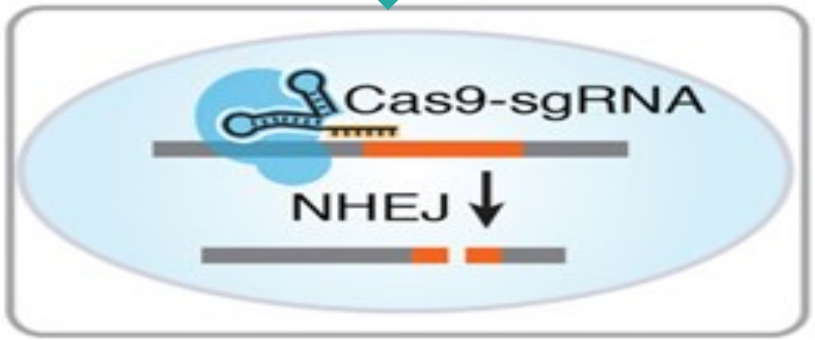
Altria
Altria Client Services

CRISPR Mutagenesis

DNA damage with CRISPR



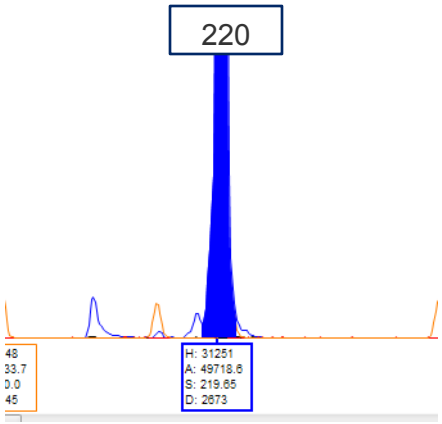
DNA repair with non-homologous end joining



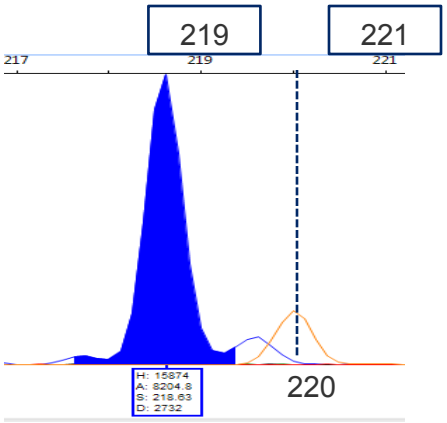
CRISPR Mutated PR50 in Tobacco

- **PR50**
  PR50-3418

3418-WT

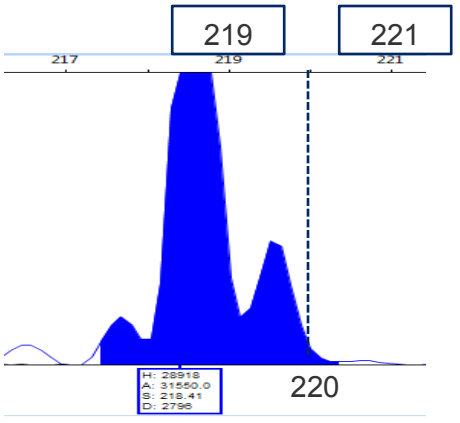


3418-agPR50[1]



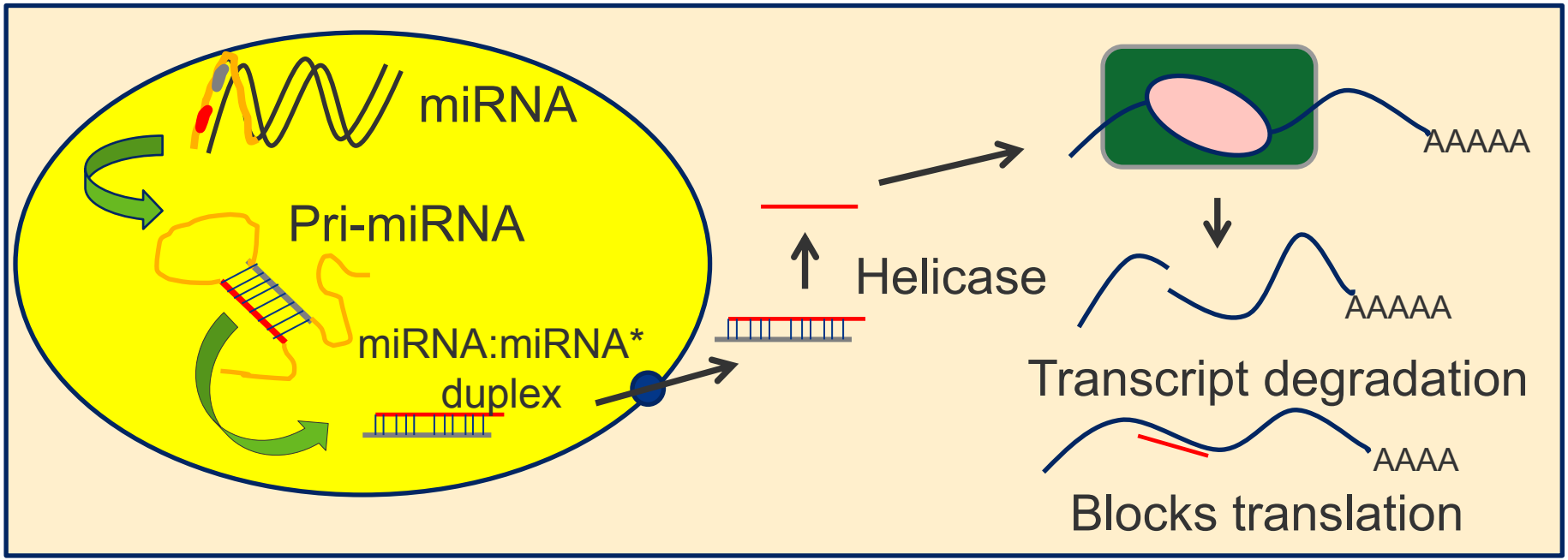
-1 homozygous

3418-agPR50[6]



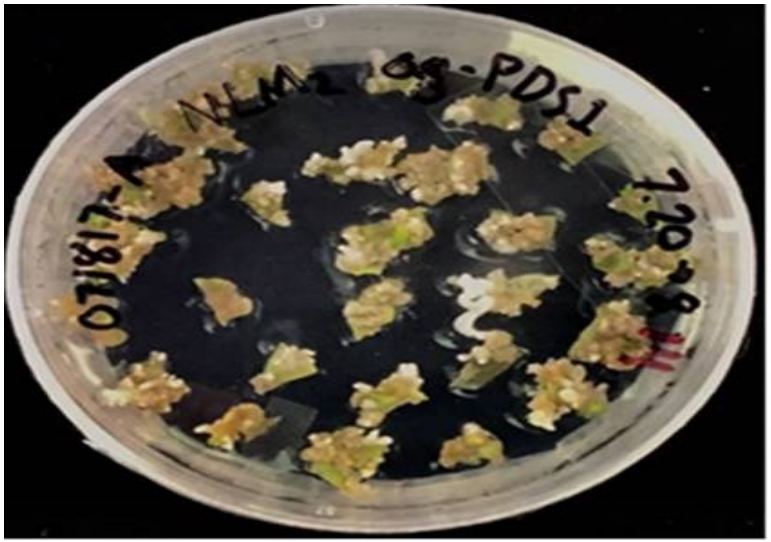
0/-1 heterozygous

MicroRNA - miRNA



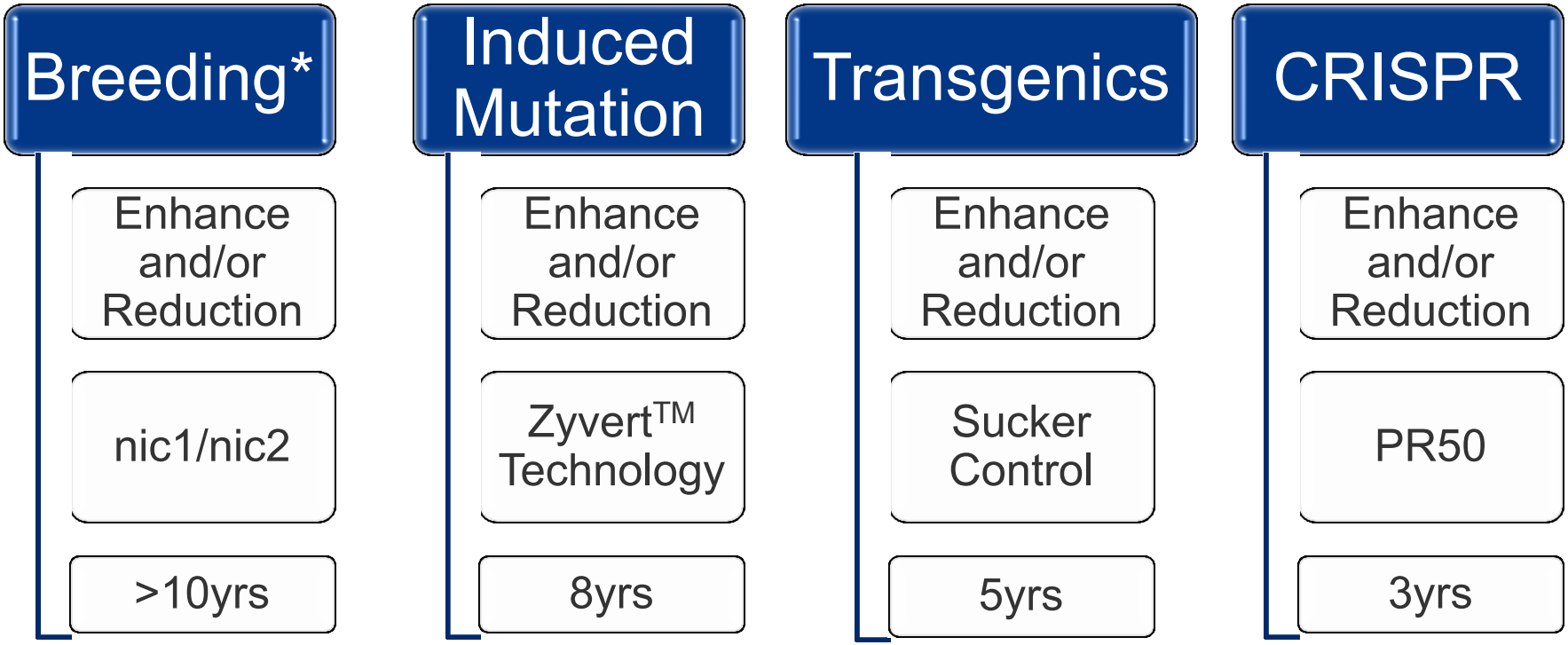
miRNA Silencing- *Phytoene desaturase*

Nta-miR6147



Transgenic plants developed white leaves: Successful miRNA design

Time Required for Commercial Trait Development



*No foreground or background selection

Altria
Altria Client Services

Regulatory Status of NBTs

Technology	Regulatory Status	
	USA	EU
EMS mutagenesis	*non GMO*	*non GMO*
Cisgenic	GMO	GMO
Intragenic	GMO	GMO
Transgenic	GMO	GMO
CRISPR	*non GMO*	GMO

Key Takeaways

- Tobacco agriculture and industry continue to encounter global challenges
- New Breeding Technologies (NBTs) offer a compelling way to address current agronomic and future regulatory issues
- The lack of NBTs acceptance and regulatory status harmonization significantly limits future trait development
- Fragmentation of tobacco's current global commoditization status is a real possibility

Acknowledgement

- Dongmei Xu
- Ujwala Warek
- Yanxin Shen
- Jesse Frederick
- Chengal Kudithipudi
- Jaemo Yang
- Tony Qi
- Marcos Lusso
- Whit Morris
- Sree Pramod
- Andrew Adams
- Kenny Lion
- Greg Davis

Altria
Altria Client Services

Reducing risk. Expanding choice.

Altria.

Website at www.altria.com/alcs-science