Proficiency Testing for Detection of Transgenic Tobacco Sub-Group

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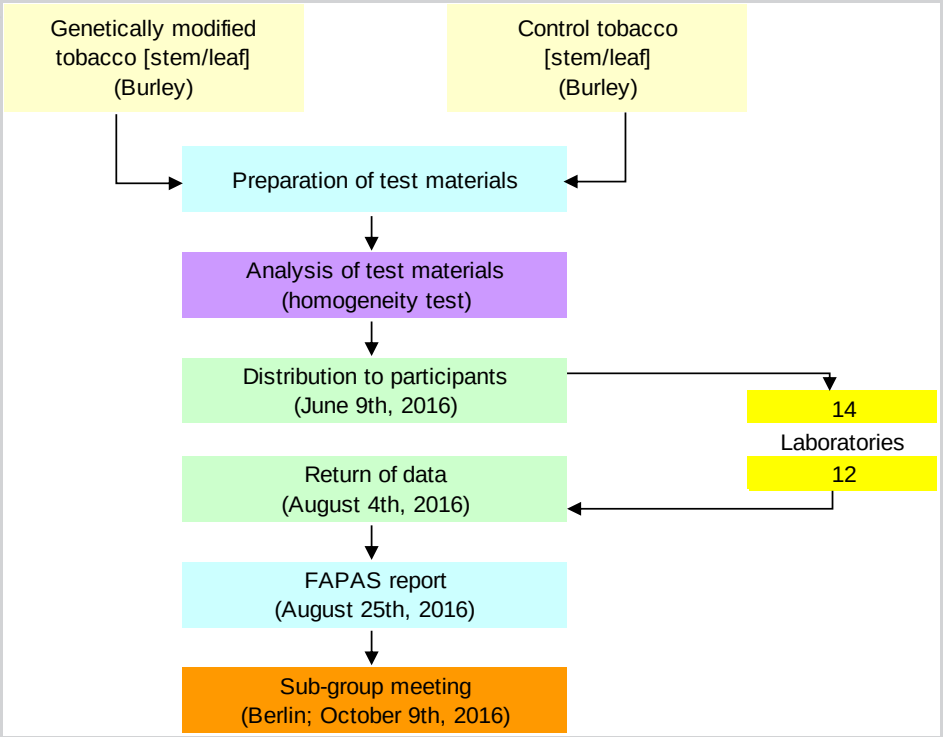
Objectives

- To provide an independent assessment of the quality of data being produced by participating laboratories, through the Tobacco Proficiency Testing.
- To study routine procedures used in participating laboratories.

CORESTA-FAPAS PT “GeM ST03”

❖ Course of events



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- 14 participating laboratories from 10 countries

Laboratory	Country
ADGENE Laboratoire	France
Biolytix AG	Switzerland
Central Lab for Food and Feed	Egypt
Chinwong Food Co., Ltd	Thailand
Dr. Blasy –Dr. Busse	Germany
Eurofins GeneScan GmbH	Germany
Guizhou Academy of Tobacco Science of CNTC	China
Global Laboratory Service, Inc.	USA
Heilongjiang Branch, Mudanjiang Tobacco Science Research Institute of CNTC	China
Japan Tobacco Inc. -Tochigi	Japan
Landesamt für Umweltschutz Sachsen Anhalt	Germany
Synergy Health Laboratory Services Ltd	UK
Tobacco Research Board	Zimbabwe
Microbac Laboratories, Inc.	USA

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- Test round contents
  - 3 test materials and 4 control materials (positive & negative stem/leaf tobacco) were distributed.
  - Participants requested to determine the presence of 35S promoter CaMV (*p35S*) and nopaline synthase terminator (*tNOS*) in the test materials.
  - Participants invited to record qualitative and quantitative data.
  - Electronic submission of the results and method information.
  - Identity of specific laboratories is not listed in the report issued by FAPAS.
  - A |*z*-score| of less than or equal to 2 is considered satisfactory.

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Qualitative results

- Test Material GeM ST03A: Positive for *p35S* (82% consensus)
  [stem tobacco] Positive for *tNOS* (82% consensus)
- Test Material GeM ST03B: Positive for *p35S* (91% consensus)
  [leaf tobacco] Positive for *tNOS* (100% consensus)
- Test Material GeM ST03C: Positive for *p35S* (100% consensus)
  [leaf tobacco] Positive for *tNOS* (100% consensus)

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❖ Quantitative results

Test material	Type	Analyte	Assigned value, x_a %	Number of satisfactory scores: |z|≤2	Total number of scores	Satisfactory (|z|≤2) %
GeM ST03A	Stem	*p35S*	0.41	3	4	75
		tNOS	0.68	3	4	75
GeM ST03B	Leaf	*p35S*	0.54	4	5	80
		tNOS	0.52	4	5	80
GeM ST03C	Leaf	*p35S*	5.95	4	5	80
		tNOS	5.79	4	5	80

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- Issues
  - One member had a registration problem.
  - Results were submitted by 12 participants (86%) before the closing date.
  - Only three laboratories participated in all three rounds of this GMT proficiency test consecutively.

Next test

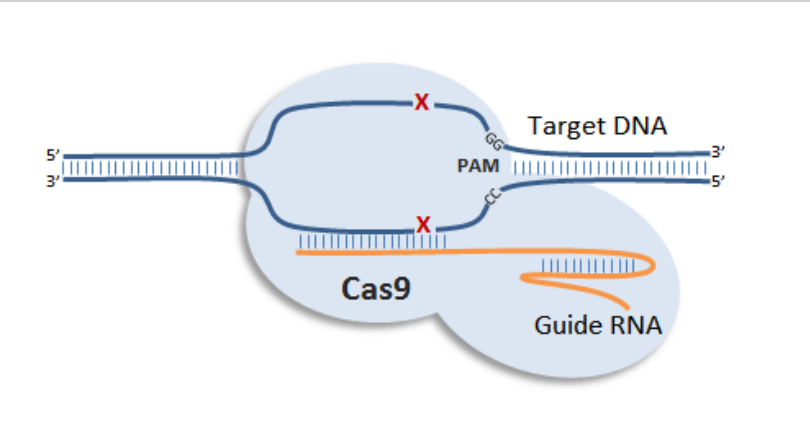
- Periodicity: one proficiency test per year
- Scheme: qualitative and/or quantitative test
- Targets: *p35S* and *tNOS*
- New GM sample:
  - Guizhou Academy of Tobacco Science of CNTC developed one GMT (Flue-cured) with single copy of *p35S*, *tNOS* and *NPT II* as their Standard Reference Material.
  - This GMT will be the materials for 2017's test.

New Breeding Techniques, GMOs or not?

- New Breeding Techniques (NBTs) with gene-editing paved the way to a new generation of crops.
- In January 2015, during the Scientific Commission, it was proposed to do an assessment of the new breeding techniques by ACAC with the support of the experts of the PTDTT sub-group.
- Presentation on new Breeding Techniques by the coordinator of the PTDTT sub-group during the ACAC meeting (June 2015, Strasbourg).

CRISPR/Cas9, probably the most promising NBT

- CRISPR/Cas9 is a new technique to create directed mutants.
- Nucleases guided by a specific RNA.
- High accuracy and frequency (can reach 90%).
- Mutants on *N. tabacum* (Gao et *al.*, 2014), bi-allelic mutants can be obtained.

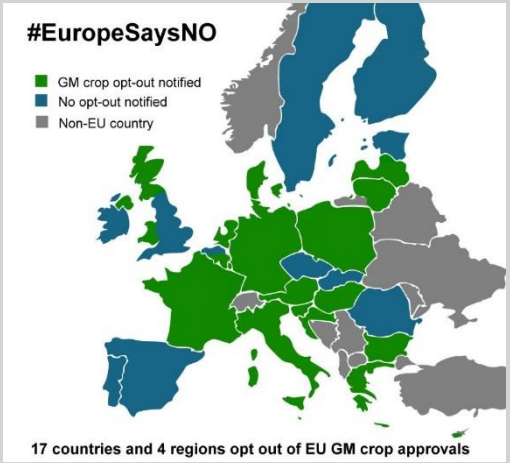


GMO regulation: differences between the US and the EU

- In the US, GMOs are not systematically tested and can even be placed on the market without any form of testing. Labelling is not required.
  - **Gene-edited plants and animals are mostly unregulated**.
- The EU has a set of GMO laws that require case-by-case risk assessment, detectability and labelling of GMOs.
  - **Gene-edited plants and animals should be covered by the law.**
  - **EU commission postponed its decision on NBTs status in April 2016**.

Acceptance by the public opinion

- In France, only 3% of the population knows what is the CRISPR/Cas9, but 68% wants a regulation on this technique (Ifop Institute, May 2016).
- Greenpeace and GeneWatch UK called the NBTs « new GMOs ». They push to obtain a regulation in EU.



Patent covers CRISPR

- Battle in USA to obtain the property on this technology.
- The Broad Institute has succeeded in getting the first patent for CRISPR/Cas9 (Zhang, 2014, USPTO 8,697,359).
  - Tobacco: We prohibit the use of the licensed technology to modify tobacco for any use other than (i) in the context of a model organism for research not directed to the commercialization of tobacco, and (ii) for manufacturing purposes of non-tobacco products.

New breeding techniques, GMOs or not?

- GMOs or not, the status of the varieties created with the new breeding techniques stays unclear in EU.
- There is no method of detection.
- Acceptance by public opinion? Not positive yet.
- The situation on patents is unclear in USA for CRISPR/Cas9.
- The breeders need visibility to use this technology.

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