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Spotlights

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> EDITORIAL

Testing for bioengineered crops, also referred to as Genetically Modified Organisms (GMOs), has become a necessity in light of legislation imposed in member countries of the European Union, Japan, South Korea, Australia, New Zealand, and in an increasing number of other countries. They established labelling laws for approved bioengineered crops while prohibiting the import of unapproved varieties. For approved varieties, common tolerance thresholds are established below which a product does not have to be labeled as containing or derived from GMOs. These thresholds vary for each legislation.

Lateral flow test strips have been in existence for over 10 years as a quick testing method. As more and more traits come into existence, testing becomes increasingly difficult. For instance, some traits become an issue when they lack approval in some countries. Many companies are turning to these test strips to ensure that these traits are not present so they can direct their crops appropriately.

Recent developments allow lateral flow test strips to be used as a qualitative method or as a semi-quantitative method when using an LFD reader. This allows for accurate and precise handling of grain from seed development to local elevators.

Mark Kimble

Genetically Modified Organisms (GMOs) have undergone alterations to their genetic material in a process known as genetic engineering. These alterations result in the organism expressing a trait or traits that would not naturally occur in that organism. GMOs currently exist in bacteria, animals, and plants, and are used in a wide range of applications. These include biological and medical research, production of pharmaceuticals, and agriculture.

GMO Plants – Global Usage and Methods of Detection

One of the most widely adopted uses of GMOs is in agriculturally important crops. In these plants, alterations to the genetic material are often accomplished by insertion of DNA material from a different organism into the target organism. This results in the plant expressing novel traits, such as herbicide tolerance or insect resistance. Seed harvested from these plants will also contain the modification. Modifications have been introduced that allow plants to be tolerant to herbicides such as glyphosate or glufosinate. This allows a field to be sprayed with the herbicide, resulting in destruction of the weeds but no harm to the crop. In addition, many modifications confer insect resistance to the plant. In these traits, known as "Bt", the plant has acquired a trait or traits that allow it to produce endotoxins, originating from the soil bacterium *Bacillus thuringiensis*. These proteins also have a history of use as spray-on insecticides since the 1920's. They are reported to have specific action against certain insect species with no affect on non-target species such as humans, wildlife, and beneficial insects. When ingested, these proteins form pores in the midgut epithelium of the larvae of susceptible insect species (which feed on the crops, causing damage). This causes paralysis of the gut, and the affected insect stops feeding and succumbs to starvation. Non-target species have no receptors in the gut for the protein, and thus the protein has no effect on them.

The transformation of plants may be accomplished in several ways. Two of the most commonly used in agricultural crops are the use of *Agrobacterium tumefaciens*, or biolistics (the "gene gun"). The bacterium *Agrobacterium tumefaciens* provides a natural mechanism for transformation. This bacterium infects injured plant tissue and transfers its Ti plasmid to the plant's chromosome. The Ti plasmid naturally contains genes that cause the plant tissues to overexpress plant hormones and nutrients for the bacteria, leading to plant tumors. The Ti plasmid may be modified to delete these unwanted effects and add a desirable trait, along with a selectable marker, which will then be integrated into the plant's chromosome during infection with the bacteria. However, not all species are susceptible to infection by this bacterium. A second method of transformation, which has been showing increased usage over *Agrobacterium* within the last decade, is biolistics (the "gene gun") method. In this method, plasmid DNA is coated onto small tungsten or gold beads. These micron-sized beads are then "shot" into the plant tissue. Some of the cells in the plant tissue may successfully take up the new DNA and integrate it into a chromosome. This method has been shown effective for integrating DNA into the cell nucleus as well as into organelles such as chloroplasts, and is effective on almost all species researched. Other methods that have been used include microinjection and elctroporation.

GMOs may be referred to in one of three ways. They may be identified by their event name, which is the name of the unique DNA recombination experiment that occurred in the laboratory in which one plant cell successfully incorporated a desired gene. That cell is then subsequently used to regenerate whole plants and is the "foundation" of a GMO strain. For example, one event name for herbicide-tolerant corn is NK603. GMOs may also be identified by the unique protein they express. In the case of event NK603, the protein expressed is CP4 EPSPS. Finally, the GMO may be identified by the trade name under which it is sold commercially.

Current GMO crop production mainly comprises four crops: soybeans, maize, cotton, and rapeseed / canola. Global trade of these crops, and of their main derivatives, is dominated by material of GMO origin. This includes approximately 90 % of the soybean trade, 80 % of the maize trade, 70 % of the rapeseed trade and 45 % of the cottonseed trade. Within these crops, there are several GMO proteins currently important to the grain and seed trade. These include the following:

• CP4 EPSPS

The expression of CP4 EPSPS transgenic protein in plants results in glyphosate herbicide tolerance. This protein is expressed in commercial varieties of creeping bentgrass, sugarbeet, rapeseed/ canola, soybean, cotton, alfalfa, potato, wheat, and corn.

• Bt-Cry1F

The expression of Bt-Cry1F transgenic protein results in insect resistance. This protein is effective against the larvae of lepidopteran pests such as the tobacco budworm, beet armyworm, soybean looper, cotton bollworm/corn earworm, European corn borer, southwestern corn borer, fall armyworm, and black cutworm. This protein is expressed in commercial varieties of corn and cotton.

• Bt-Cry34Ab1

The expression of Bt-Cry34Ab1 transgenic protein in plants results in insect resistance. This protein is effective against the larvae of coleopteran pests such as the corn rootworm. This protein is expressed in commercial varieties of corn.

• Bt-Cry1Ab / 1Ac

The expression of Bt-Cry1Ab/Cry1Ac transgenic protein results in insect resistance. The proteins are effective against the larvae of lepidopteran pests such as the European corn borer, tobacco budworm, cotton bollworm/corn earworm, pink bollworm, beet armyworm and soybean looper. These proteins are expressed in commercial varieties of corn, cotton, and tomato.

• Bt-Cry3Bb1

The expression Bt-Cry3Bb1 transgenic protein results in insect resistance. The protein is effective against the larvae of coleopteran pests such as the corn rootworm. This protein is expressed in commercial varieties of corn.

• Bt-Cry2Ab

The expression of Bt-Cry2Ab transgenic protein results in insect resistance. The protein is effective against the larvae of lepidopteran pests such as the cotton bollworm, pink bollworm and tobacco budworm. This protein is expressed in commercial varieties of cotton.

• PAT

The expression of PAT transgenic protein in plants results in Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium. It is also often used as a selectable marker for genetic transformation. This protein is expressed in commercial varieties of corn, rapeseed/canola, cotton, chicory, sugarbeet, and rice.

• VIP3A

The expression of VIP3A transgenic protein in plants results in insect resistance. This protein is effective against the larvae of lepidopteran pests such as the cotton bollworm/corn earworm, tobacco budworm, pink bollworm, fall armyworm, beet armyworm, soybean looper, cabbage looper, cotton leaf perforator, black cutworm, and the western bean cutworm. This protein is expressed in commercial varieties of corn and cotton.

• PMI

The PMI protein (phosphomannose isomerase) is expressed by a gene derived from *E. coli*. This protein allows growth on mannose and is often used as a selectable marker in GMO corn.

The cultivation of GMO plants is increasing globally, as is the utilization of stacked traits, including two or more novel traits in the same plant. The first field trials of GMO plants began in the United States and France in 1986, with herbicide-tolerant tobacco. The first country to allow commercialized GMO plants was China, which introduced a virus-resistant tobacco in 1992. The first GMO crop approved for sale in the US was the *FlavrSavr* tomato in 1994. In that year, the European Union also approved its first GMO plant for sale, which was a herbicide-tolerant tobacco. Commercialized cultivation of GMO plants such as corn and cotton began in 1996. As of 2009, 11 different types of GMO crops were commercially grown on 330 million acres (134 million hectares) worldwide, in 25 different countries. As shown in the ISAAA brief 41-2009, "Global Status of Commercialized Biotech/GM Crops: 2009", the global cultivation of GMOs in 2009 was as shown in Table 1. Adoption rates of GMO crops in the countries where they are planted are often high. In the United States, based on USDA survey data, in 2011 herbicide-tolerant soybeans comprised 94 % of the planted acreage, herbicide-tolerant cotton comprised 73 % of the planted acreage, and herbicide-tolerant corn comprised 72 % of the planted acreage.

Plantings of Bt insect-resistant cotton in the United States comprise 75 % of the planted acreage, and corn containing a Bt trait comprises 65 % of the planted acreage. These percentages include stacked traits, which have both herbicide resistance and insect tolerance. In Brazil, approximately 70 % of the soybean acreage is herbicide-tolerant. In Canada, >90 % of the rapeseed/canola acreage is herbicide tolerant. In China, approximately 70 % of the cottonseed acreage planted is Bt (insect resistant), and in India approximately 90 % of cotton planted is Bt. Apart from the 25 GMO growing countries 32 other countries do not cultivate GMO crops, but have approved them for import as food and feed. Thus, GMO crops are approved for food, feed, or release into the environment in a total of 57 countries worldwide. The most commonly approved GMOs are herbicide-tolerant soybean (event GTS-40-3-2; protein CP4 EPSPS) which has approvals in 23 countries (European Union counted as one, but has 27 separate approvals), followed by herbicide-tolerant corn (event NK603, protein CP4 EPSPS) which has 21 approvals. The most commonly approved Bt is insect-resistant corn event MON810 (protein Bt-Cry1Ab) with approvals in 21 countries. Insect-resistant cotton event MON531/757/1076 (protein Bt-Cry1Ac) has 16 approvals.

Table 1: *Global cultivation of GMOs in 2009 (ISSAAA brief 41-2009)*

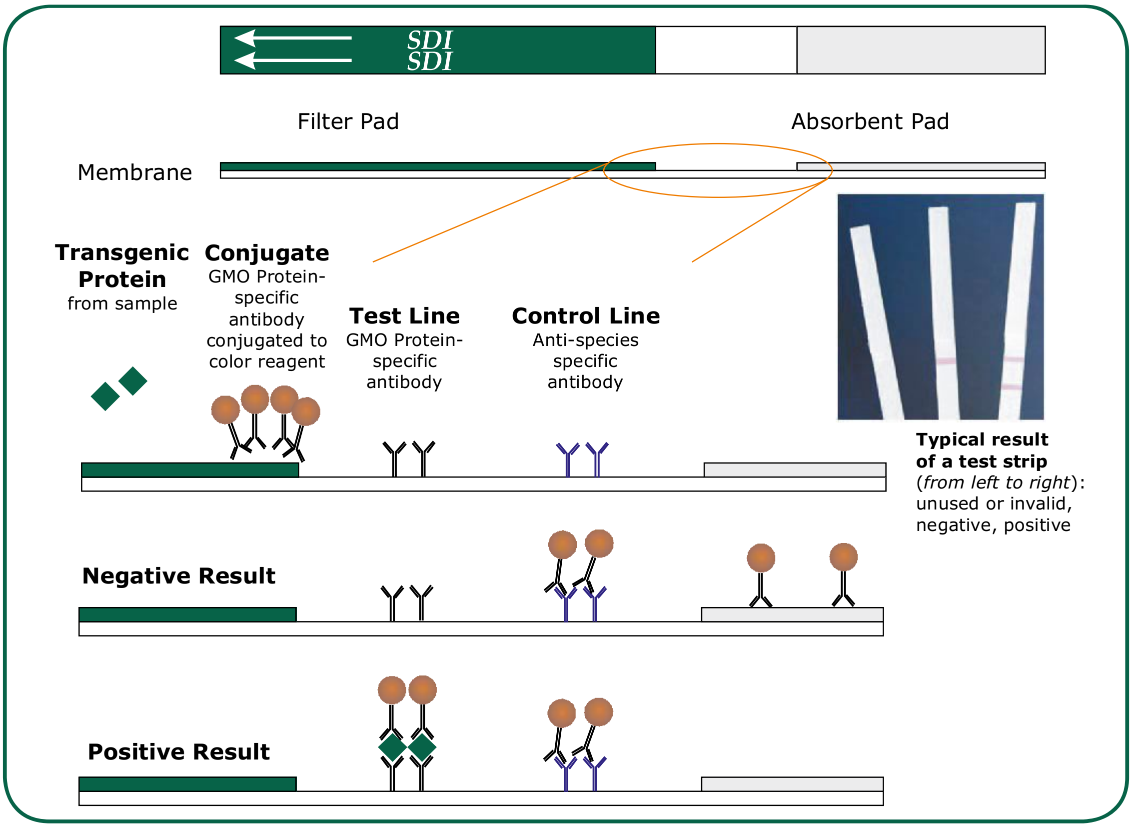
Rank	Country	Area (million hectares)	GMO Crops
1	United States	64.0	Soybean, corn, cotton, canola, squash, papaya, alfalfa, sugarbeet
2	Brazil	21.4	Soybean, corn, cotton
3	Argentina	21.3	Soybean, corn, cotton
4	India	8.4	Cotton
5	Canada	8.2	Rapeseed/canola, corn, soybean, sugarbeet
6	China	3.7	Cotton, tomato, poplar, papaya, sweet pepper
7	Paraguay	2.2	Soybean
8	South Africa	2.1	Soybean, corn, cotton
9	Uruguay	0.8	Soybean, corn
10	Bolivia	0.8	Soybean
11	Philippines	0.5	Corn
12	Australia	0.2	Cotton, rapeseed/canola
13	Burkina Faso	0.1	Cotton
14	Spain	0.1	Corn
15	Mexico	0.1	Cotton, soybean
16	Chile	<0.1	Soybean, corn, rapeseed/canola
17	Columbia	<0.1	Cotton
18	Honduras	<0.1	Corn
19	Czech Republic	<0.1	Corn
20	Portugal	<0.1	Corn
21	Romania	<0.1	Corn
22	Poland	<0.1	Corn
23	Costa Rica	<0.1	Cotton, soybean
24	Egypt	<0.1	Corn

Plants are tested for GMOs for several reasons. The seed or leaf material may be tested by seed companies for the purposes of R&D and quality control of their products. The bulk grain material may be tested by grain companies, marketers, growers, exporters and others for the purposes of the non-GMO market, identity preservation, global trade, and detection of unapproved traits. Various countries have a variety of requirements regarding which traits they will accept on import, and at which levels, in addition to labeling requirements for products containing GMO or products which are to be labeled as GMO-free.
Tests for GMOs may be performed by methods for detecting the actual modified DNA, or the protein that is expressed by the GMO plant. PCR methods detect the actual DNA inserted in the GMO plant. PCR stands for "polymerase chain reaction", and in this method the specific target DNA is replicated and detected. PCR may be either qualitative or quantitative. Assays are also often performed that detect the presence of a GMO (based on promoter/terminator testing) but do not identify which specific GMO is present. These methods provide a wide range for quantitation, are very sensitive and can be specific, but require a laboratory environment, highly trained staff, and expensive equipment to perform. They also take more time than protein assays. Protein assays utilize antibodies to capture and detect the novel protein expressed by the GMO plant. These assays are typically used in two varieties: Lateral flow devices and ELISA plates. Both typically use a sandwich-ELISA technology. On the lateral flow devices, a capture antibody on the device binds to the GMO protein of interest.

A detector antibody, conjugated to a color particle (such as colloidal gold), binds to a second site on the target protein. In the case of lateral flow devices, a visible line will form on the device in the presence of the GMO protein of interest. These tests have been traditionally used in the field for rapid, qualitative identification of specific GMO proteins. However, these methods may also be modified for semi-quantitative use by incorporating a strip reader, such as the AgraStrip XReader.

In ELISA plates, capture antibodies bind to the GMO protein of interest. An enzyme conjugate of a second detector antibody binds to a second site on the protein. Then, an enzyme-catalyzed reaction occurs which will change the color in the ELISA wells. Higher levels of the GMO of interest result in darker colors in the wells. These methods may be quantitative or qualitative.
Such methods are, however, very specific for the protein of interest and are best used in raw grain samples. Processing steps which typically occur in food processing may denature the proteins, rendering them undetectable by the antibodies. Specific antibodies are required for detection of these denatured proteins, such as the one used in the AgraStrip RUR TM test (which detects the denatured CP4 EPSPS protein in toasted meal and other processed products). The protein methods are rapid, fast, and reliable, and do not require a laboratory environment or any expensive equipment to operate.

Romer Labs offers a variety of protein-based test methods for GMOs, including lateral flow tests and ELISA plates which cover most of the registered GMO traits. These test kits were developed by SDIX, our partner with longtime experience in GMO testing, and are sold exclusively by Romer Labs.



AgraStrip GMO Principle

Romer Labs® GMO SeedChek™ Products

Product	Item No.	Protein detected
AgraStrip® Cry2Ab SeedChek	3000007	Bt-Cry2Ab
AgraStrip® LL Cotton SeedChek	3000008	PAT
AgraStrip® EPSPS SeedChek	3000091	EPSPS (Event GA21)
AgraStrip® Cry1Ac SeedChek	3000094	Bt-Cry1Ac
AgraStrip® RUR-HS Comb SeedChek	3000184	CP4 EPSPS
AgraStrip® PMI SeedChek	7000052	PMI
AgraStrip® RUR SeedChek	7800010	CP4 EPSPS
AgraStrip® Cry3Bb SeedChek	7800011	Bt-Cry3Bb
AgraStrip® Cry34Ab1 SeedChek	7800012	Bt-Cry34Ab1
AgraStrip® RUR-HS SeedChek	7800013	CP4 EPSPS
AgraStrip® Triple Trait SeedChek	7800014	CP4 EPSPS, Bt-Cry1Ab, Bt-Cry3Bb
AgraStrip® Cry1F SeedChek (water extract) - corn	7800015	Bt-Cry1F
AgraStrip® Vip SeedChek	7800016	Vip3A
AgraStrip® Cry1Ab SeedChek	7800017	Bt-Cry1Ab
AgraStrip® Cry1F SeedChek (buffer extract) - cotton	7800018	Bt-Cry1F
AgraStrip® LL SeedChek	7800019	PAT
AgraStrip® RUR Comb SeedChek	7801100	CP4 EPSPS
AgraStrip® Btk SeedChek	7802000	Bt-Cry1Ab, Bt-Cry1Ac
AgraStrip® Btk Comb SeedChek	7802100	Bt-Cry1Ab, Bt-Cry1Ac
AgraStrip® BR SeedChek	7803000	CP4 EPSPS, Bt-Cry1Ac
AgraStrip® BR Comb SeedChek	7803100	CP4 EPSPS, Bt-Cry1Ac
AgraStrip® LL Comb SeedChek	7804100	PAT
AgraStrip® B2R SeedChek	7805000	CP4 EPSPS, Bt-Cry1Ab, Bt-Cry1Ac
AgraStrip® B2R Comb SeedChek	7805100	CP4 EPSPS, Bt-Cry1Ab, Bt-Cry1Ac
AgraStrip® Triple Trait Comb SeedChek	7806100	CP4 EPSPS, Bt-Cry1Ab, Bt-Cry3Bb

Romer Labs® GMO ELISA Products

Product	Item No.	Protein detected	Range
AgraQuant® Cry1F SeedChek	7020000	Bt-Cry1F	n/a
AgraQuant® EPSPS SeedChek	7020100	CP4 EPSPS	n/a
AgraQuant® Vip3A SeedChek	7020105	Vip3A	n/a
AgraQuant® DAS Cry1Ac SeedChek	7140220	Bt-Cry1Ac	n/a
AgraQuant® Soya Toasted Meal GMOChek	7099999	CP4 EPSPS	n/a
AgraQuant® RUR Soya Grain GMOChek	7100000	CP4 EPSPS	0.3 – 2.5 %
AgraQuant® Cry1Ab Maize GMOChek	7110000	Bt-Cry1Ab	0.15 – 2.0 %
AgraQuant® Cry9C Maize GMOChek	7110030	Bt-Cry9C	0.0075 – 0.10 %

Romer Labs® GMO TraitChek™ Products

Product	Item No.	Protein detected	LOD
AgraStrip® RUR-HS Bulk Grain TraitChek	7000011	CP4 EPSPS	0.125 % in corn 0.1 % in canola 0.167 % in alfalfa
AgraStrip® Cry9C Bulk Grain TraitChek	7000012*	Bt-Cry9C	0.125 % in corn
AgraStrip® RUR Bulk Grain TraitChek	7000014	CP4 EPSPS	0.1 % in soy and sugarbeets in 5 min; 0.143 % in soy in 3 min
AgraStrip® RUR Seed and Leaf TraitChek	7000017	CP4 EPSPS	qualitative
AgraStrip® Cry1F Bulk Grain TraitChek (buffer extract)	7000018	Bt-Cry1F	0.9 % in corn
AgraStrip® Cry1Ab Bulk Grain TraitChek	7000025	Bt-Cry1Ab	0.9 % in corn
AgraStrip® Cry1Ab Seed and Leaf TraitChek	7000026	Bt-Cry1Ab	qualitative
AgraStrip® Cry1F Seed and Leaf TraitChek	7000028	Bt-Cry1F	qualitative
AgraStrip® Cry3Bb Bulk Grain TraitChek	7000041	Bt-Cry3Bb	0.125 % in corn
AgraStrip® Cry3Bb Seed and Leaf TraitChek	7000042	Bt-Cry3Bb	qualitative
AgraStrip® LL Bulk Grain TraitChek	7000043	PAT	0.9 % in corn and sugarbeet, 2 % in canola
AgraStrip® Cry1Ab/LL Bulk Grain Combo Trait-Chek	7000044	Bt-Cry1Ab, PAT	0.9 % in corn
AgraStrip® LL Seed and Leaf TraitChek	7000045	PAT	qualitative
AgraStrip® LL Bulk Grain for Rice TraitChek	7000048*	PAT	0.05 % in LLRice62 2 % in LLRice61
AgraStrip® Cry1F Bulk Grain TraitChek (water extract)	7000053	Bt-Cry1F	0.9 % in corn
AgraStrip® Triple Trait Seed and Leaf TraitChek	7000054	CP4 EPSPS, Bt-Cry1Ab, Bt-Cry3Bb	qualitative
AgraStrip® Cry34Ab1 Bulk Grain TraitChek	7000055*	Bt-Cry34Ab1	0.125%in corn
AgraStrip® Vip Seed and Leaf TraitChek	7000092	Vip3A	qualitative
AgraStrip® Vip Bulk Grain TraitChek	7000093*	Vip3A	0.33 % in corn
AgraStrip® RUR Toasted Meal TraitChek	7120050	CP4 EPSPS	0.9 % in toasted soy
AgraStrip® Triple Trait Bulk Grain TraitChek	7806000	CP4 EPSPS, Bt-Cry1Ab, Bt-Cry3Bb	0.5 % in corn, 0.9 % in corn, 0.5 % in corn
AgraStrip® Corn Comb 7 Traits	7880610	CP4 EPSPS PAT, Bt-Cry9C Bt-Cry1Ab, Bt-Cry3Bb, Bt-Cry1F Bt-Cry34Ab1	depends on the trait

*****approved by USDA/GIPSA*

Trait™

Romer Labs® Semi-quantitative GMO TraitChek™ Products

Product	Item Number	Protein detected	Quantification Range
AgraStrip® RUR Bulk Grain TraitChek™	7000014	CP4 EPSPS	0.1 to 4.0 % RUR soy
AgraStrip® RUR HS Bulk Grain TraitChek™	7000011	CP4 EPSPS	0.1 to 4.0 % RUR corn
AgraStrip® Cry1Ab Bulk Grain TraitChek™	7000025	Bt-Cry1Ab	0.5 to 4.0 % Cry1Ab corn
AgraStrip® Cry3Bb1 Bulk Grain TraitChek™	7000041	Bt-Cry3Bb1	0.1 to 4.0 % Cry3Bb1 corn
AgraStrip® Cry34Ab1 Bulk Grain TraitChek™	7000055	Bt-Cry34Ab1	0.1 to 4.0 % Cry34Ab1 corn
AgraStrip® Cry1F Bulk Grain TraitChek™	7000053	Bt-Cry1F	0.5 to 4.0 % Cry1F corn
AgraStrip® Cry9C Bulk Grain TraitChek™	7000012	Bt-Cry9C	0.1 to 4.0 % Cry9C corn
AgraStrip® LL Bulk Grain TraitChek™	7000043	PAT	0.5 to 4.0 % LL corn
AgraStrip® VIP3A Bulk Grain TraitChek™	7000093	VIP3A	0.1 to 4.0 % VIP3A corn
AgraStrip® Semi-Quantitative Corn Comb Bulk Grain TraitChek™	7880620	CP4 EPSPS, Cry1Ab, Cry3Bb1, Cry1F, PAT, Cry9C, Cry34Ab1, VIP3A	Analyte dependent

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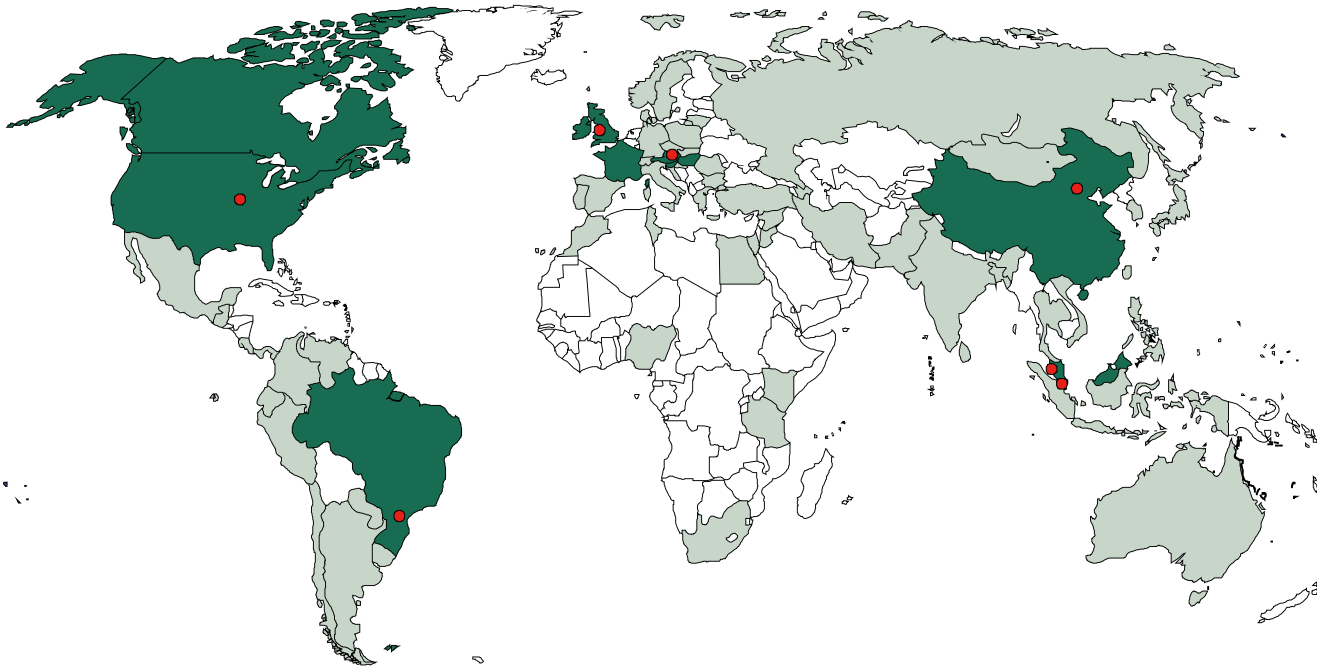

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