2017 ACORN Conference & Trade Show
Best Western Glengarry - Truro, NS

Workshop Title: New Genetic Engineering Techniques

Speaker: Dr. Ricarda Steinbrecher

Executive Summary: Dr. Ricarda Steinbrecher works for EcoNexus and The European Network of Scientists for Social and Environmental Responsibility (ENSSER). She spoke about the advancements in genetic engineering technologies and made the case that even with full cheques making sure edit appears successful, our understanding is limited and full consequences will likely never be known.

Detailed Notes:

Introduction:

- Lucy Sharratt gave an intro to Dr. Ricarda, a synthetic biologist from the EU. Dr. Ricarda works for ENSSE, who recently published a statement on this topic.
- Secondary title to the presentation: New and Different? Or Same and Risky?
- Must remember science is not how things are, it's how we see them.
- Science's views on Genes have been changing. An example: they thought there to be 100,00 genes, but that number keeps being downsized and is now at 18 thousand and some. The concept of one gene = one protein is not applicable anymore.

Views of the community:

- Dr. Ricarda spoke about early moments when everything seemed possible.
- Simplistic views of a new fact or discovery make us think it will solve all our problems, but in reality they over-assume how much change our proven techniques will actually provide.

Overview of the Genome (view slides):

- These techniques are largely valid for all: microorganisms, plants, animals, including insects and humans.
- Cells have nucleus' which store chromosomes made from DNA that encode the information of genes with nucleotides found together as basepairs: A-T & C-G
- You do not have one gene right next to the other, useful information is sporadic on the DNA strand.

- They look linear when shown, but genes are complex. Every part of the chromosome interacts from folding and bonds; thus, things influence each other even if they do not seem to be in close proximity
- Gene to protein: In most cases, more than one RNA/protein is produced from a given gene. DNA transcripts to RNA (copy) then gets processed (altered/spliced) into mRNA which is then translated into a protein.
- Techniques for gene splicing will affect mRNA stage.

Old Techniques for Editing:

- Example: Making rice insect resistant
  1. Isolate resistance gene (such as Bt-toxin gene from Bacillus Thuringiensis)
  2. Make a gene construct that will work in the plant or animal. For each gene to work, it must commence and finish with a regulatory sequence of nucleotides. Called a promoter to start and a terminator to complete. These can be added to the gene from other sources.
     - Plants: often CaMV (virus)
     - GE salmon: from ocean pout antifreeze protein gene (AFP)
     - GE Innate potato: from potato genes pAgp and pGbss
     - Must have the right switch (promoter) to have gene work
  3. Prepare plant cells or tissues
  4. Transform plant cells
     - How do you get the new gene into the cell/chromosomes.
     - Bacterium used as shuttle; or put it on a neutral metal and shoot it into the chromosomes (particle bombardment) (random integration) results in 100-1000's of mutations.

Mutations:

- Can be bad, good or neutral
- Can have unintended effects (problematic or deadly)
- Size of mutation does not predict scale of effect. Ex. a one letter mutation can cause hemophilia, sickle-cell anemia, or cystic fibrosis
- Point mutations can knock out the expression of a whole gene

New Techniques for Editing:

- The idea is to design & program special nucleases (molecular scissors) to go and cut the DNA at a determined place.
- New Breeding Techniques (NBTs)

  1. Oligoneucleotide dependent mutagenesis
  2. ZFN (zink fingernucleases)

- The idea is you can just go in and edit it. But in order to edit it, you need to know what it is originally saying, which we do not necessarily know.
- Targeting is now used. You target a site, sequence it (ATTCGTA), take a nuclease (to cut the DNA), and make the cut specific by adding a strand of RNA that is complementary to the target site (mirror image in order to bond/dock).
- Cutting makes the double-stranded break and results in degradation of both strands by endogenous enzymes, thus enlarging the area of the damage before the body can fix/attach the strand back together. In trying to prevent this, we add a DNA template for homologous repair.
- CRISPR - very fast, very easy, very cheap (compared to old technology)
- Unpredictability and risks
    - Sometimes the cut will make indels (small insertions or deletions) resulting in off-target effects. This can result in
    - Changes of protein function
    - Changes in gene expression
    - Increased presence of toxins
    - Absence of important proteins
    - The only way to know this is to sequence the entire Genome after Editing
    - Sometimes they only resequence similar looking sections.
    - You can also have unintended effects from on-target alterations. Changing one protein can affect other functions down the line. Genes are like an ecosystem (similarities to gardening). It is false if you are too focused on immediate results, science at this level needs holistic views/intuition
    - Organisms resulting from these techniques need a full risk assessment as well should be labelled.

<u>Thoughts On The Future:</u>

- Easy to use on single gene traits, though that is very rare for most traits.
- Stress tolerance traits are complex.
- There exists multi gene traits.
- CRISPR/Cas9 are good research tools.
- These techniques are not going to solve the problems of our time.

<u>Questions From Audience:</u>

Q) When using CRISPR in plant, how does it get into the cell wall.

1. Nuclease is protein, still shoot it in, produce the scissors, and the cut where it is supposed to. Then you deselect the progeny that the scissors are remaining in.
2. New type, already produce protein, than strip cell wall, then incubate and grow plants from that.

Q) Why has Europe been successful for mandatory labelling of GMOs, while CANADA and US do not require this?

- In Europe they have enshrined a constitution of cautionary principle. Late lessons from early warnings are easier traced.

Q) How is this more risky than conventional genome sequencing

- Cobalt in the field and you would select, now it is in the lab (chemical induced mutational breeding). Because of protein folding old ways would get hit a lot less and now with CRISPR you can go/change anywhere. And now multiplex altering happens (up to 40 changes at once).