HUDSON RIVER

Bio technology

Technologies for Better Crops

Plant Regeneration Solutions

What drives us

Our bioeconomy and agricultural system faces many challenges: How do we provide plant-based products for a growing world population in a sustainable manner? How can we create new crop varieties that amaze consumers? How do we increase yields and productivity to improve farmers' lives?

From our oxygen and vegetables, to our vaccine development, and to the rubber in our car tires and the biofuel heating our houses. **We cannot escape our need for plants.**

But with a growing world-population, the effects of climate change, declining soil quality, and competing demands on land, we are running into the limitations of our bioeconomy.

How can we utilize technology to solve these challenges?

Hudson River Biotechnology intends to deliver on the promise that crop improvement can address the demands of a resilient, sustainable bioeconomy.

We solve plant production challenges across the value chain. To do this, we deliver technologies that are needed to develop and grow crops of higher quality, more efficiently and sustainably.

We focus on two important aspects:

- Speeding up the development of plant varieties through targeted editing of traits and single-cell regeneration;
- Increasing the efficacy of field application to enable efficient use of crop input through nanotechnology.

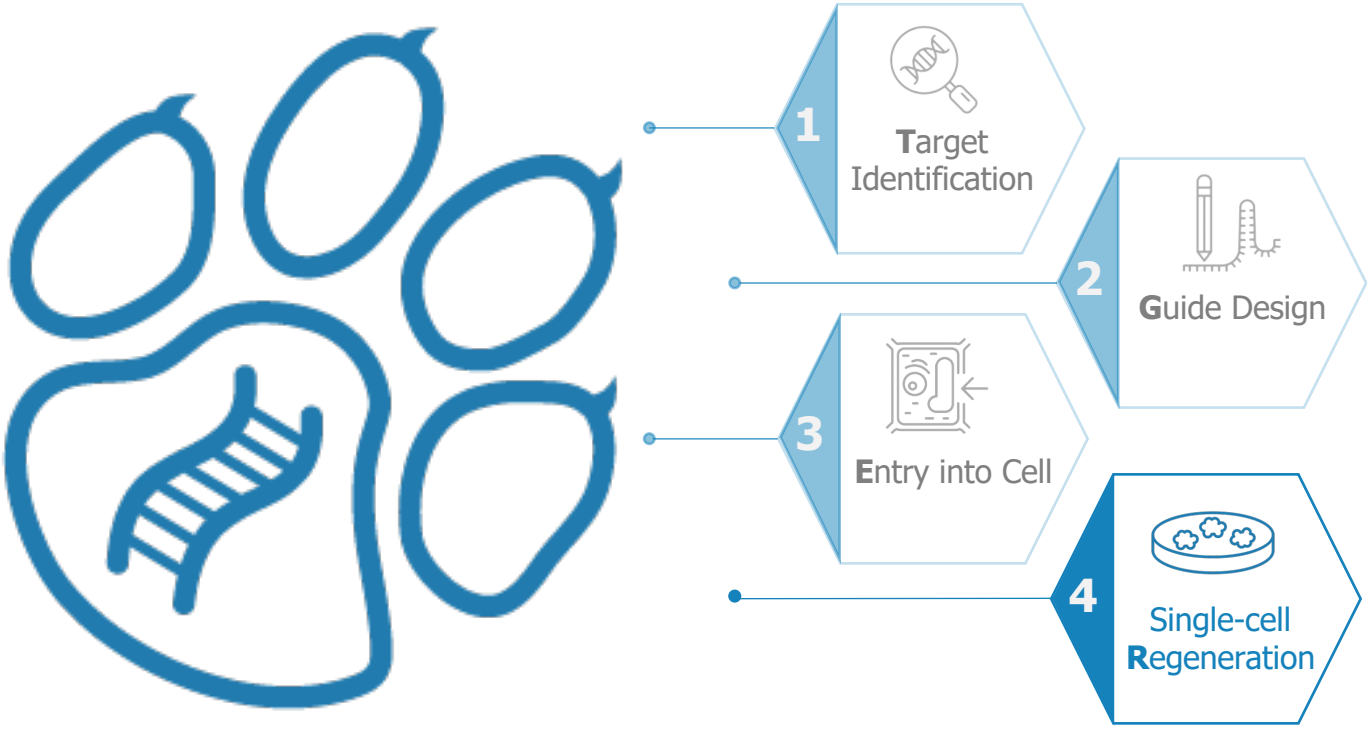
By providing these technologies to our partners, we contribute to better plants and higher plant yields while reducing the inputs necessary. Ultimately decreasing the impact on our natural ecosystems. We can help you greatly reduce the time and cost of plant breeding, and accelerate crop improvement in terms of **adaptation**, **resilience**, and **end-use**.

Our proprietary **Regeneration Approach** helps to remove the hurdles which are usually encountered in plant tissue regeneration, leading to a faster and more efficient process.

In this whitepaper we tell you more about our proven approach.

TiGER Workflow

HRB's TiGER workflow establishes genetically homogeneous plants from CRISPR editing events in an end-to-end fashion (*see our TiGER whitepaper*). As part of the workflow, HRB performs DNA-free and selection marker-free editing. After successful genome editing, *in vitro* cells need to be regenerated back into a plant, ready for the next phase of plant breeding. This fourth and final step needs a specialized approach and can also be performed as a stand-alone procedure.



Regeneration at a glance

HRB regenerates plants in a clonal fashion from individual cells – single-cell regeneration. We have validated protocols for regeneration of multiple species and are in various stages of development for many more. While regeneration approaches depend on the needs of the plant tissue, most often regeneration follows the classical organogenesis route.

Organogenesis regeneration: *Protoplasts extracted from specific plant tissue are edited, diluted and embedded in a hydrogel, after which the single cells proliferate and form calli. In the following steps, hormone treatments induce production of shoots and production of roots. These plantlets can then be used for further purposes.*

HRB

How to address the challenges of conventional regeneration

Successful regeneration enables the conversion of (CRISPR-)edited cells into market-ready crops. However, current approaches to regenerate plant material from modified in-vitro plant tissue is associated with several technical barriers. Within its TiGER workflow and regeneration approach, HRB tackles the most pressing challenges associated with Regeneration:

HIGH COMPLEXITY	Regeneration is a multi-step approach and technological hurdles are found at every point.
UNIQUE SPECIES	Every species or variety is unique in its regeneration requirements and a generalized approach often doesn't work.
LOW EFFICIENCY	Lengthy and costly projects are needed to determine the best regeneration protocol.
EDITED CHIMAERAS	Conventional regeneration methods often create genetically heterogenous products.

HRB's proprietary regeneration approach yields fully grown, genetically homogenous plantlets within 6-18 months (timeline highly dependent on species and variety). We address common challenges with an innovative **single-cell regeneration approach** performed by a **specialized regeneration team,** with the use of **adaptive protocols and proprietary technologies.** Single-cell protoplast regeneration makes sure we can provide a homozygous end-product and avoid edited chimaeras.

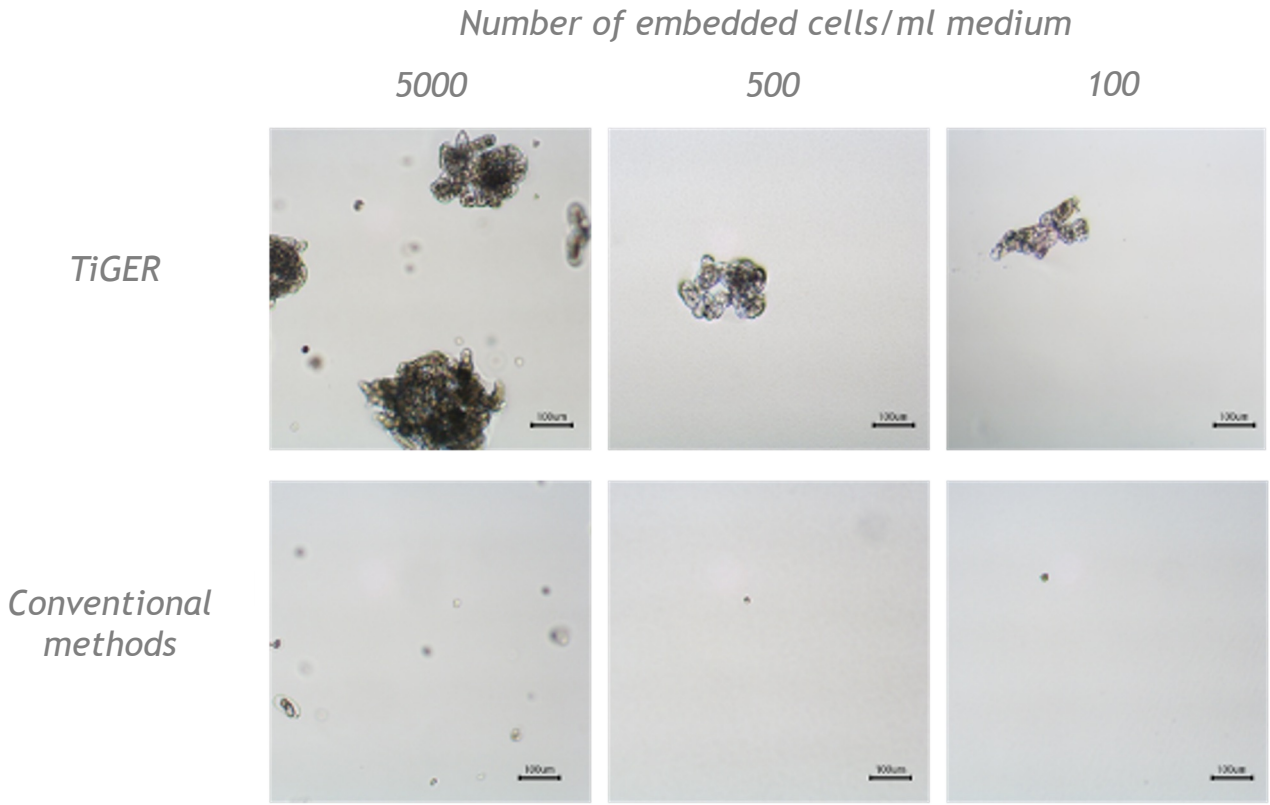
Our in-house regeneration team consists of expert scientists who have extensive experience with every step of the regeneration process. To be able to adapt protocols as needed for the regeneration of different species, HRB has access to an array of highly different regeneration solutions. These range from physical to chemical to biological, in order to customize where needed. Our proprietary technologies enable or improve the efficiency and speed of starting up protoplast cell divisions, callus regeneration and differentiation. HRB's solutions include multiple patented or patent-pending technologies.

Our single-cell regeneration approach

The most common method to generate CRISPR-edited plants involves the use of transgenes delivered via Agrobacterium. However, this results in unwanted genetic chimaeras: plants with sectors/clusters of edited and non-edited cells or tissues. These may also still contain a transgene that needs to be removed. Another approach is to regenerate plants from individually edited cells. As protoplasts need signals from surrounding cells to initiate growth, conventional approaches need to embed these edited protoplasts at a sufficiently high density. Because of this, expanding microcalli rapidly incorporate multiple protoplasts, leading to genetically chimeric end-results.

By regenerating plants from diluted single cells, HRB can circumvent the risk of chimaeras and establish genetically homogeneous plants.

For our single-cell regeneration approach, HRB has optimized media composition and regeneration protocols and materials, adding several proprietary components. These enable us to lower the concentration at which protoplasts need to be seeded in the gelling agent for effective regeneration by 1,000 to 10,000-fold (also when using alginate) compared with commonly used protoplast regeneration protocols.



Using the TiGER single-cell regeneration approach, protoplasts can be diluted down to ensure single-cell regeneration: *Microcalli derived from embedded cells (images taken after 3 weeks post transfection and embedding). At these dilutions, microcalli can only be seen forming with the TiGER approach. Using conventional methods, the concentration of embedded cells needs to be increased by 1,000 tot 10,000x to allow microcallus formation; at this concentration, expanding microcalli rapidly incorporate multiple protoplasts.*

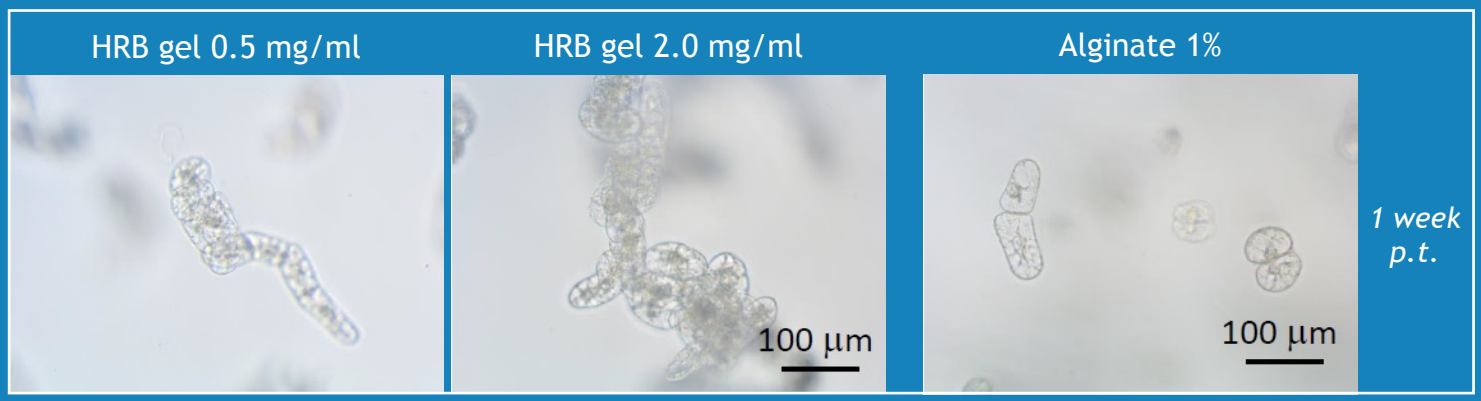
Our key regeneration technologies

HRB continuously integrates new technologies to further innovate our single-cell regeneration. These include multiple patented or patent-pending technologies that enable or improve the efficiency of starting up protoplast cell divisions, callus regeneration and differentiation. Two of our key technologies are explained below.

1 HRB gel technology improves callus induction

Protoplast embedding is crucial for supporting the cell during regeneration. Hydro Regeneration Booster (HRB) synthetic polymers are proven, highly effective gelling agents in the field of mammalian organoid culture.

Compared with classical alginate, HRB gels can enable **faster onset of cell divisions** in protoplasts towards micro-calli formation. HRB gel IP is covered by multiple patents owned by Radboud University Nijmegen, and HRB has an exclusive license for agricultural use of these gels.



Embedding in HRB gel enhances overall survival of the recovering protoplasts and enables cell divisions sooner, compared with Alginate: *Protoplasts from a commercially relevant dicot crop, transfected and afterwards embedded in HRB gel or Alginate. Cell wall regeneration and cell division can be seen in the HRB gel 5 days post transfection while most cells in the alginate have remained protoplasts.*

2 Kinase inhibitors promote plant cell de-differentiation and regeneration

HRB has access to several small compounds that can promote plant cell de-differentiation, regeneration and shoot formation. Most notably are our kinase inhibitors. One example entails two related kinase inhibitors that **enhance the efficiency of embryonic induction by up to 27.5%**, as well as **improve germination capacity**. HRB is currently optimizing their application in callus regeneration. The molecules are covered by multiple patent applications by CSIC, and HRB has an exclusive license.

HRB

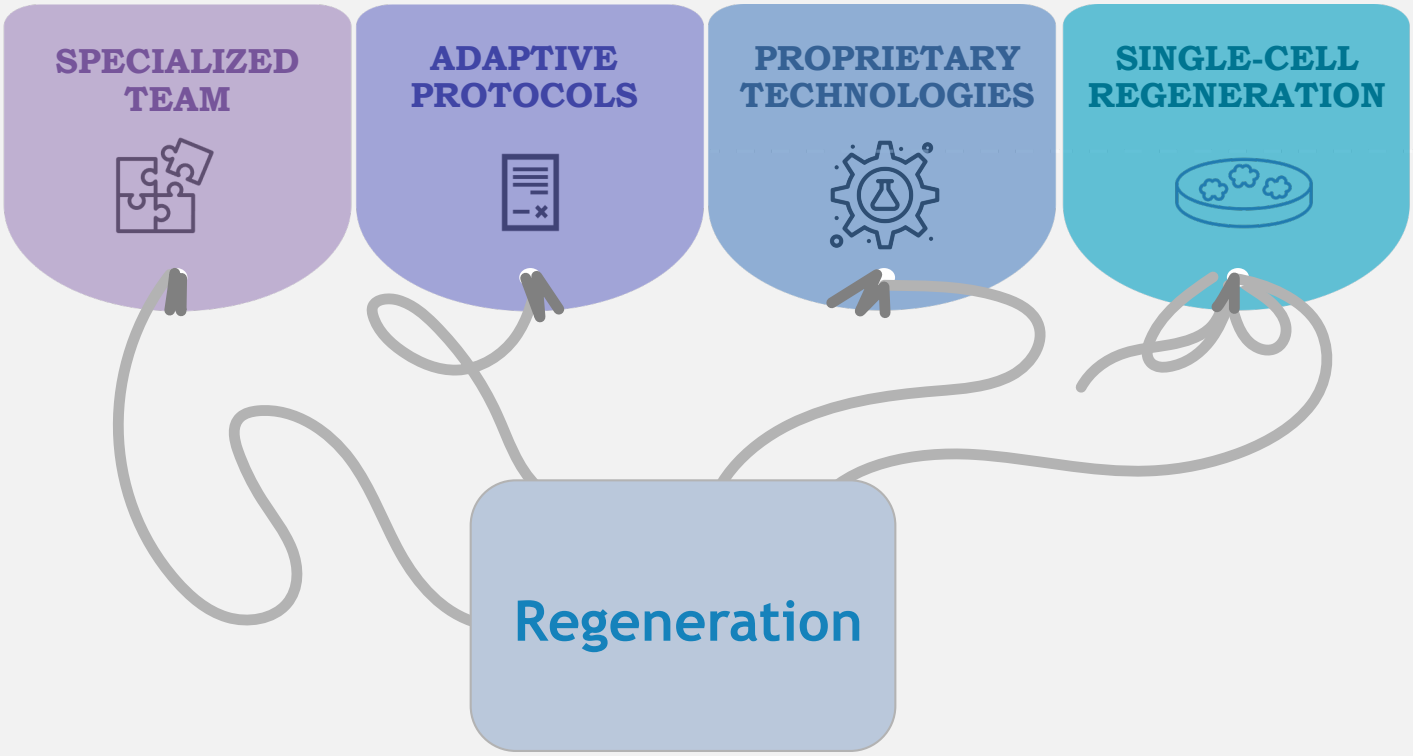
The benefits of our regeneration approach

HRB's single-cell regeneration stands out from other conventional regeneration approaches. Our validated technologies and protocols significantly increase the chance of successful regeneration, and we have a wide toolbox for developing solutions to regenerate recalcitrant species.

Ultimately, our regeneration solutions increase efficiency, and reduce time and costs for your plant breeding program.

Some of HRB's validated results:

- Regeneration protocols that work for multiple species
- Gelling agents enable 1,000 to 10,000 fold dilution of regenerating protoplasts
- 10% of edited protoplasts form micro-callus at low cell densities
- Faster onset of cell division through HRB embedding
- Kinase inhibitors enhance embryogenic induction efficiency



Boost your plant breeding program - start collaborating

If you would like to learn more about our approach reach out to Gabino Sanchez at gabino.sanchez@hrb.bio

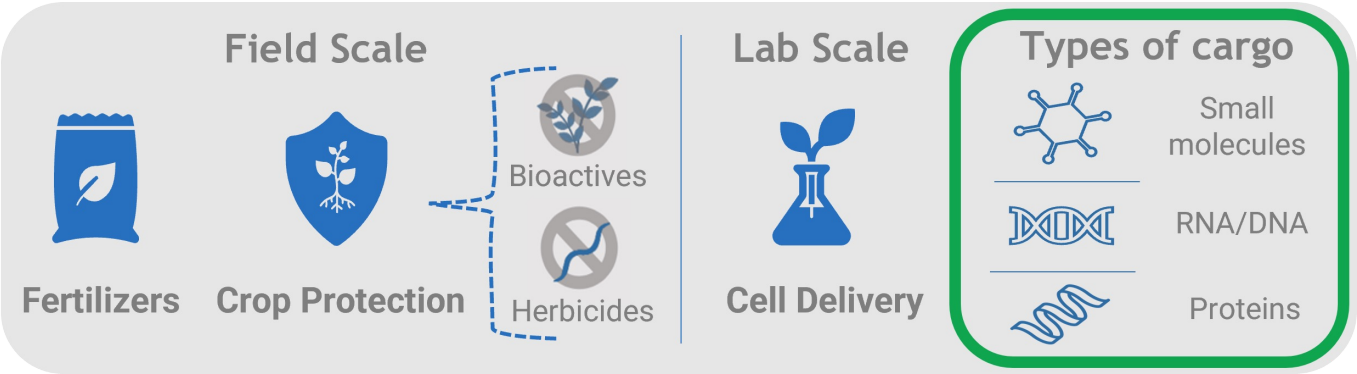
Highlight

Plentrance™

Nanotechnology for efficient delivery of biologicals and agrochemicals inside plants

New and better molecules for plant treatment are currently being brought on the market. But efficient delivery remains a challenge, especially for field application.

Building on the applications in our TiGER workflow, we have now crossed over to applying nanoparticles for delivery of agro-biologicals in plant tissue. This can be used in our TiGER workflow to simplify delivery of editing agents to plant cells but is also highly relevant for field practices by enabling efficient use of crop inputs.

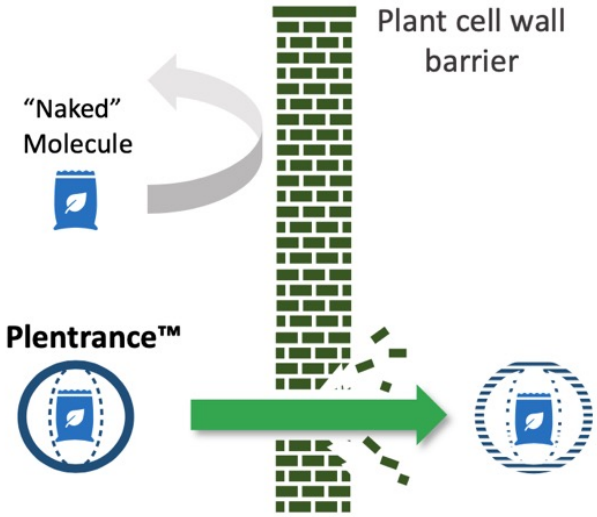


Plentrance™ nanoparticle formulations are **scalable**, **biodegradable** and provide **better control and efficiency**

How does it work?

Plentrance™ nanoparticles **penetrate the plant cell wall** and make active ingredients available in the right place at the right time.

Together with you, we develop **tailor-made nanoparticles** that increase the efficacy of the delivery, significantly lowering the dosage requirements. This lowers the associated costs for farmers and decreases the strain on our natural ecosystems. Our solutions include multiple patented formulations for delivery of various substances, such as herbicides, fertilisers and nucleotides.



The main benefits of our Plentrance™ nanoparticles:

- Optimization of field application to enable efficient use of active ingredients
- Customized and controlled release over time
- Biodegradable and compatible with ecological agriculture
- Increased bio-availability leads to less waste

HRB

Ferdinand Los
CSO Hudson River Biotechnology

About us

Hudson River Biotechnology is an independent technology provider, specialized in plant breeding, crop production, genetics, and biotechnology. Since HRB's founding in 2015, we have established a name in the market as a go-to player for disruptive innovations and cutting-edge technological developments. As our first portfolio technology, we have worked with a variety of companies to develop new traits and solve technological barriers in gene editing with CRISPR. In 2021 we have expanded our portfolio to offer delivery of biologicals and small molecules in field applications through nanotechnology.

Our customers range from large multinationals to family-owned businesses in, for example, the food, pharmaceuticals, plant breeding and biotech industries. Our goal is to make plant-focused technologies accessible, tailor-made to each customer and create mutual success. In short: HRB is perfectly positioned to become your strategic technology partner.

If you would like to learn more or discuss possibilities for collaboration reach out to Gabino Sanchez at gabino.sanchez@hrb.bio