BioEcoAgro

UCCS
UNITÉ DE CATALYSE ET CHIMIE DU SOLIDE

CRIStAL
Centre de Recherche en Informatique, Signal et Automatique de Lille

Brings catalysis over lightspeed

REALCAT

High-throughput strain identification and production of fungal enzymatic cocktails for the valorisation of lignocellulosic biomass

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The perfect enzyme cocktail for biomass degradation

Virtual lab tour:
https://www.vip-studio360.fr/galerie360/visites/vv-centrale-lille/vv-realcat-c.html

The REALCAT platform

REALCAT

- **Advanced High-Throughput Technologies Platform for all types of experiments in Chemistry and Biology, dedicated to biomass valorization.**

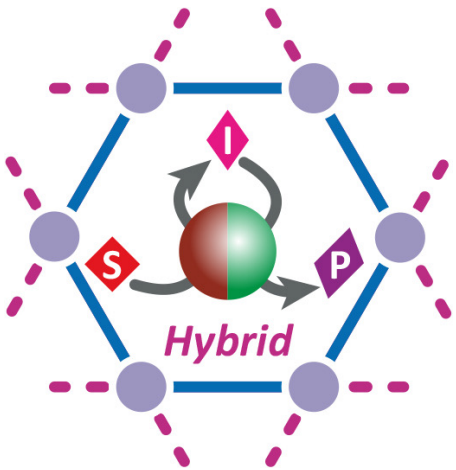
3 main areas of expertise

Brings catalysis over lightspeed

REALCAT

- **Catalysis**
  - **Chemical catalysis: homogeneous and heterogeneous**
  - **Biocatalysis: enzyme and fermentation**
- **Structural and compositional characterisation of chemical and biological materials used/generated (catalysts, metabolites, polymers, enzymes, etc.)**
- **Side activities in biology**
  - **Proteomics, NRPomics**
  - **Search for new antimicrobial molecules**
  - **Production of secondary metabolites**

Combination of chemo & Bio



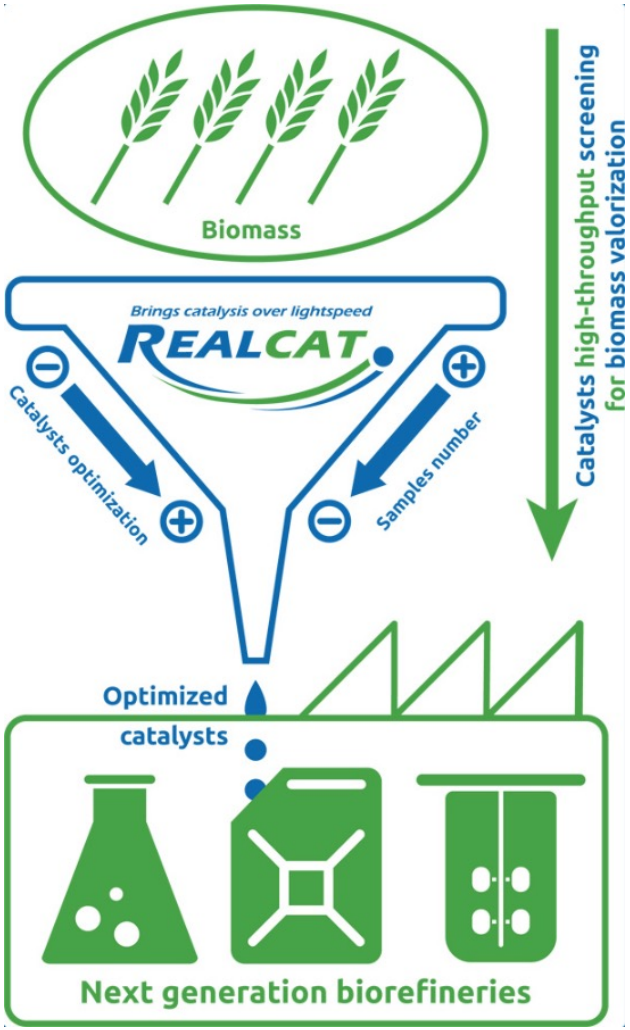
The REALCAT platform

3 main types of equipment dedicated to :

- **Synthesis** of chemical and biological materials (catalysts, strains, enzymes, metabolites, etc.)
- **Testing** of catalytic properties (catalytic, nutritional, antimicrobial, etc.)
- **Characterization** (structure, composition, physico-chemical properties, etc.)

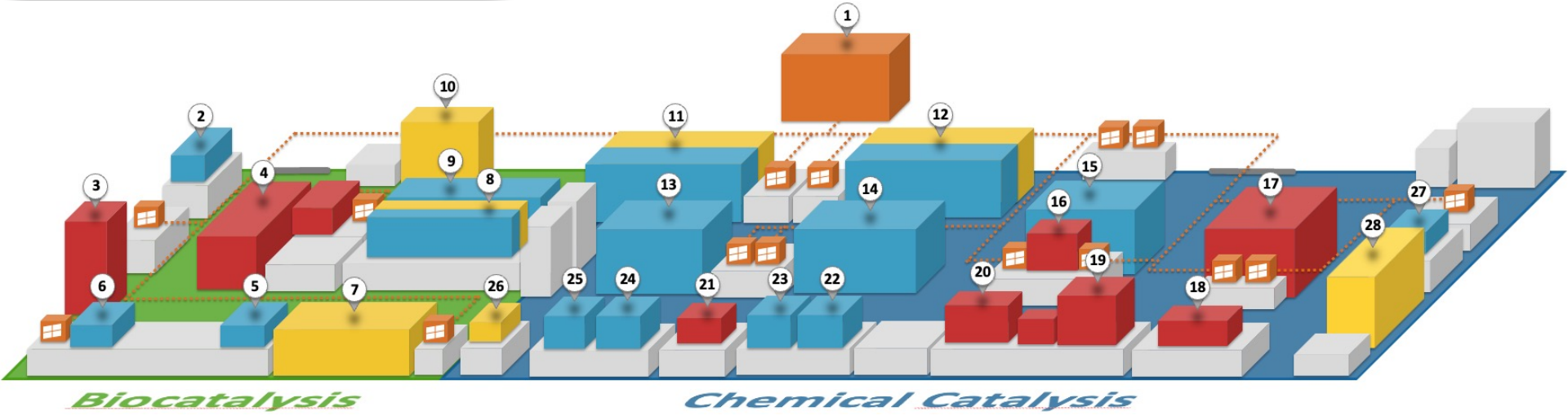
Our final objectives :

- **Accelerate each step of the experimental phase** of a chemistry and biotechnology research project to significantly reduce the consumption of money and time
- Define new ways to **valorise biomass**



REALCAT

REALCAT floorplan



More than 30 automated devices

+ some other robots, technical rooms and offices.

- All fully dedicated to high-throughput experiments
- Very high modularity allowing a large variety of subjects
- Fully integrated to allow synergy between machines

https://

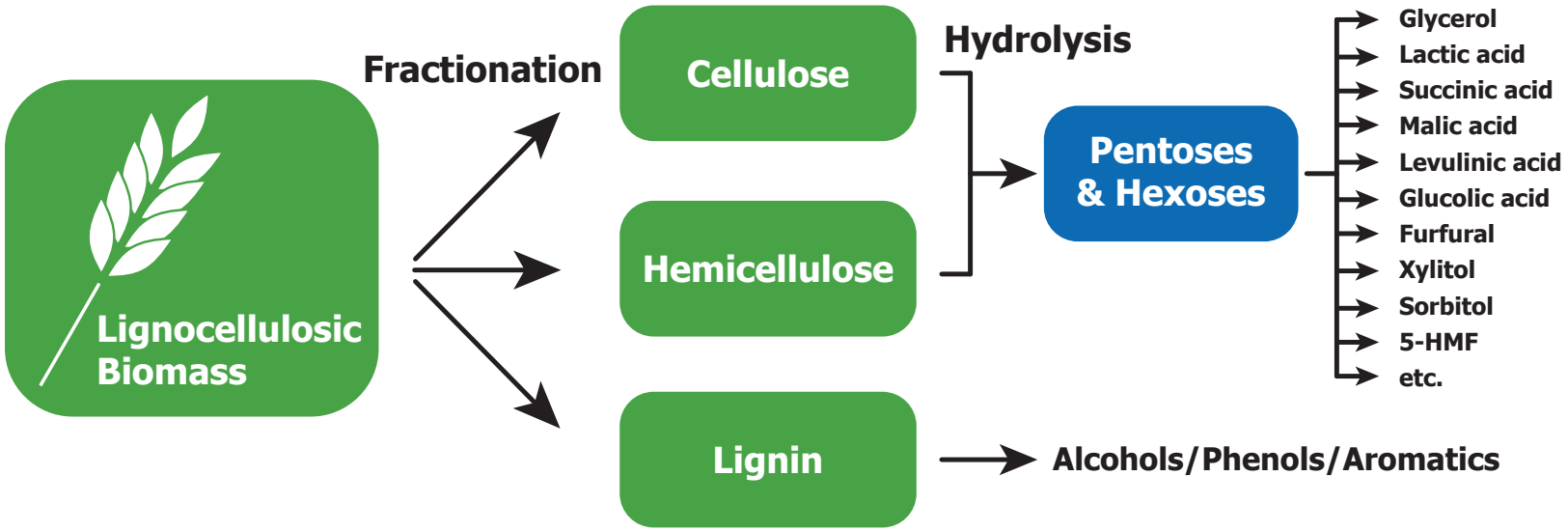
www.realcat.fr

1	Central server - DELL	15	Flowrence - Avantium
2	Biomek NXp + BioLector Pro - Beckman Coulter/M2PLabs	16	M4 Tornado - Bruker
3	Autoflex Speed - Bruker	17	D8 Discover - Bruker
4	Acquity UPLC Synapt G2-Si HDMS - Waters	18	Tensor 37-HTS-XT - Bruker
5	Cary 3500 - Agilent	19	ICP-OES - Agilent
6	BioLector - M2pLabs	20	Vulcan 42S - Questron Technologies/Horiba
7	QPix 460 - Molecular Devices	21	XploRa - Horiba Jobin Yvon
8	Biomek FXp - Beckman Coulter	22	GC-FID-2010 Plus AF - Shimadzu
9	Biomek FXp - Beckman Coulter	23	GC-FID-MS-QP2010 Ultra EI - Shimadzu
10	Laminar flow hood - Aquaria	24	HPLC-UV-IR – Shimadzu
11	Catimpreg – Chemspeed	25	HPLC-DAD-MS - Shimadzu
12	Autoplant – Chemspeed	26	Calcination oven - Dislab
13	Flowrence - Avantium	27	Screening Pressure Reactor (SPR) - Freeslate
14	Flowrence - Avantium	28	Fume hood - Asem

Valorization of lignocellulosic biomass

Target: Biomass decomposition into biofuels, building blocks for fine chemistry and polymers:

- **Production of bioethanol from the fermentation of extracted sugars**
- **Production of 20 platform molecules from C5 and C6 sugars**

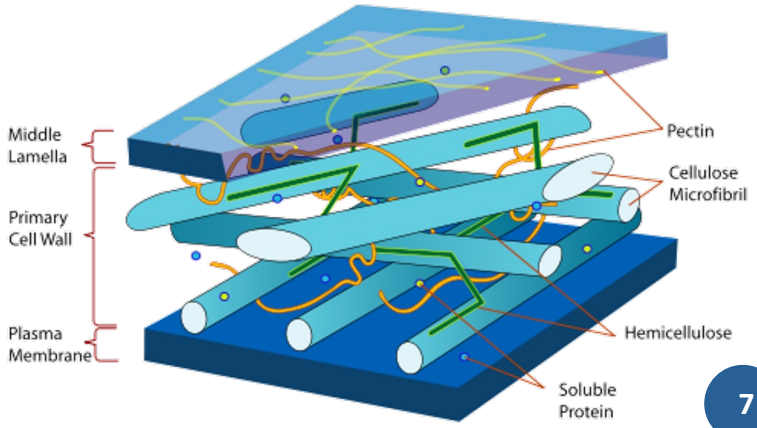


- **+ alcohols/phenols/aromatics from lignin: methanol, benzoic acid, catechol, cinnamic acid, etc.**

Valorization of lignocellulosic biomass

The challenge: designing the perfect enzyme cocktail for lignocellulose degradation

- **Ideal case: no pre-treatment (mimic nature)**
- **No "universal" bacterial/fungal strain efficient on cellulose, hemicellulose AND lignin => Find the perfect enzyme combination**
- **Many available approaches: metagenomics, low throughput fermentation screenings, *de novo* design, commercial blends…**
- **Numerous drawbacks:**
  - **Very time and money consuming**
  - **High systems complexity (specially in biodiversity mixtures)**
  - **Model substrates are not representative**
  - **Lack of synergy and thermodynamic equilibriums issues**



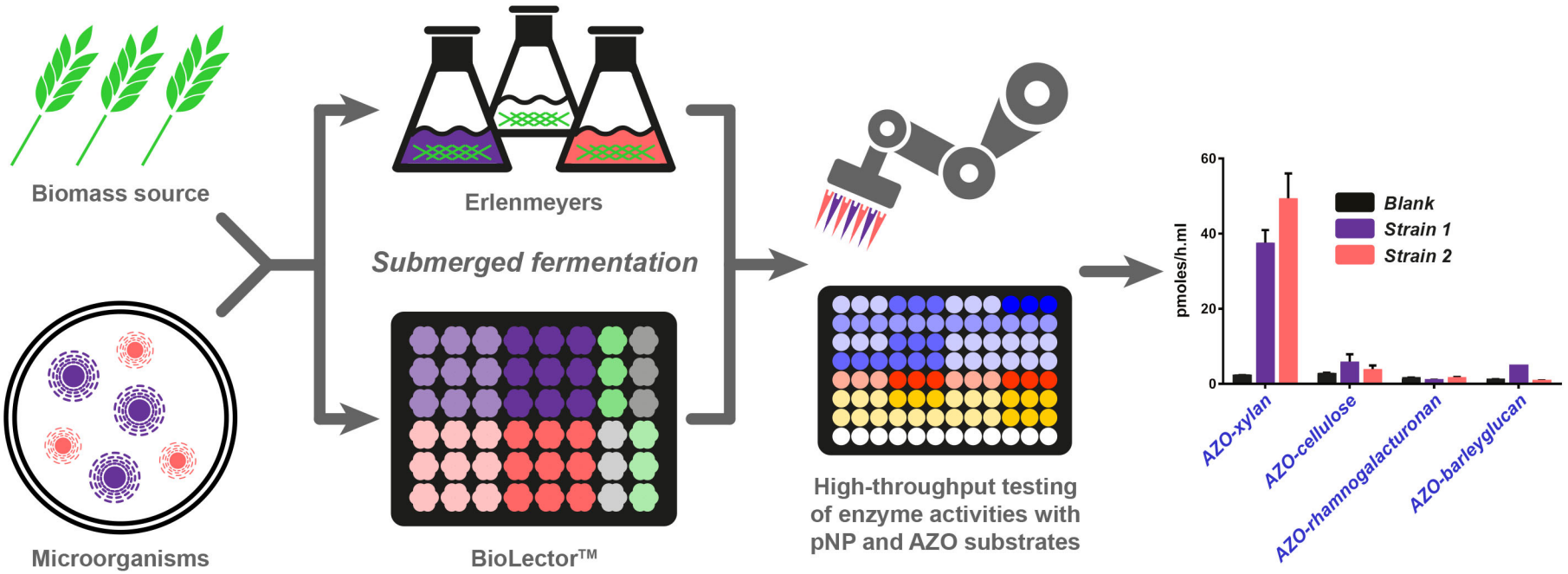
HT approach for CWDE screening

Harvesting enzyme from microorganisms living on target substrate

- **Premise 1: Organisms that develop on biomass are the best equipped to degrade it**
- **Premise 2: No single organism can proceed to a complete degradation**

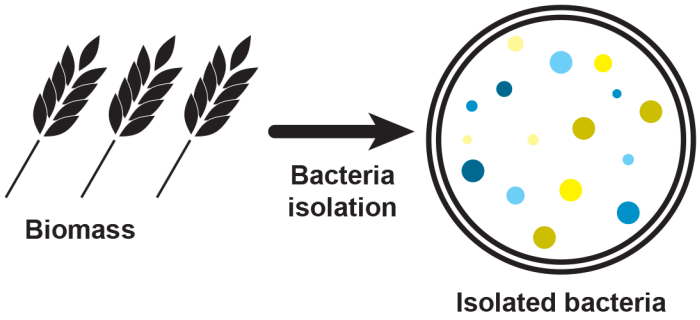
⇒ **Need for harvesting and combining numerous strains**

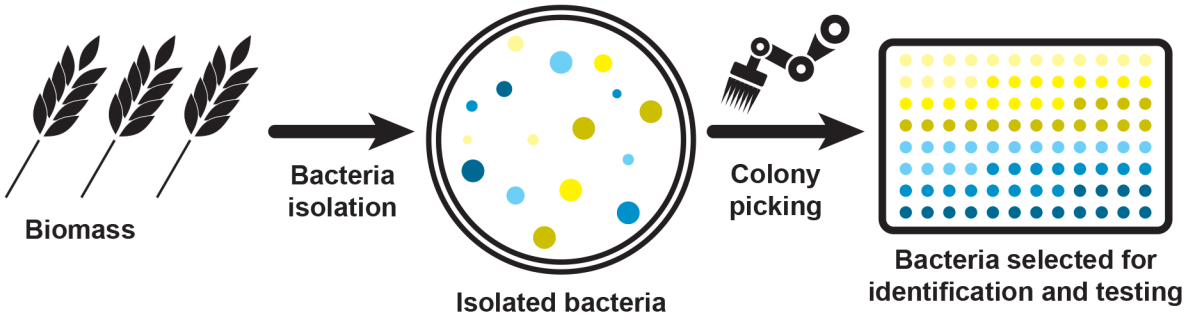
- **First semi-automated method presented in 2018**

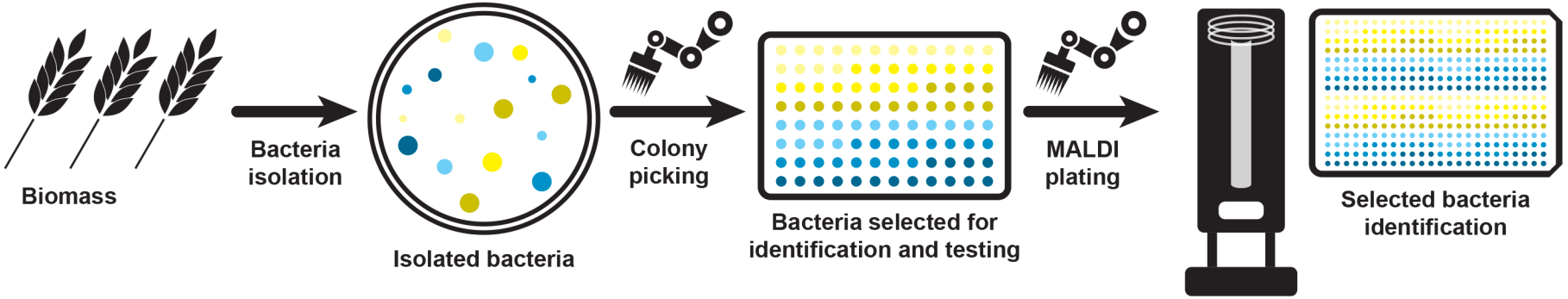


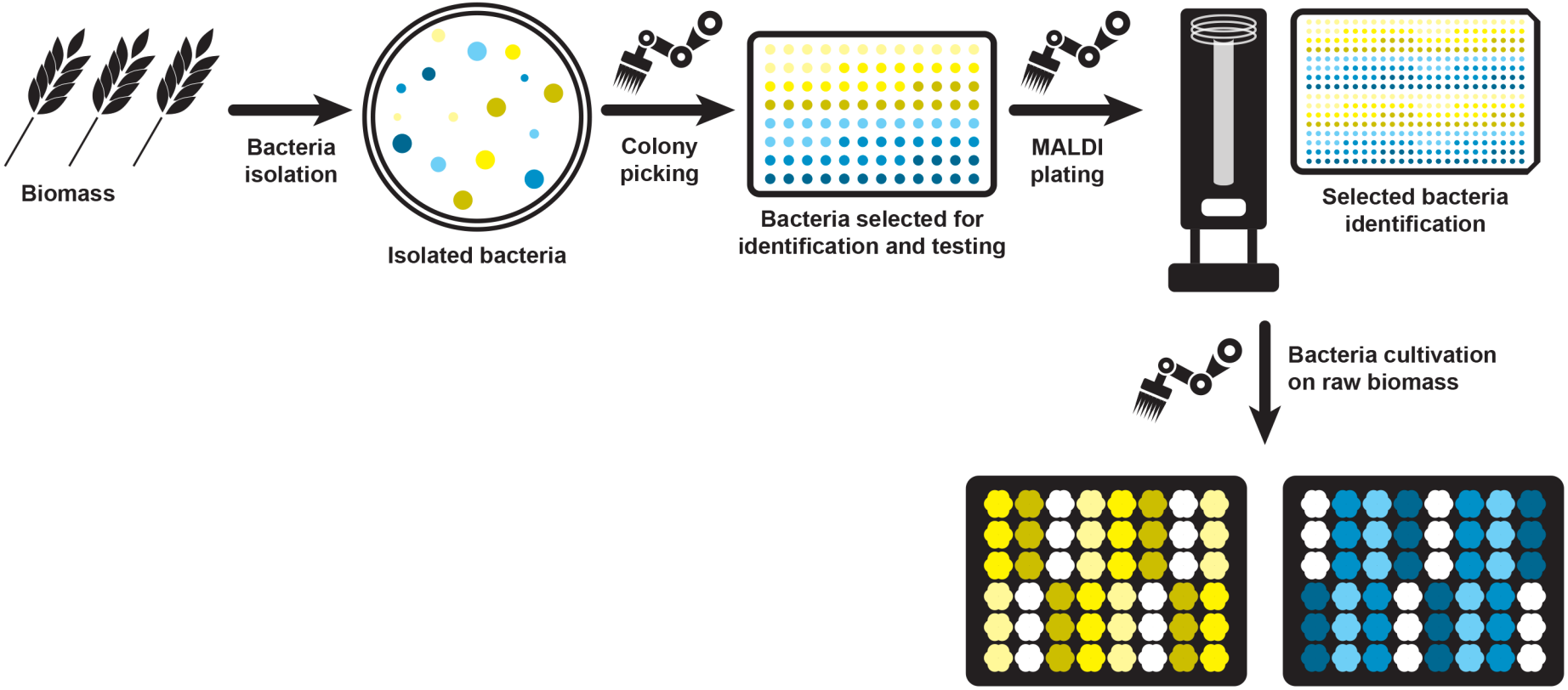
Raulo *et al.*, Microb. Biotechnol. 2019, 12 (6), 1286.

Biomass


Biomass
Bacteria
isolation
Isolated bacteria


Biomass
Bacteria isolation
Isolated bacteria
Colony picking
Bacteria selected for identification and testing


Biomass
Bacteria isolation
Isolated bacteria
Colony picking
Bacteria selected for identification and testing
MALDI plating
Selected bacteria identification


Biomass
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Colony picking
Bacteria selected for identification and testing
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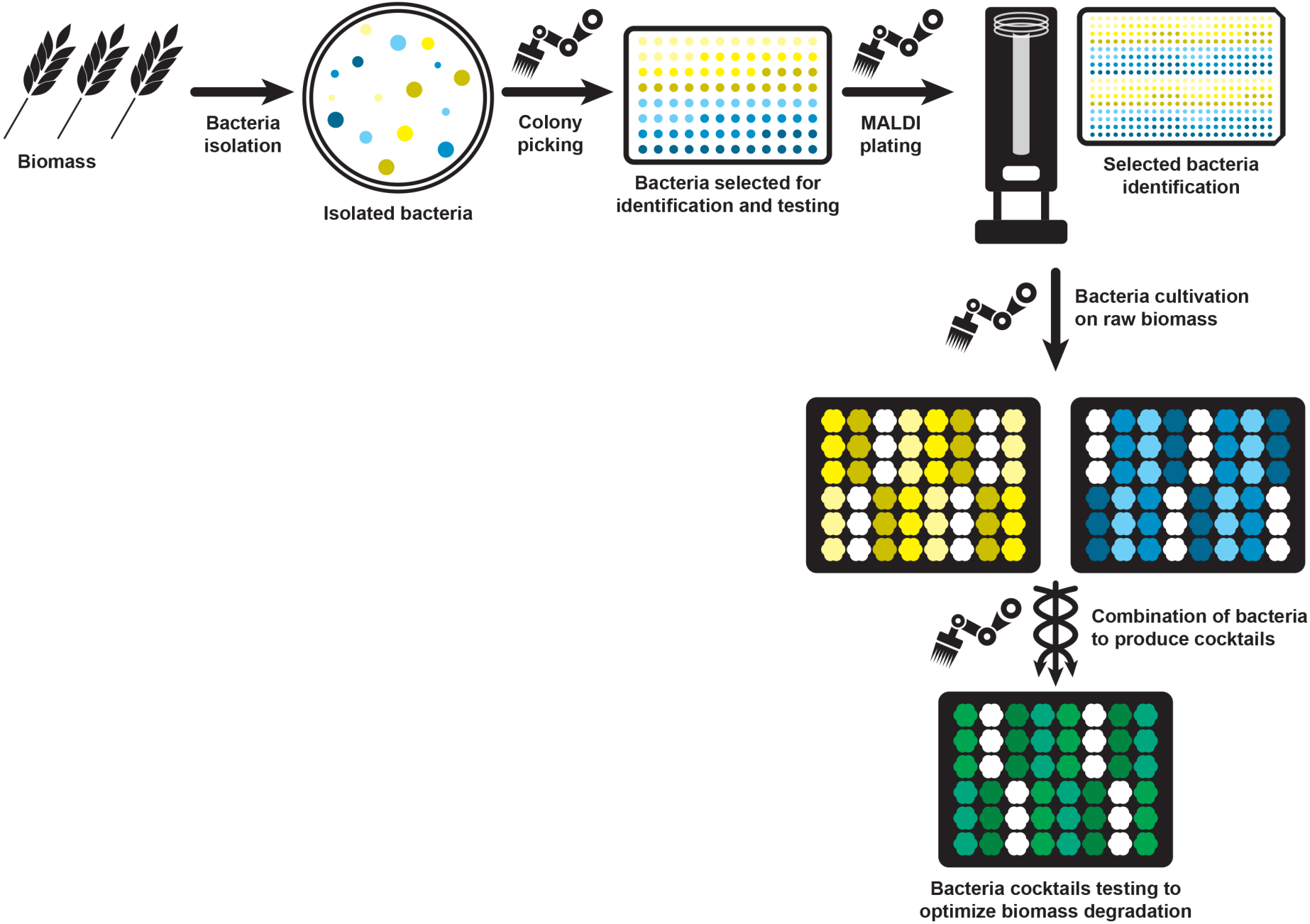
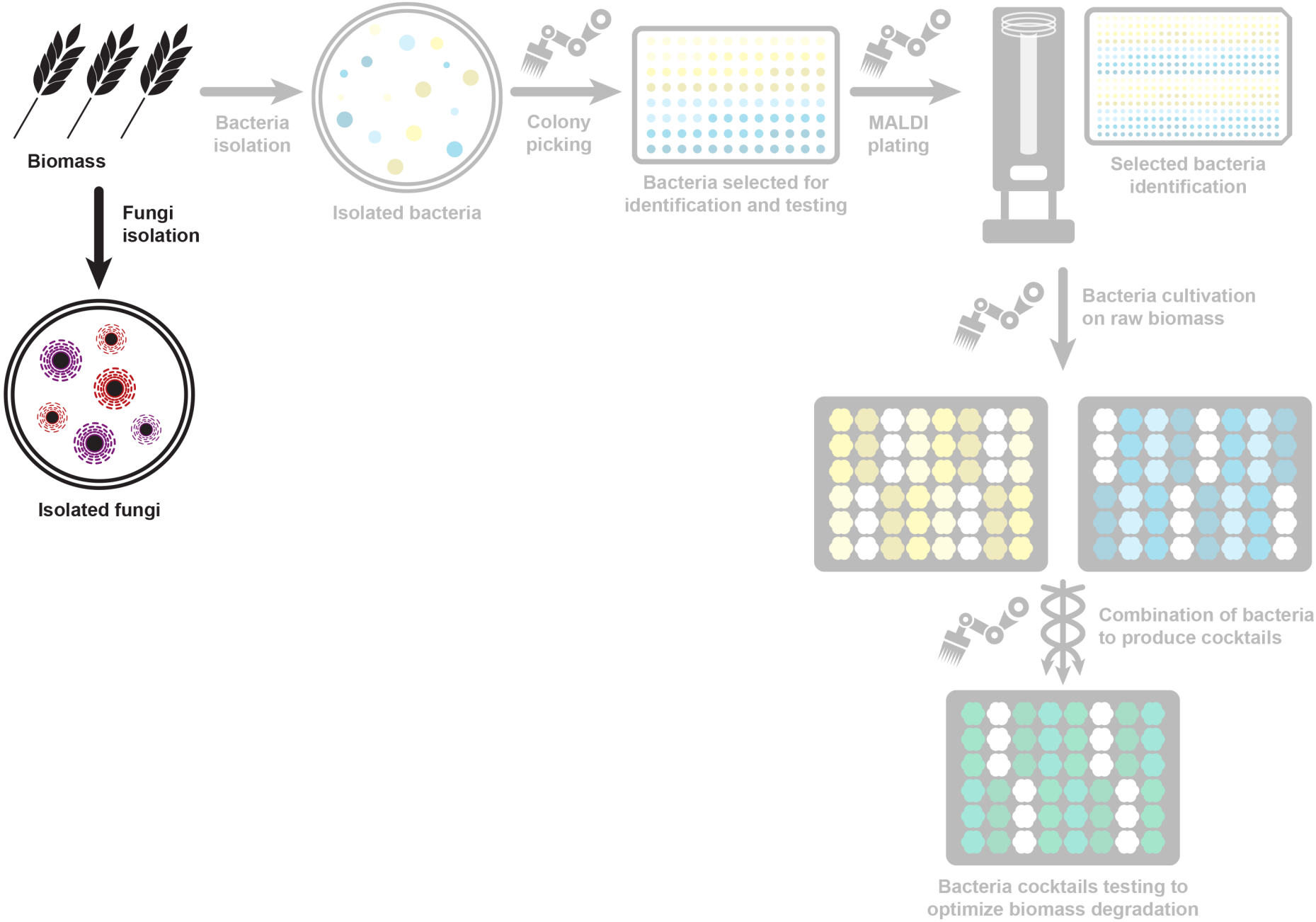
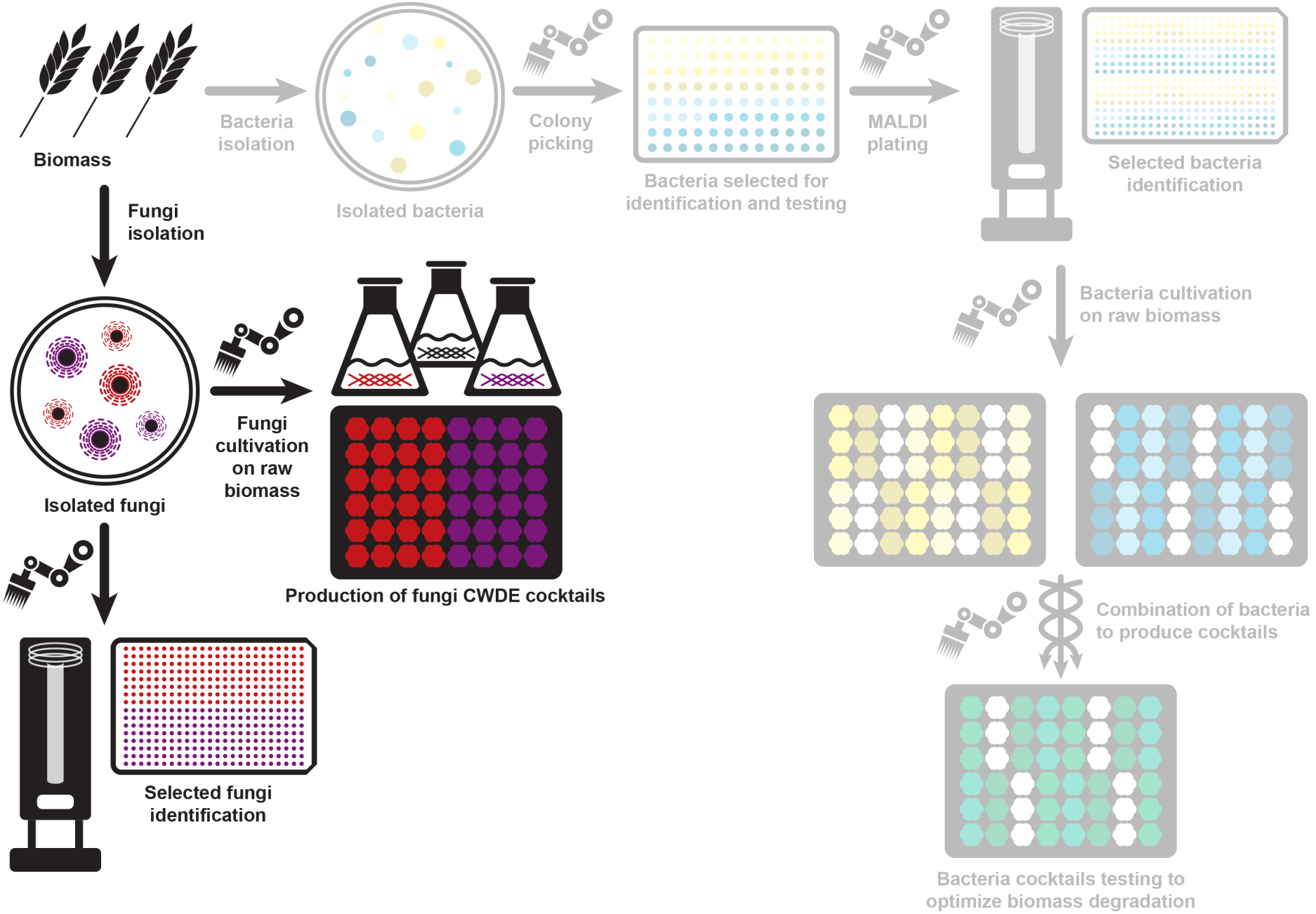
Bacteria cultivation on raw biomass


Biomass
Bacteria isolation
Isolated bacteria
Colony picking
Bacteria selected for identification and testing
MALDI plating
Selected bacteria identification
Bacteria cultivation on raw biomass
Combination of bacteria to produce cocktails
Bacteria cocktails testing to optimize biomass degradation


Biomass
Bacteria isolation
Isolated bacteria
Colony picking
Bacteria selected for identification and testing
MALDI plating
Selected bacteria identification
Fungi isolation
Isolated fungi
Bacteria cultivation on raw biomass
Combination of bacteria to produce cocktails
Bacteria cocktails testing to optimize biomass degradation


Biomass
Bacteria isolation
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Bacteria selected for identification and testing
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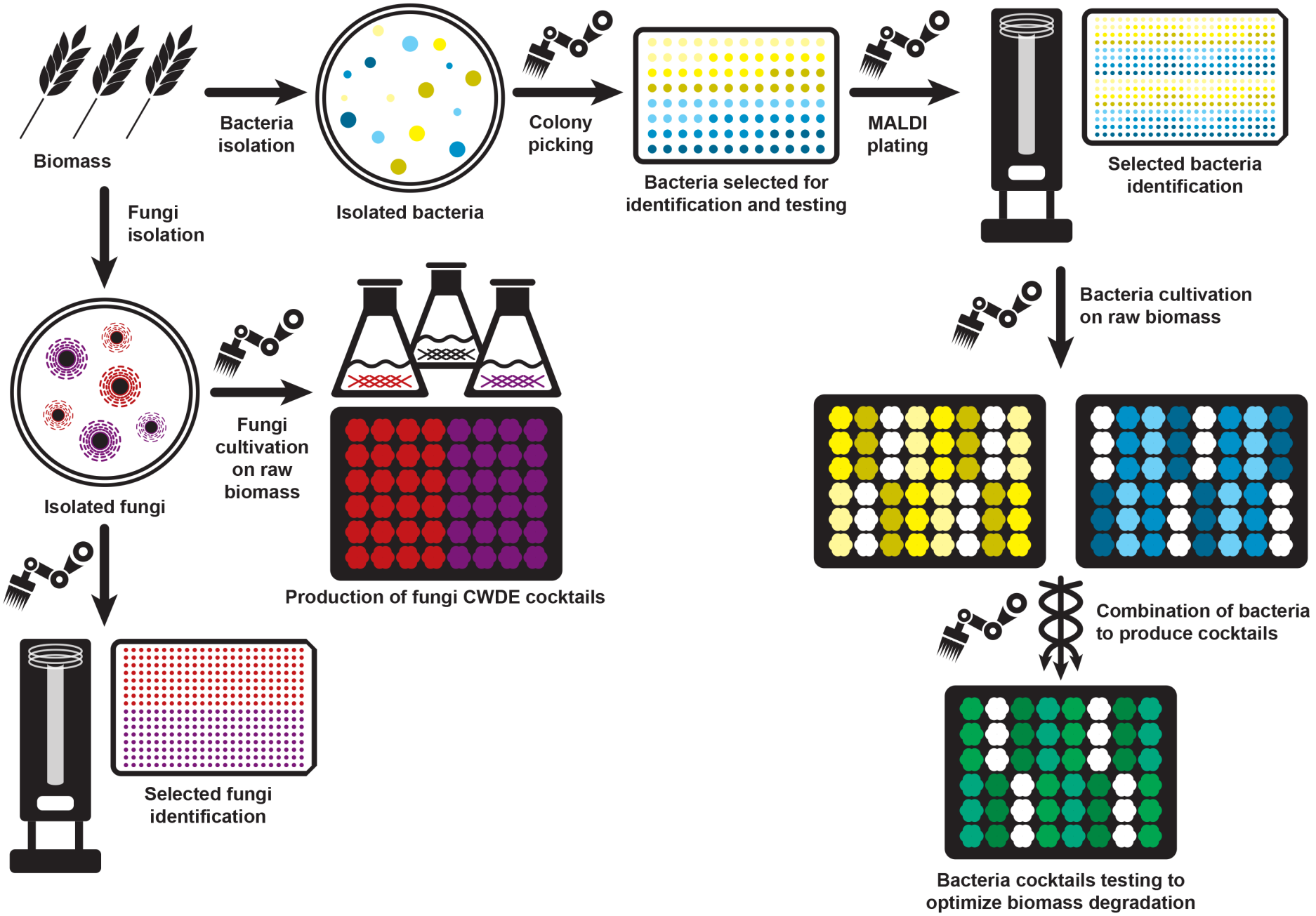
Fungi isolation
Isolated fungi
Fungi cultivation on raw biomass
Production of fungi CWDE cocktails
Bacteria cultivation on raw biomass
Selected fungi identification
Combination of bacteria to produce cocktails
Bacteria cocktails testing to optimize biomass degradation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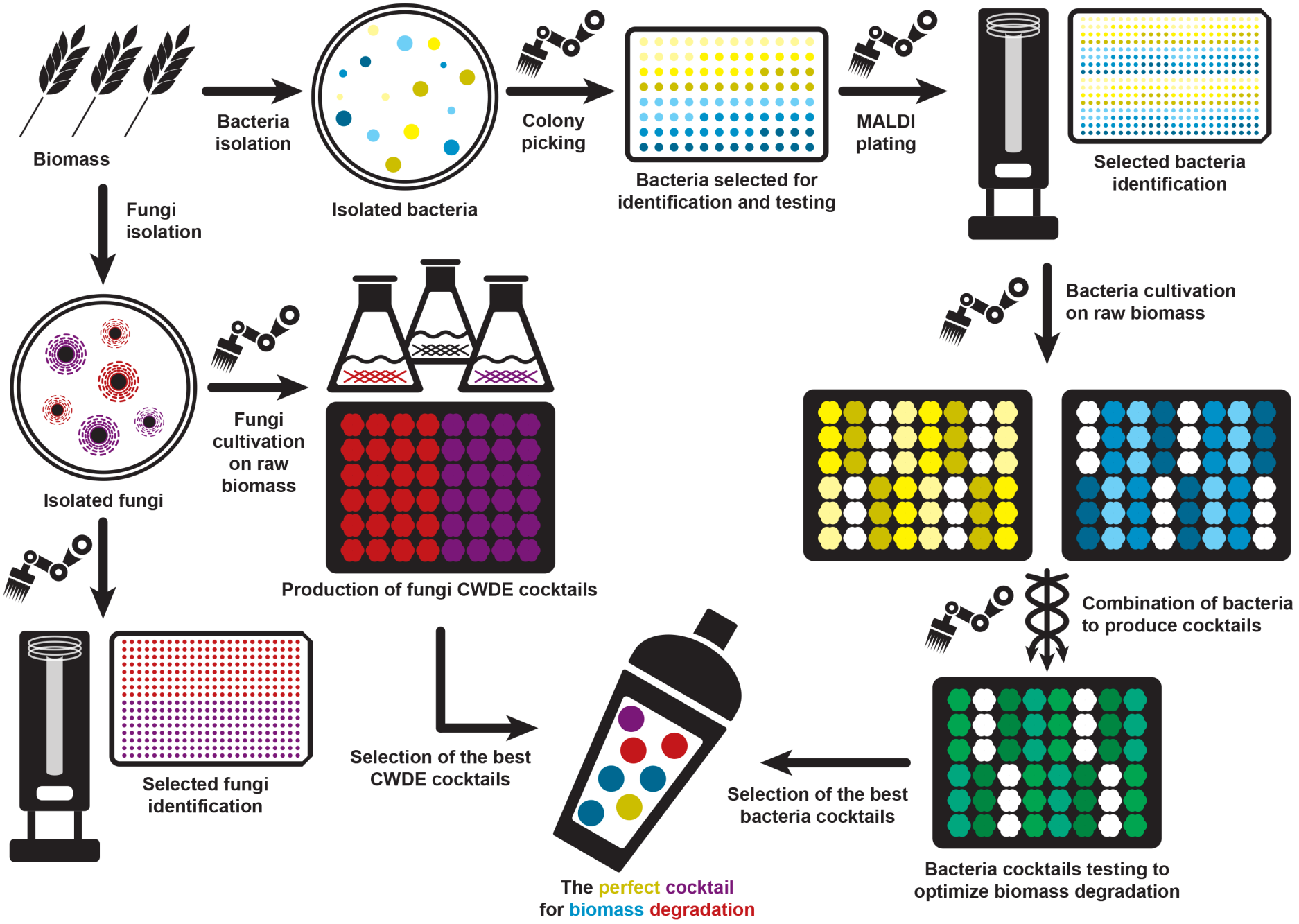
Fungi isolation
Isolated fungi
Fungi cultivation on raw biomass
Production of fungi CWDE cocktails
Bacteria cultivation on raw biomass
Selected fungi identification
Combination of bacteria to produce cocktails
Bacteria cocktails testing to optimize biomass degradation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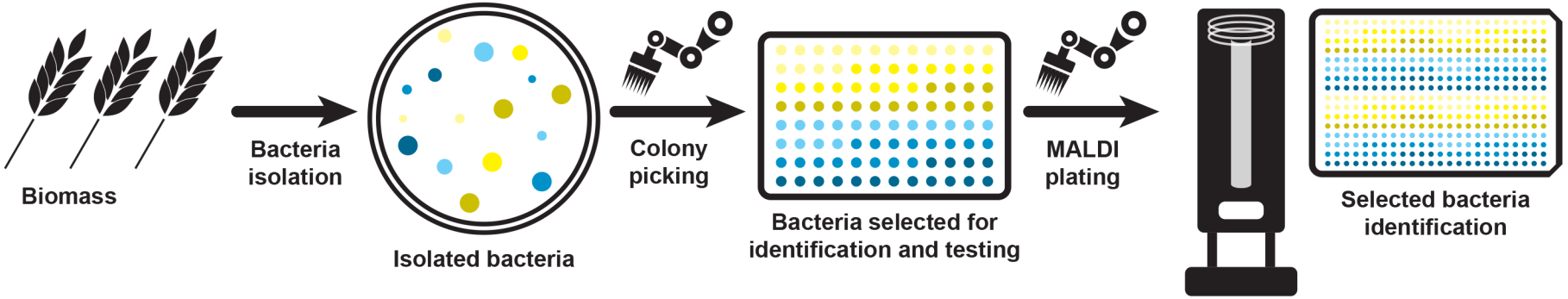
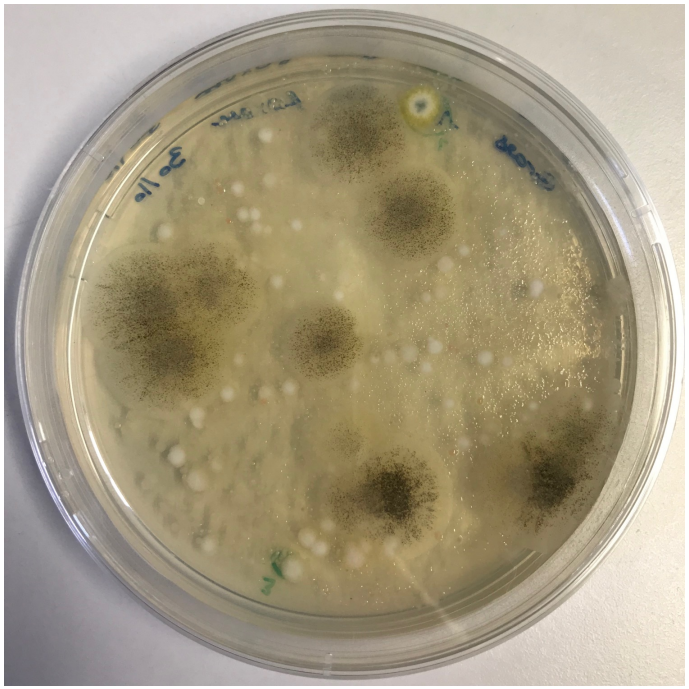
Isolated fungi
Fungi cultivation on raw biomass
Production of fungi CWDE cocktails
Bacteria cultivation on raw biomass
Combination of bacteria to produce cocktails
Bacteria cocktails testing to optimize biomass degradation
Selection of the best bacteria cocktails
Selection of the best CWDE cocktails
Selected fungi identification
The perfect cocktail for biomass degradation

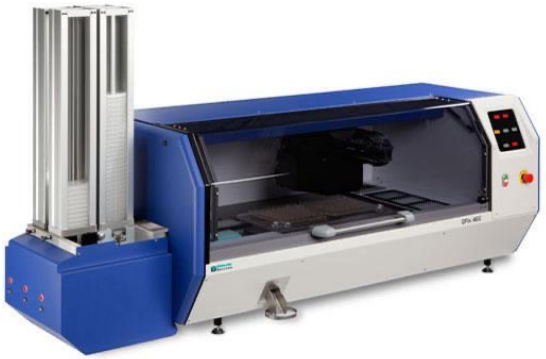

Biomass
Bacteria isolation
Isolated bacteria
Colony picking
Bacteria selected for identification and testing
MALDI plating
Selected bacteria identification

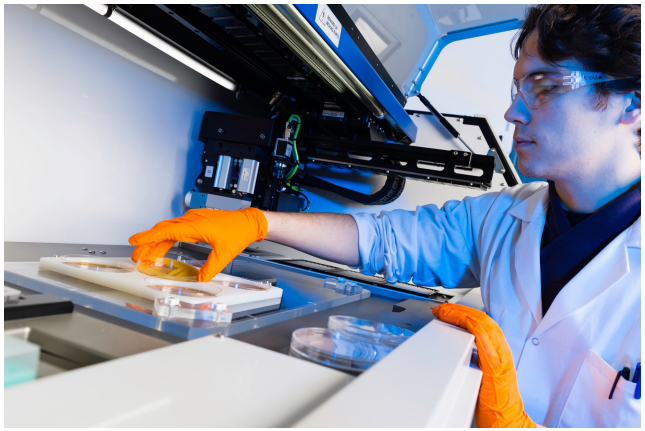
Step 1 – Colony picking

Manual and automated colony recovery: using the QPix 460 :

- 96-needle head (approx. 2000 colonies/hour)
- Up to 10 Petri (or 2 Q-Trays) dishes and 40 destination plates per batch
- "Intelligent" optical recognition software







Qpix 460 – Molecular devices

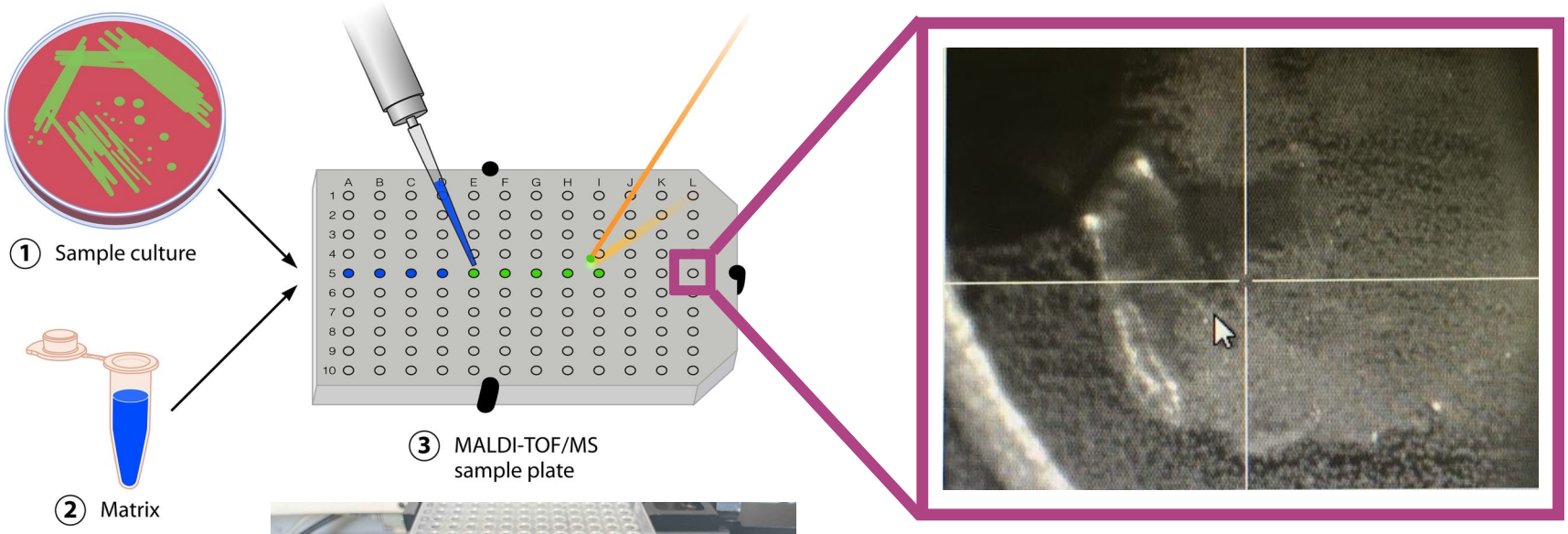
Step 1 – Colony picking

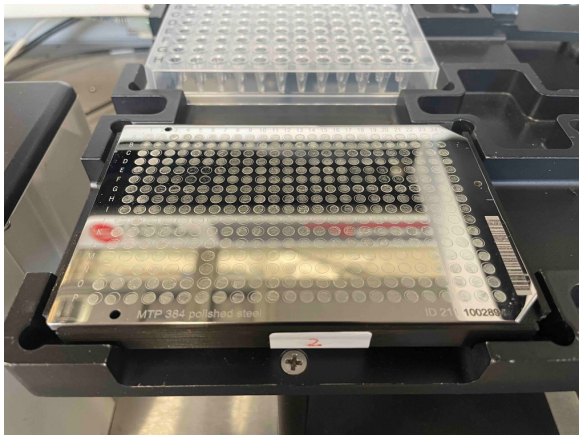
Automated colony picking

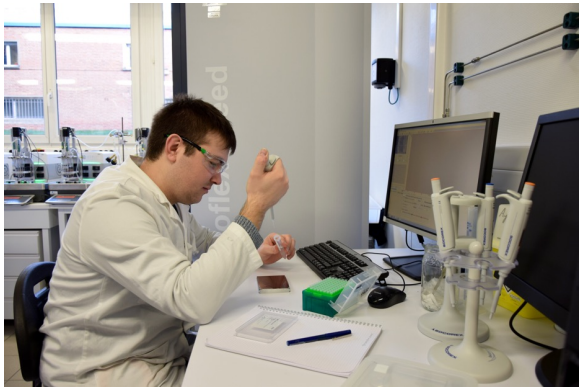
Step 2 – MALDI BioTyper

⇒ **First rapid identification using a MALDI-TOF mass spectrometer**

⇒ **Confirmation by 16s / ITS RNA sequencing or API galleries if required**







Autoflex Speed – Bruker

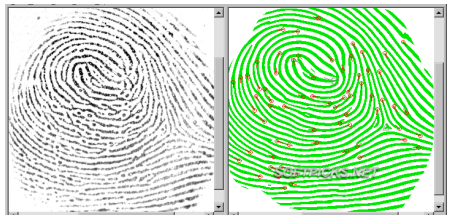
Step 2 – MALDI BioTyper

Principle of MBT identification: ***Digital fingerprinting technique based on the membrane proteins of microorganisms***

=

1 person

1 unique fingerprint



Comparison in a database with other prints already collected

Human fingerprinting

Step 2 – MALDI BioTyper

Principle of MBT identification: ***Digital fingerprinting technique based on the membrane proteins of microorganisms***

=

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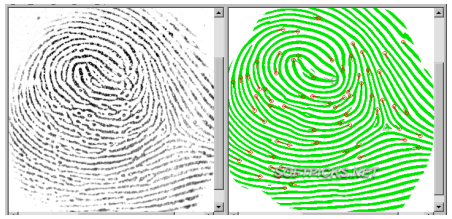
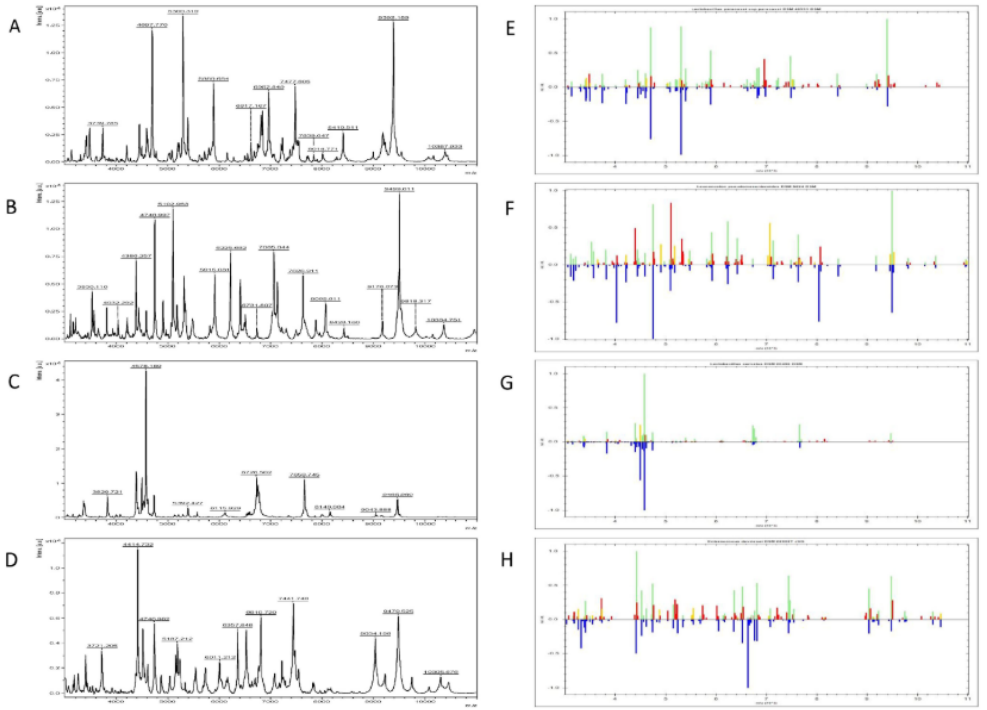


Comparison in a database with other prints already collected

Human fingerprinting



Mass spectra

Comparison with our in-house + Bruker databases (> 10000 strains)

Molecular fingerprinting

Step 2 – MALDI BioTyper

Principle of MBT identification: ***Digital fingerprinting technique based on the membrane proteins of microorganisms***

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
N9 (+++) (C)	8 (standard)	Lactobacillus plantarum	2.40	Lactobacillus plantarum	2.39
N10 (+++) (C)	9 (standard)	Lactobacillus pentosus	2.37	Lactobacillus pentosus	2.29
N11 (+++) (C)	10 (standard)	Lactobacillus plantarum	2.24	Lactobacillus plantarum	2.24
N12 (+++) (C)	11 (standard)	Lactobacillus pentosus	2.07	Lactobacillus pentosus	2.03
N13 (+++) (C)	12 (standard)	Lactobacillus plantarum	2.47	Lactobacillus plantarum	2.45
N14 (+++) (C)	13 (standard)	Lactobacillus brevis	2.13	Lactobacillus brevis	2.13

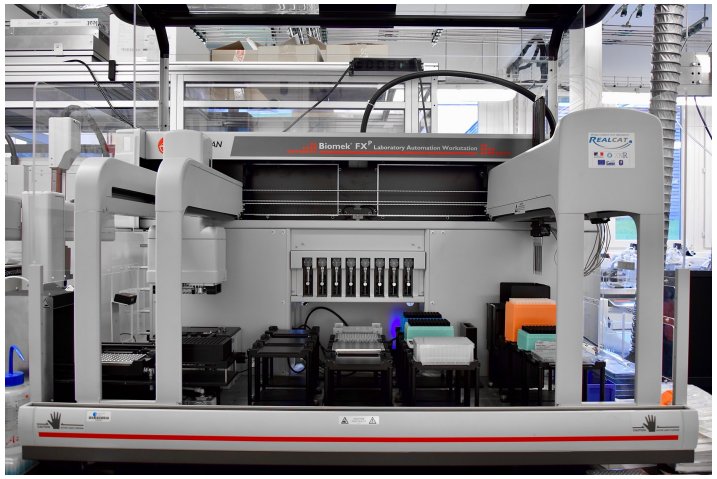
Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

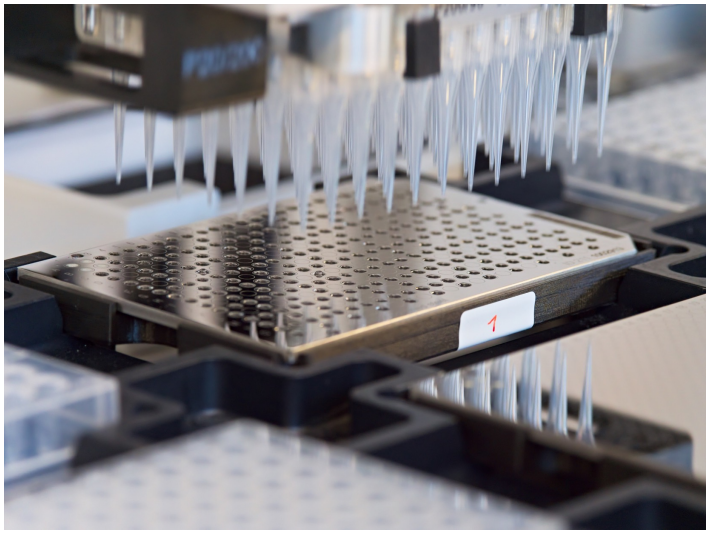
Step 2 – MALDI BioTyper

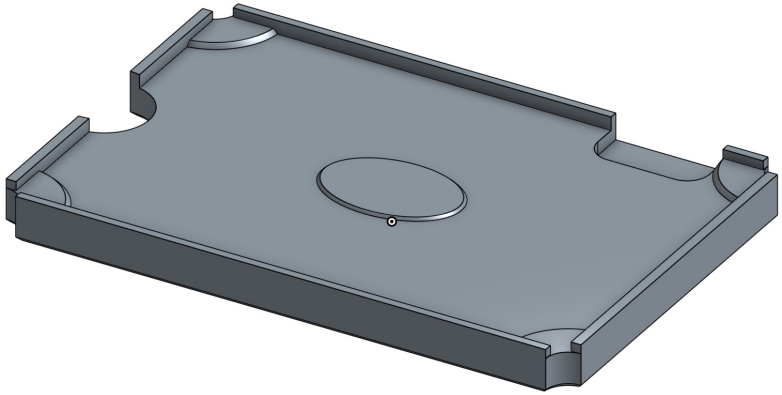
Use of a liquid handling robot : Automated protein extraction and MALDI target preparation

- **3 main steps:**
  - ⇒ **Cryo-stocks preparation in 30% glycerol solution for bank conservation**
  - ⇒ **Cells washing and protein extraction**
  - ⇒ **Mixing the protein extracts with the matrix and depositing on the target**



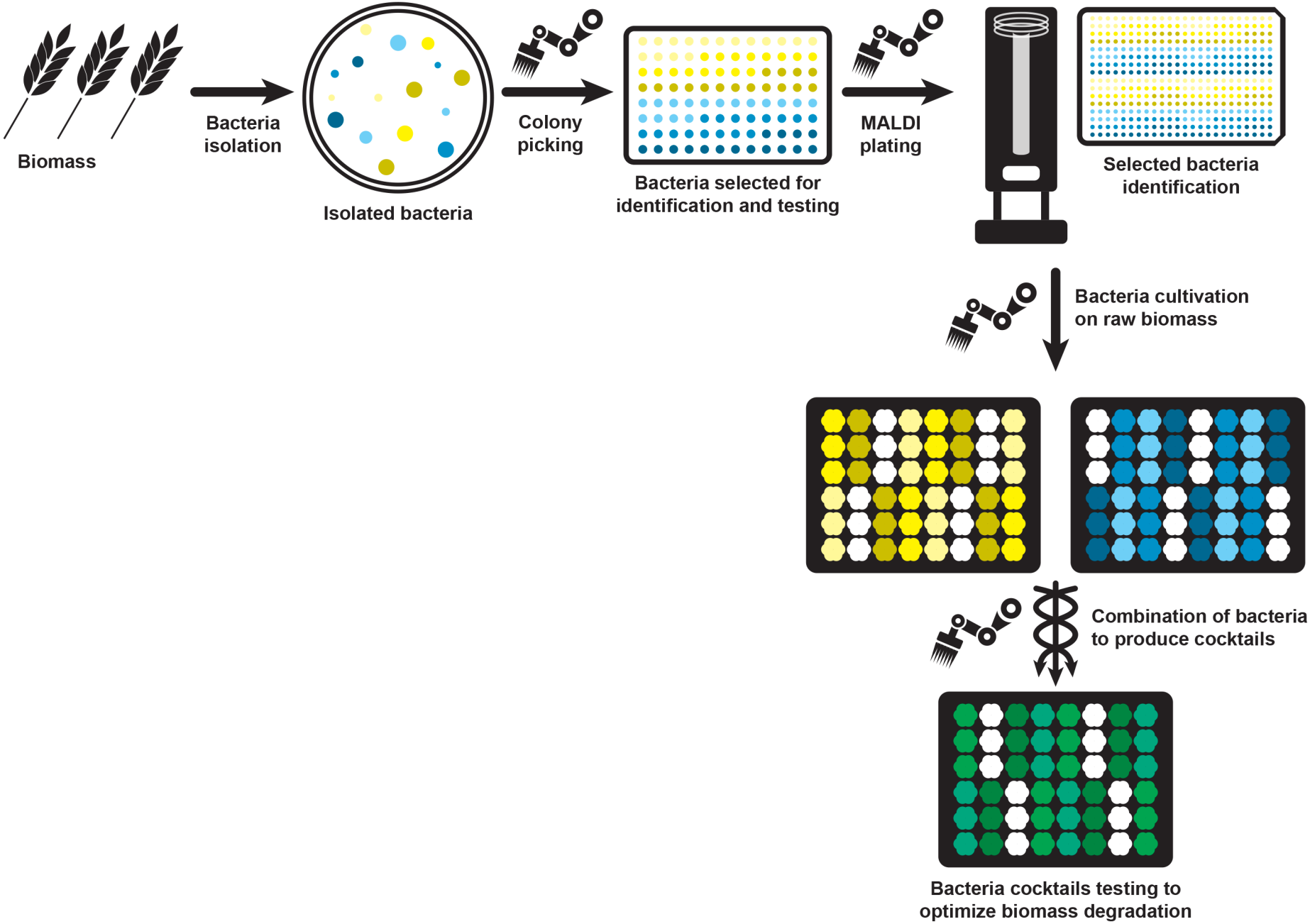
Biomek FXp – Beckman Coulter





Step 2 – MALDI BioTyper

Deposition of the sample/matrix mixture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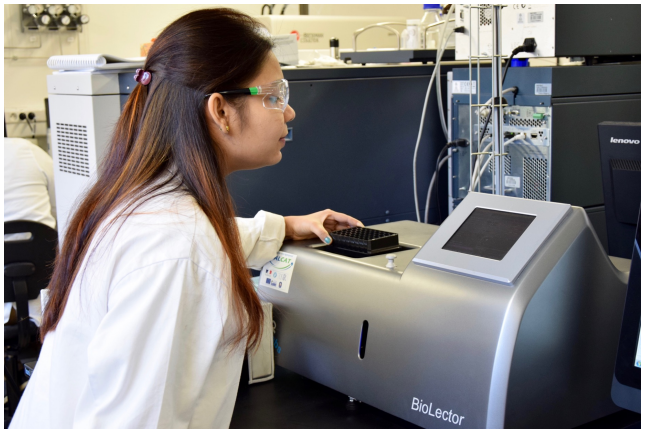
Step 1 – Cultivation and CWDE induction

Use of a high throughput culture device: The BioLector

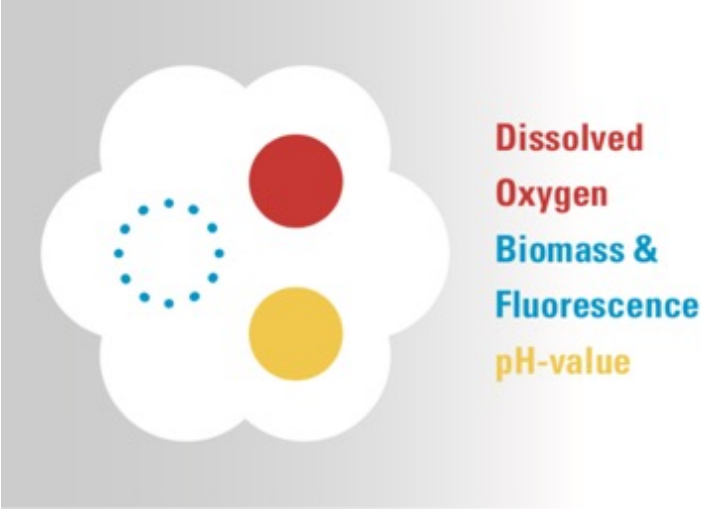
⇒ **48 x 1mL parallel fermentations**

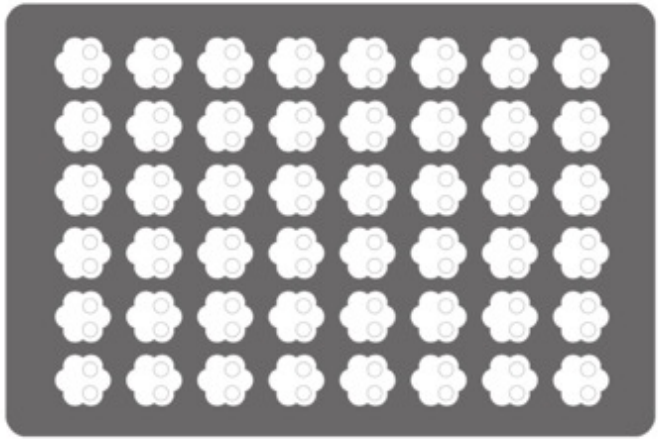
⇒ **On-line monitoring and control of pH, pO_2 and biomass**

⇒ **Temperature, humidity and atmosphere control (aerobic and anaerobic fermentation)**



BioLector – M2PLabs





Step 1 – Cultivation and CWDE induction

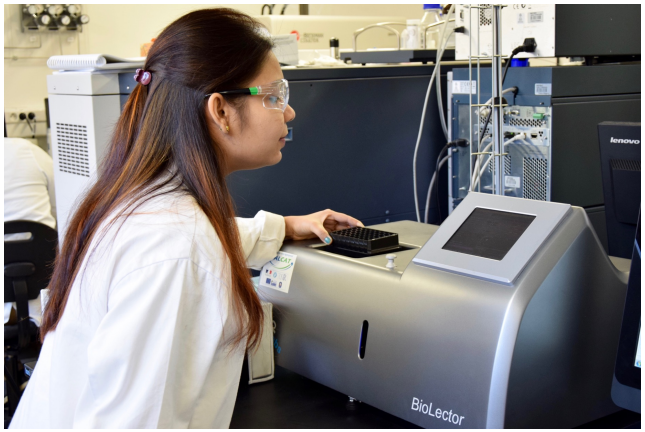
Use of a high throughput culture device: The BioLector

⇒ **48 x 1mL parallel fermentations**

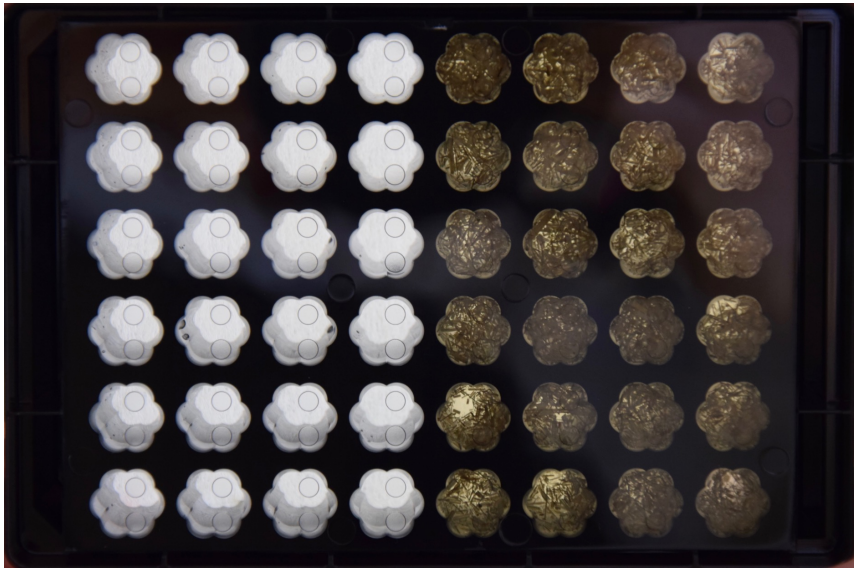
⇒ **On-line monitoring and control of pH, pO_2 and biomass**

⇒ **Temperature, humidity and atmosphere control (aerobic and anaerobic fermentation)**

⇒ **Adaptation to offer the possibility to use raw materials directly (straw, stover, etc.)**



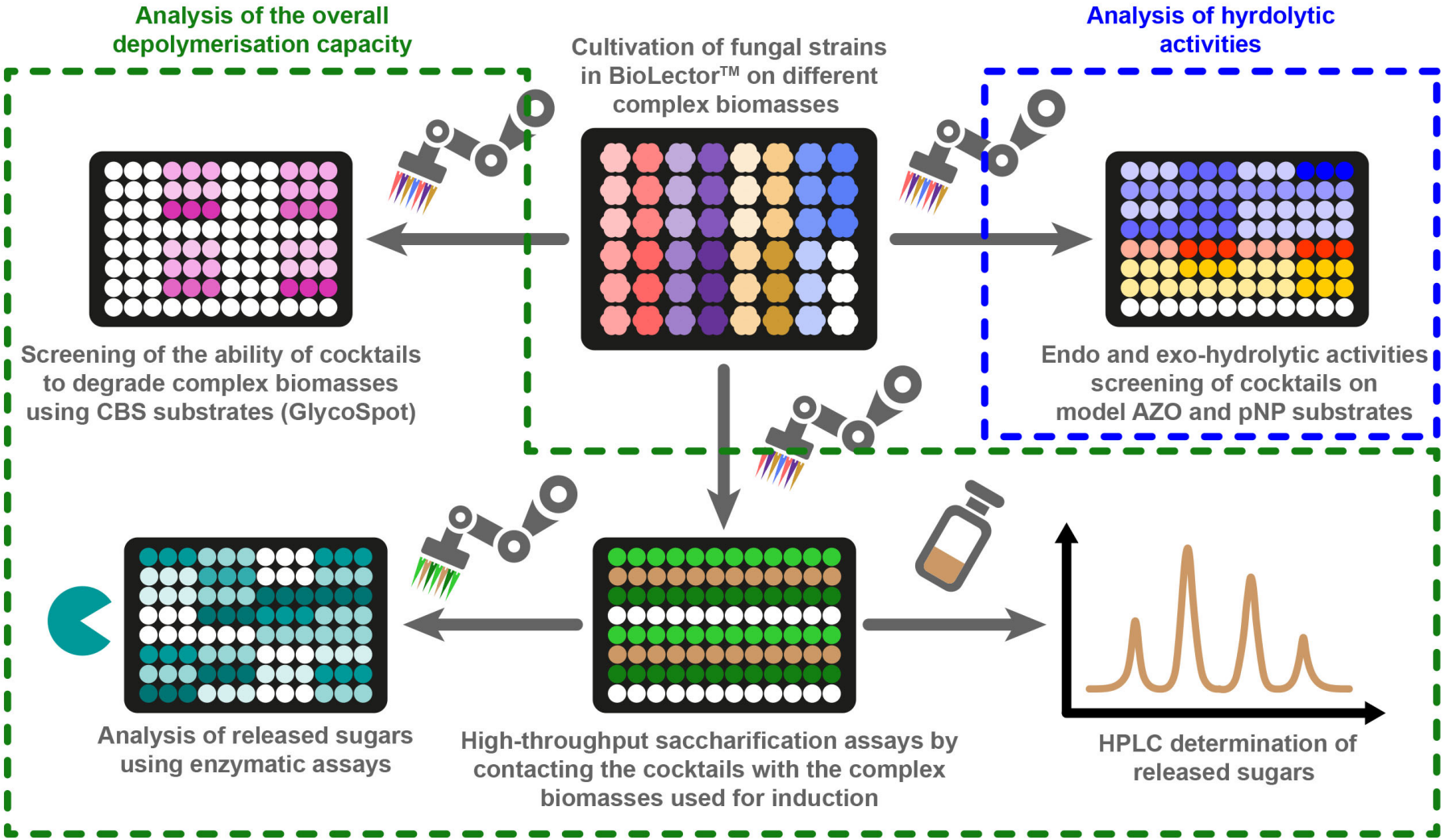
BioLector – M2PLabs



Raulo *et al.,* Microb. Biotechnol. 2019, 12 (6), 1286.

Step 2 – CWDE activity measurement

Extension of the activity measurement panel

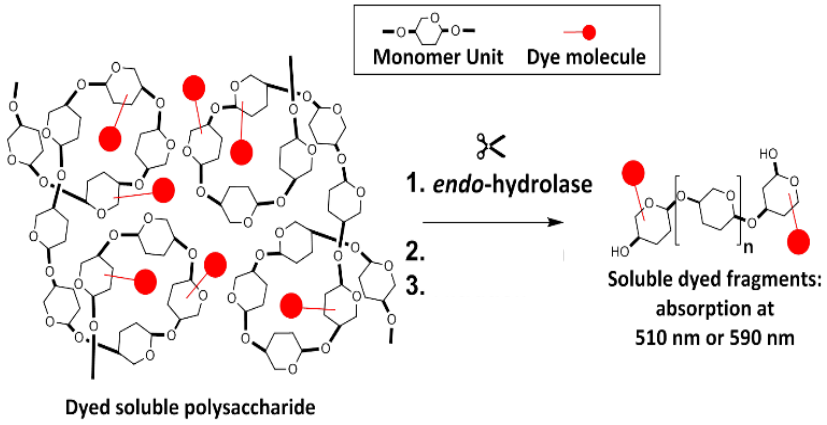


Raulo *et al.,* Biotechnol. Lett. 2021, 43, 2283. – Heuson *et al.,* Revue IAA. 2022, 20220102-30-35.

Model biomass substrates

1st Screen – Model substrates for general activities measurement and classification

Substrate	Enzyme family targetted
pNP-glucopyranoside	α-glucosidases (exo)
pNP-xylopyranoside	β-xylosidase (exo)
AZO-xylan	β-xylanases (endo)
AZO-cellulose	β-glucanases (endo)
AZO-rhamnogalacturonan	rhamnogalacturonanases (endo)
AZO-barley glucan	β-glucanases (endo)



Real biomass substrates

2nd Screen - Insoluble Chromogenic Biomass substrates (ICB, Glycospot)

⇒ *Substrates in 96-wells plates format*

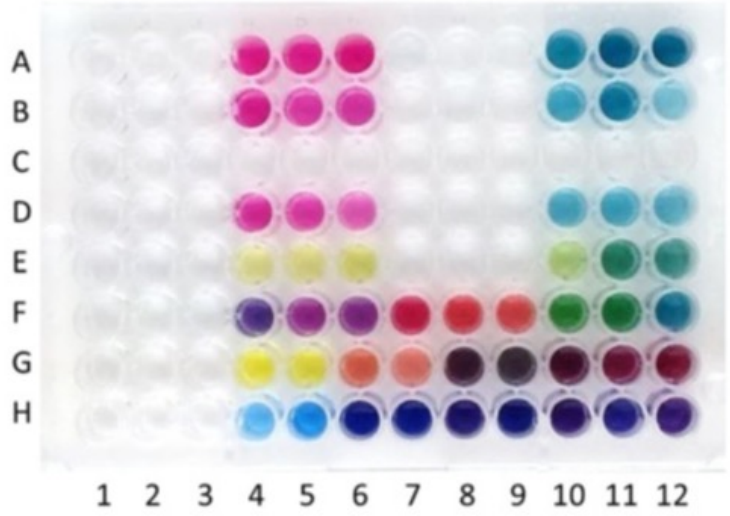
⇒ *4 different dyes (absorption at 404 nm, 517 nm, 595 nm and 630 nm)*

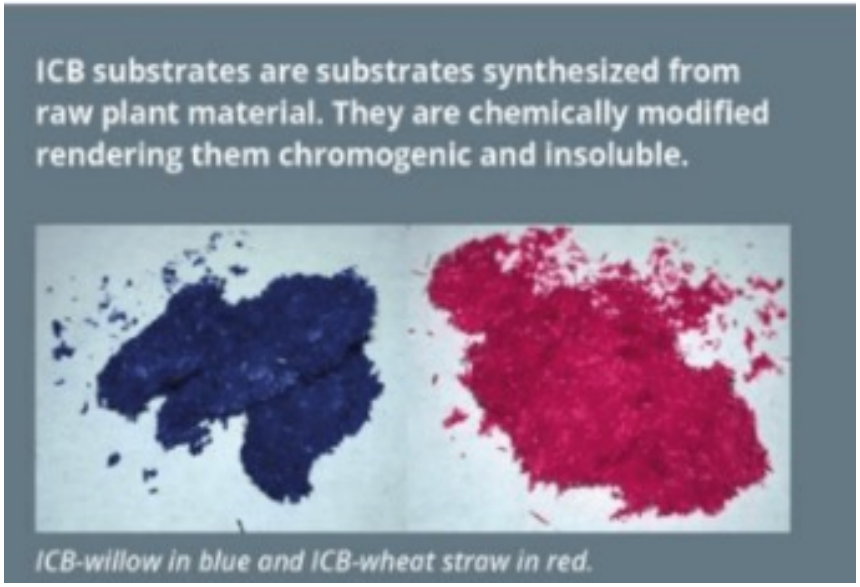
⇒ *Complex substrates (custom-made from numerous raw biomass sources)*

⇒ *Perfect testing of "real" Biomass*

⇒ *Easy removing by filtration*

GlycoSpot





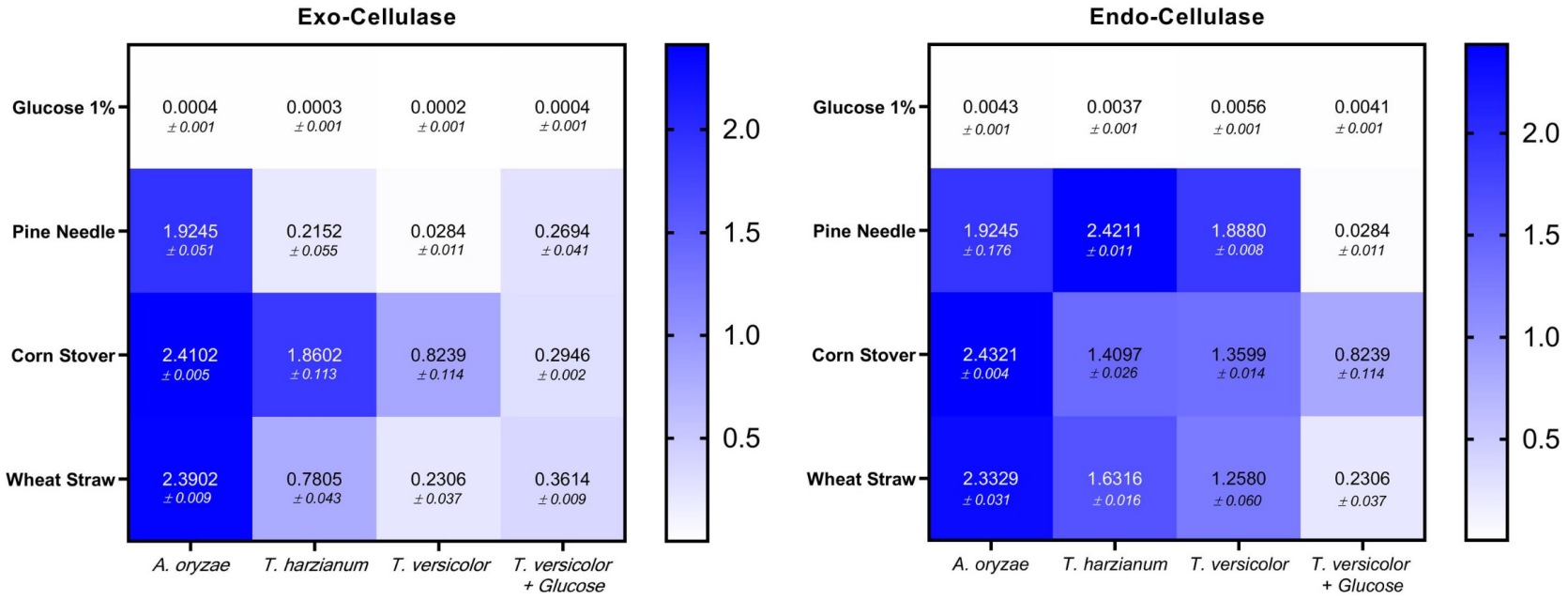
https://www.glycospot.dk/

Results obtained from the first screenings

Results for CWDE activity families

⇒ ***Confirmation via sugar quantification by HPLC***

⇒ ***Cellulases:***

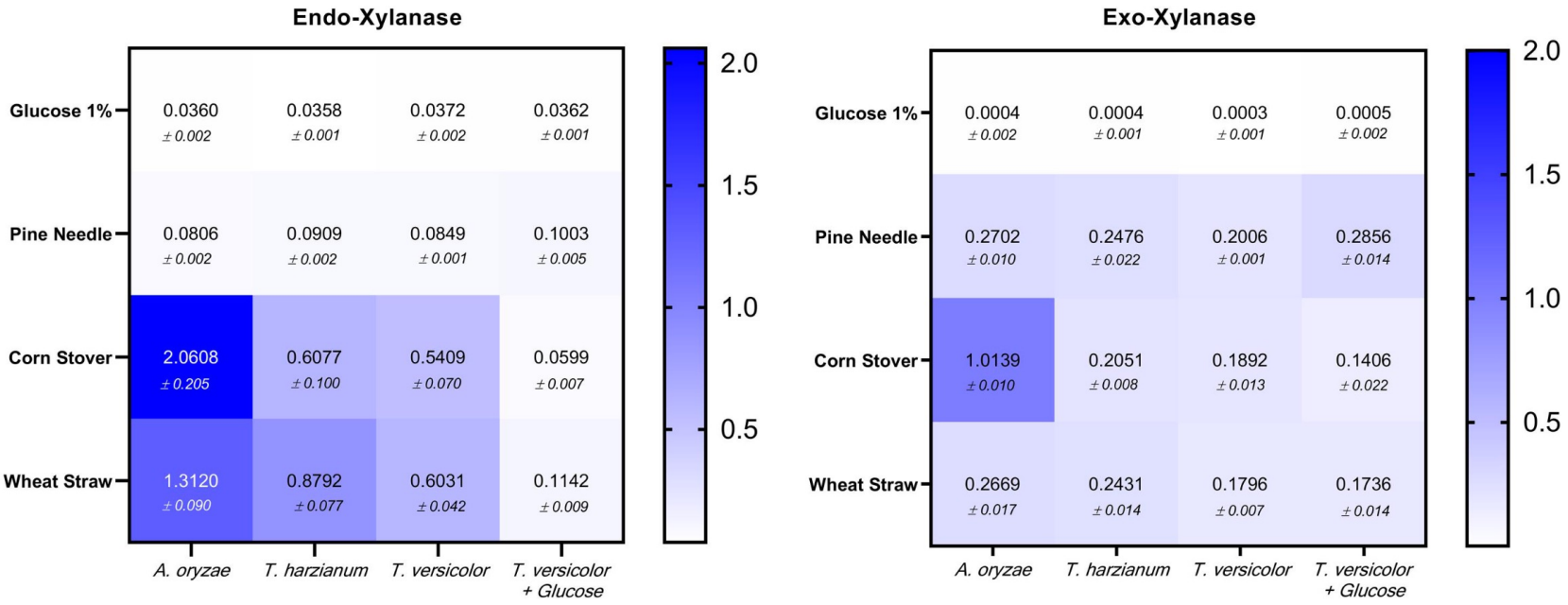


Results obtained from the first screenings

Results for CWDE activity families

⇒ ***Confirmation via sugar quantification by HPLC***

⇒ ***Xylanases:***

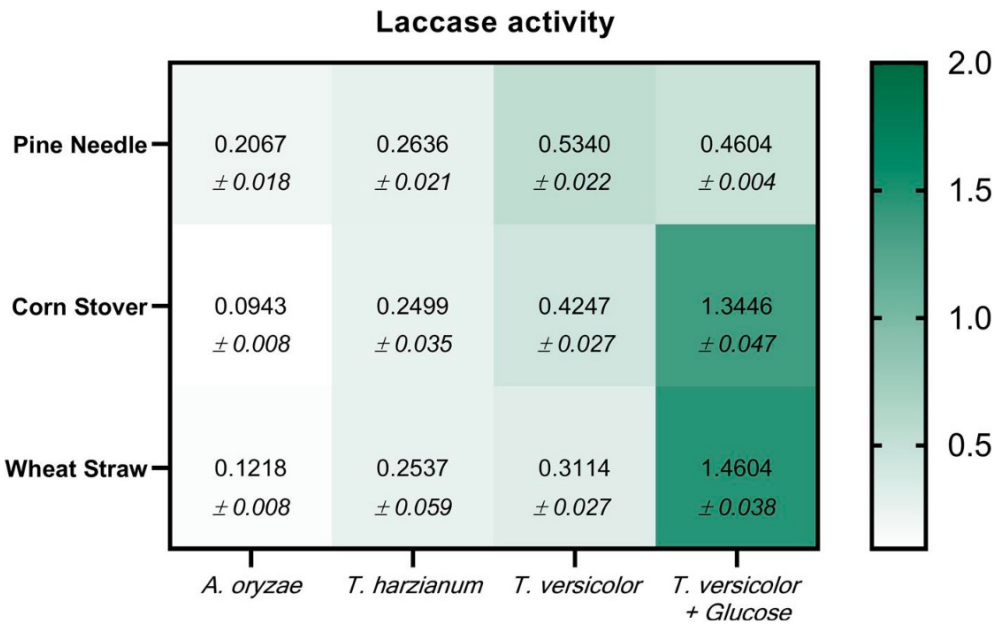


Results obtained from the first screenings

Results for CWDE activity families

⇒ ***Confirmation via sugar quantification by HPLC***

⇒ ***Laccases:***

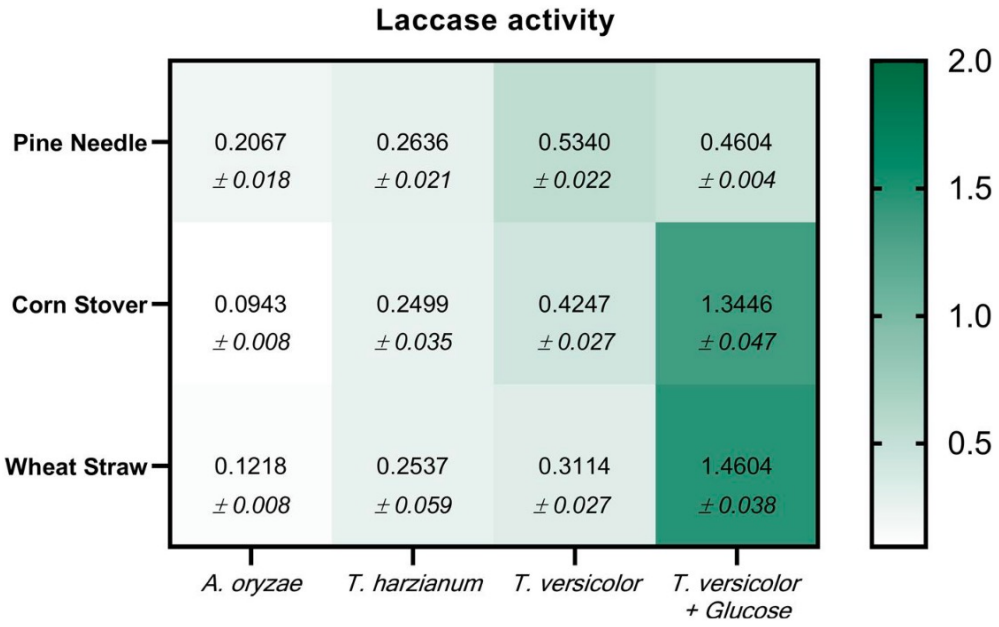


Results obtained from the first screenings

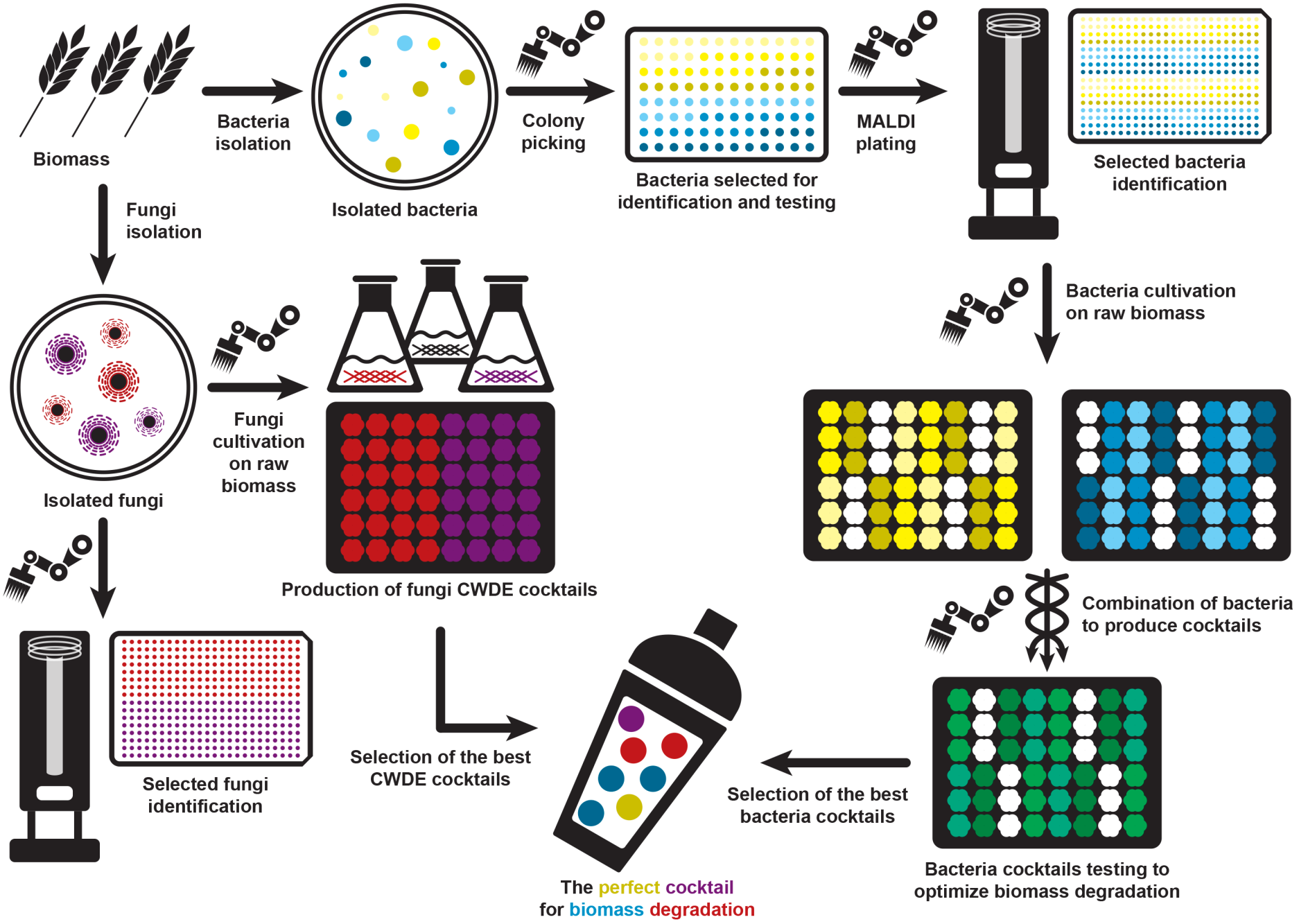
Results for CWDE activity families

⇒ ***Confirmation via sugar quantification by HPLC***

⇒ ***Laccases:***



⇒ ***Further studies on dark fermentation (short chain organic acid production) using the cocktails as biomass pre-treatment***


Biomass
Bacteria isolation
Isolated bacteria
Colony picking
Bacteria selected for identification and testing
MALDI plating
Selected bacteria identification
Fungi isolation
Isolated fungi
Fungi cultivation on raw biomass
Production of fungi CWDE cocktails
Bacteria cultivation on raw biomass
Combination of bacteria to produce cocktails
Bacteria cocktails testing to optimize biomass degradation
Selection of the best bacteria cocktails
Selection of the best CWDE cocktails
Selected fungi identification
The perfect cocktail for biomass degradation

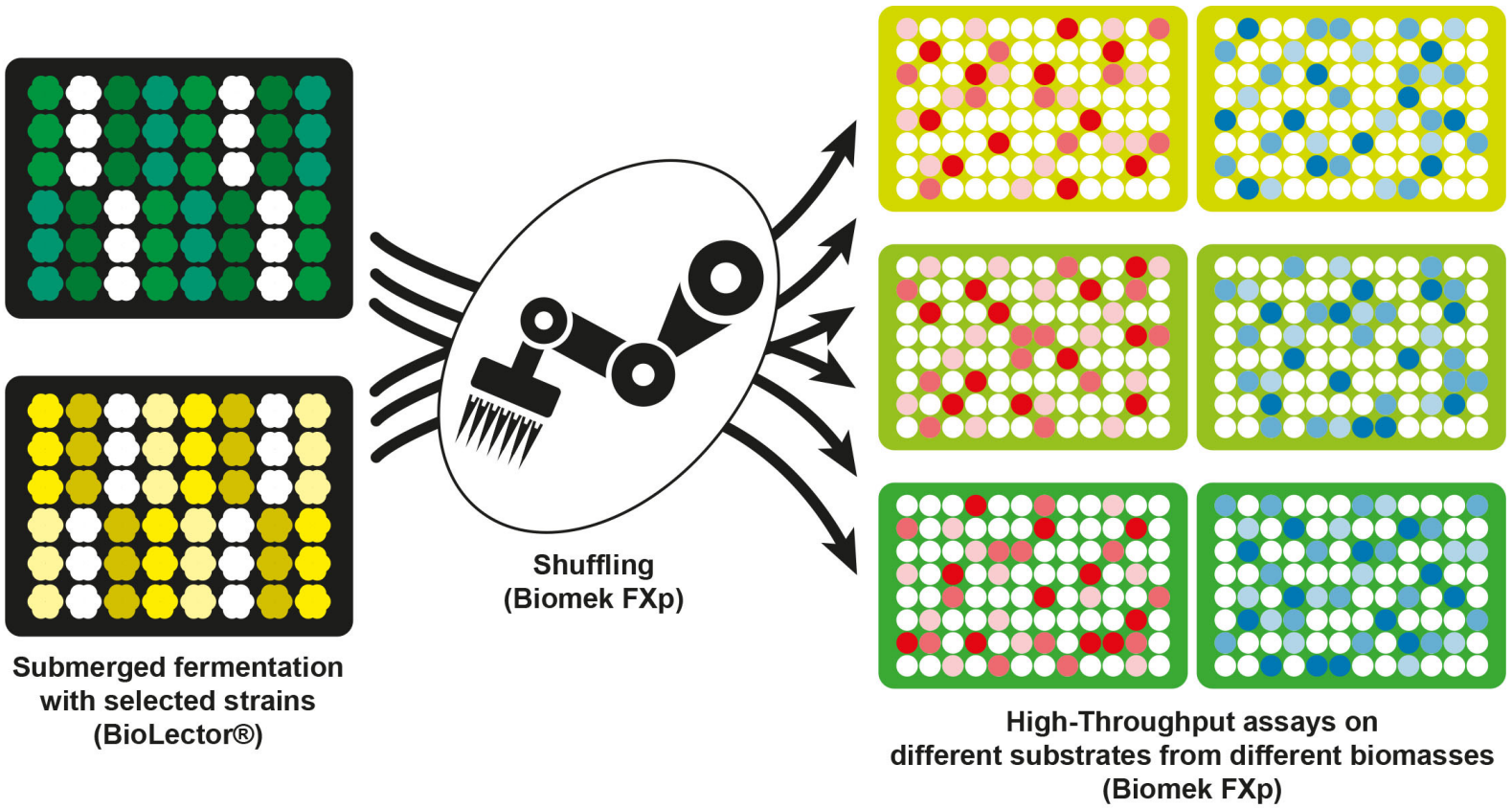
Cocktails production

Step 3 – Secreted CWDE mixing

Rational mixing using fully automated workflow using Biomek FXp

⇒ **384 cocktails combinations created from each batch of 3 strains / 1 inducing biomass / 2 tested substrates**

⇒ **New automation compatible DOE program developed**



CWDE production

Overall results & perspectives

Results for strain identification and enzymes production:

⇒ **55 Bacteria isolated / 22 identified - 23 Fungi isolated / 14 identified from 5 biomass sources**

⇒ **Screenings of 3 Fungi + 3 inducing biomass sources for CWDE production**

⇒ **Correlation of enzyme activities detected with complementary analytical methods**

⇒ **Strong correlation between biomass composition and CWDE composition**

⇒ **Successful use of CWDE cocktails to increase the production of short chain organic acids by bacterial dark fermentation, including the implementation of a Plug Flow Reactor (PASS-BIO)**

FACCE SURPLUS
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PASS-BIO
plug-flow acid fermentation

New developments in progress:

⇒ **Analysis of the protein composition of cocktails (proteomics)**

⇒ **Attempt to correlate the enzyme composition of the cocktails with the screening parameters (biomass, conditions, strains, etc.) by machine learning.**

⇒ **Creation of artificial cocktails with additional heterologous enzymes**

TWIST BIOSCIENCE

Thank you for your attention!

Brings catalysis over lightspeed

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... au Nord, c'étaient les Corons!