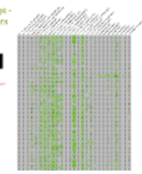
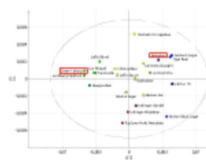
Testing for individual glycans
- Time consuming
- Expensive
- Low-throughput

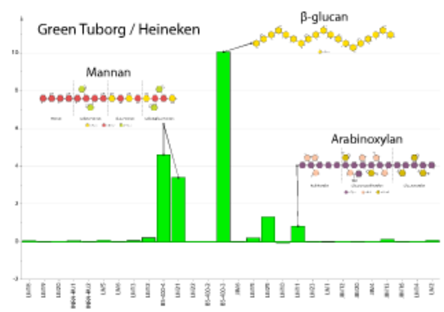
Carbohydrate microarrays

Output

Proof of concept - analysis of beers







Future

More sophisticated data analysis and integration with other information about the brewing process (SIMCA)

Analysis of not just final product but throughout the brewing process to be able to track the fate of polysaccharides

Internal standards to determine the fibre amount

Non-alcoholic beers, enhanced fibre content?

Acknowledgement

kennis instituut bier

Aafje Sierksma

William Willats
Louise Nancke
Peter Ulvskov
Henrik Siegumfeldt

Fibres in beer:
The relevance for health?

Microarray-based high throughput polysaccharide profiling in brewing

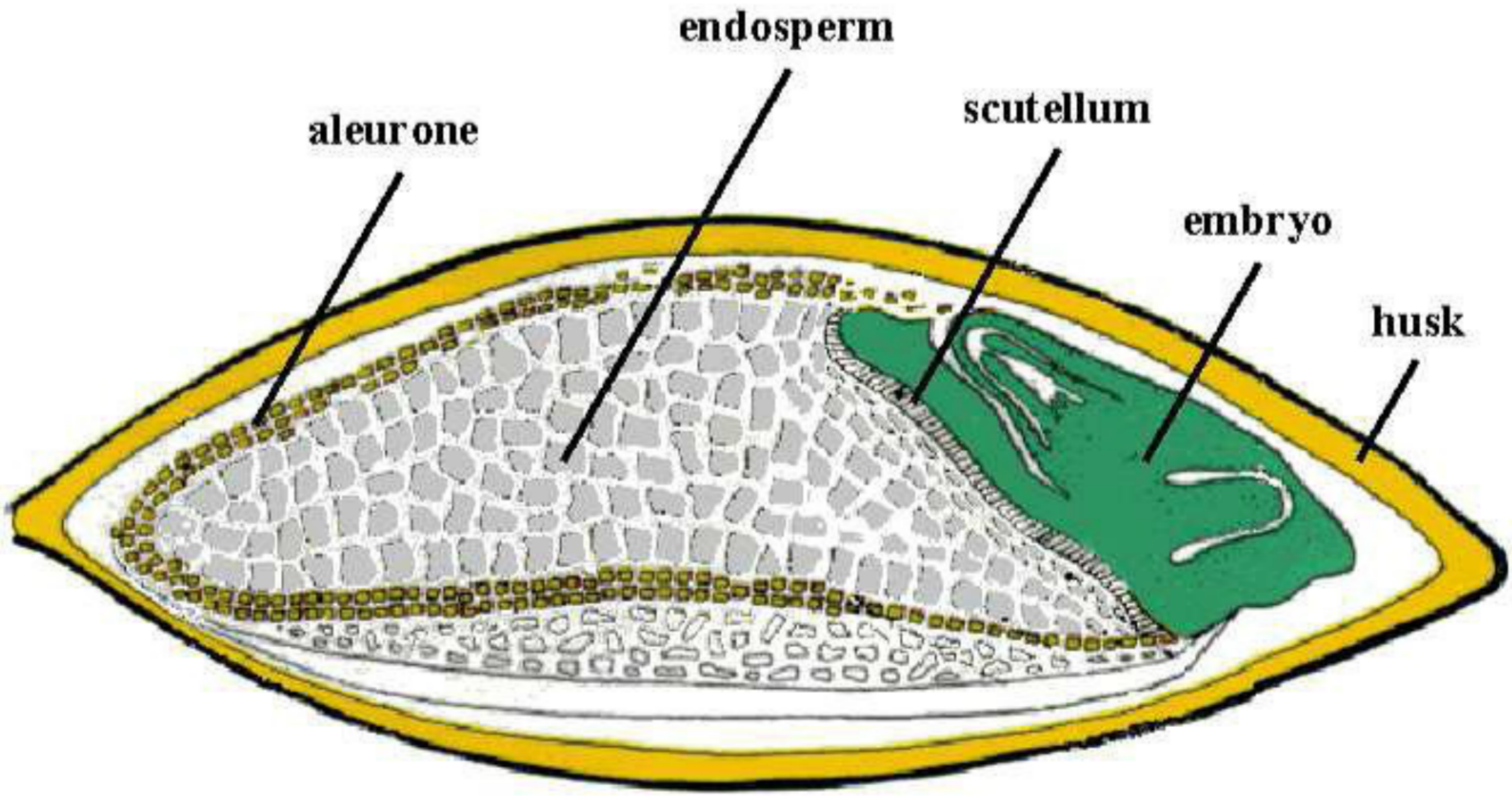
Jonatan U. Fangel
Department of Plant and Environmental Science
University of Copenhagen

Beer and Health
THE 7TH EUROPEAN
BEER AND HEALTH SYMPOSIUM

Outline

- Background
- Carbohydrate microarray technology
- Proof of concept analysis of beers
- Future

'Fibre' : Polysaccharides that are resistant to digestion and absorption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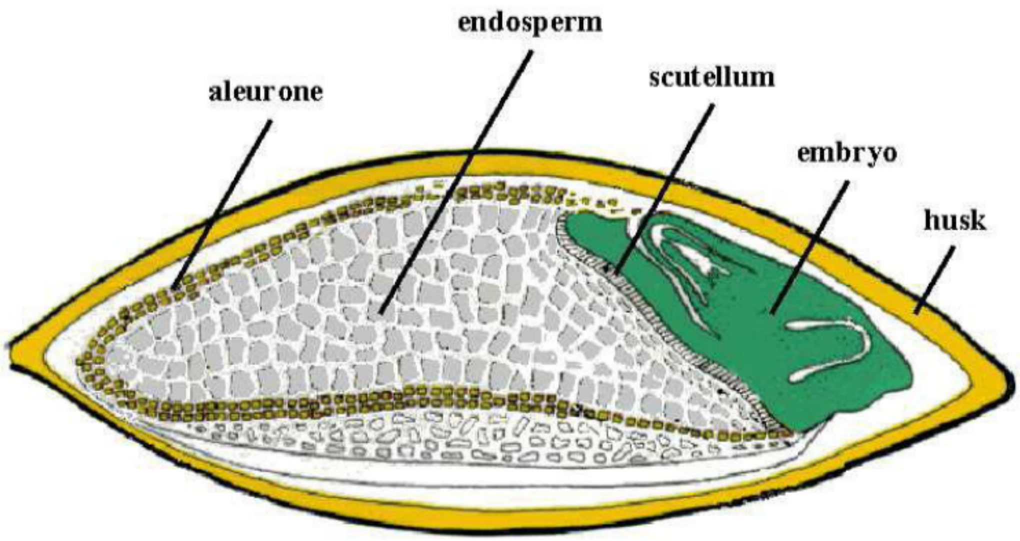
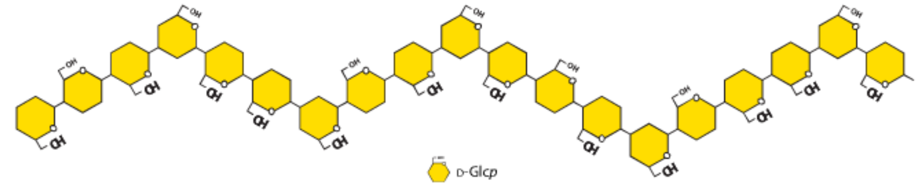
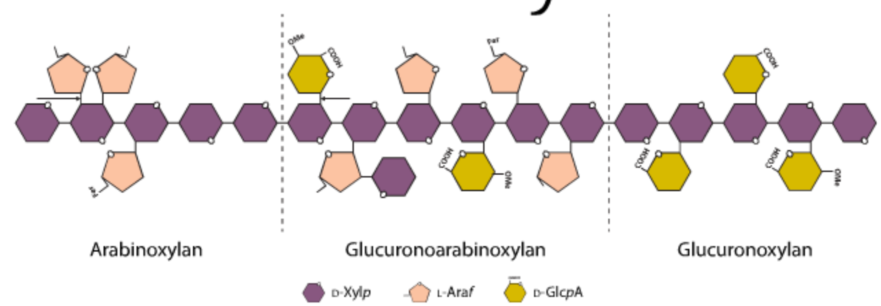
aleur one
scutellum
embryo
husk



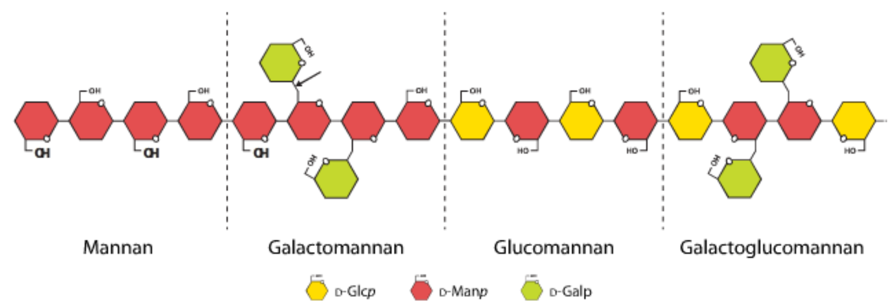
β-glucan



Arabinoxylan



Mannan



Brewing

Grain

Polysaccharides → Polysaccharides?

NUTRITION JOURNAL

REVIEW **Open Access**

Immunomodulatory dietary polysaccharides: a systematic review of the literature

Jane E Ramberg*, Erika D Nelson, Robert A Sinnott

Table 1 Immunomodulatory Glucan Extracts: Oral Animal Studies

Source	Extract	Animal	Dose/day	Duration of study	Treatment	Effects	Reference
Agaricus (*A. blazei*) *subrufescens*	α-1,6 and α-1,4 glucans	8-week ♀ C3H/He mice (5/group)	100 mg/kg IG every 3 days	1 month	Healthy animals	↑ #s splenic T lymphocytes (Thy1.2, CD4+ and CD8+)	[24]
	Aqueous	7-9-week ♂ Balb/cByJ mice (40/group)	1 ml 0.45N, 0.6N, or 3N aqueous extract	2 months		All doses ↑ serum IgG levels, CD3+ T cell populations and PML phagocytic activity	[22]
		7-9-week male Balb/cByJ mice (40/group)	1 ml 0.45N, 0.6N, or 3N aqueous extract	10 weeks	IP injection of OVA at 4 weeks	0.6N and 3N ↑ levels of OVA-specific serum IgG 28 days post-immunization; all doses ↑ delayed-type hypersensitivity and TNF-α secreted from splenocytes at 10 weeks; 0.6N ↑ splenocyte proliferation at 10 weeks	
		5-6 -week ♀ BALB/cHsdOla mice (8/group × 2)	One 200 μl extract day 1, orogastric intubation	1 week	Injected IP fecal solution day 2	↓ CFU in blood of mice with severe peritonitis & improved overall survival rate in all peritonitis groups	[46]
		6-week BALB/c nu/nu mice (7/group)	2.5 mg extract days 20-41, drinking water	41 days	Injected SC Sp-2 myeloma cells day 1	↓ tumor size & weight after 21 days treatment	[65]

		4-8-week BALB/c mice (10/group)	50, 100 or 200 mg/kg, oral	10 days	Injected SD Sarcoma 180 cells	↓ of tumor weight was dose dependent: 27.7, 55.8, 66.7%, respectively	[67]
Ganoderma lucidum (mycelia)	Aqueous	7-week ♂ F344/Du Crj rats (16/group)	1.25% or 2.5% of diet	6 months	Injected SC AOM once a week, weeks2-5	Both doses ↓ colonic adenocarcinoma incidence; 2.5% ↓ total tumor incidence; both doses ↓ nuclear staining of β-catenin and cell proliferation	[68]
Ganoderma tsugae	Aqueous	8-week ♀ BALB/cByJNarl mice (14/group)	0.2-0.4% of diet (young fungi); 0.33 or 0.66% of diet (mature fungi)	5 weeks	Injected IP OVA days 7, 14, 21; aerosolized OVA twice during week 4	In splenocytes, both doses of both extracts ↑ IL-2 and IL-2/IL-4 ratios, 0.2% young extract and 0.66% mature extract ↓ IL-4; in Mø, 0.66% mature extract ↑ IL-1β, both doses of both extracts ↑ IL-6	[53]
Grifola frondosa	D fraction	Mice: 1) ICR, 2) C3H/HeN, 3) CDF_1 (10/group)	1.5 mg every other day, beginning day 2	13 days	Implanted SC: 1) Sarcoma-180, 2) MM-46 carcinoma, or 3) IMC carcinoma cells	↓ tumor weight & tumor growth rate: 1) 58%, 2) 64%, and 3) 75%, respectively	[71]
		5-week ♂ BALB/c mice (10/group)	2 mg, days 15-30	45 days	Injected in the back with 3-MCA, day 1	↓ (62.5%) # of animals with tumors; ↑ H_2O_2 production by plasma Mø; ↑ cytotoxic T cell activity	[72]
Hordeum vulgare	β-1,3;1,4 or β-1,3;1,6-D-glucans	Athymic nu/nu mice (4-12/group)	40 or 400 μg IG for 4 weeks	31 weeks	Mice with human xenografts (SKMel28 melanoma, A431 epidermoid carcinoma, BT474 breast carcinoma, Daudi lymphoma, or LAN-1 neuroblastoma) ± mAb (R24, 528, Herceptin, Rituximab, or 3F8, respectively) therapy twice weekly	400 μg + mAb ↓ tumor growth & ↑ survival; higher MW ↓ tumor growth rate for both doses	[75]
	β-1,3;1,4-D-glucans	Athymic BALB/c mice	4, 40, or 400 μg for 3-4 weeks	1 month	Mice with neuroblastoma (NMB7, LAN-1, or SK-N-ER) xenografts, ± 3F8 mAb therapy twice weekly	40 and 400 μg doses + mAB ↓ tumor growth; 400 μg dose ↑ survival. Serum NK cells required for effects on tumor size	[76]
		C57BL/6 WT and CR3-deficient mice (10/group)	0.4 mg for 3 weeks	100 days	Injected SC RMA-S-MUC1 lymphoma cells day 1 ± IV 14.G2a or anti-MUC1 mAb every 3rd day	±mAB ↓ tumor diameter; ↑ survival	[73]
	β-glucans	♀ Fox Chase ICR immune-deficient (SCID) mice (9/group)	400 μg days 1-29	50 days	Mice with human (Daudi, EBV-BLCL, Hs445, or RPMI6666) lymphoma xenografts, ± Rituximab mAb therapy twice weekly	+mAB ↓ tumor growth and ↑ survival	[74]
Laminaria digitata	Laminarin	♂ ICR/HSD mice (3/group)	1 mg	1 day	Healthy animals	↑ Mø expression of Dectin-1 in GALT cells; ↑ TLR2 expression in Peyer's patch dendritic cells	[29]

			challenge (day 1)				
	β-1,3;1,6 glucans (particulate)	3 and 8-week ♀ BALB/c mice (15/group)	50, 100 or 250 μg	1-2 weeks	Injected murine mammary carcinoma (Ptas64) cells into mammary fat pads 2 weeks before treatment	↓ tumor weight	[27]
	β-1,3-glucan				Healthy animals	All 3 doses ↑ phagocytic activity of blood monocytes & neutrophils & ↑ spleen cell IL-2 secretion	
		WT or CCD11b$^{-/-}$ C57BL/6 mice (2/group)	0.4 mg for 3 weeks	100 days	Injected SC RMA-S-MUC1 lymphoma cells ± 14.G2a or anti-MUC1 mAb IV injection every 3rd day	↓ tumor diameter when included with mAb; ↑ survival with and without mAb	[73]
		C57BL/6mice (4/group)	25 mg	1 week	Healthy animals	↑ # intestinal IELs; ↑ # TCRαβ +, TCR γδ+, CD8+, CD4+, CD8αα+, CD8αβ+ T cells in IELs; ↑ IFN-γ mRNA expression in IELs and spleen	[28]
Sclerotinia sclerotiorum	SSG	6-8-week specific pathogen-free ♂ CDF$_1$ mice (3/group)	40 or 80 mg/kg days 1-10	2 weeks	Healthy animals	10 mg dose ↑ acid phosphatase activity of peritoneal Mø (day 14)	[30]
			40, 80 or 160 mg/kg days 2-6	35 days	Implanted SC Metha A fibrosarcoma cells day 1	80 mg dose ↓ tumor weight	
		6-8-week specific pathogen-free ♂ CDF$_1$ mice (10/group)	40, 80 or 160 mg/kg days 2-11		Injected ID IMC carcinoma cells day 1		
		6-8-week specific-pathogen free ♂ mice of BDF1 and C57BL/6 mice (7/group)	0.5, 1, 2, or 4 mg days 1-10	2-3 weeks	Injected IV Lewis lung carcinoma (3LL) cells	2 mg ↓ # of 3LL surface lung nodules at 2 weeks	[83]
Sclerotium rofsii	Glucan phosphate	♂ ICR/HSD mice (3/group)	1 mg	1 day	Healthy animals	↑ systemic IL-6; ↑ Mø expression of Dectin-1 in GALT cells; ↑ TLR2 expression in dendritic cells from Peyer's patches	[29]
Trametes (*Coriolus*) *versicolor*	PSP	6-8-week ♂ BALB/c mice (10/group)	35 μg days 5-29 in drinking water	29 days	Implanted SC Sarcoma-180 cells day 1	↓ tumor growth & vascular density	[94]

β-glucan

The Genetics and Transcriptional Profiles of the Cellulose Synthase-Like *HvCslF* Gene Family in Barley[1[OA]]

Rachel A. Burton, Stephen A. Jobling, Andrew J. Harvey, Neil J. Shirley, Diane E. Mather, Antony Bacic, and Geoffrey B. Fincher*

Australian Centre for Plant Functional Genomics (R.A.B., A.J.H., N.J.S., G.B.F.) and Molecular Plant Breeding Cooperative Research Centre (D.E.M.), School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, South Australia 5064, Australia; Commonwealth Scientific and Industrial Research Organization, Food Futures Flagship, Australian Capital Territory 2601, Australia (S.A.J.); and Australian Centre for Plant Functional Genomics, School of Botany, University of Melbourne, Parkville, Victoria 3010, Australia (A.B.)

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Food

Federal Register - 71 FR 29248 May 22, 2006: Health Claims; Soluble Dietary Fiber From Certain Foods and Coronary Heart Disease; Final Rule

[Federal Register: May 22, 2006 (Volume 71, Number 98)]
[Rules and Regulations]
[Page 29248-29250]
From the Federal Register Online via GPO Access [wais.access.gpo.gov]
[DOCID:fr22my06-4]

FDA considered the scientific evidence presented in the petition as part of its review of the scientific literature on barley beta-glucan soluble fiber and CHD risk, as well as information previously considered by the agency on the relationship of consumption of beta-glucan containing oat foods and blood (serum or plasma) cholesterol levels. The agency summarized this evidence in the interim final rule (70 FR 76150 at 76153--76155). Based on the available evidence, FDA concluded that consuming whole grain barley and dry milled barley products that provide at least 3 grams of beta-glucan soluble fiber per day, is effective in lowering blood total and LDL cholesterol, and that the cholesterol-lowering effects of beta-glucan soluble fiber in dry milled barley products is comparable to that of the oat sources of beta-soluble glucan fiber now listed in Sec. 101.81(c)(2)(ii)(A). Consequently, FDA amended Sec. 101.81 to broaden the health claim to include whole grain barley and dry milled barley products as an additional source of beta-glucan soluble fiber eligible for the health claim.

The Economist explains

Why are sales of non-alcoholic beer booming?

Aug 11th 2013, 23:50 by E.H.

The Economist

ACROSS most of the world the consumption of alcohol is falling. In some places the trend is most marked among the young: in Britain, ten years ago 70% of 16- to 24-year-olds claimed to have had a drink in the previous week, whereas by 2010 just 48% had. Many Western teenagers are playing on games consoles or chatting on Facebook rather than illicitly swigging cider in the park. But alongside this trend (which is not universal, with many Eastern European countries, such as Russia and Moldova, glugging away) another has appeared. Last year 2.2 billion litres of non-alcoholic beer were drunk, 80% more than five years earlier. Why are sales of non-alcoholic beer booming?

Non-alcoholic beer, which is also sometimes branded as "light" or "low-alcoholic" beer, is normally fermented beer that is then boiled to reduce the alcohol within it. It became popular around the time of prohibition in America, which set a limit of 0.5% alcohol by volume (ABV). Most mainstream lager brands have a lighter alternative. Now non-alcoholic beer is the fastest-growing category in a market that is pretty static or declining slightly, according to Sean Durkan of Bavaria beer, an independent brewery that sells 0.00% ABV beer and lager shandy along with lighter alcoholic beers. For one thing, people are more aware than before of the damaging effects of alcohol. Governments have stepped up health campaigns and chivvied the drinks industry into promoting low-alcohol alternatives to their usual products. In Japan an ageing population, mindful of its health but fond of a tipple, has started to take up non-alcoholic beer. And better technology means that it is tastier than before, Mr Durkan claims.

One chunk of the market is taking off for other reasons. The Middle East now accounts for almost a third of the worldwide sales by volume of non-alcoholic beer. In 2012 Iranians drank nearly four times as much of it as they did in 2007. It is popular in Saudi Arabia and the United Arab Emirates, where alcohol is either wholly or partially banned. Partly this is for religious reasons. After Hamas, a Palestinian Islamist movement, won a landslide election victory in Gaza in 2005, a local brewer launched an alcohol-free "halal" version of its beer. But it also taps into growing consumer aspirations. As a statement of a globalised lifestyle beer, even if non-alcoholic, may be more potent than Coca-Cola. Non-alcoholic lager is slowly being drunk more in bars and restaurants, rather than just being consumed at home. Prominent Saudi and Egyptian clerics have issued fatwas declaring it permissible to drink zero-alcohol beer.

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U.S. Food and Drug Administration

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Food

Home > Food > Labeling & Nutrition > Label Claims

Labeling & Nutrition

Label Claims

Health Claims Meeting Significant Scientific Agreement (SSA)

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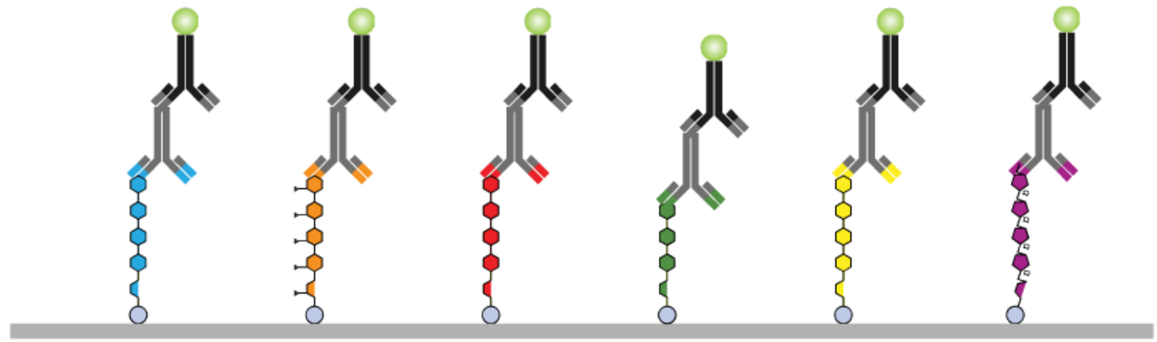
Testing for individual glycans

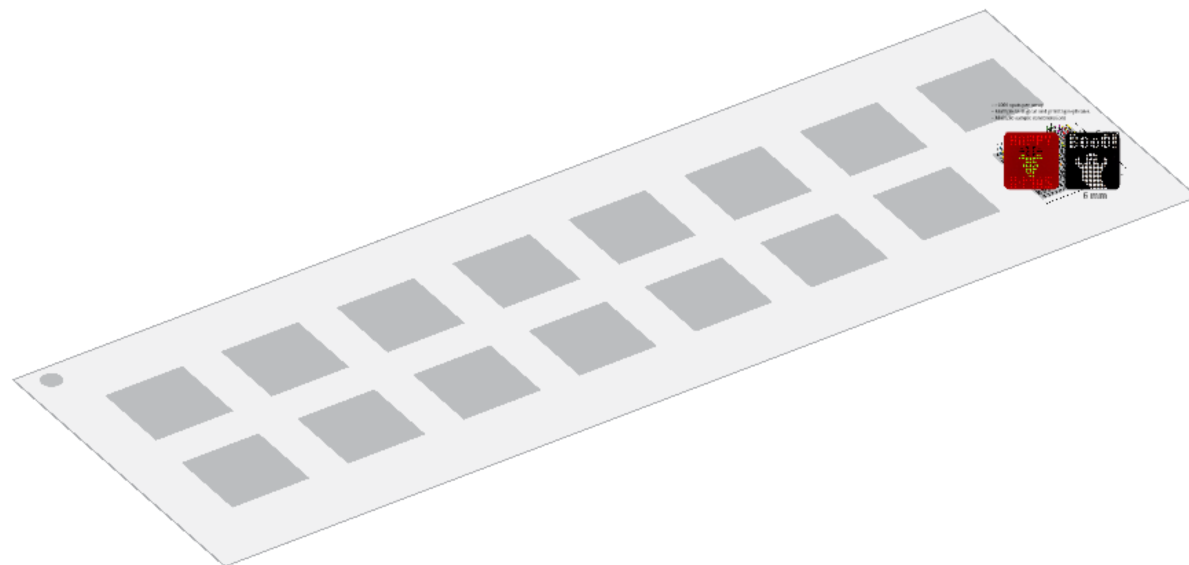
- Time consuming
- Expensive
- Low-throughput

Carbohydrate microarrays

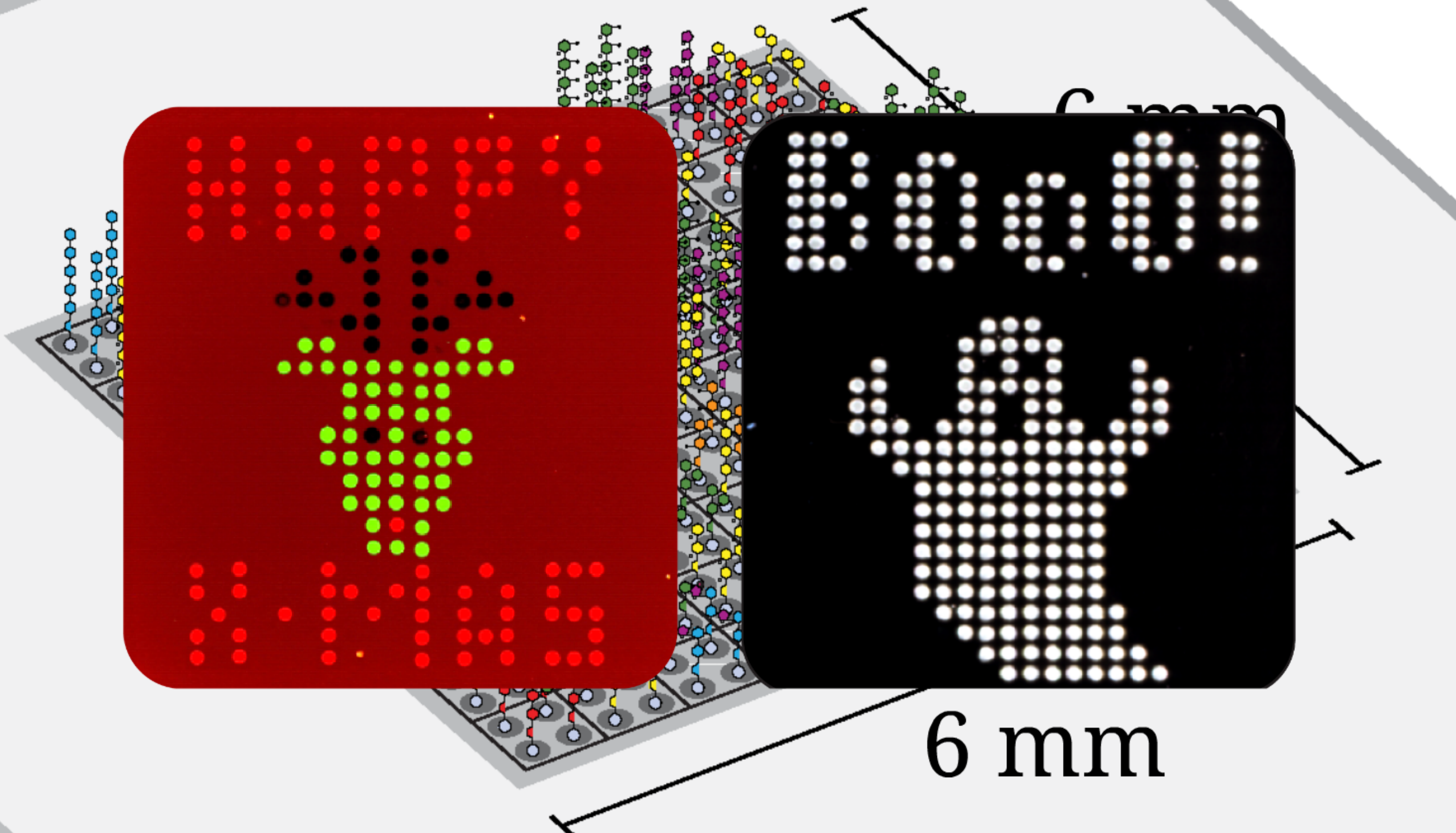
Carbohydrate microarrays

- Transcriptomics
- Gene discovery
- Pathway ellucidation
- Protein interactions





- >1000 spots per array
- Multiple biological and printing replicates
- Multiple sample concentrations



Molecular probes for polysaccharides

Polysaccharide	Code	Epitope
Pectin	JIM5	HG partially/de-esterified
Pectin	JIM7	HG partially esterified
Pectin	LM18	HG partially/de-esterified
Pectin	LM19	HG partially/de-esterified
Pectin	LM20	HG partially esterified
Pectin	LM8	Xylogalacturonan
RG-I related	INRA-RU1	Backbone of rhamnogalacturonan I
RG-I related	INRA-RU2	Backbone of rhamnogalacturonan I
RG-I related	LM5	(1→4)-β-D-galactan
RG-I related	LM6	(1→5)-α-L-arabinan
RG-I related	LM13	Linearised (1→5)-α-L-arabinan
RG-I related	LM16	JPK Processed (1→5)-α-L-arabinan
RG-I related	LM12	Feruloylate on any polymer
Mannan	BS-400-4	(1→4)-β-D-(galacto)mannan
Mannan	LM21	(1→4)-β-D-(galacto)(gluco)mannan
Mannan	LM22	(1→4)-β-D-(gluco)mannan
Glucan	BS-400-2	(1→3)-β-D-glucan
Glucan	BS-400-3	(1→3)(1→4)-β-D-glucan
Xyloglucan	CCRC-M1	Fucosylated xyloglucan
Xyloglucan	LM15	Xyloglucan (XXXG motif)
Xyloglucan	LM24	Xyloglucan
Xyloglucan	LM25	Xyloglucan
Xylan	LM10	(1→4)-β-D-xylan
Xylan	LM11	(1→4)-β-D-xylan/arabinoxylan
Xylan	LM23	(1→4)-β-D-xylan
Cellulose	CBM3a	Celulose (crystalline)
Cellulose	CBM4-1	Cellulose (amorphous)
Extensin	LM1	Extensin
Extensin	JIM12	Extensin
Extensin	JIM19	Extensin
Extensin	JIM20	Extensin
AGP	JIM4	AGP
AGP	JIM13	AGP
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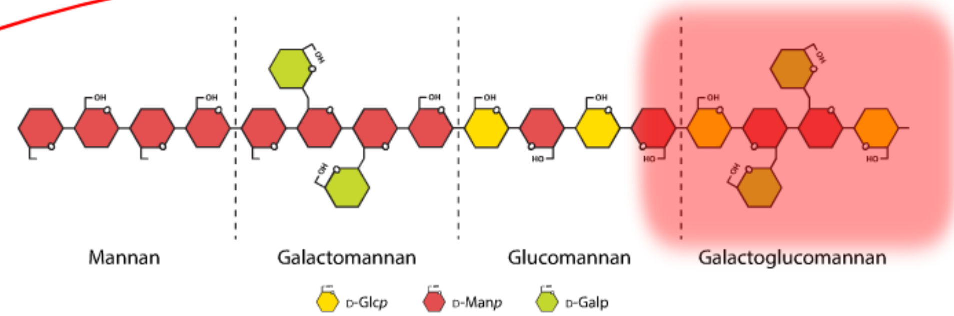
AGP	JIM17	AGP
AGP	LM14	AGP

mAb LM21
Galactoglucomannan



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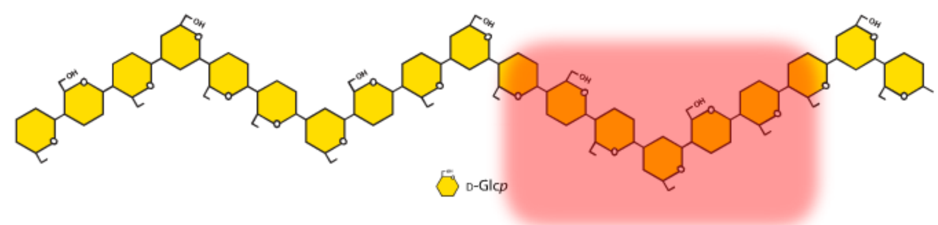
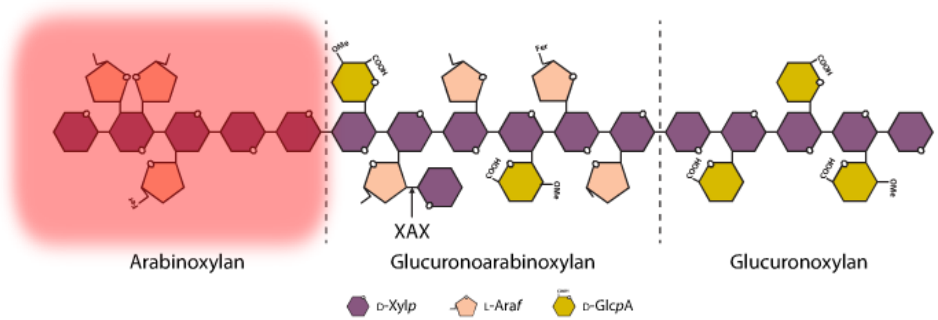
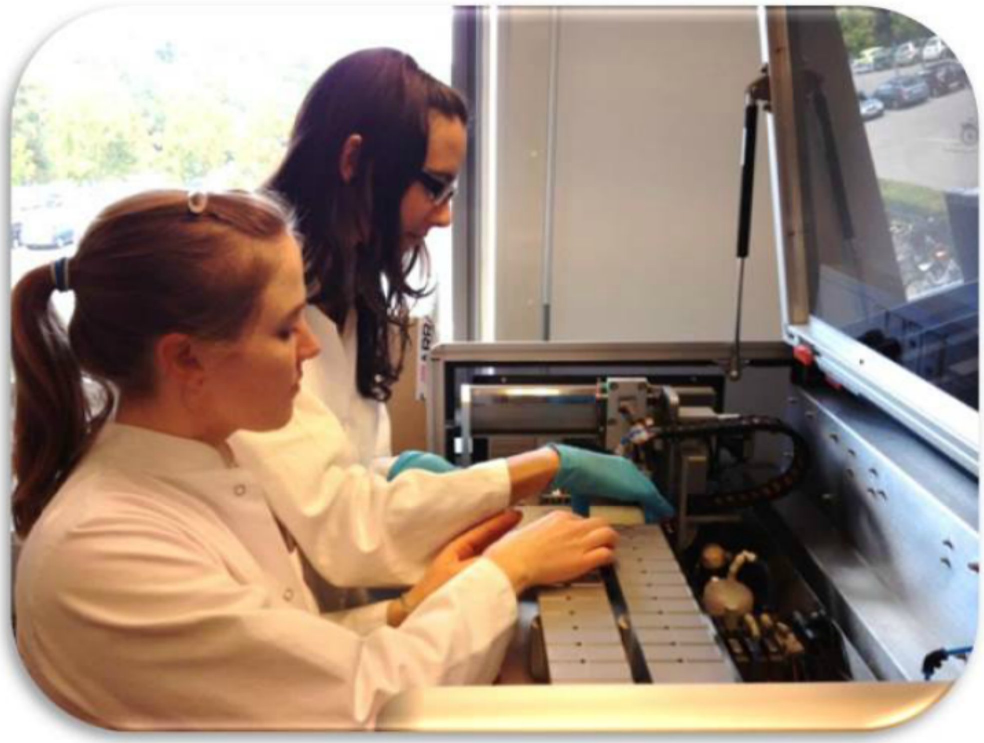
(1→3)(1→4)-β-D-glucan

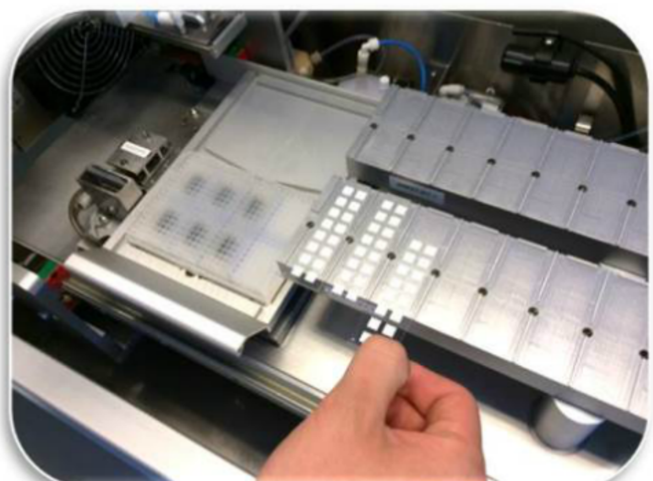


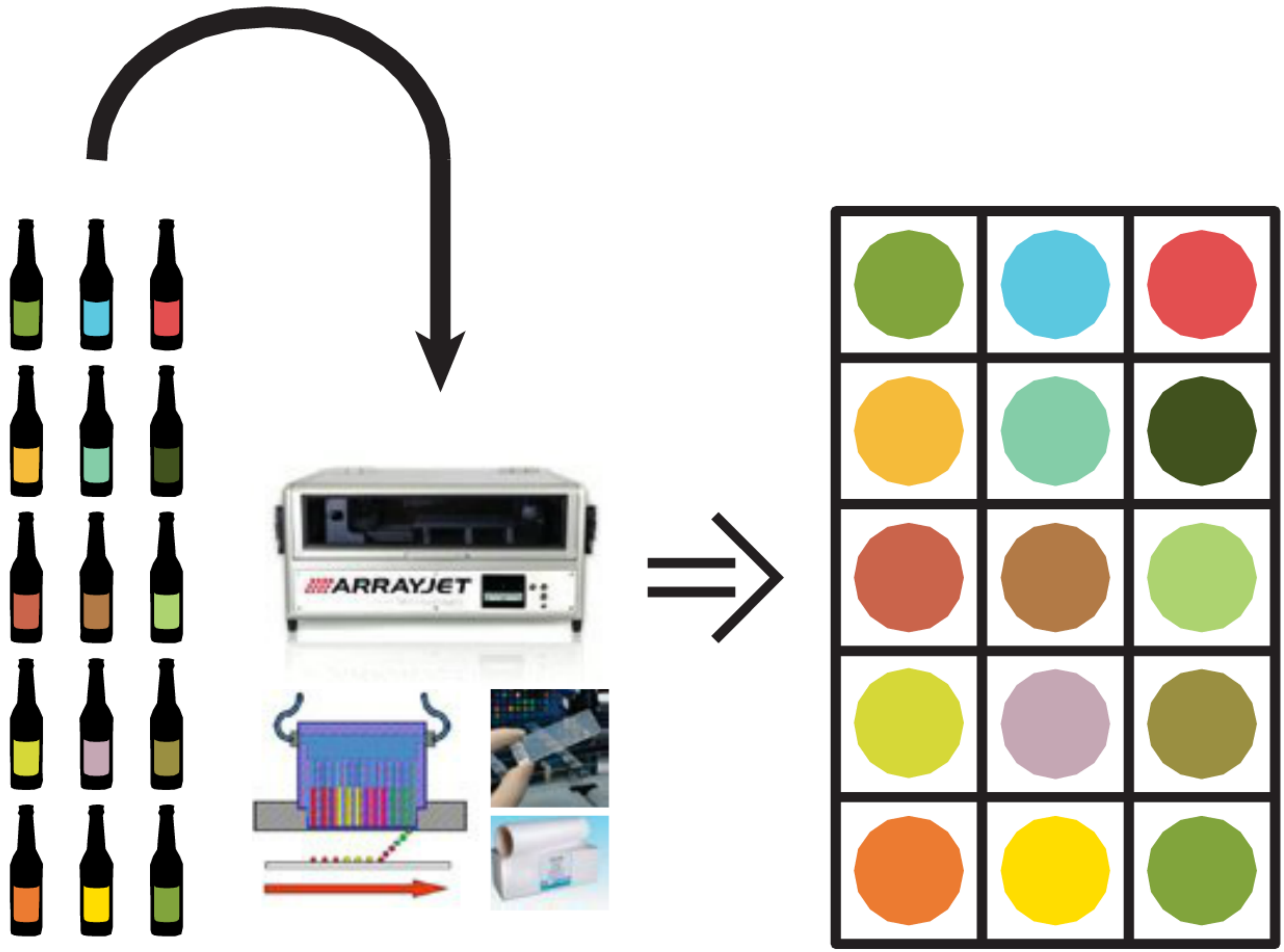
mAb LM11
(1→4)-β-D-xylan/arabinoxylan



Carbohydrate microarrays



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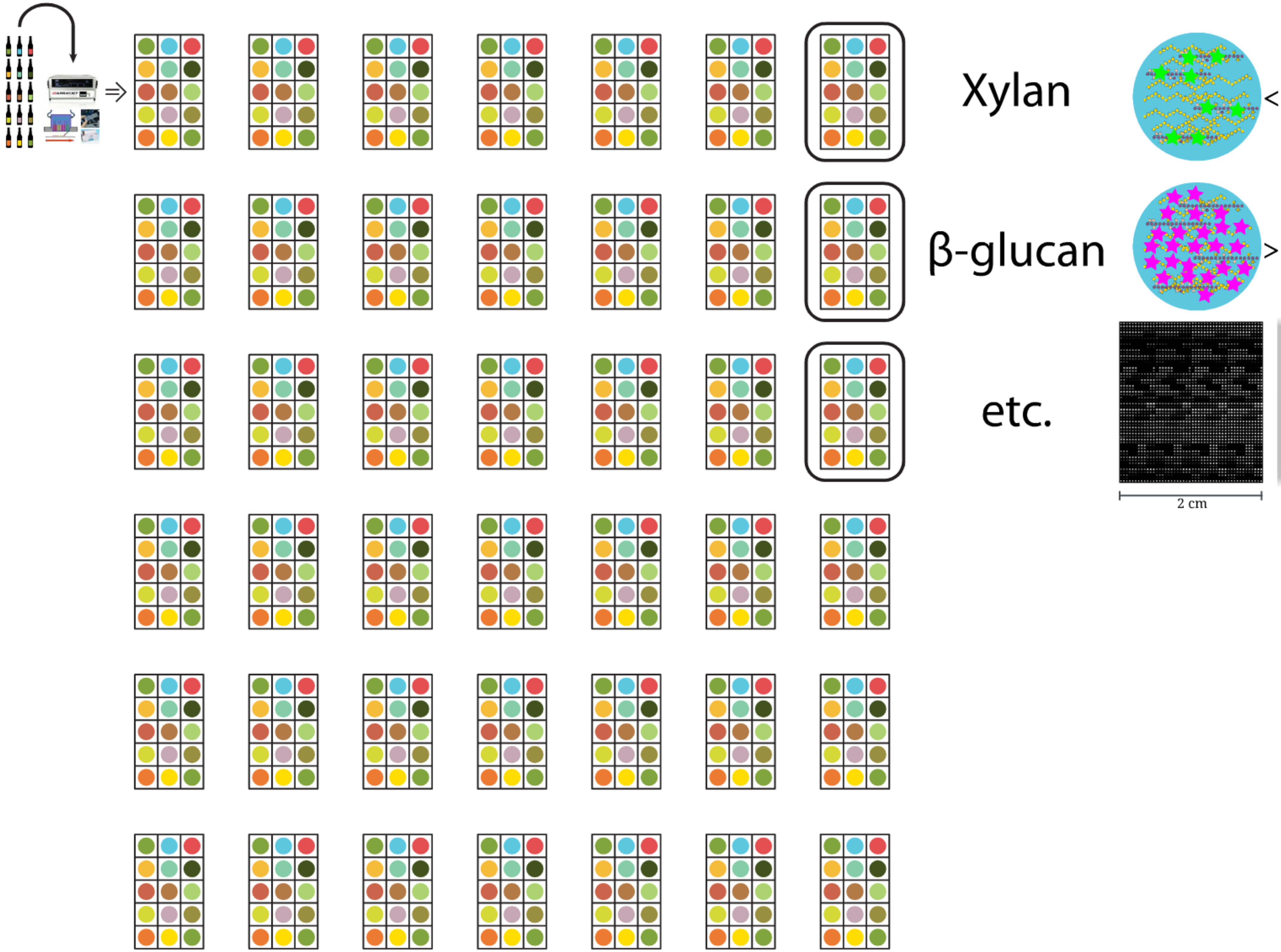
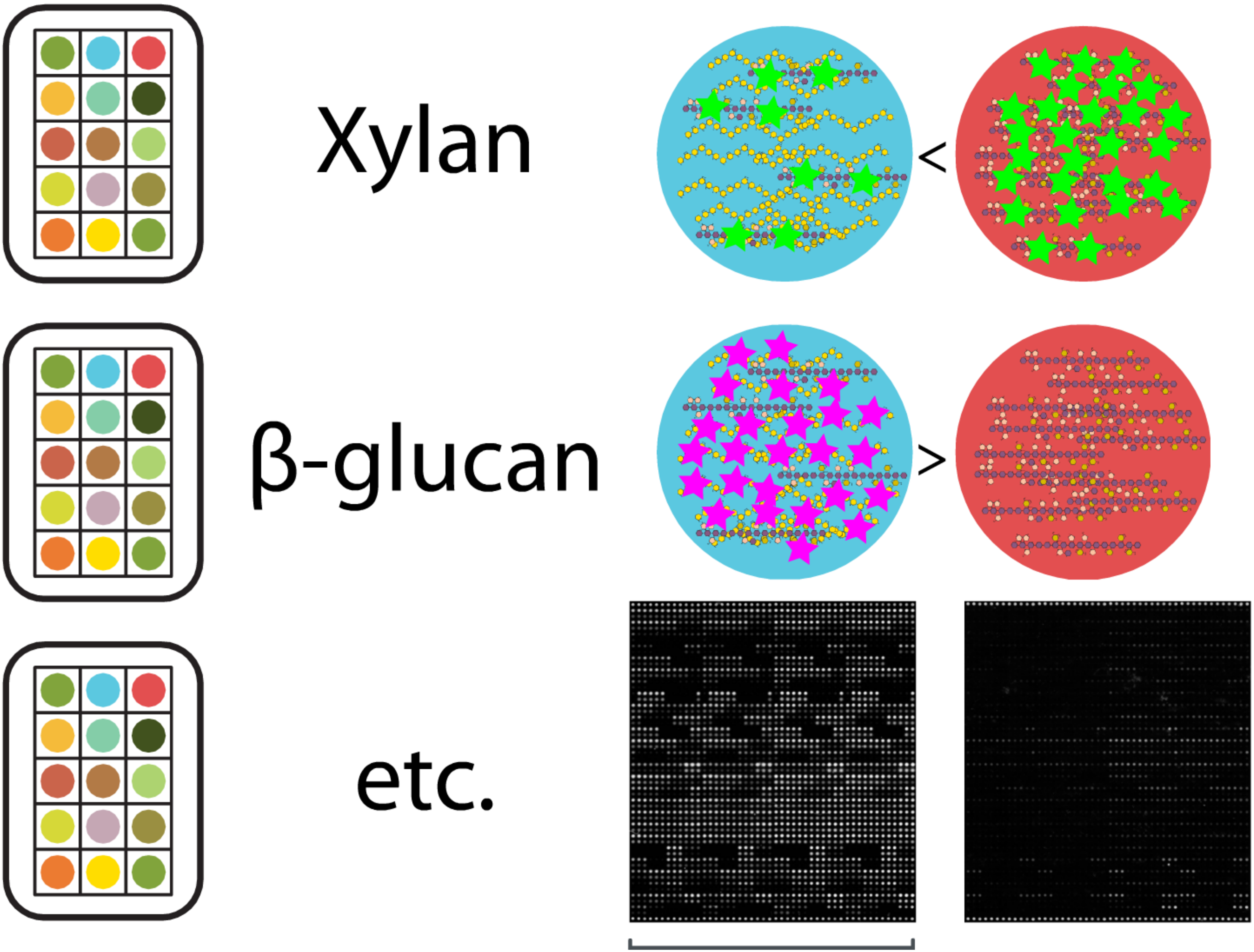
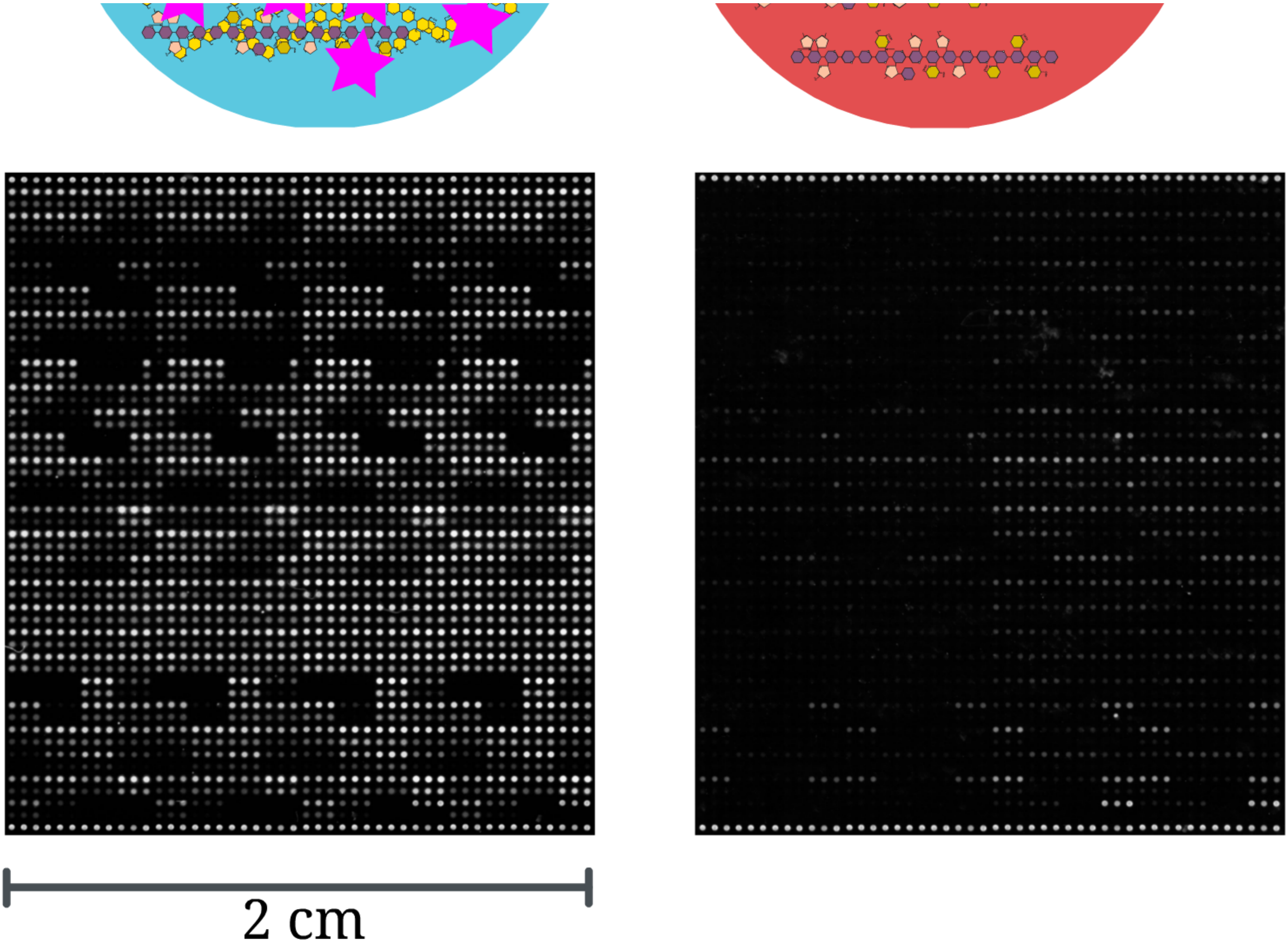
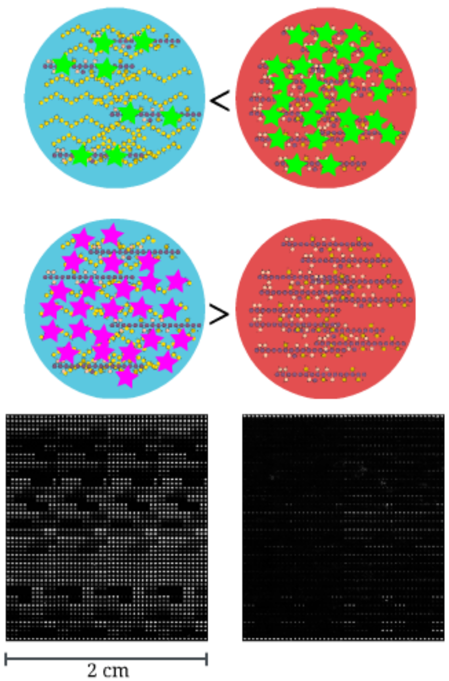


Xylan
β-glucan
etc.
2 cm



Xylan
<
β-glucan
>
etc.


2 cm



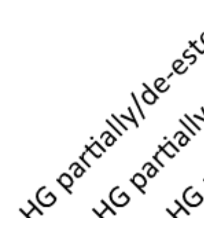
Output

Semi-quantitative information about the relative abundance of polysaccharides in beer samples.

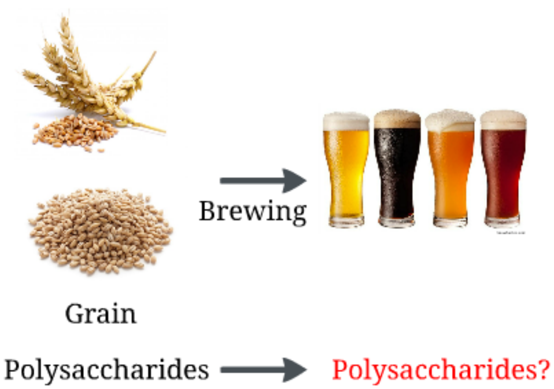
Quantitative with internal polysaccharides standards

Throughput: 350 samples in 10 hours using 20 volume (ul)

Proof of concept - analysis of beers



Proof of concept - analysis of beers



HG partially/de-esterified (mAb LM18)	HG partially/de-esterified (mAb LM19)	HG partially esterified (mAb LM20)	Backbone of rhamnogalacturonan I (mAb INRA-RU1)	Backbone of rhamnogalacturonan I (mAb INRA-RU2)	(1→4)-β-D-galactan (mAb LM5)	(1→5)-α-L-arabinan (mAb LM6)	Linearised (1→5)-α-L-arabinan (mAb LM13)	Feruloylate on any polymer (mAb LM12)	(1→4)-β-D-(galacto)mannan (mAb BS-400-4)	(1→4)-β-D-(galacto)(gluco)mannan (mAb LM21)	(1→4)-β-D-(gluco)mannan (mAb LM22)	(1→3)-β-D-glucan (mAb BS-400-2)	(1→3)(1→4)-β-D-glucan (mAb BS-400-3)	Xyloglucan (XXXG motif) (mAb LM15)	Xyloglucan / unsibstituted β-D-glucan (mAb LM25)	(1→4)-β-D-xylan (mAb LM10)	(1→4)-β-D-xylan/arabinoxylan (mAb LM11)	(1→4)-β-D-xylan (mAb LM23)	Extensin (mAb LM1)	Extensin (mAb JIM12)	Extensin (mAb JIM20)	AGP (mAb JIM4)	AGP (mAb JIM13)	AGP (mAb JIM16)	AGP (mAb LM14)	AGP, β-linked GlcA (mAb LM2)
0	0	0	7	0	16	24	13	32	12	11	0	0	58	0	31	0	0	0	0	0	0	0	13	0	0	0
0	0	0	10	0	20	24	17	32	33	32	0	0	66	0	30	0	0	0	0	0	0	0	15	0	0	0
0	0	0	0	0	14	22	10	37	27	26	0	0	61	0	36	0	9	0	0	0	0	0	12	0	0	0
0	0	0	0	0	15	22	11	32	34	32	0	0	64	0	35	0	7	0	0	0	0	0	16	0	0	0
0	0	0	8	0	17	32	11	38	0	0	0	0	24	0	33	0	0	0	0	0	0	0	20	0	0	0
0	0	0	0	0	14	15	12	26	23	21	0	0	14	0	17	0	0	0	0	0	0	0	10	0	0	0
0	0	0	0	0	11	19	8	36	16	14	0	0	0	0	29	0	0	0	0	0	0	0	13	0	0	0
0	0	0	0	0	9	15	0	35	16	14	0	0	13	0	28	0	0	0	0	0	0	0	10	0	0	0
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0	0	0	28	21	28	34	17	36	0	0	0	0	6	0	19	0	0	0	0	0	13	0	22	0	0	11
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0	0	0	0	0	9	16	0	34	0	0	0	0	0	0	23	0	0	0	0	0	0	0	12	0	0	0
0	0	0	0	0	17	21	12	40	42	39	0	0	60	0	34	0	8	0	0	0	0	0	17	0	0	0
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0	0	0	0	0	0	13	0	31	0	0	0	0	0	0	17	0	0	0	0	0	0	0	14	0	0	0
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0	0	0	9	0	15	27	15	35	44	35	0	0	57	0	28	11	9	0	0	0	0	0	14	0	0	0
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0	0	0	0	0	10	19	9	34	44	23	0	0	38	0	21	0	8	0	0	0	0	0	11	0	0	0
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0	0	0	18	8	17	35	22	37	31	28	0	0	30	0	22	8	18	0	0	0	0	0	19	0	0	0
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0	0	0	27	10	14	32	15	34	0	0	0	0	48	0	20	17	33	0	0	0	8	0	17	0	0	0
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0	0	0	12	0	21	30	17	46	45	39	0	0	72	0	33	0	15	0	0	0	0	0	12	0	0	0
0	0	0	7	0	0	16	0	46	41	0	0	0	61	0	22	0	26	0	0	0	0	0	8	0	0	0
0	0	0	0	0	16	32	0	37	13	11	0	0	10	0	31	0	0	0	0	0	0	0	16	0	0	9
0	0	0	8	0	17	35	8	41	13	10	0	0	32	0	31	9	9	0	0	0	9	0	18	0	0	9
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0	0	0	11	0	16	28	12	50	11	8	0	9	64	0	27	0	17	0	0	0	8	0	24	0	0	0
0	0	0	7	0	14	26	8	55	0	0	0	0	49	0	29	0	9	0	0	0	12	0	24	0	0	0
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0	0	0	10	0	14	29	15	44	47	42	0	0	26	0	30	0	13	0	0	0	9	0	22	0	0	0
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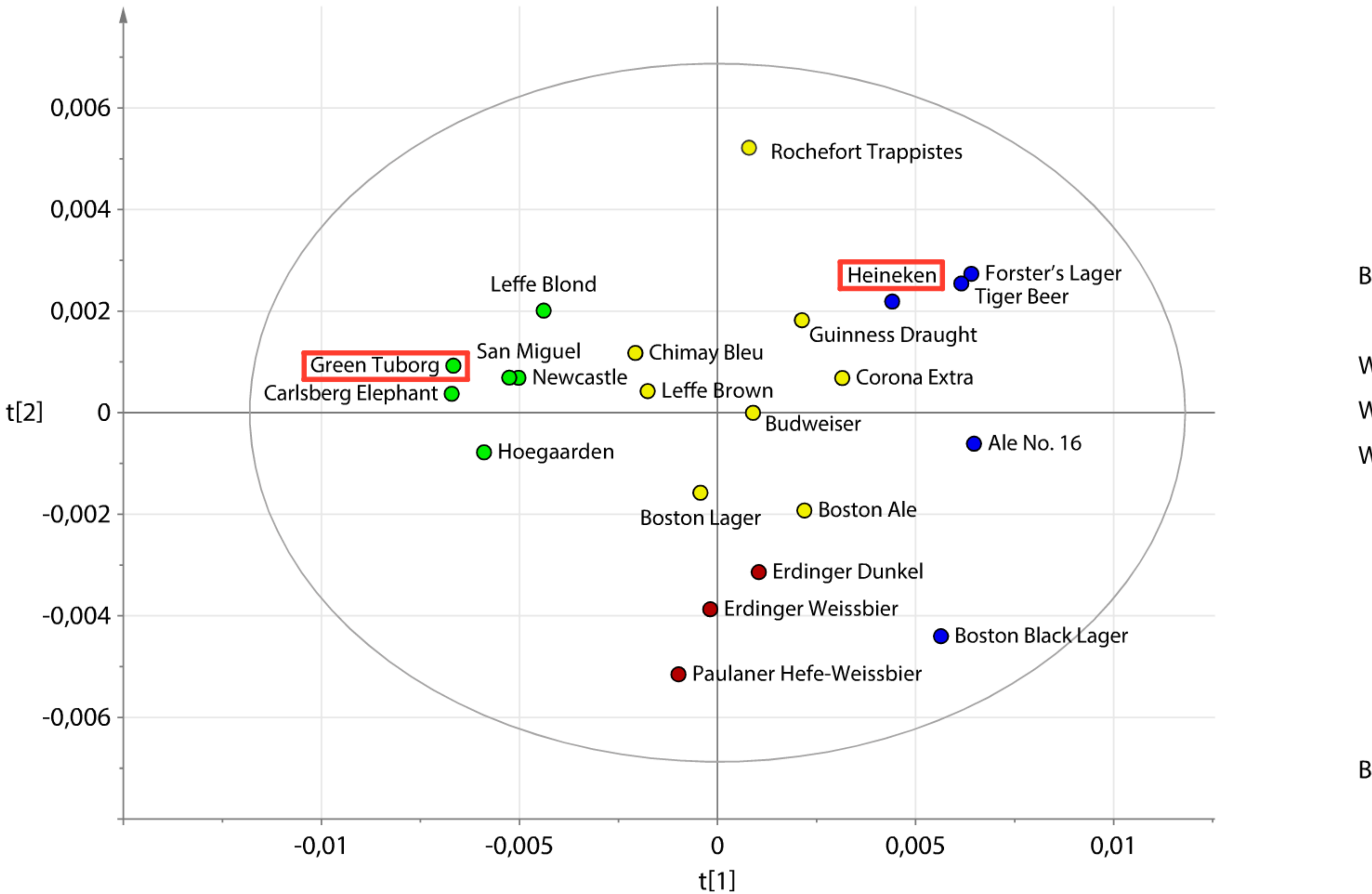

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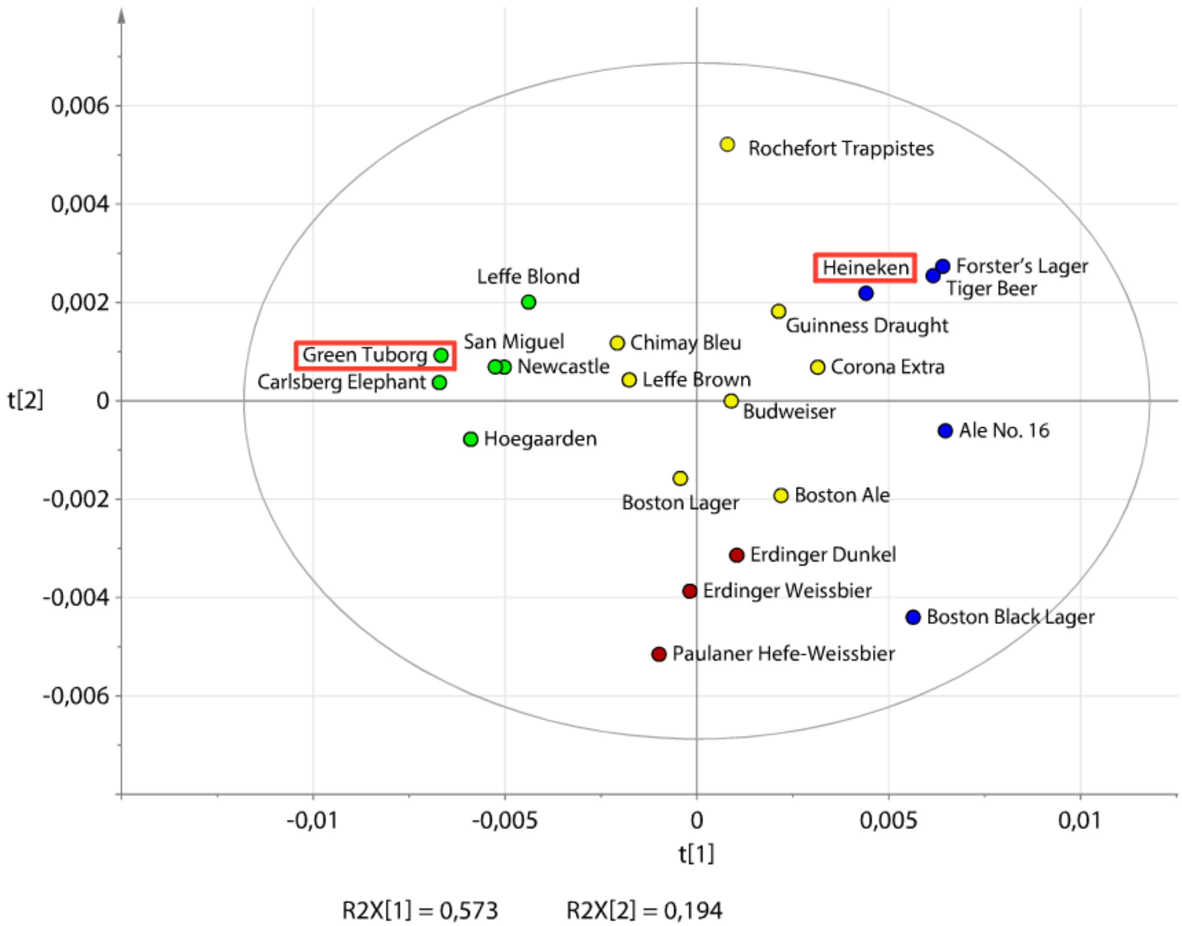
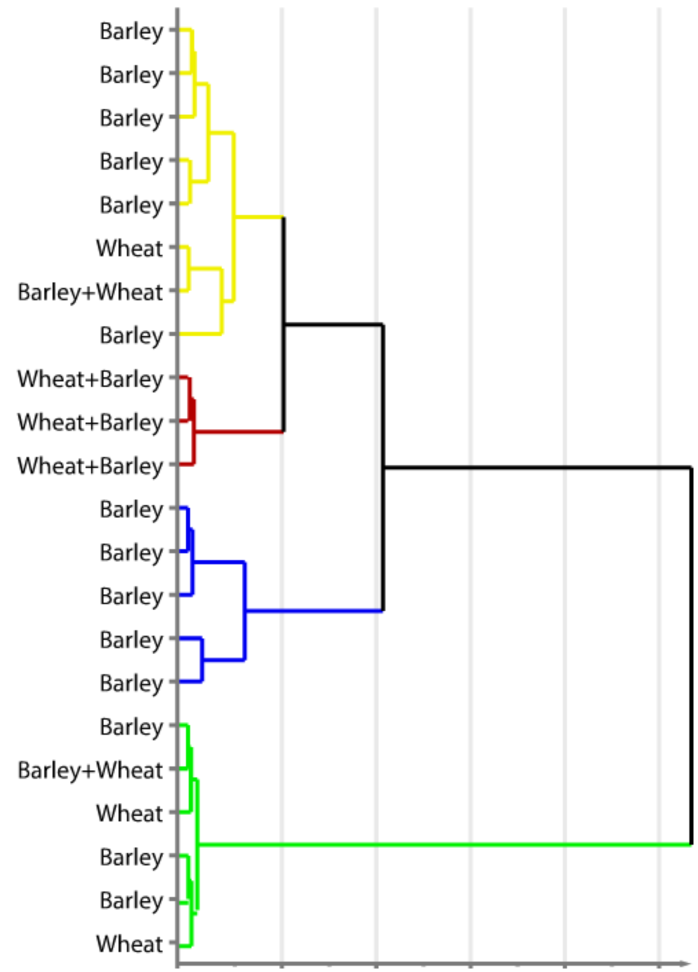
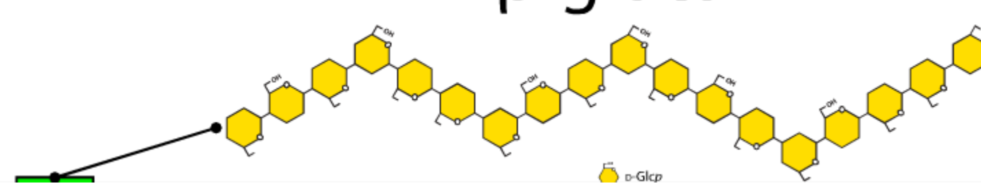
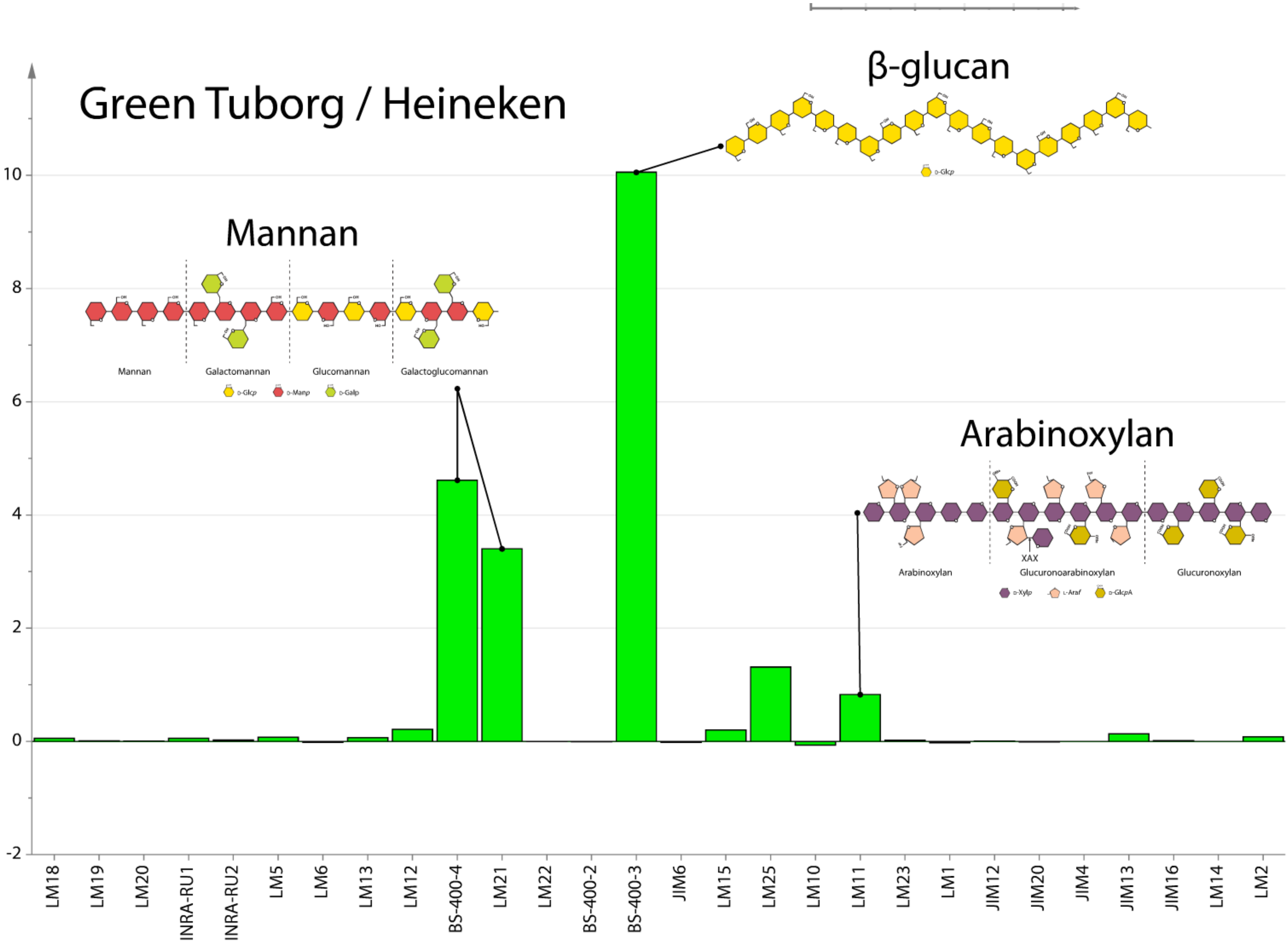




Green Tuborg / Heineken

β-glucan





Green Tuborg / Heineken
β-glucan
D-Glcp
Mannan
Mannan
Galactomannan
Glucomannan
Galactoglucomannan
D-Glcp
D-Manp
D-Galp
Arabinoxylan
XAX
Arabinoxylan
Glucuronoarabinoxylan
Glucuronoxylan
D-Xylp
L-Araf
D-GlcpA
10
8
6
4
2
0
-2
LM18
LM19
LM20
INRA-RU1
INRA-RU2
LM5
LM6
LM13
LM12
BS-400-4
LM21
LM22
BS-400-2
BS-400-3
JIM6
LM15
LM25
LM10
LM11
LM23
LM1
JIM12
JIM20
JIM4
JIM13
JIM16
LM14
LM2

Future

More sophisticated data analysis and integration with other information about the brewing process (SIMCA)

Analysis of not just final product but throughout the brewing process to be able to track the fate of polysaccharides

Internal standards to determine the fibre amount

Non-alcoholic beers, enhanced fibre content?

Acknowledgement

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