

Engineering the Nitrogen Symbiosis for Africa

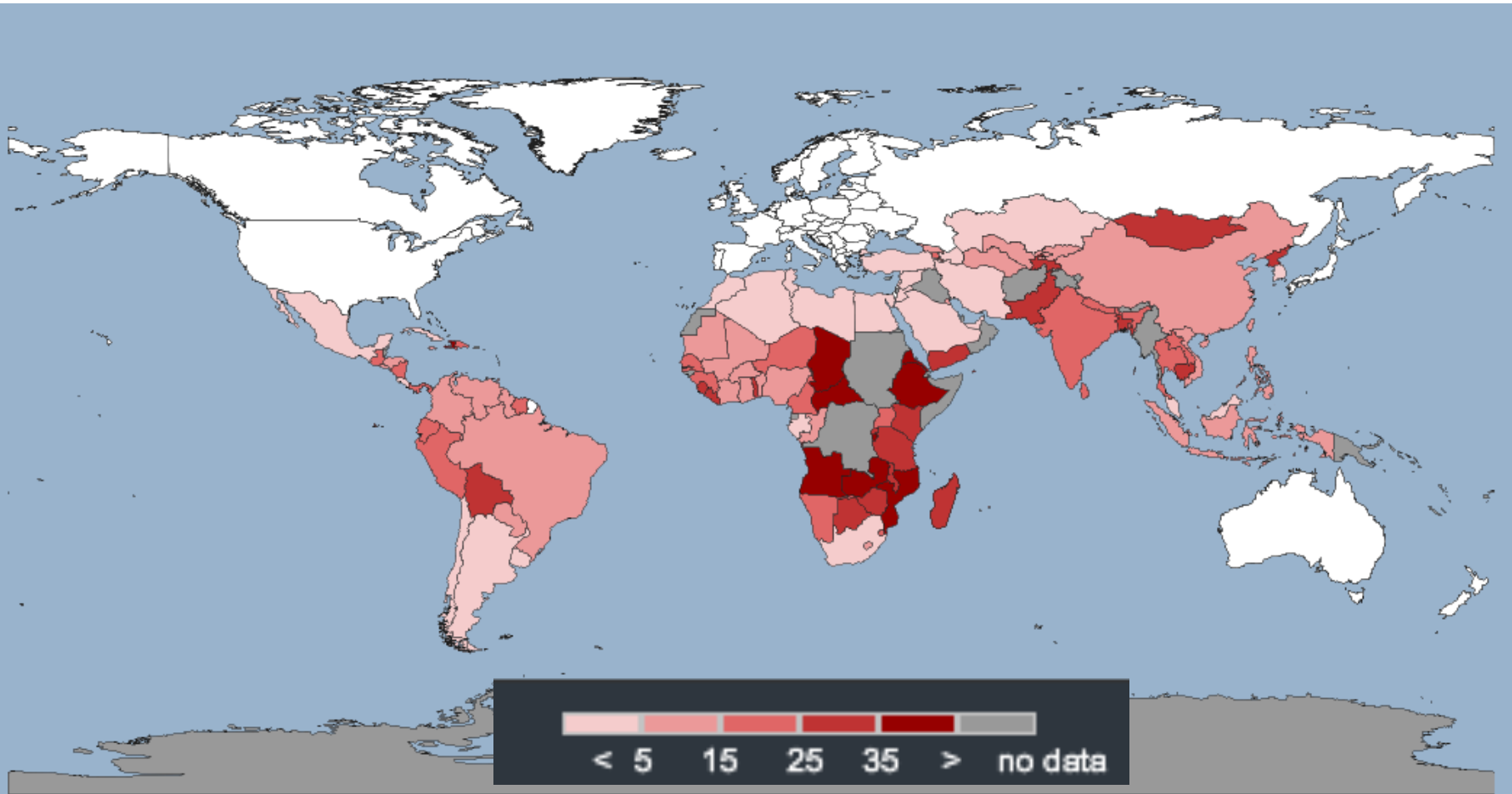


BILL & MELINDA
GATES *foundation*

>1 billion people with food insecurity



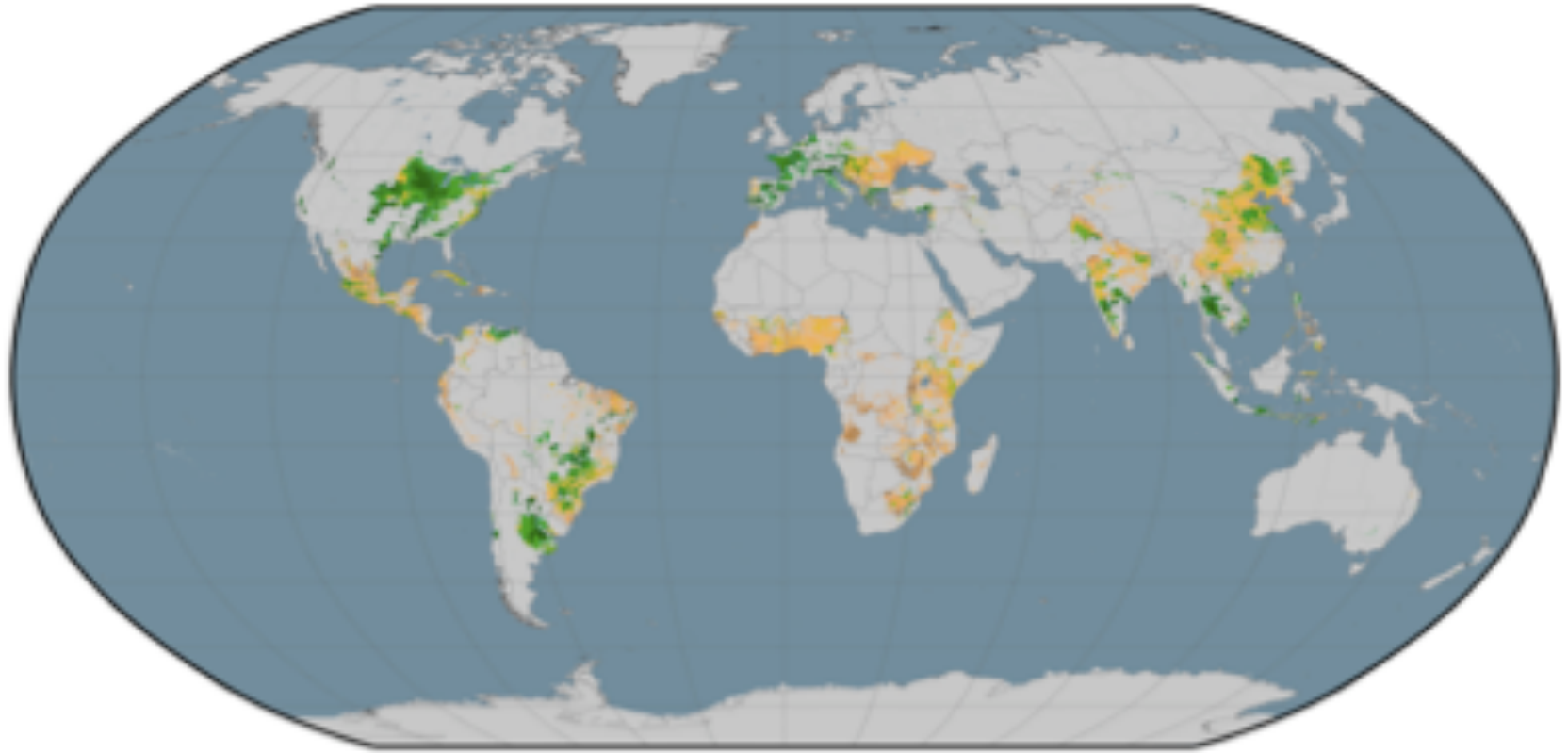
Prevalence of Undernourishment in Total Population (%)



Crop yields in Sub-saharan Africa are below potential



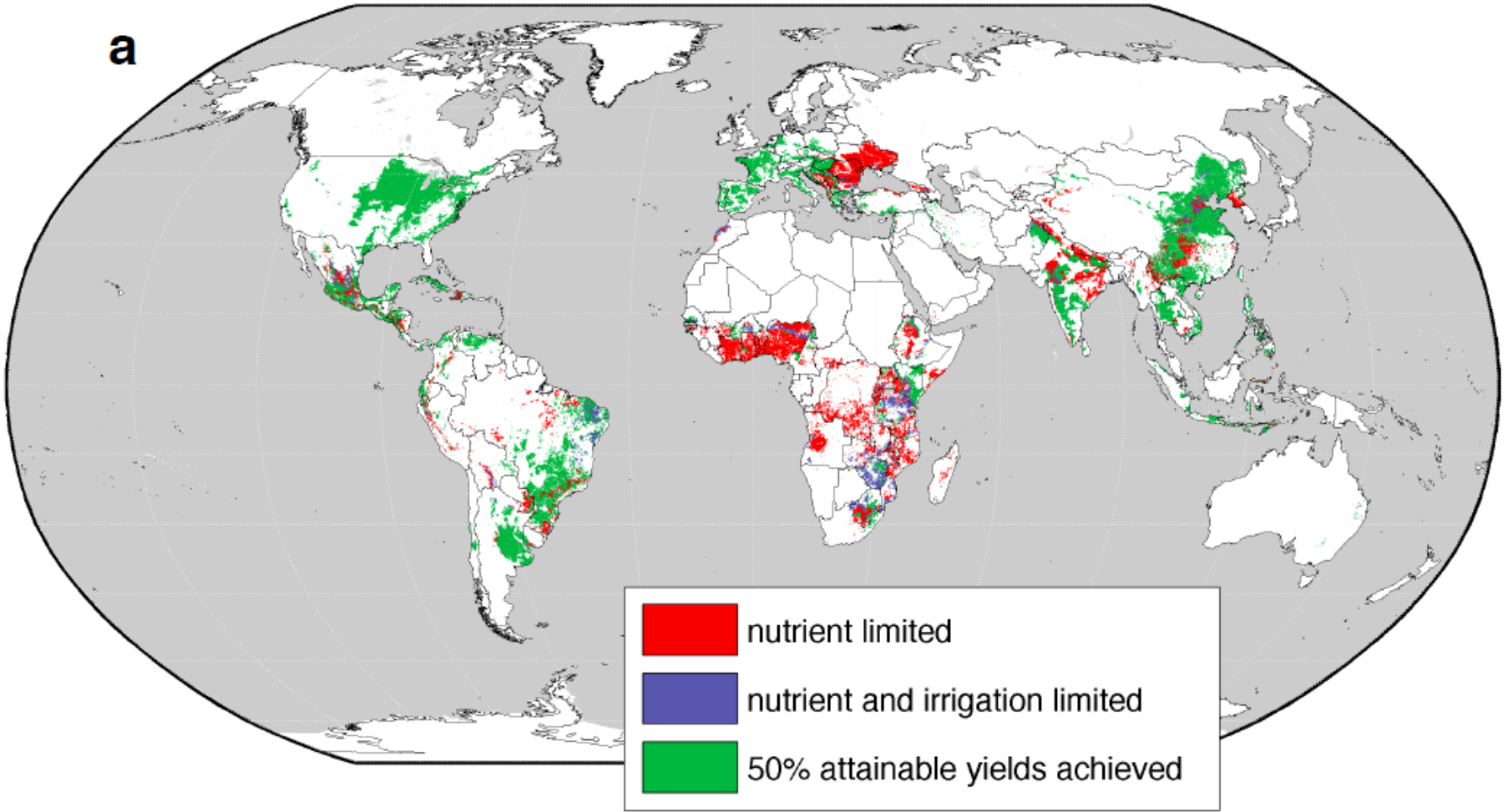
maize yield attainment



percent of potential yield attained



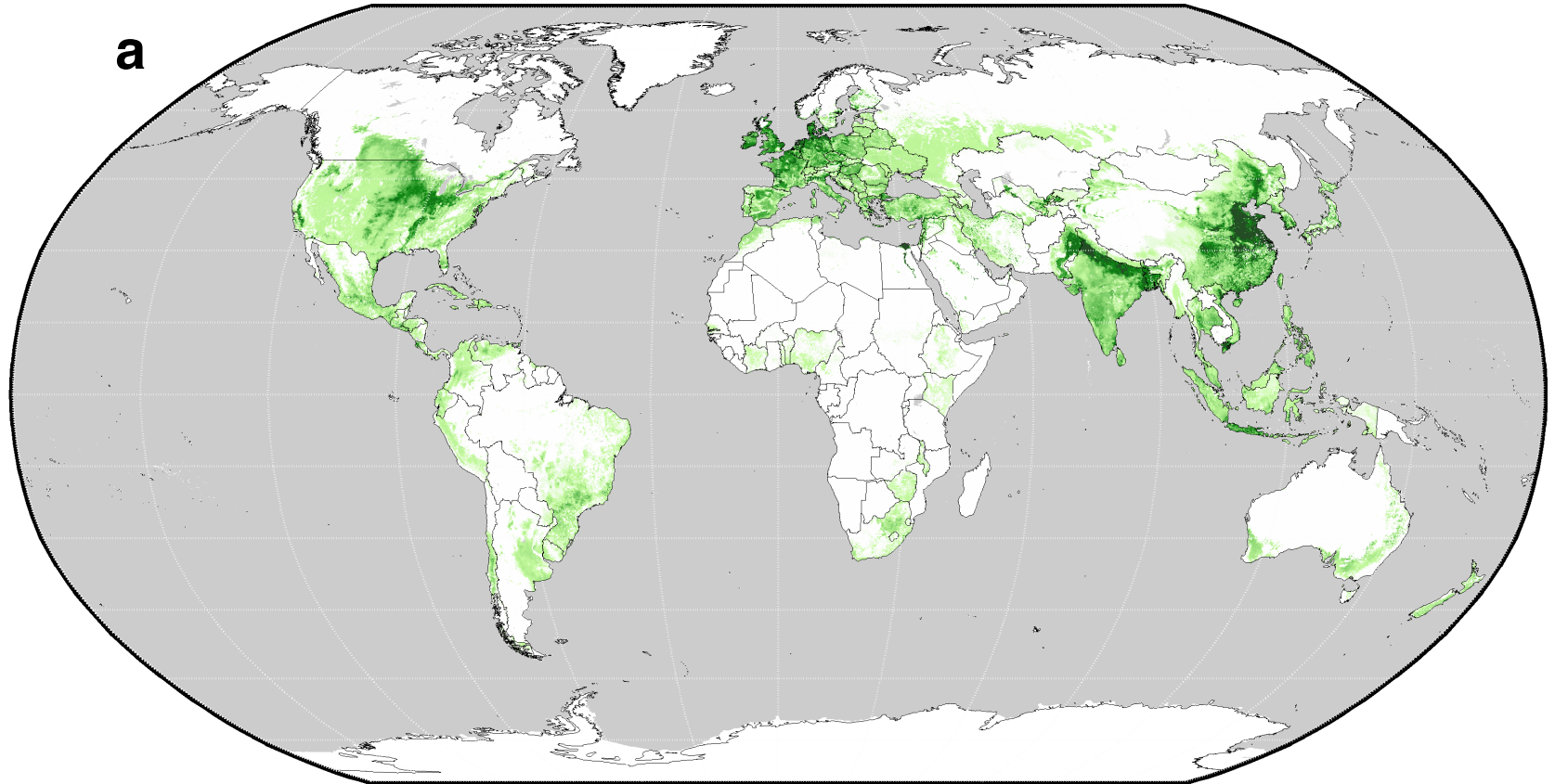
Nitrogen availability Limits Yields in Africa



Fertiliser use is very low in Africa



a



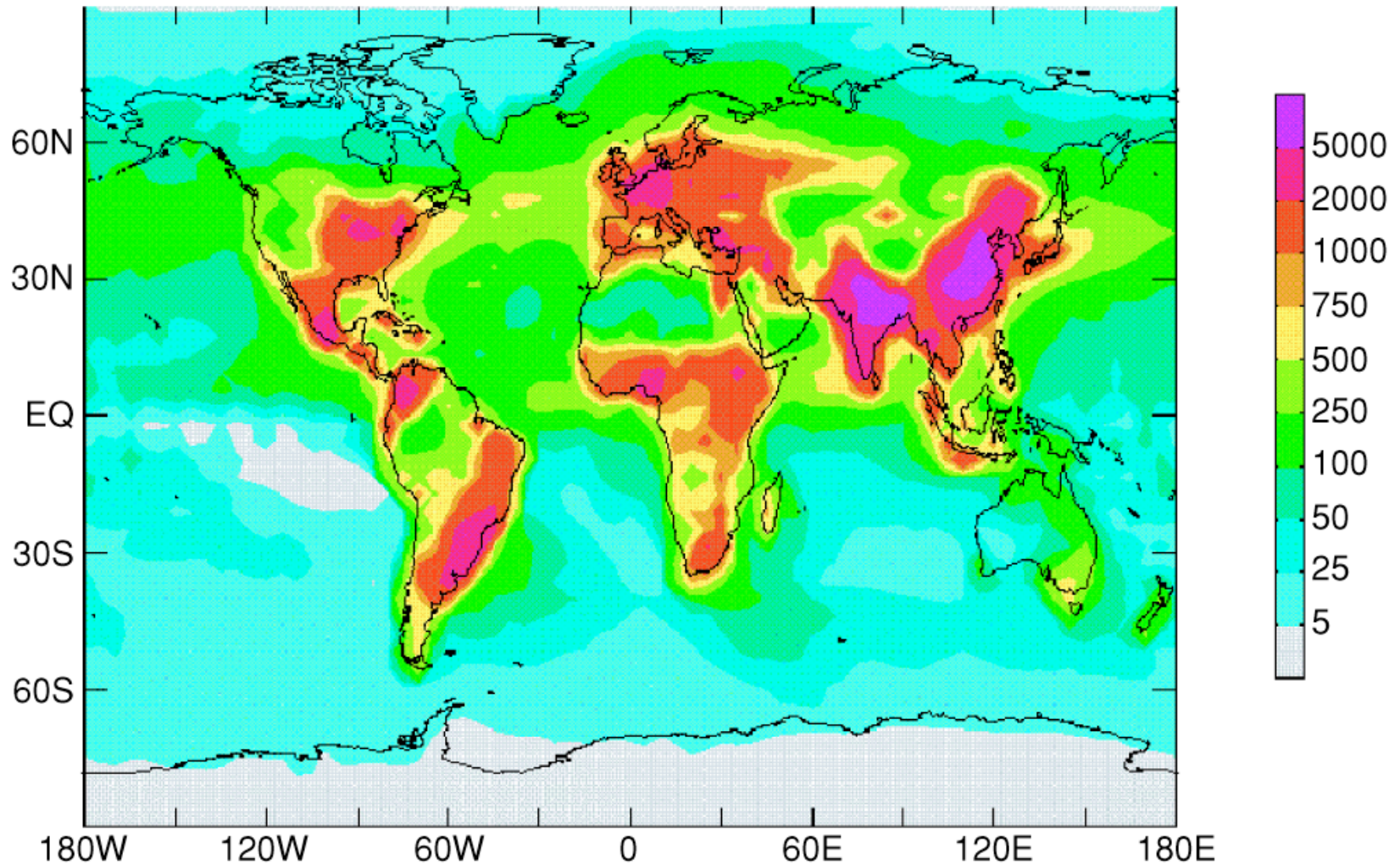
global nitrogen consumption (kg N / grid cell ha)



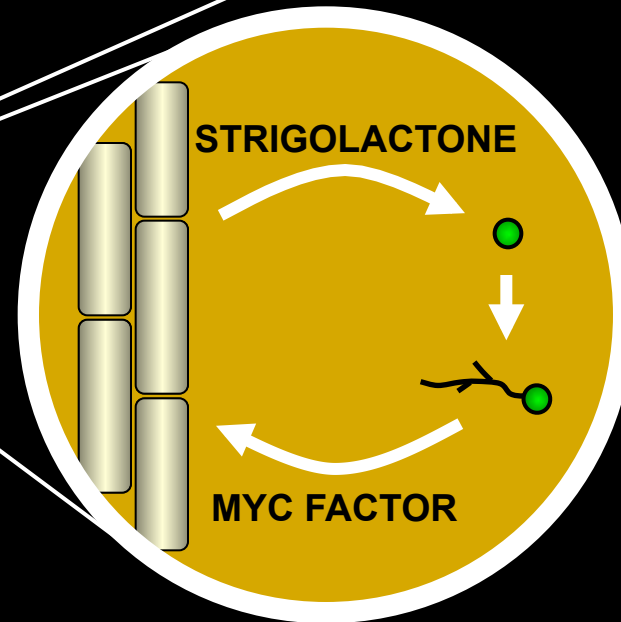
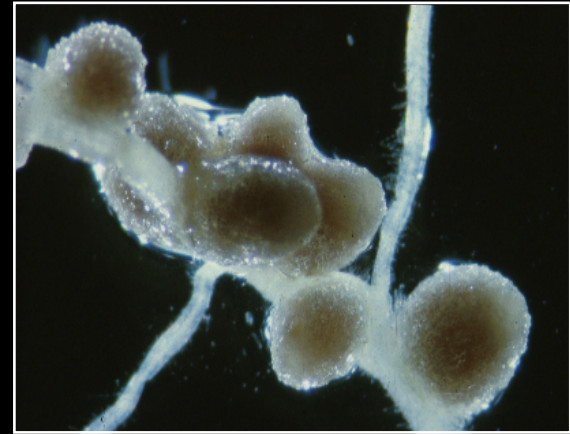
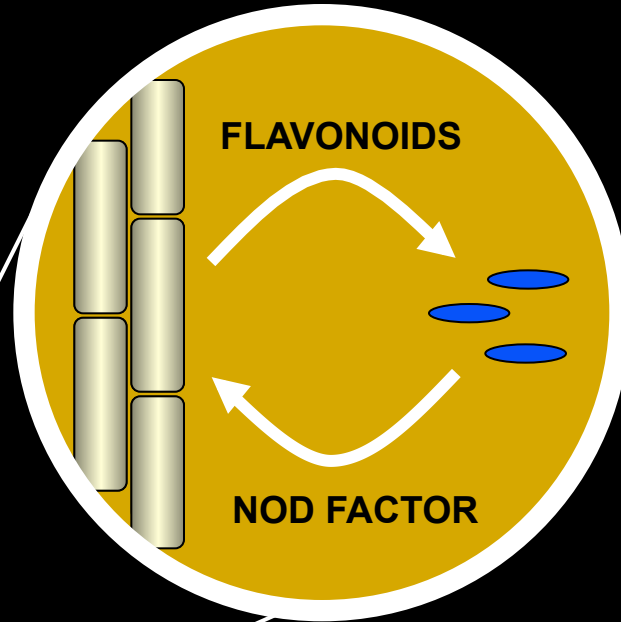
Projections for inorganic nitrogen deposition



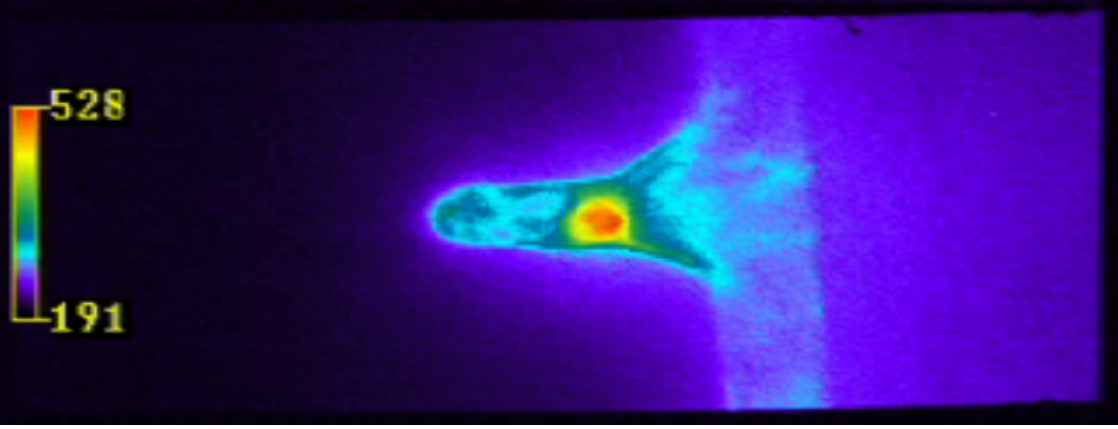
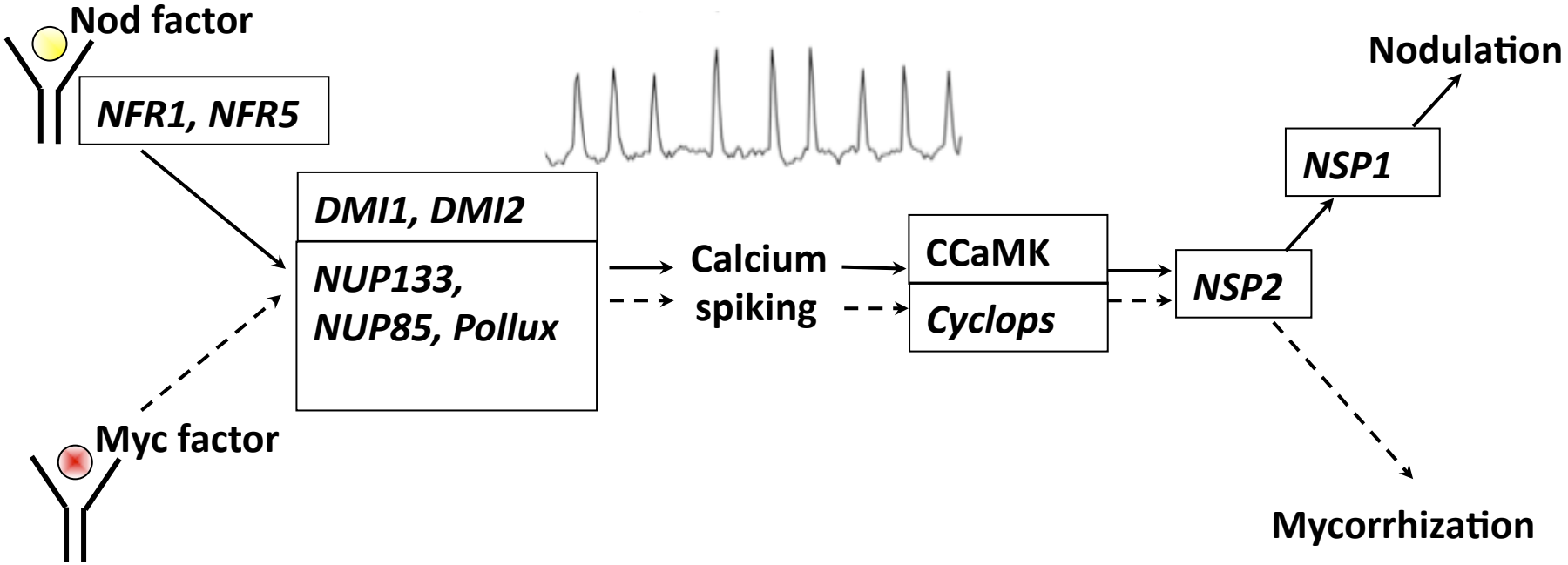
2050



The early steps in symbiosis are a molecular dialogue



The Symbiosis signalling pathway



Symbiotic N-fixation evolved from the mycorrhizal symbiosis

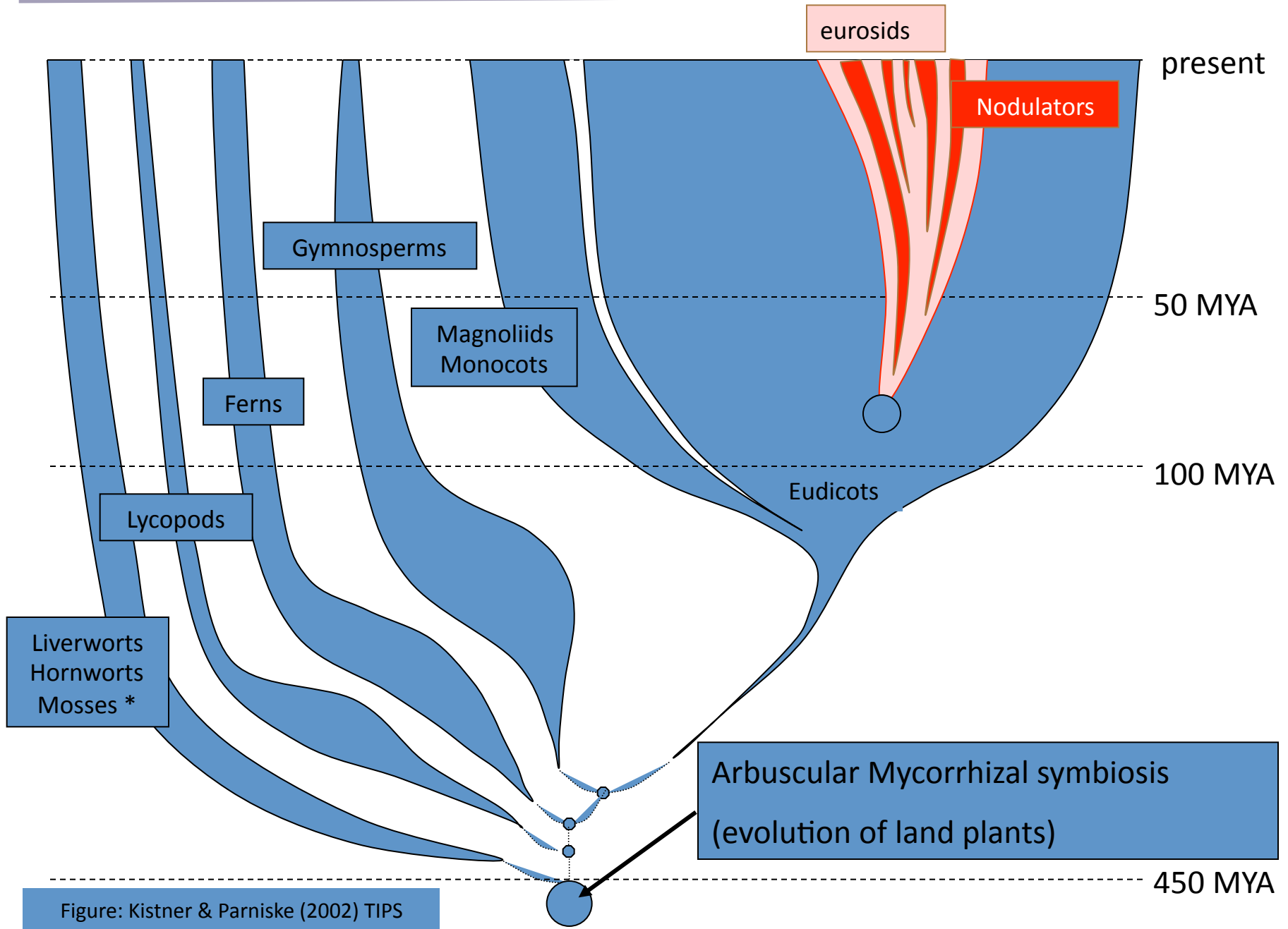
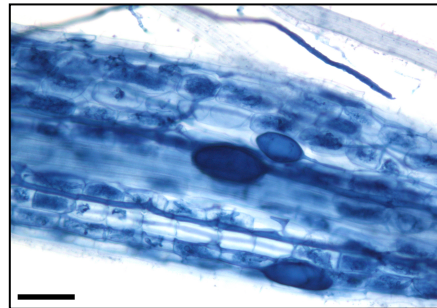


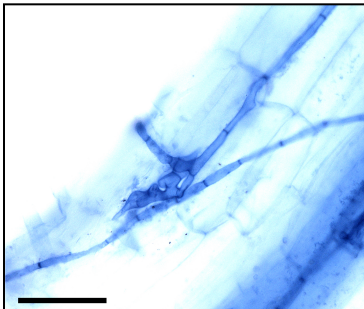
Figure: Kistner & Parniske (2002) TIPS

Rice symbiosis signalling mutants

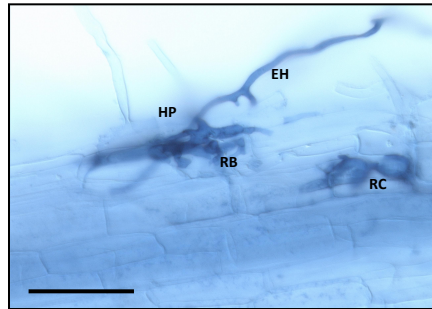
WT



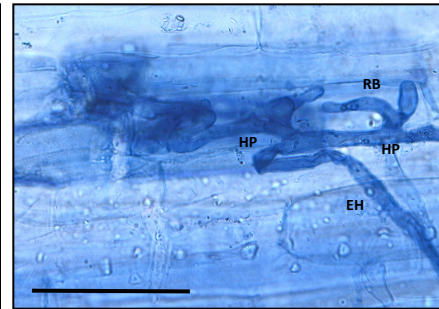
Rice *common sym* mutants



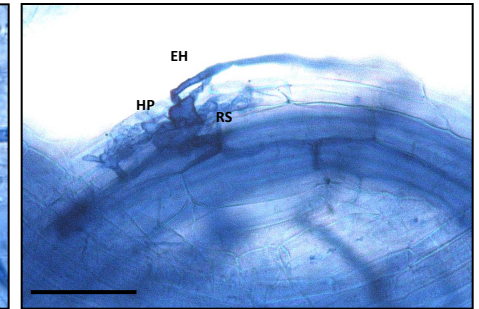
castor-1



pollux-1



ccamk-2



cyclops-3

size bars = 50 μ m

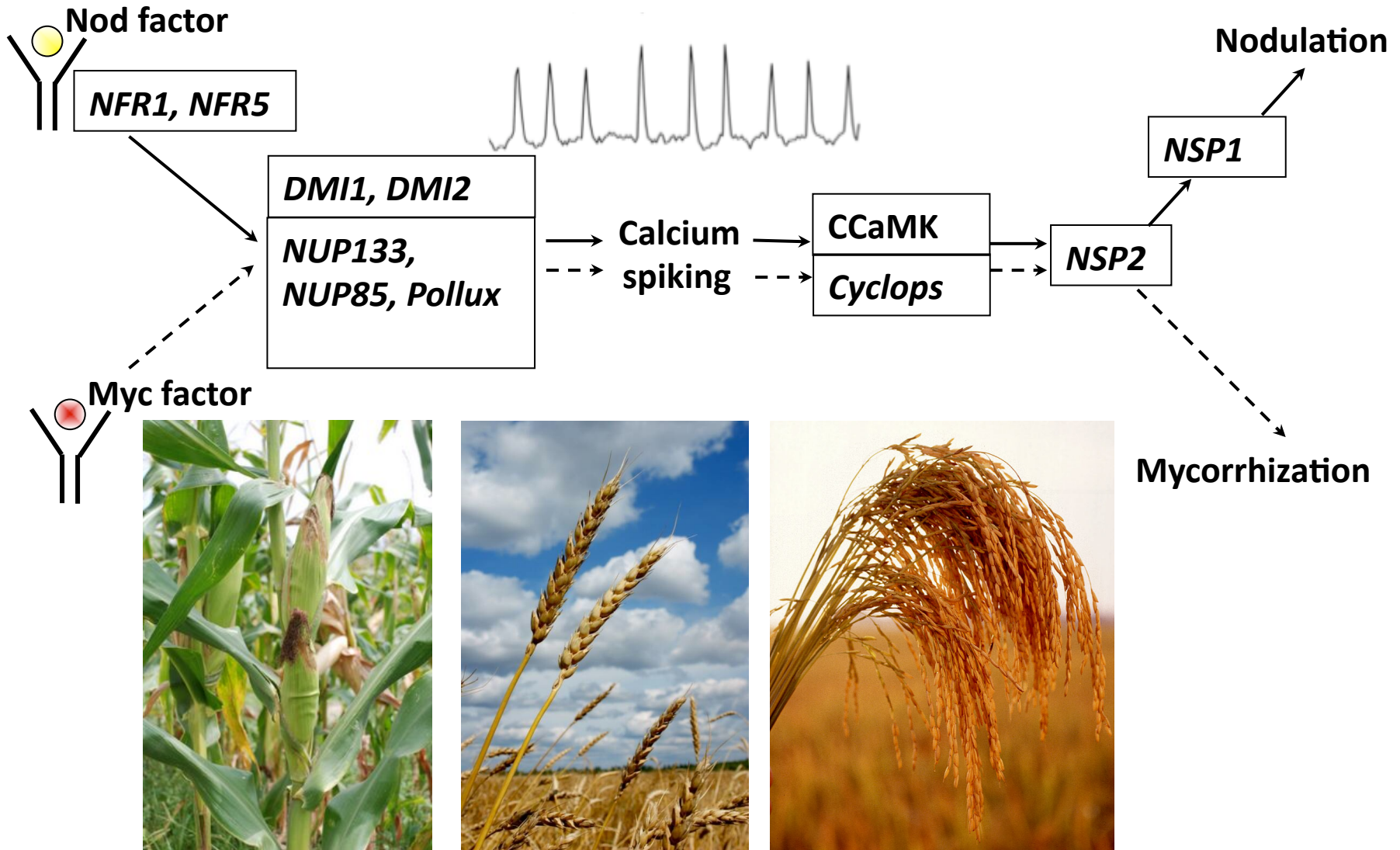
Cereals contain a functional symbiosis signalling pathway



ccamk-1 complementation
with rice CCaMK

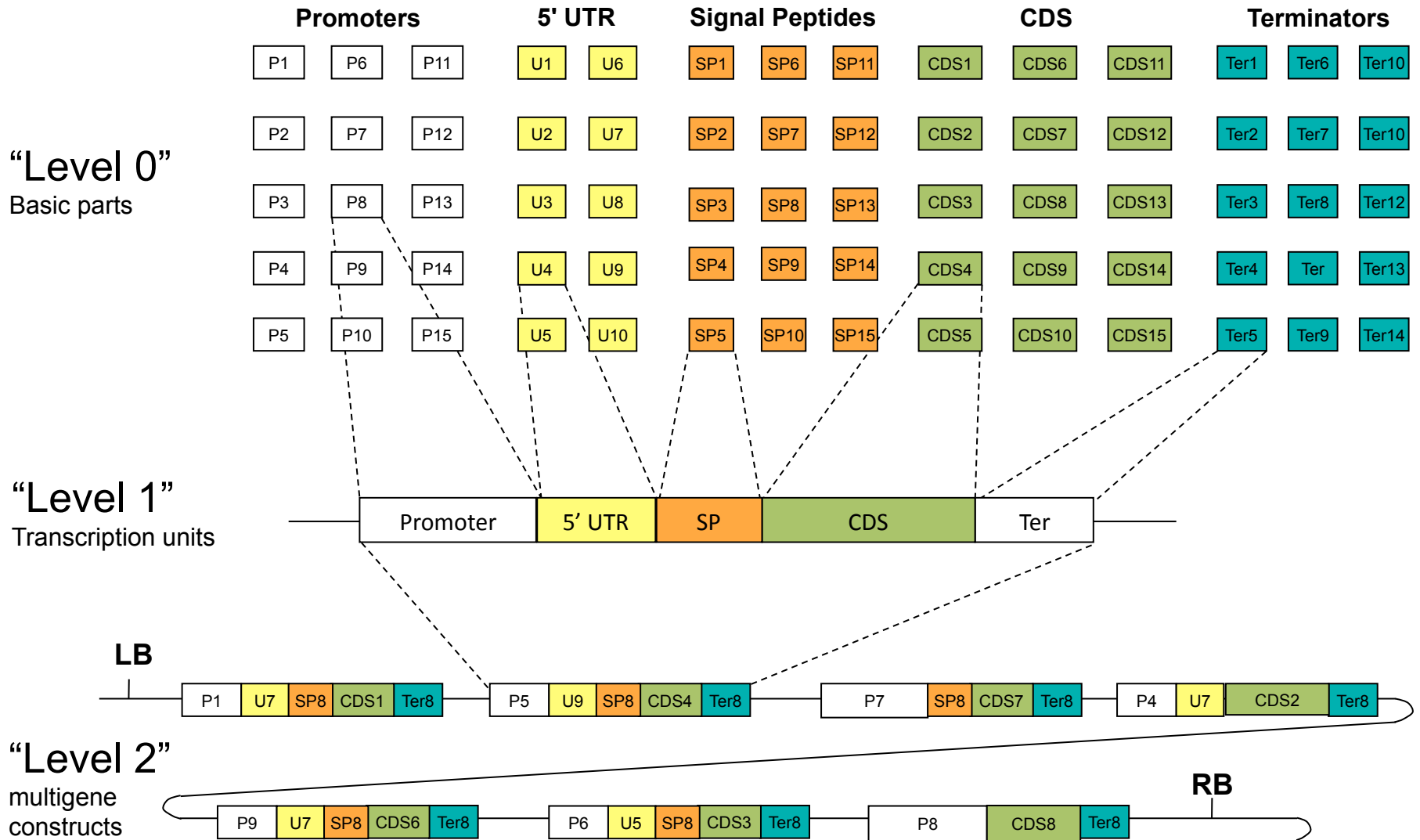


Engineering the symbiosis signaling pathway in cereals



“...redesign existing natural biological systems for useful purposes.”

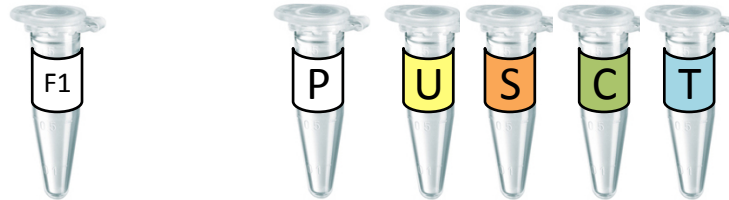
Golden Gate facilitates multi-gene vector construction



The Golden Gate Reaction

Vector backbone

Level 0 Basic Parts



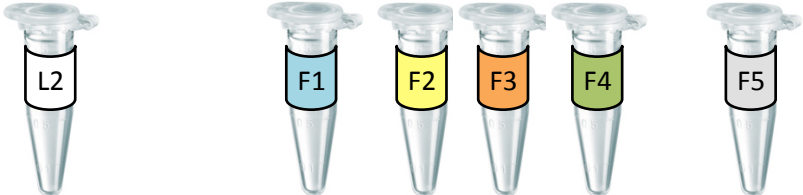
1 tube
*Bsa*I
Ligase
↓
1 hour
↓
Transformation



Creating multigene constructs



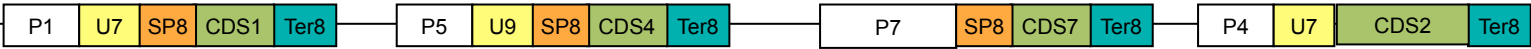
Vector backbone 4 Genes End Linker



1 tube
*Bpi*I
Ligase
↓
1 hour
↓
Transformation



LB

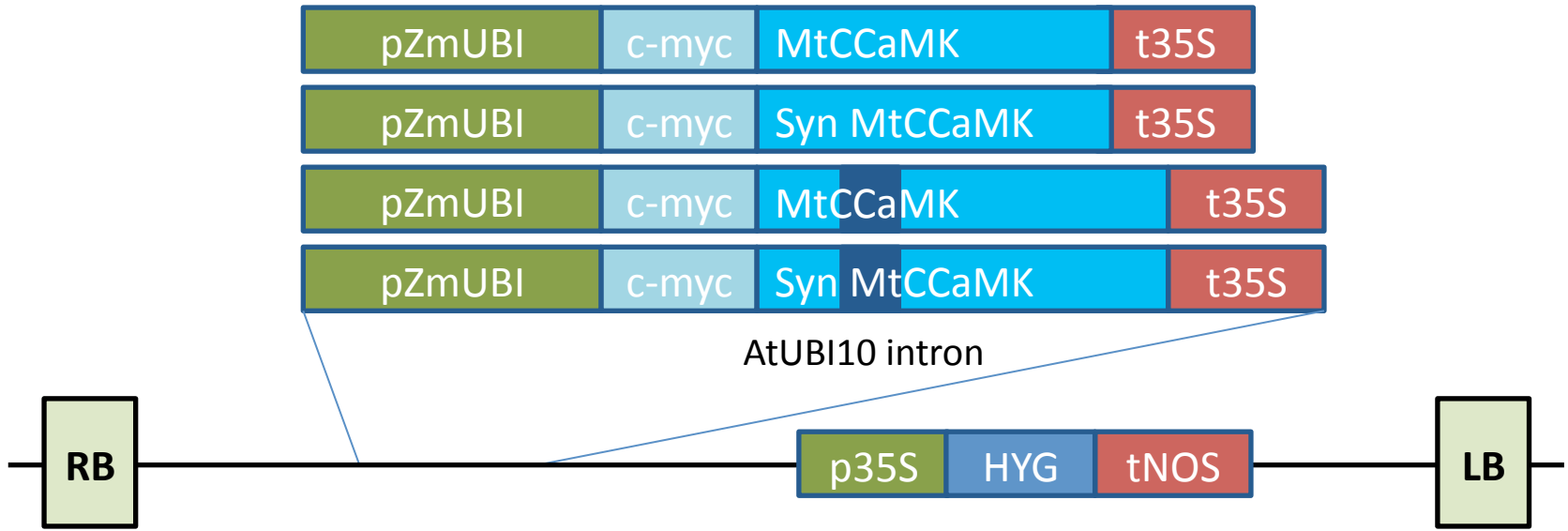


RB



Level 2
multigene
constructs

Creating multigene constructs



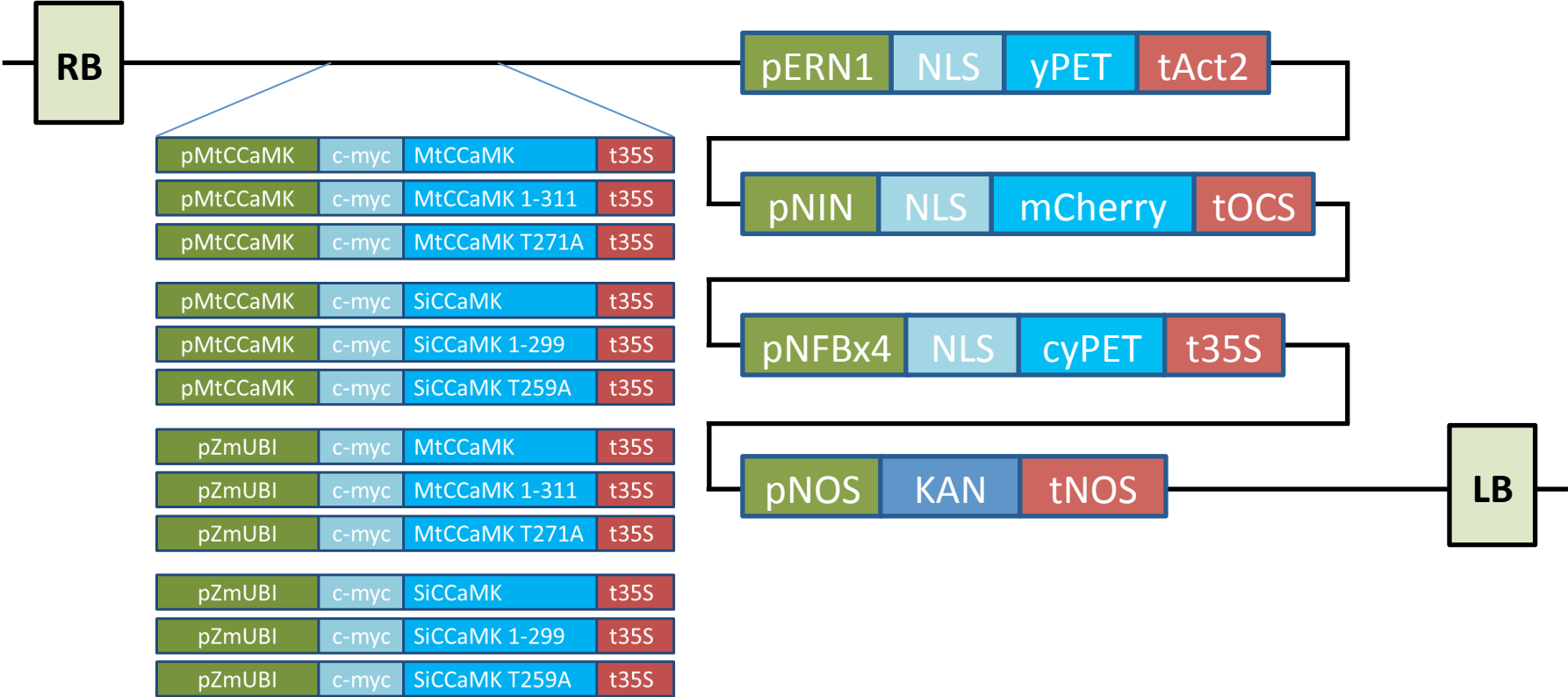
Compare expression levels in Setaria

Creating multigene constructs



PU	S	C	T
pMtCCaMK	c-myc	MtCCaMK	t35S
pMtCCaMK	c-myc	MtCCaMK 1-311	t35S
pMtCCaMK	c-myc	MtCCaMK T271A	t35S
pMtCCaMK	c-myc	SiCCaMK	t35S
pMtCCaMK	c-myc	SiCCaMK 1-299	t35S
pMtCCaMK	c-myc	SiCCaMK T259A	t35S
pZmUBI	c-myc	MtCCaMK	t35S
pZmUBI	c-myc	MtCCaMK 1-311	t35S
pZmUBI	c-myc	MtCCaMK T271A	t35S
pZmUBI	c-myc	SiCCaMK	t35S
pZmUBI	c-myc	SiCCaMK 1-299	t35S
pZmUBI	c-myc	SiCCaMK T259A	t35S

Creating multigene constructs



“Level 0” Basic parts

Promoters			5' UTR		Signal Peptides			CDS			Terminators		
P1	P6	P11	U1	U6	SP1	SP6	SP11	CDS1	CDS6	CDS11	Ter1	Ter6	Ter10
P2	P7	P12	U2	U7	SP2	SP7	SP12	CDS2	CDS7	CDS12	Ter2	Ter7	Ter10
P3	P8	P13	U3	U8	SP3	SP8	SP13	CDS3	CDS8	CDS13	Ter3	Ter8	Ter12
P4	P9	P14	U4	U9	SP4	SP9	SP14	CDS4	CDS9	CDS14	Ter4	Ter	Ter13
P5	P10	P15	U5	U10	SP5	SP10	SP15	CDS5	CDS10	CDS15	Ter5	Ter9	Ter14

Synthesising basic parts:

Single 2kb sequence: ~ £450

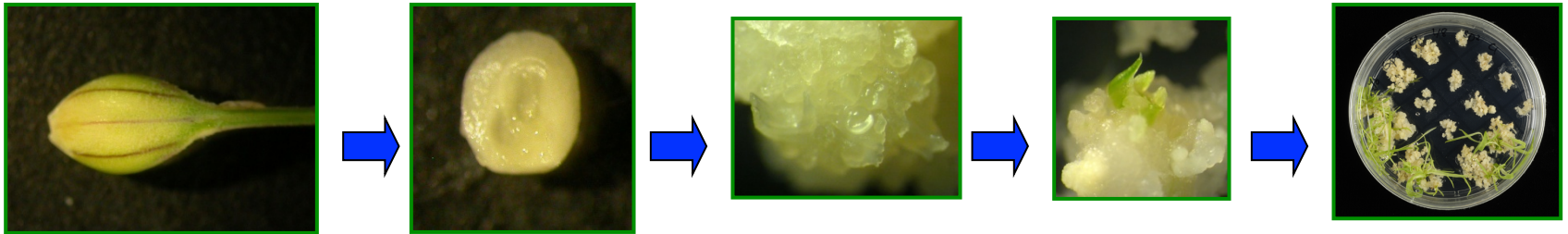
Average cost of a 2kb sequence: £230

9-20 days, subcloned, sequence verified,

DNA prep, glycerol stock.

Transformation has become the limiting factor....

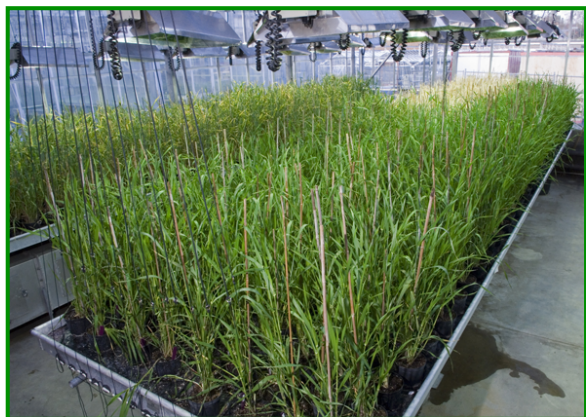
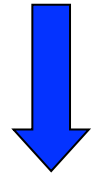
Streamlining transformation



Barley



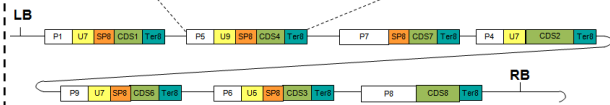
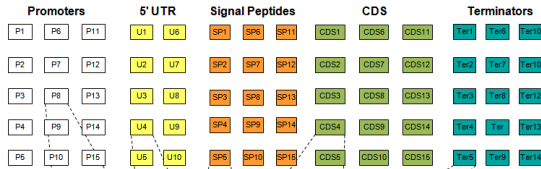
BRACT – **B**iot**e**chnology **R**esources for **A**rable **C**rop **T**ransformation



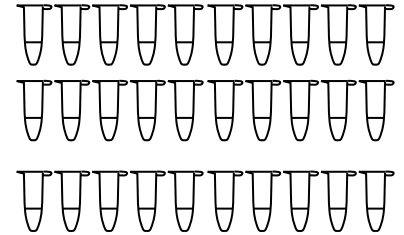
Tracking constructs, milestones and plants



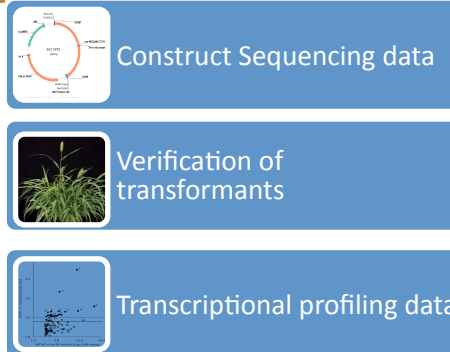
Related Golden Gate constructs



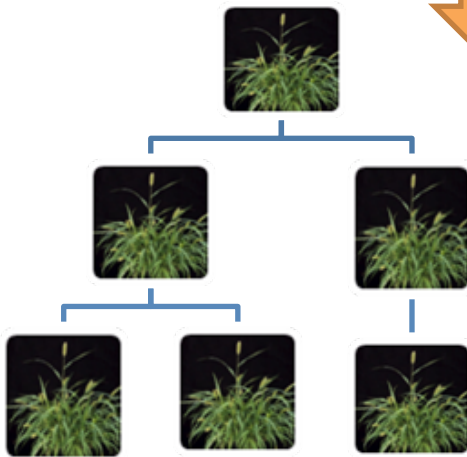
Bacterial stock related to LCOs and constructs



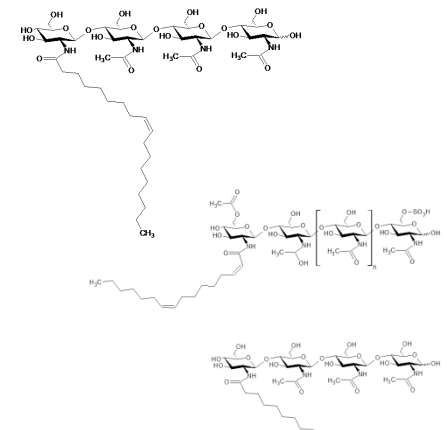
Data Archive



Generations of related transformed plant lines



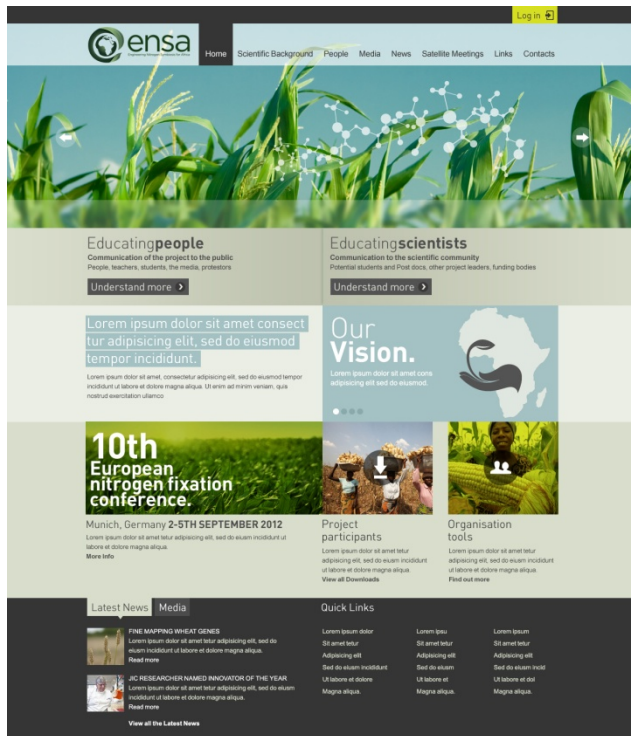
Multiple LCO structures



Tracking constructs, milestones and plants



The ENSA public website



The ENSA project management website



Tracking constructs, milestones and plants



Search | Constructs | Plant Materials | Fileshare | Bacterial Stocks | LCOs

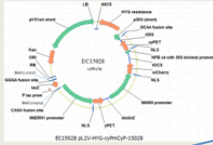
Home » Databases » Constructs » EC15028

Show Help

Edit Record | Delete Record | New Record | Request Construct | View Requests

Construct EC15028

Associate Me



Unique ENSA ID: EC15028
ENSA Standard Name: pL2V-HYG-cyPmCyP-15028
Original Name: pL2V-HYG-cyPmCyP-15028
Bacterial Antibiotic Selection: Kan
Bacterial Visual Marker: LacZ

Vector NTI files

General Description: This is a Level 2-1 intermediate vector containing the LacZ endlinker in Position 5 for downstream Golden Gate cloning. It already contain modules for HYG selection, and three fluorescent reporters for modulation signalling.

Backbone Description: The backbone is the Level 2 vector (EC50505) possessing Kan bacterial resistance.

Vector inserts Description: The insert possesses the Hyg selection (EC15030) the pNFB-cyPET reporter (EC15031) The pERN1-yPET reporter (EC15033) and the LacZ end Linker pL1F-4 (EC47761)

Golden Gate Cloning Details

Golden gate component: Level 2 Cloning vector

Cloning components into this golden gate construct

5' Fusion Site	GGAG	Enzyme	BsaI
5' Fusion Site	CGCT	Enzyme	BsaI

Cloning downstream of this golden gate construct
This construct cannot be used directly in downstream Golden Gate constructs.

Constructs (5)

EC87633	PL0M-PU-Nos-TMV-1 - piCH87633	Associate Me	View
EC86977	pL15C-BASTA - piCH86977	Associate Me	View
EC86966	pL1F2-Kan - piCH86966	Associate Me	View
EC84011	pL1M-ELR-7 - piCH84011	Associate Me	View
EC41780	pL1M-ELE-4 - piCH41780	Associate Me	View

Objective Milestones (2)

Participants (3)

Plant Materials (2)

Bacterial Stocks (0)

LCOs (0)

Fileshare Folders (1)

Vector maps and sequence data

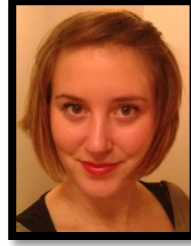
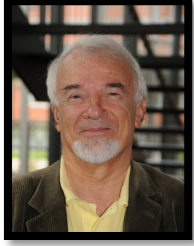
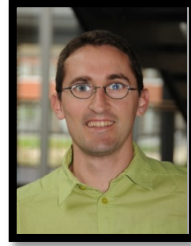
Searchable database with integrated request system

Breakdown of Golden Gate Components

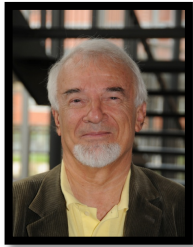
Links to:

1. Transformed plants
2. Sequencing data
3. Authorship
4. Experimental data

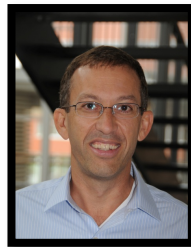
Current ENSA participants



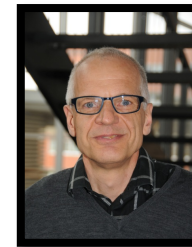
Acknowledgements



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Grenoble
Sébastien Fort



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Tom Brutnell
Hui Jiang
Quan Zhang



University of Aarhus
Jens Stougaard
Simona Radutoiu
Lene Madsen
Mickael Blaise
Dugald Reid
Ei-ici Murakami
Estelle Marchal



JIC
Giles Oldroyd
Christian Rogers
Jongho Sun

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Doug Cook
Michael Udvardi
Sharon Long



University of Wisconsin Madison
Jean-Michel Ané
Matt Crook



Ben Miller
Andrey Korolev
Eleni Soumpourou
Andy Breakspear

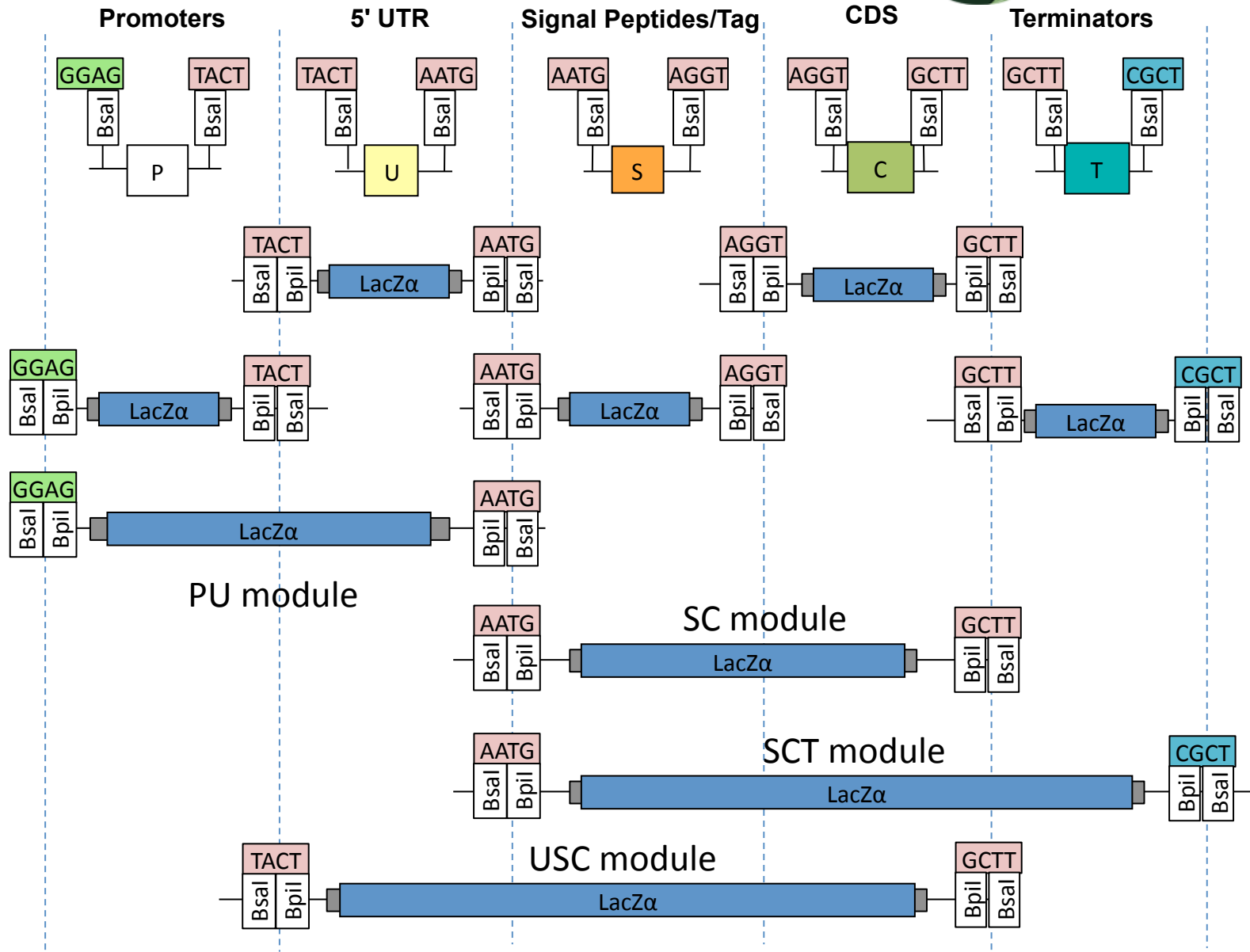
Bill & Melinda Gates Foundation

Katherine Kahn
Rachel Malinen
Nicole Lackey

BRACT

Wendy Harwood
Mark Smedley

The structure of Golden Gate is flexible allowing any number of parts to be combined



The complete set of modules allows ultimate flexibility in creating and modifying multi-gene constructs



Level 1 Vectors

