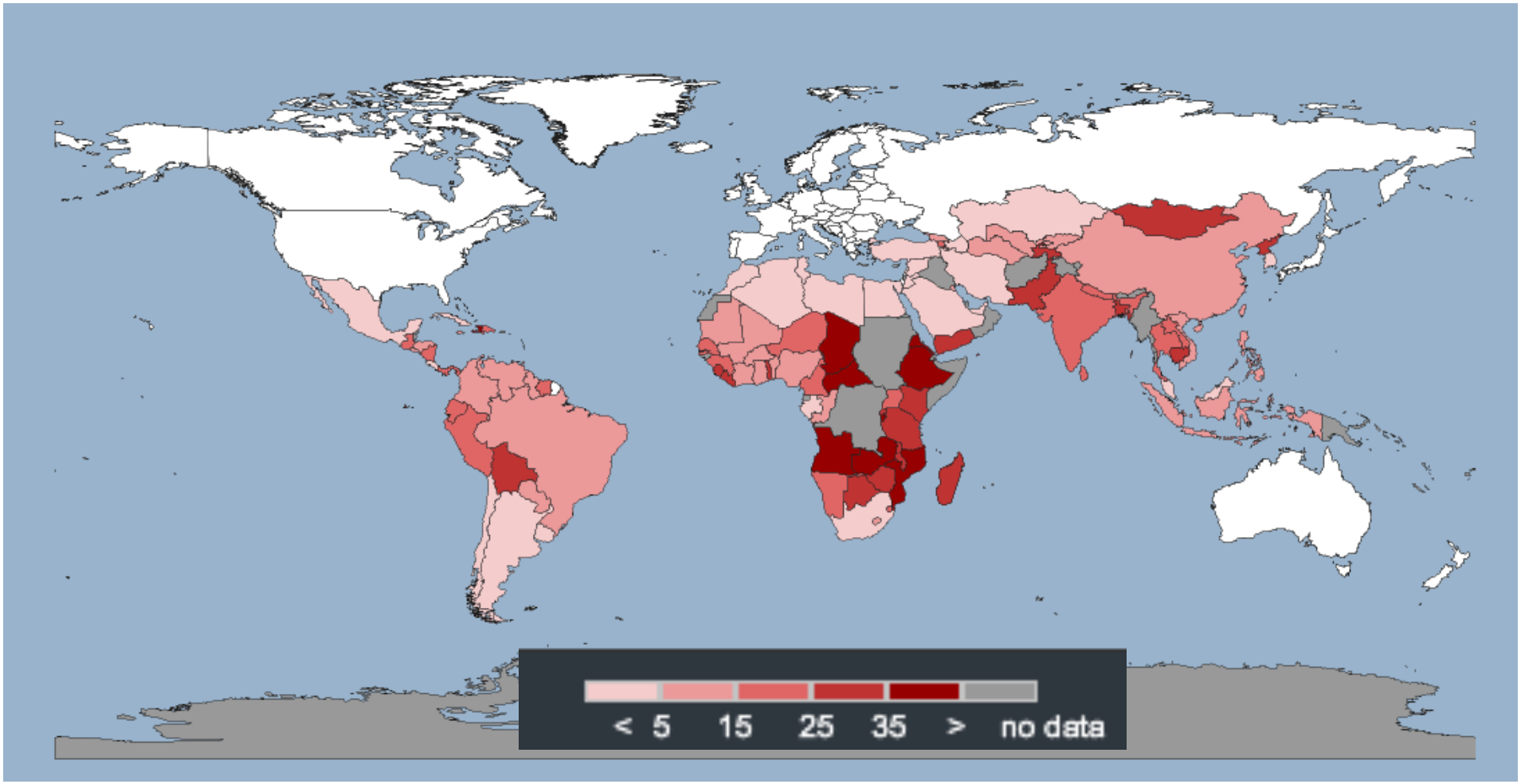
Engineering the Nitrogen Symbiosis for Africa

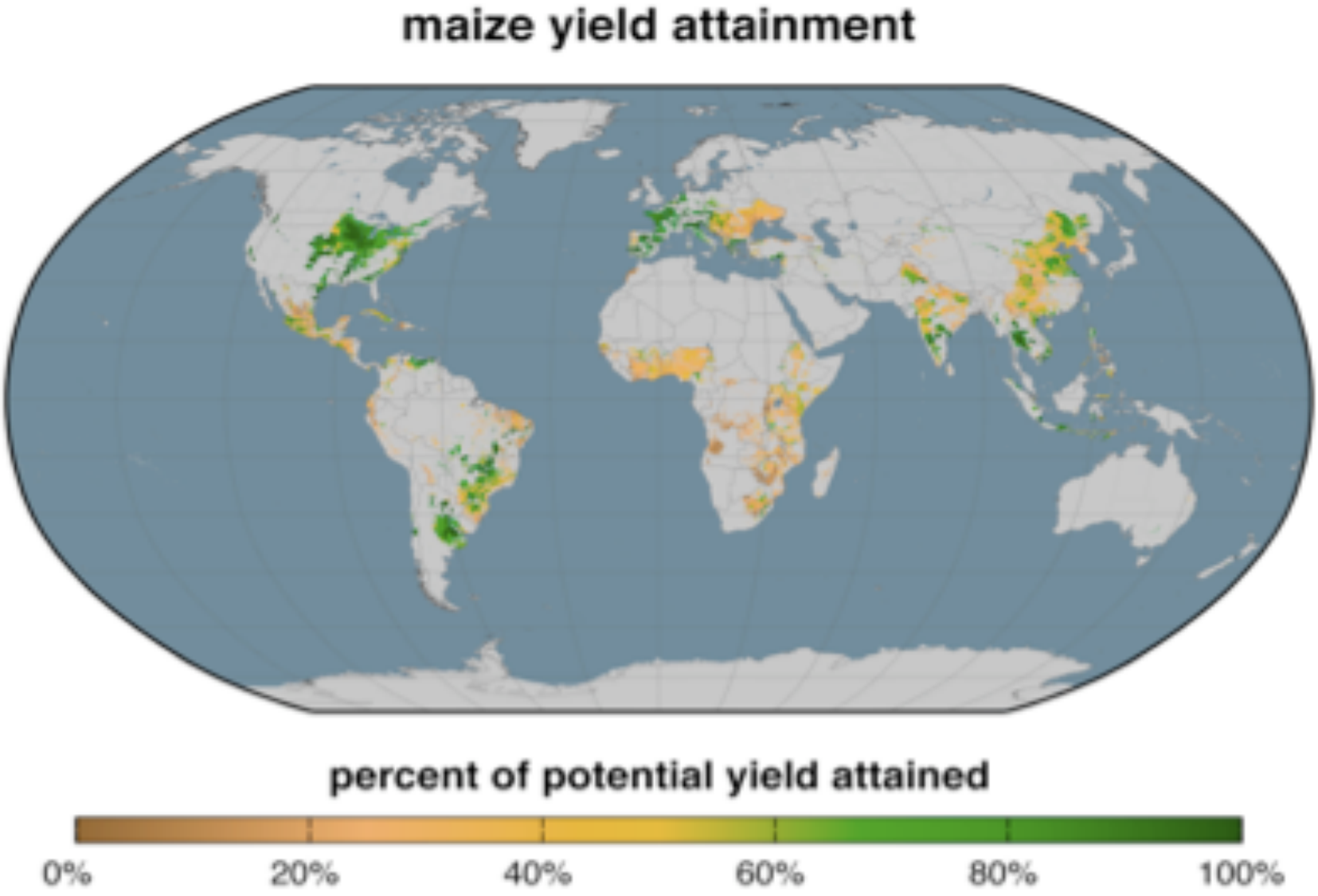
>1 billion people with food insecurity

Prevalence of Undernourishment in Total Population (%)



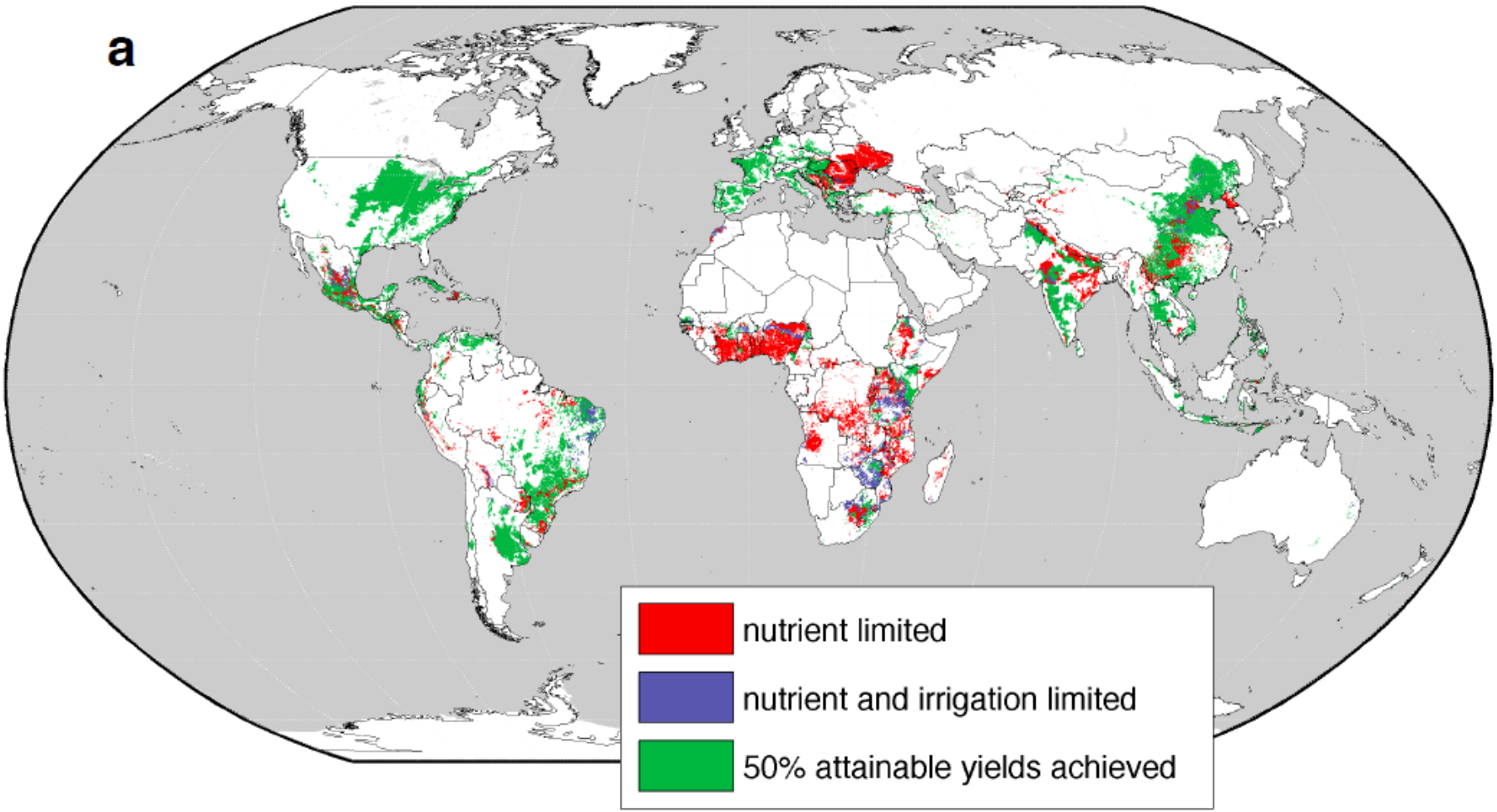
FAO STAT 2008

Crop yields in Sub-saharan Africa are below potential



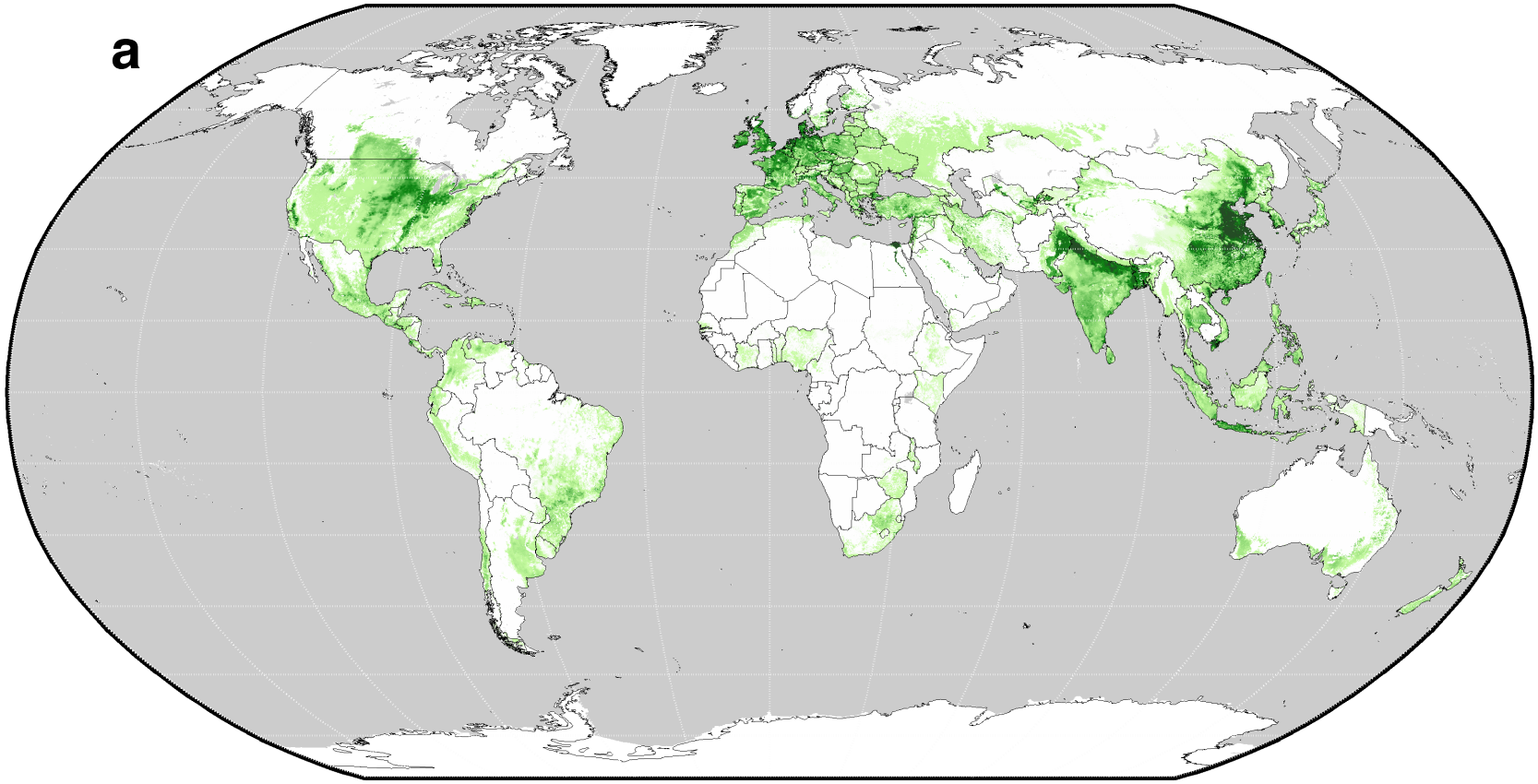
Foley et al. 2011, *Nature*

Nitrogen availibility Limits Yields in Africa



Mueller et al 2012, *Nature*

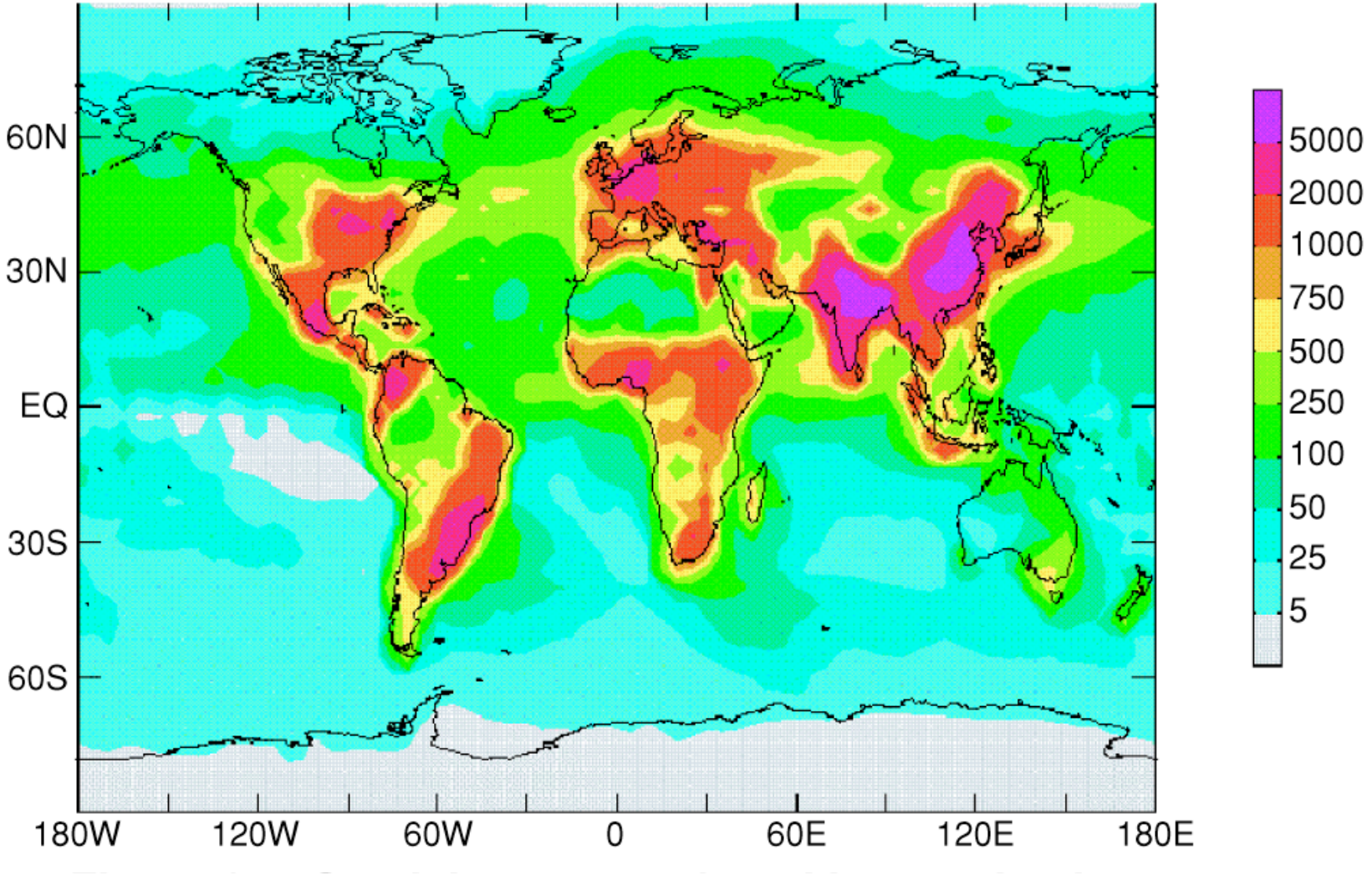
Fertiliser use is very low in Africa



Mueller et al 2012, *Nature*

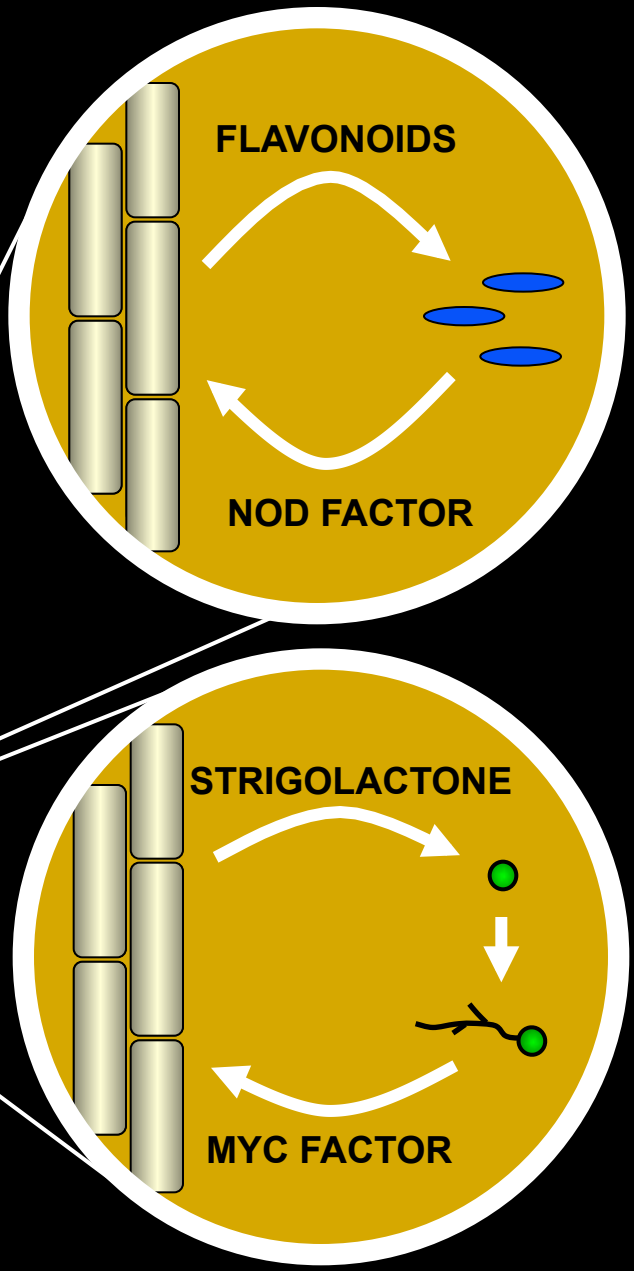
Projections for inorganic nitrogen deposition

2050

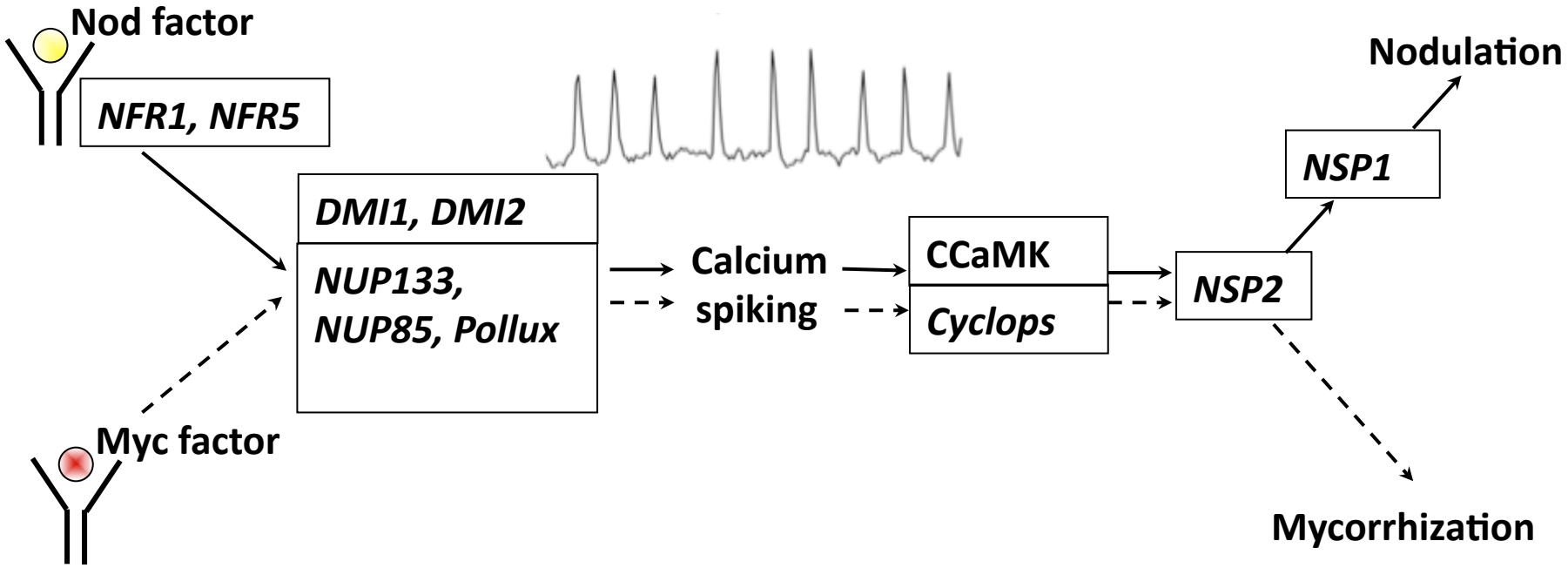


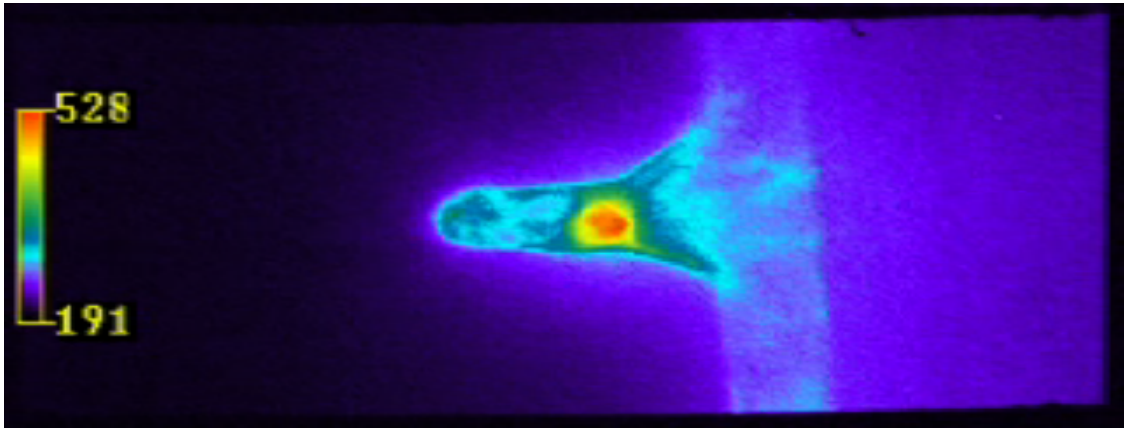
Galloway et al. Biogeochemistry 70: 153–226, 2004

The early steps in symbiosis are a molecular dialogue



The Symbiosis signalling pathway





Symbiotic N-fixation evolved from the mycorrhizal symbiosis

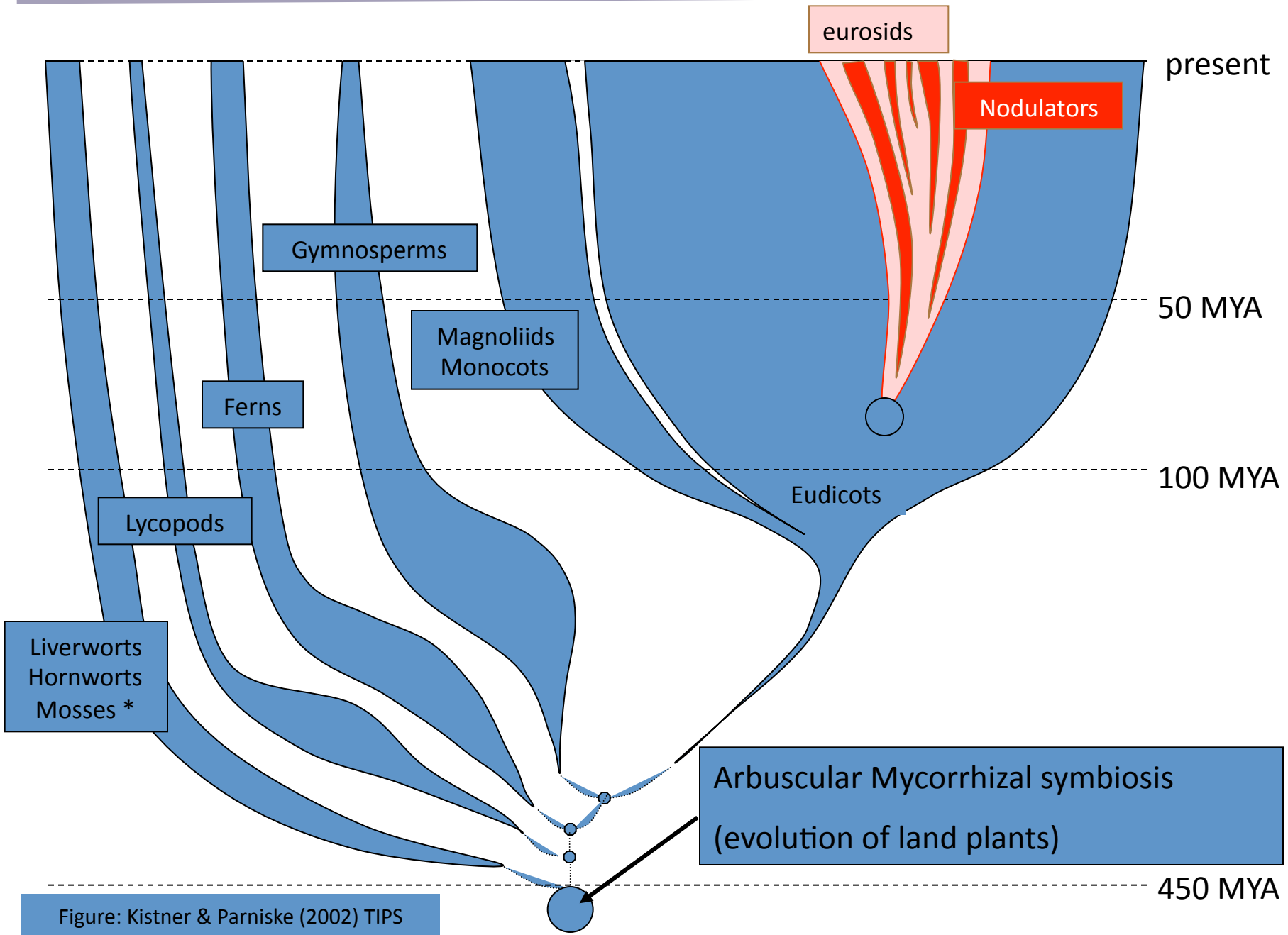
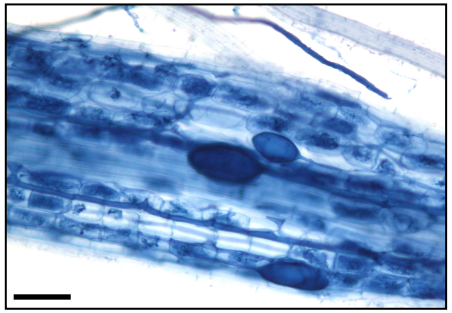


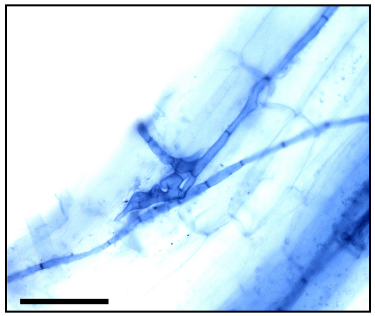
Figure: Kistner & Parniske (2002) TIPS

Rice symbiosis signalling mutants

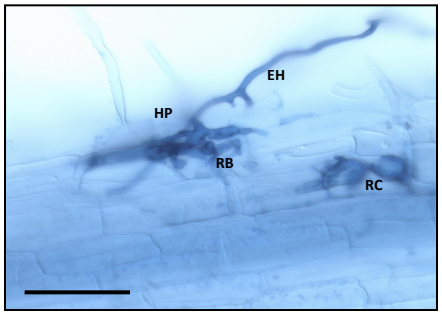
WT



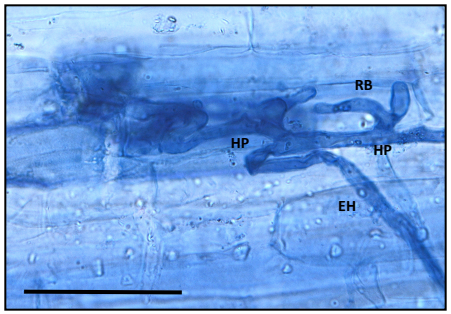
Rice *common sym* mutants



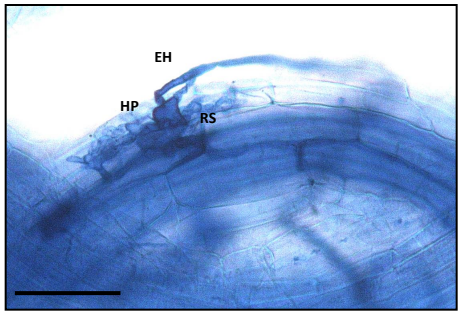
castor-1



pollux-1



ccamk-2



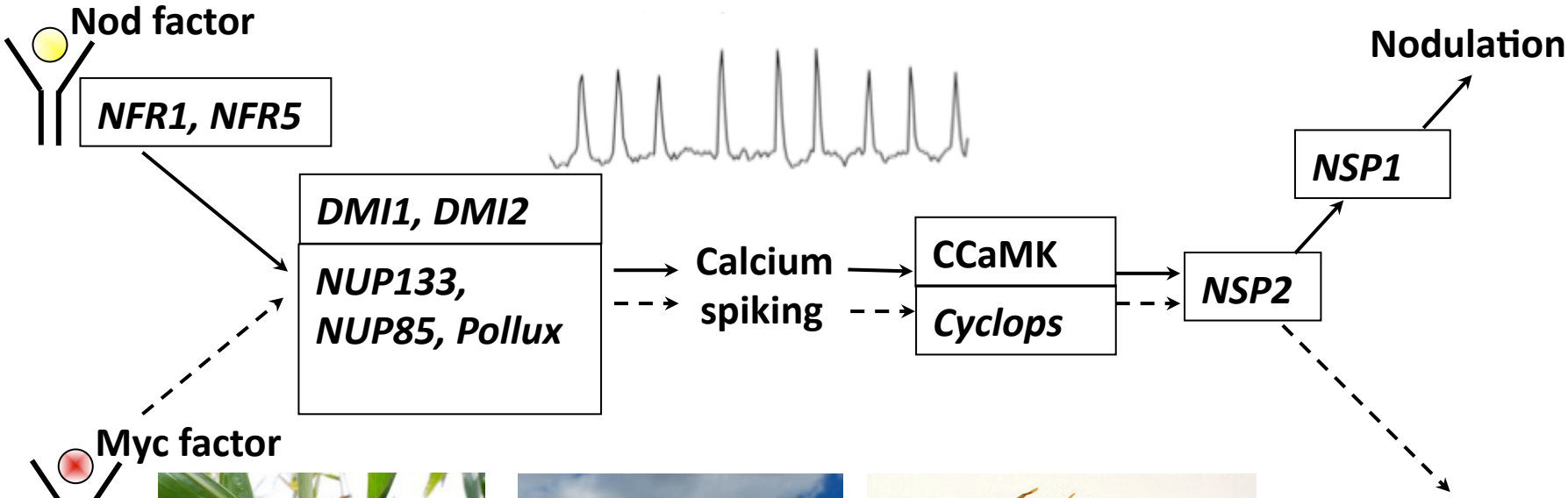
cyclops-3

size bars = 50 µm

Gutjahr et al. 2008, Plant Cell

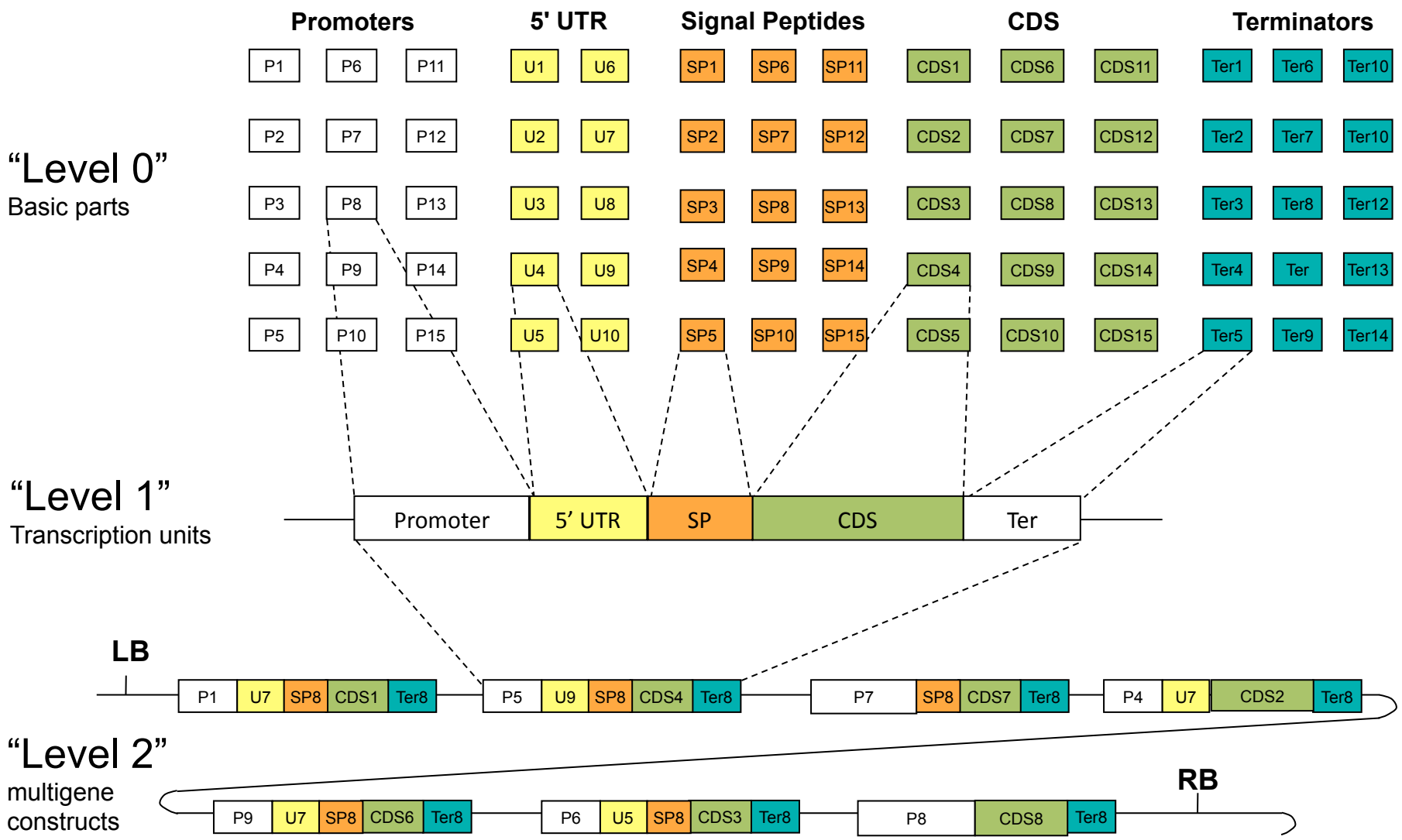
Cereals contain a functional symbiosis signalling pathway

Engineering the symbiosis signaling pathway in cereals



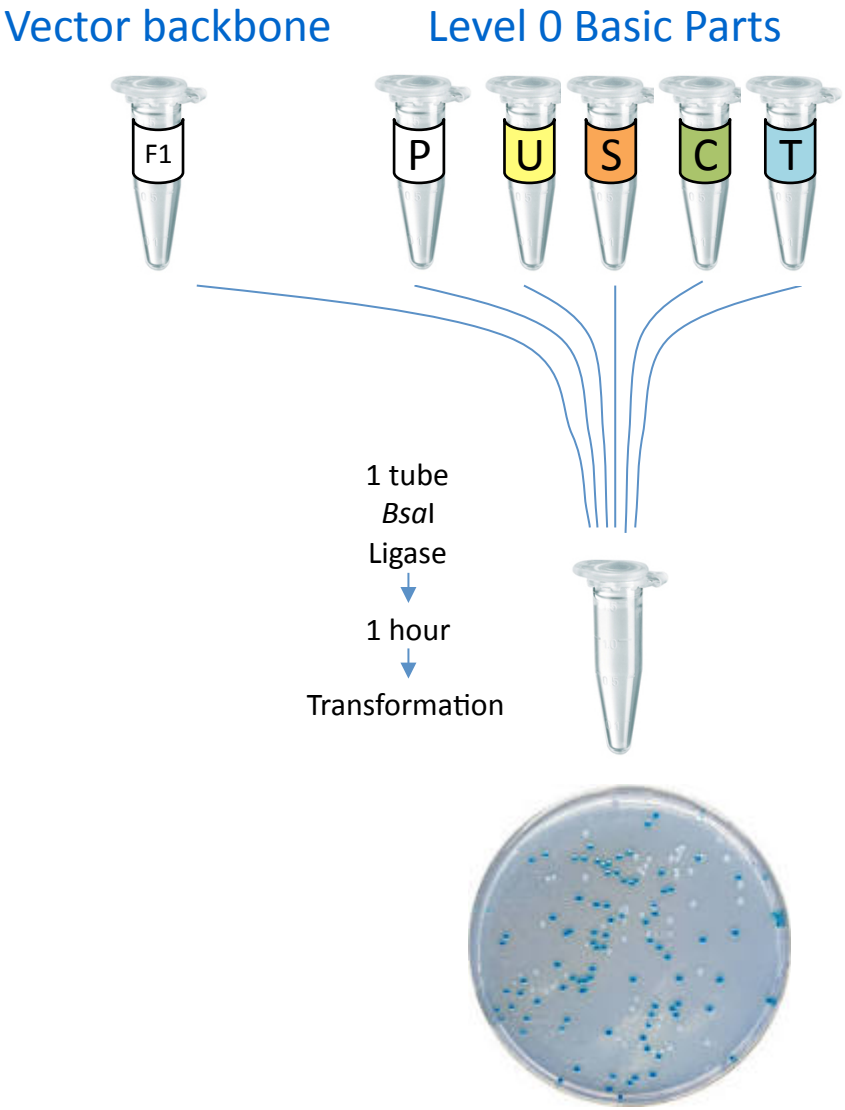
"…redesign existing natural biological systems for useful purposes."

Golden Gate facilitates multi-gene vector construction

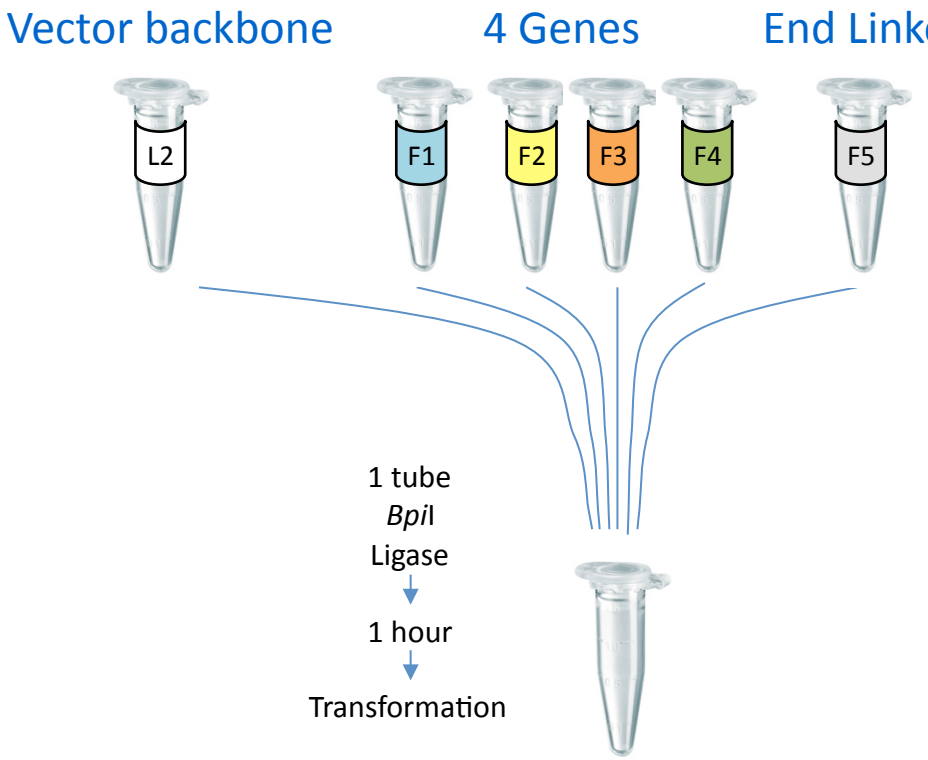


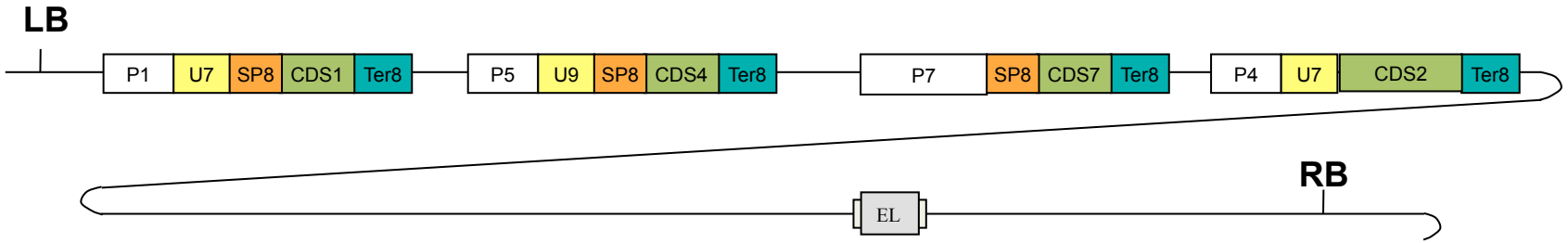
Weber 2011, *PloSone*

The Golden Gate Reaction



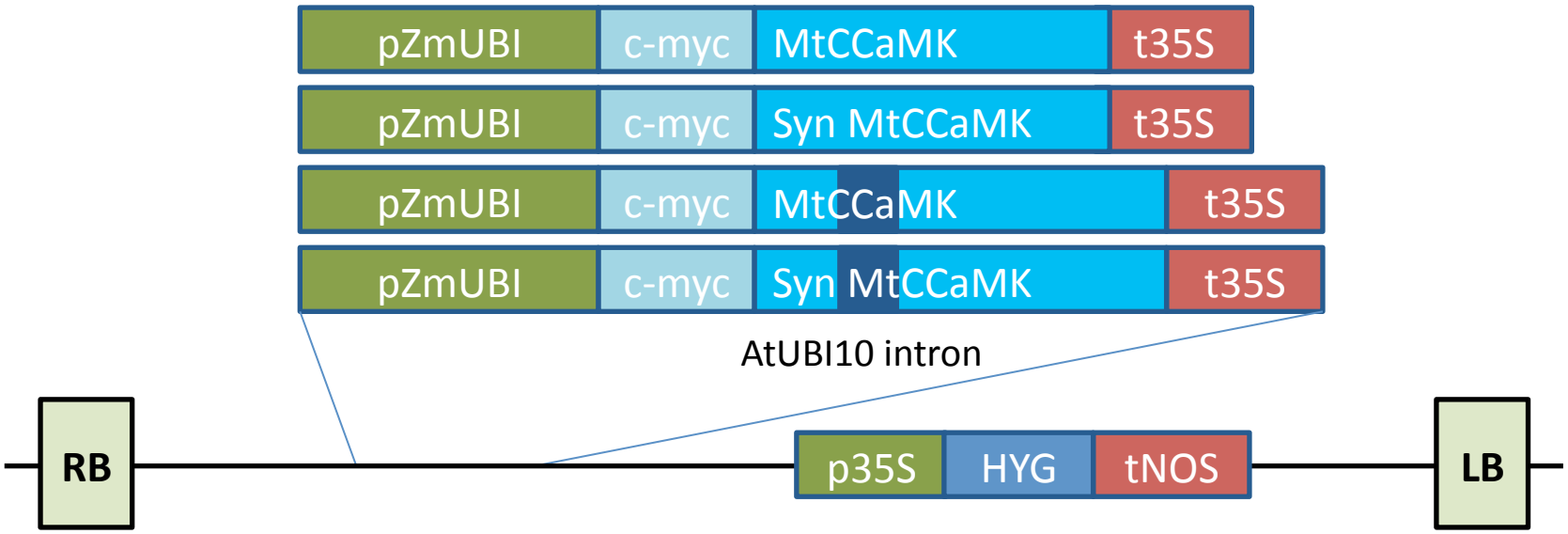
Creating multigene constructs





Level 2 multigene constructs

Creating multigene constructs

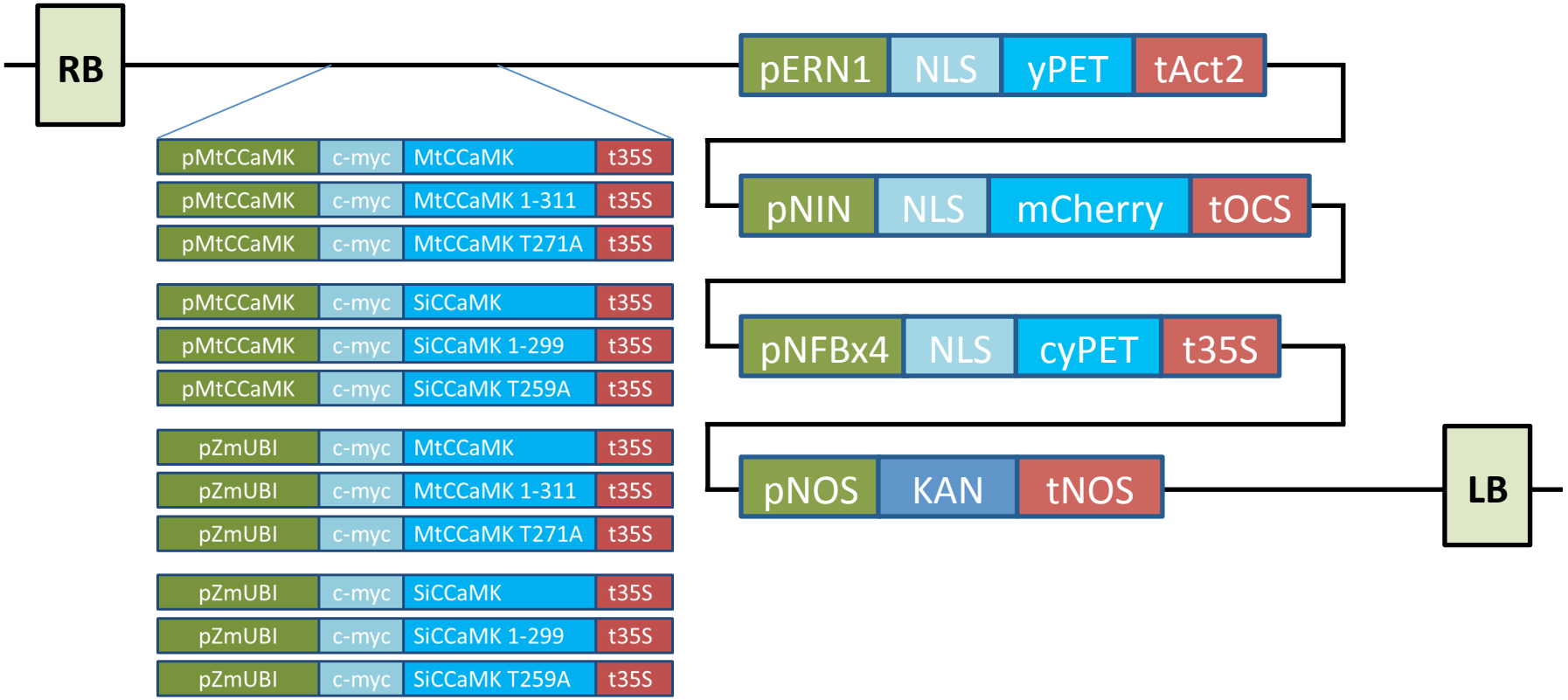


Compare expression levels in Setaria

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



ENSA project constructs: numbers and costs

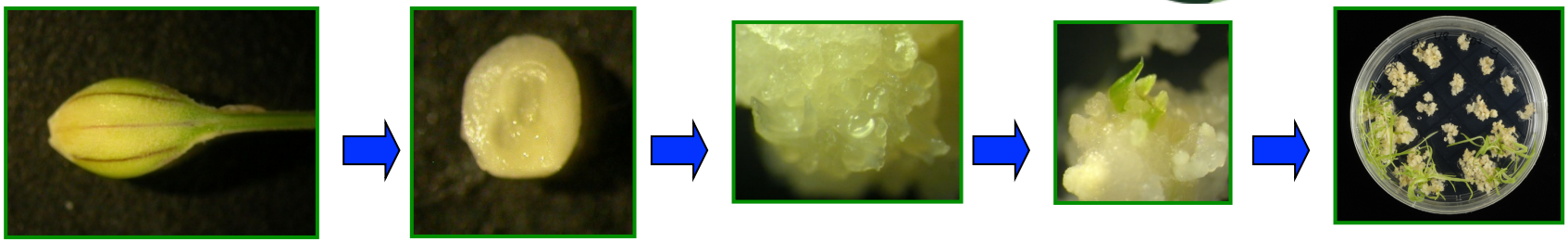
"Level 0" Basic parts

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.

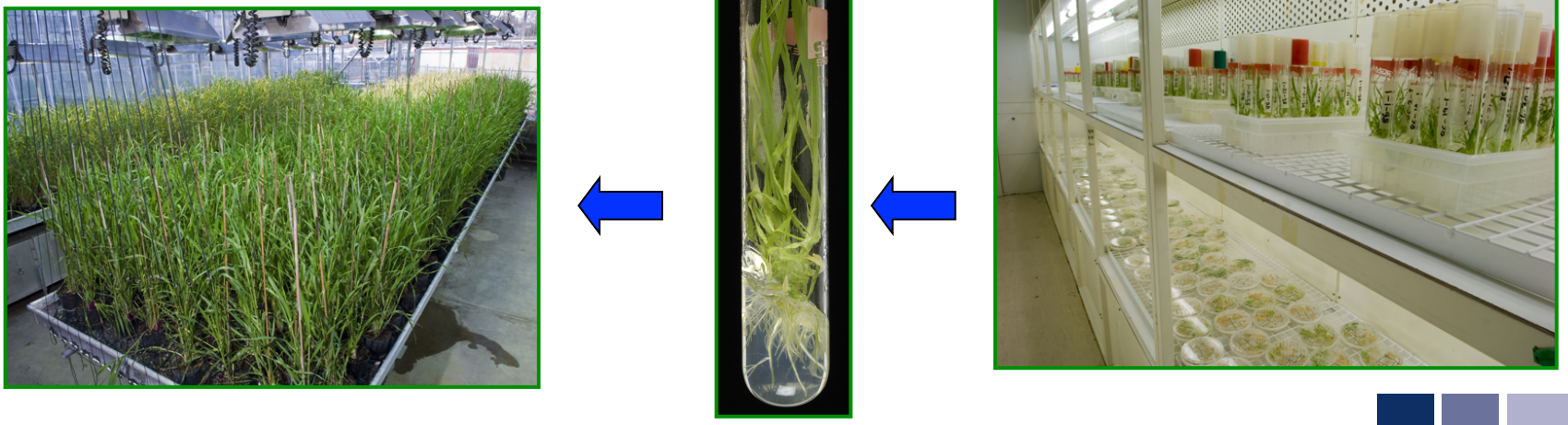
Transformation has become the limiting factor....

Streamlining transformation



Barley

BRACT – Biotechnology Resources for Arable Crop Transformation

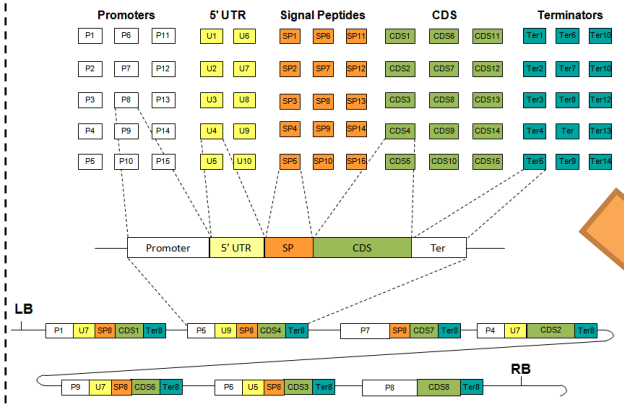


Wendy Harwood

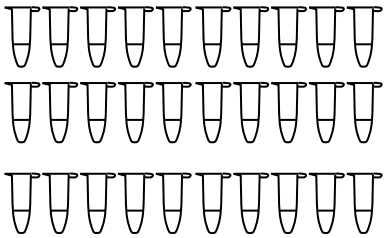
John Innes Centre

Tracking constructs, milestones and plants

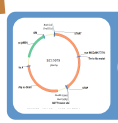
Related Golden Gate constructs



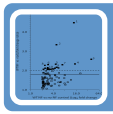
Bacterial stock related to LCOs and constructs

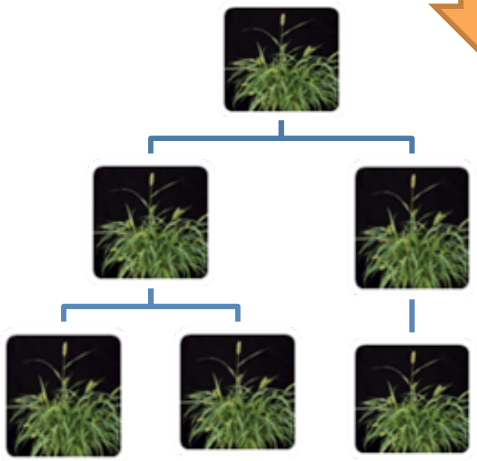


Data Archive

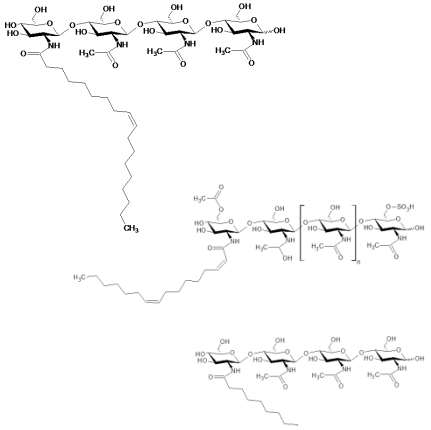

Construct Sequencing data


Verification of transformants


Transcriptional profiling data



Generations of related transformed plant lines



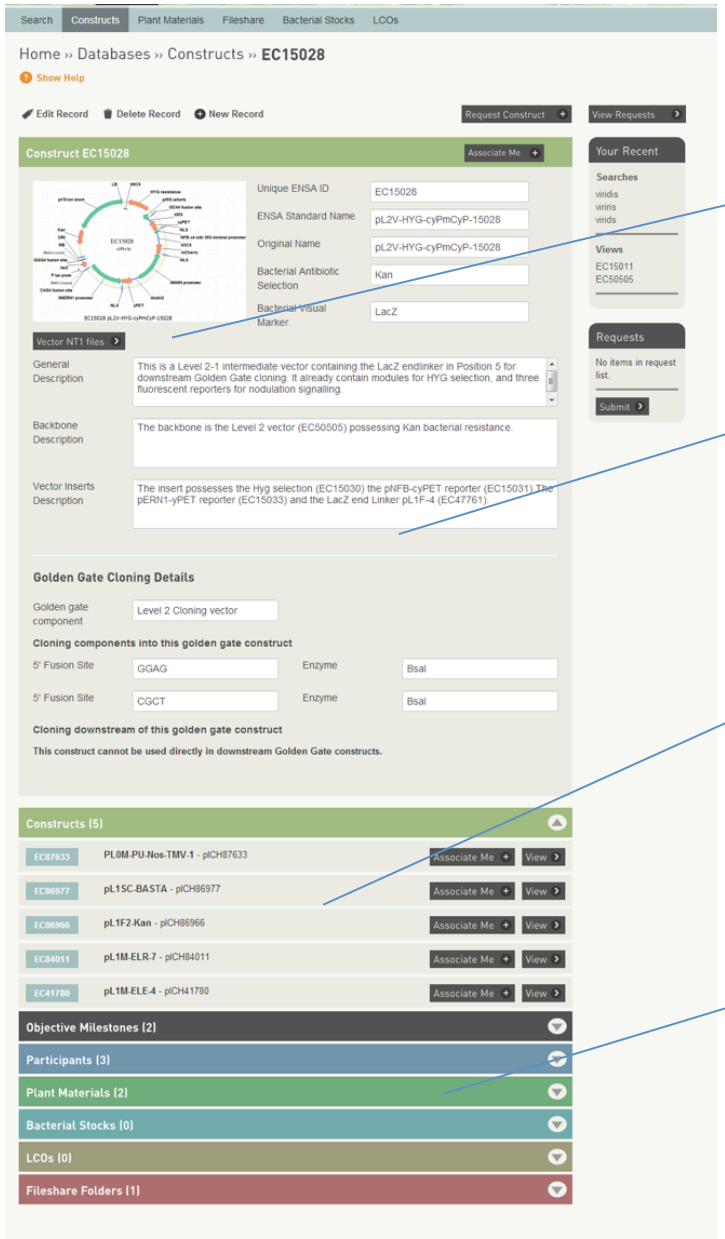
Multiple LCO structures

Tracking constructs, milestones and plants

The ENSA public website

The ENSA project management website

Tracking constructs, milestones and plants



Vector maps and sequence data

Searchable database with integrated request system

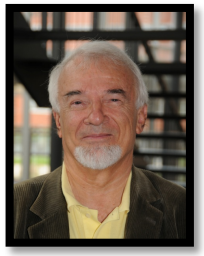
Breakdown of Golden Gate Components

Links to:

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

Current ENSA participants

Acknowledgements



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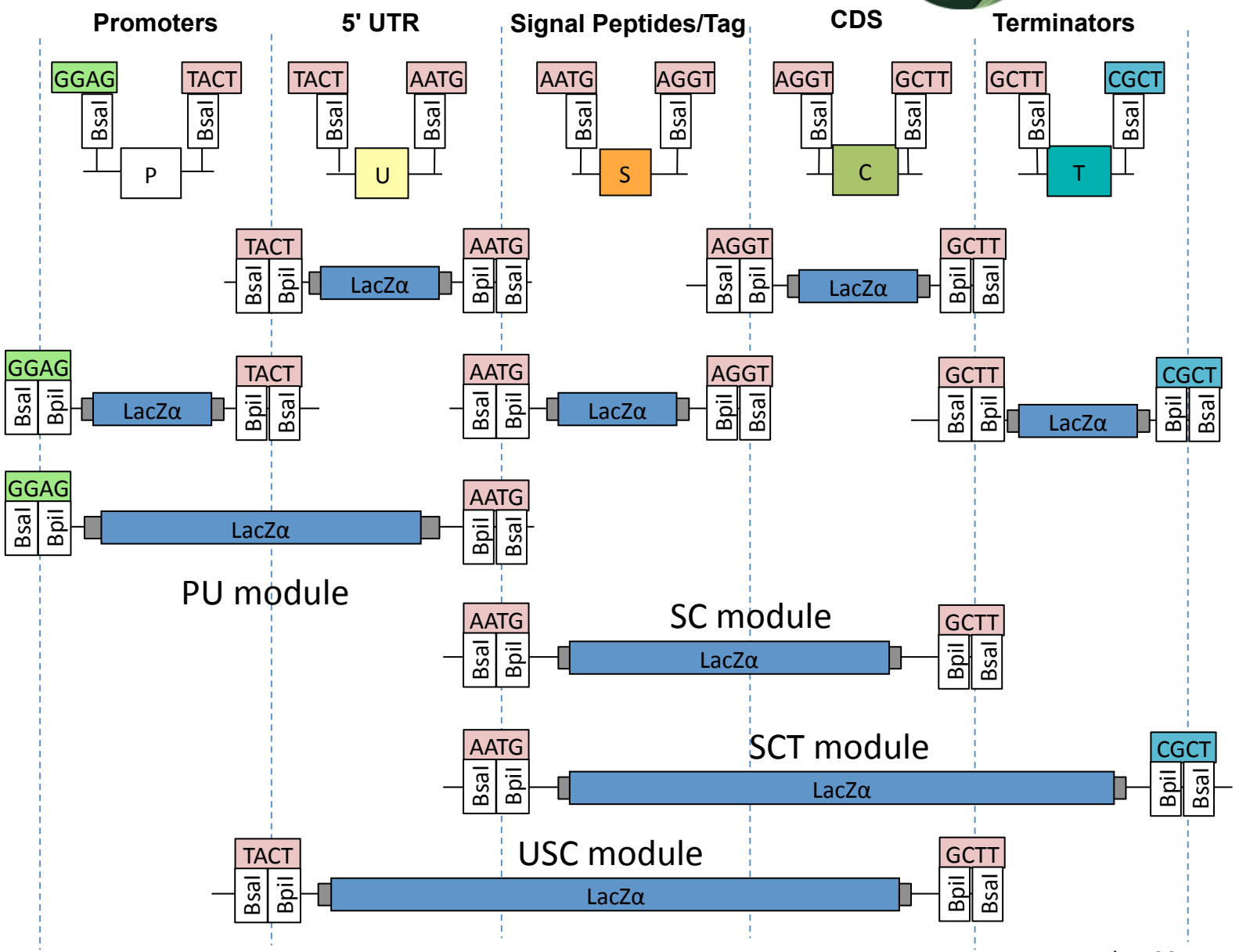
BRACT
Wendy Harwood
Mark Smedley

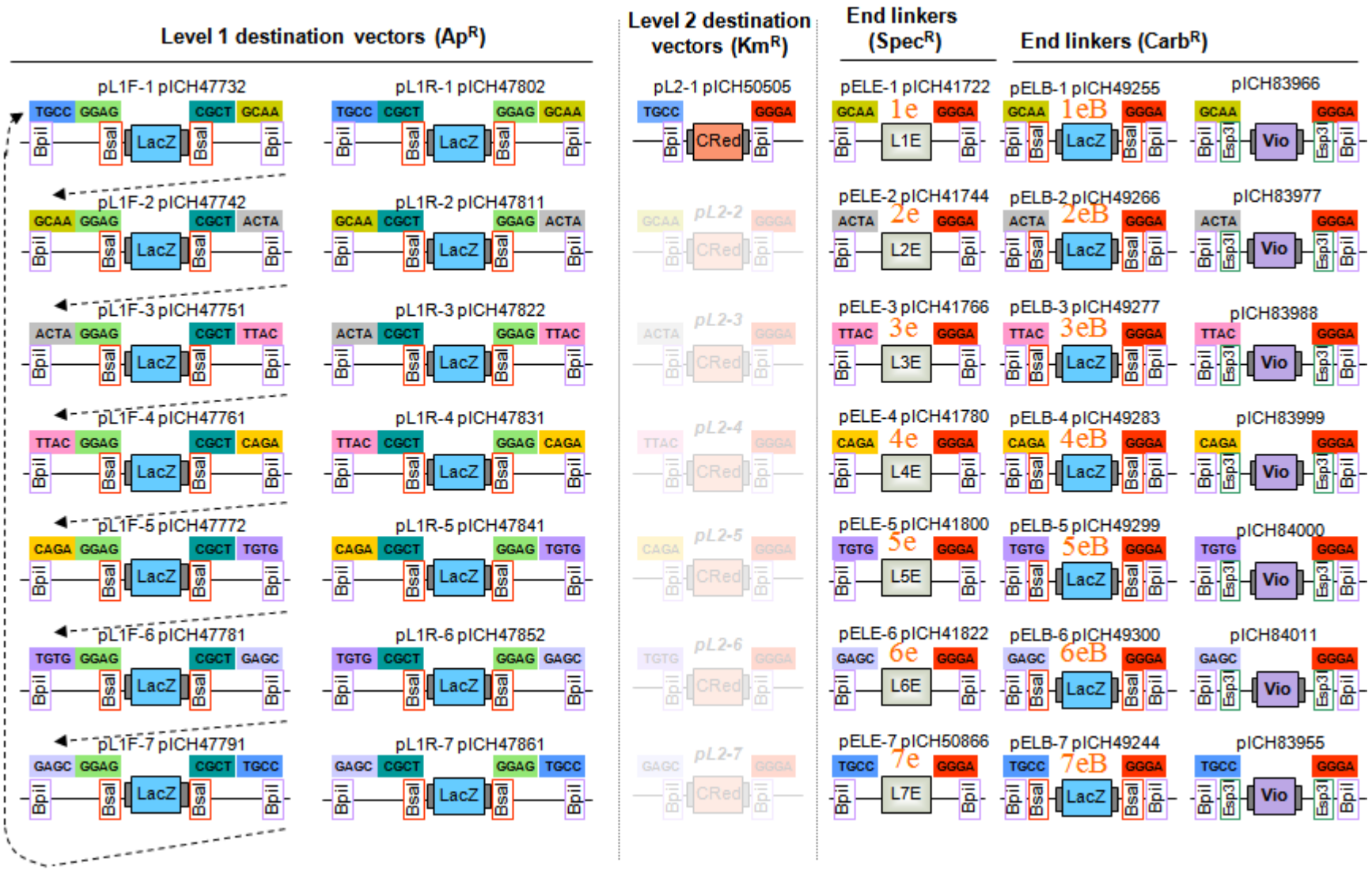
The structure of Golden Gate is flexible allowing any number of parts to be combined



Weber 2011, *PLoSone*

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

Level 1 Vectors



Weber 2011, *PLoSone*