Interactive Series for Plant Transformation Protocols

Plant GENE

Episode 2:

Guide to *Agrobacterium* Basics

- Storage
- Quality Control
- Introducing plasmids

AUGUST 6th
11 AM ET/8 AM PT

Participants

Joyce Van Eck, BTI
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zoom

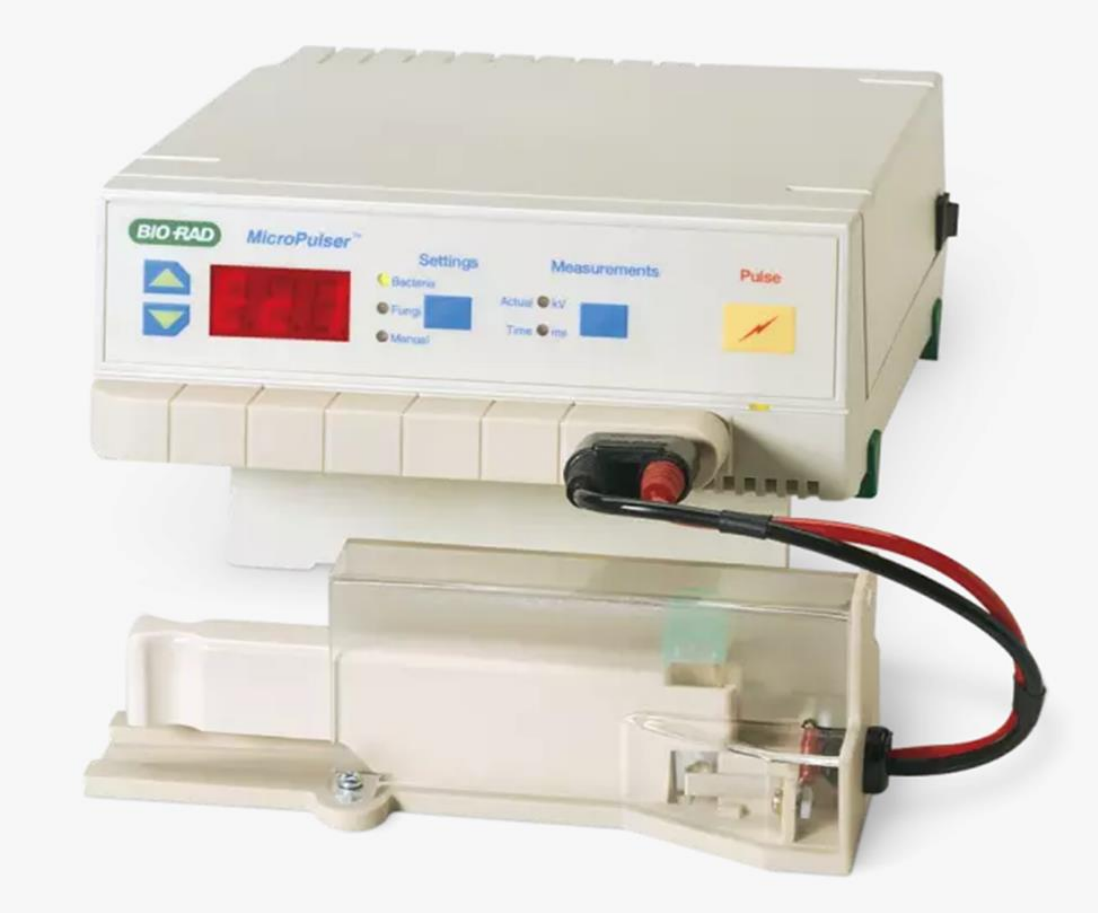
Meeting ID:
924 2219 4763
Passcode: 039150

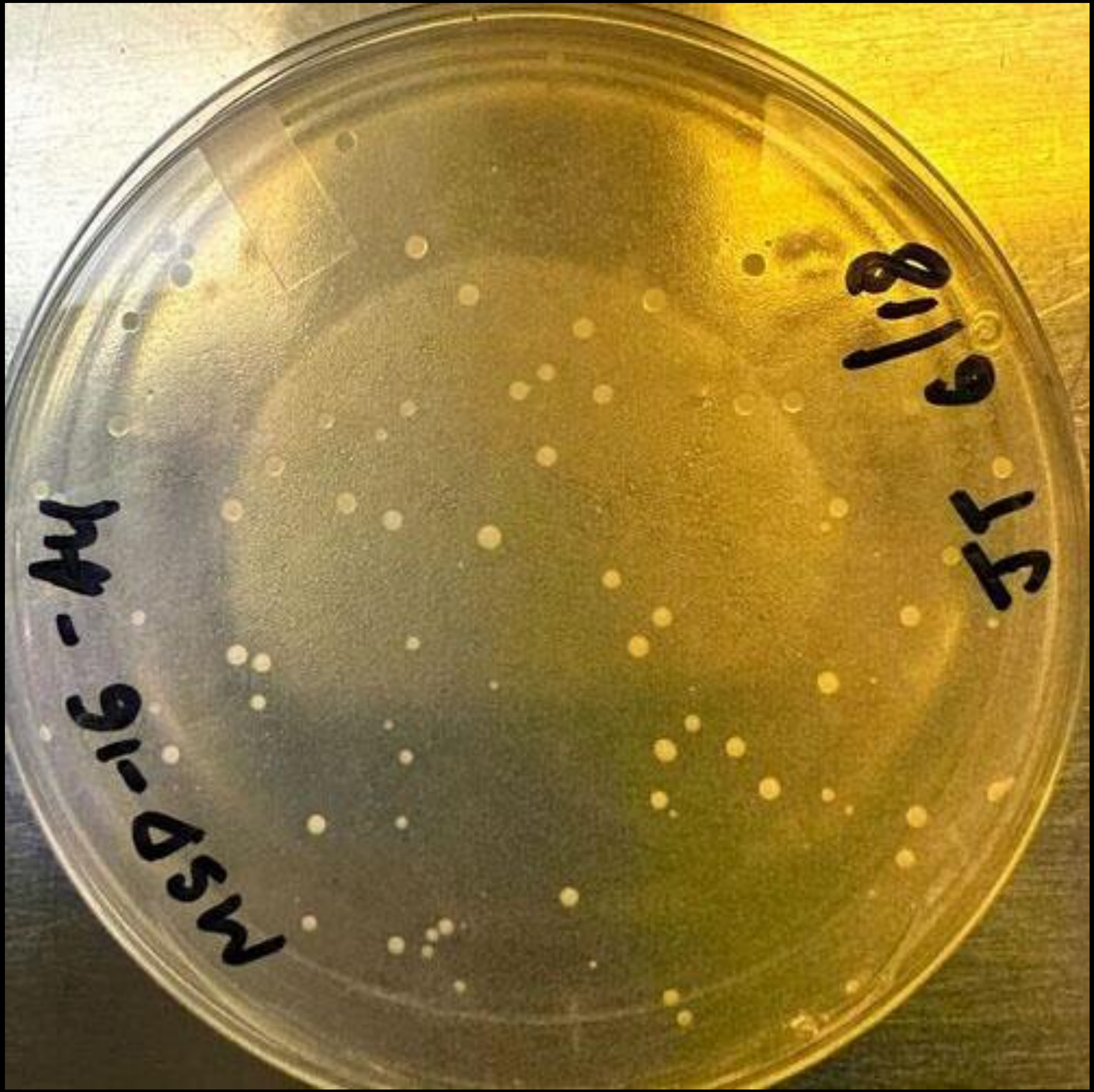
Transformation: Introducing new constructs into *Agrobacterium*

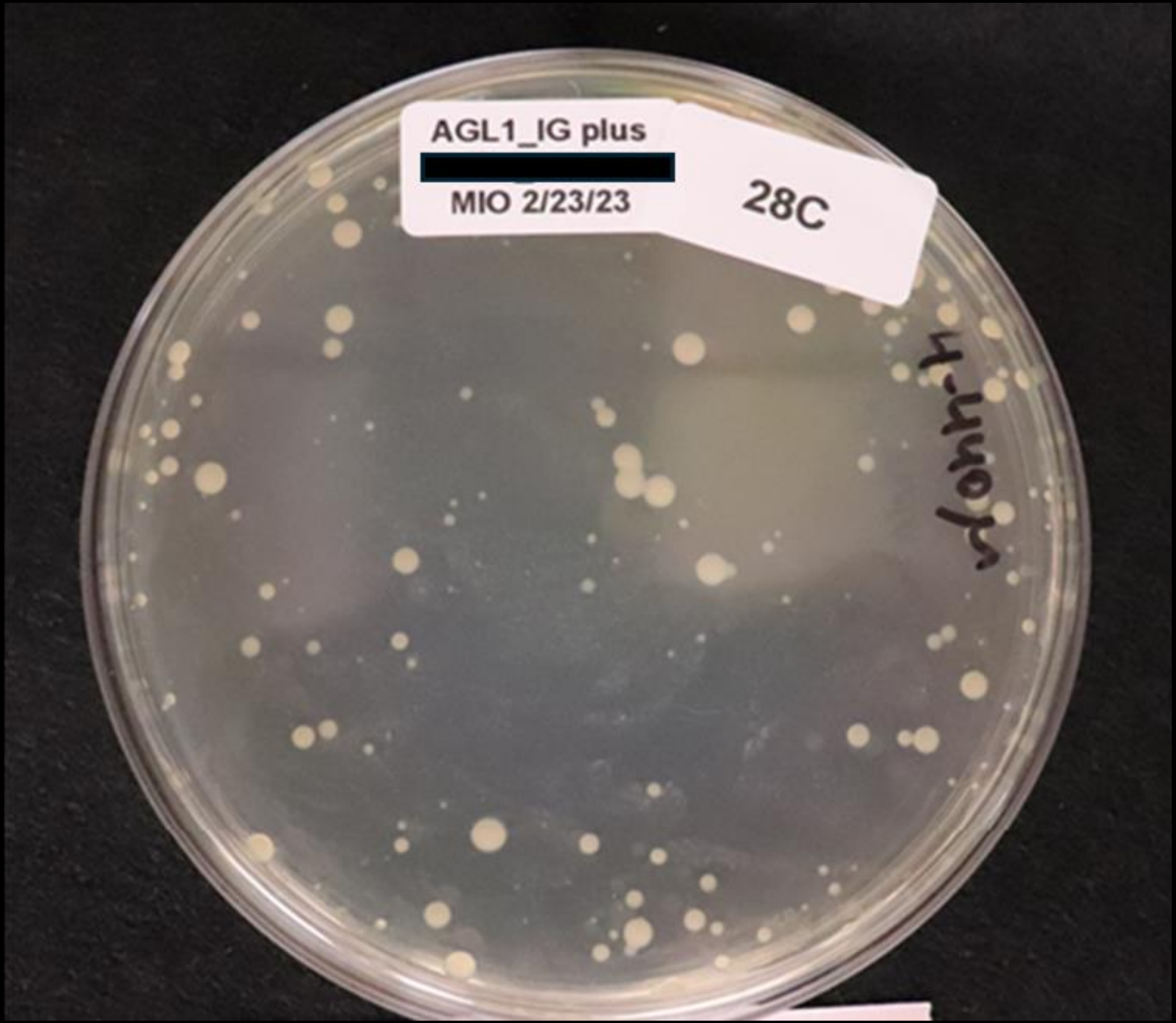
- **Electroporation**
  - Need to purchase or prepare electrocompetent cells
  - Commonly used method for *Agrobacterium*

  - **Protocol Links on website:**
    - Electroporation of Agrobacterium_Protocol 1
    - Electroporation of Agrobacterium_Protocol 2
    - Preparation of electrocompetent cells

Bio-Rad MicroPulser and electroporation cuvettes






AGL1_IG plus
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MIO 2/23/23
28C

Transformation: Introducing new constructs into *Agrobacterium*

- **Freeze and thaw**
- **Chemical competent**

  Protocol Link: Storage of Competent Cells

- **Triparental mating**
  - Alternate method
  - Useful for larger DNA fragments

Long-term storage of *Agrobacterium* strains

Stocks

- **Glycerol stock & DMSO stocks**
  - Storage (-80 °C)

- **Stab cultures**
  - Storage RT or 4 °C
  - Need to periodically prepare new stab cultures

Protocol Links on website:

- Glycerol Stock Production_Protocol 1
- Glycerol Stock Production_Protocol 2

Long-term storage of *Agrobacterium* strains

Best practices

- Make sure you are using correct strain containing correct plasmids
- Make multiple, small aliquots, avoid freeze/thaw
- **Timing**: make aliquot early (e.g., O/N grown culture), don't wait for several days. You may want to grow to an O.D. as indicated in the protocol you are following
- Proper labelling and record keeping (Track and trace)

Upon receiving…

Inoculate culture in YM + antibiotic for 1-2 days

Mix with glycerol for final glycerol concentration of 15-25%

Quality Control: Plasmid and *Agrobacterium*

QC confirm plasmids **before** and **after** introduction to *E. coli* & *Agrobacterium*

- Make sure plasmid sequence and maps are accurate
- Watch-out for point mutations, additional or deleted regions or DNA software assembly errors in the T-DNA or vector backbone compared to reference.

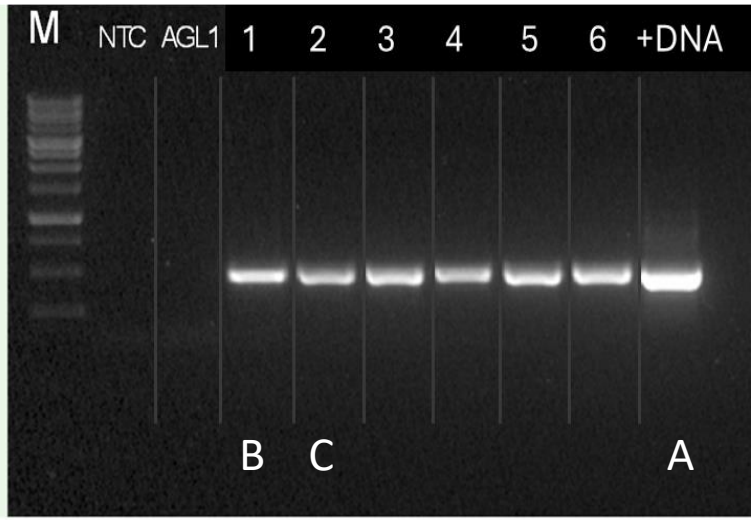
QC of plasmids from *Agrobacterium* Conventional Binary vector systems vs ternary vector system.

Do's and don't's

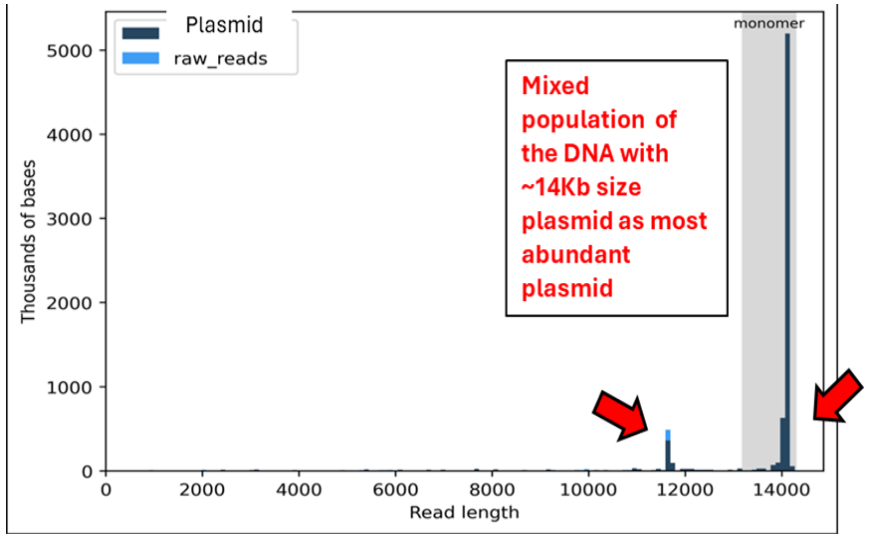
- Ensure you are working with *Agrobacterium* type plasmids and plasmid sequences you intend to use for your transformation experiments.
- If you find the errors, assess, restart or fix as needed, update maps and information

Quality Control: Plasmid and *Agrobacterium*

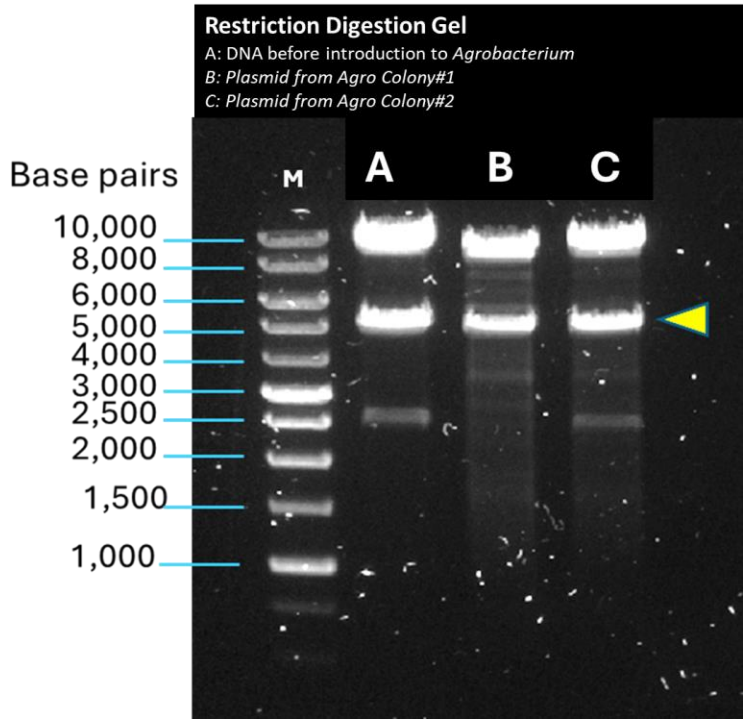
Standard PCR on *Agro* colonies



Full-Plasmid Sequence analysis



Restriction Digestion



Growing *Agrobacterium*

- **Follow the protocol** you are using for plant transformation

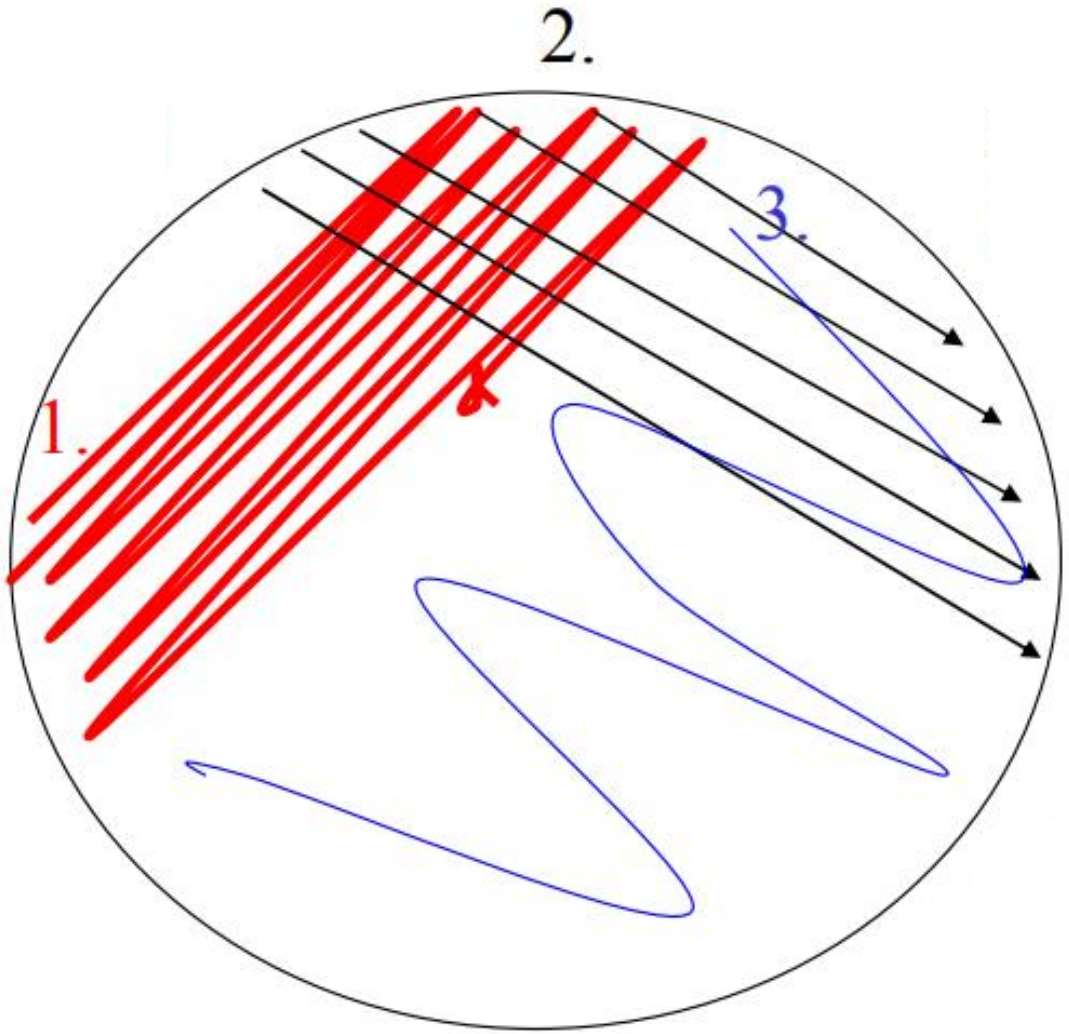
- **Growth media**
  - Solid and Liquid cultures
  - Be aware: there are different types of media. Use what is in protocol
  - Depends on protocol you are following
    - Either method can be successful

- **Use of antibiotics**
  - Be aware some strains have chromosomal markers for antibiotic resistance
  - Plasmid-specific markers for antibiotic resistance

Prepping Agro strain LBA4404 THY- for DevGene Transformation

Details matter!

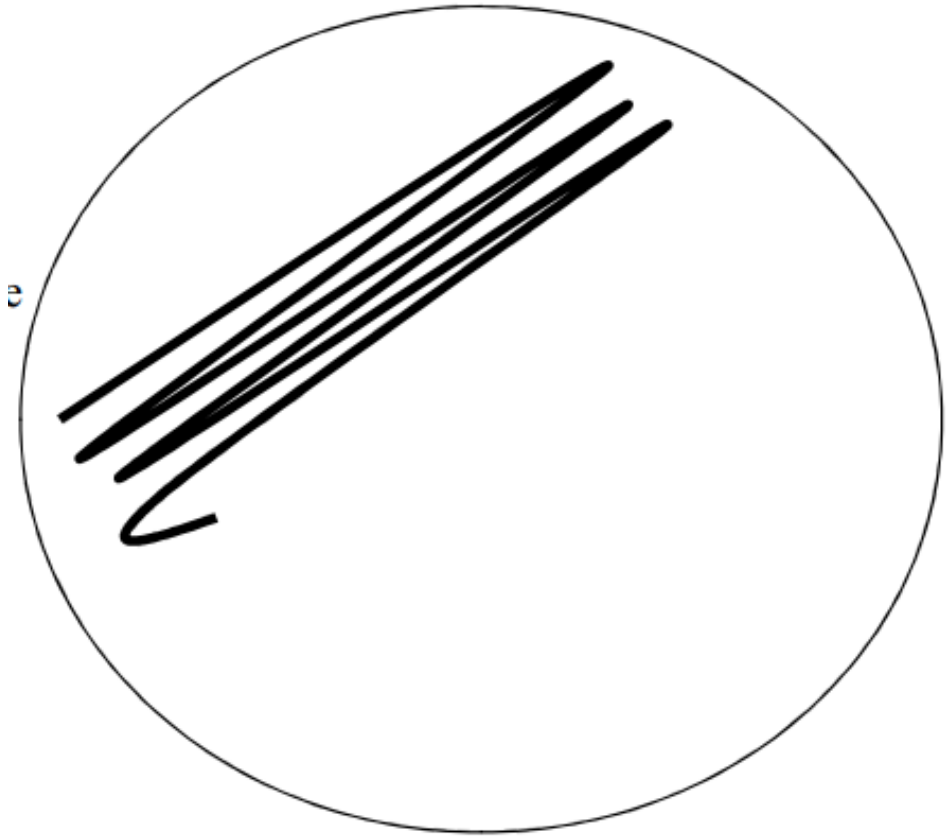
Streaking a Agro “Master Plate”



1. Streak glycerol/Agro mix onto portion of plate
2. Use new sterile loop – Drag 4-5 lines across first streak
3. Use new sterile loop – Make 1 long woven streak

Image of Agro on "Master Plate"

Streaking a “Working Plate”



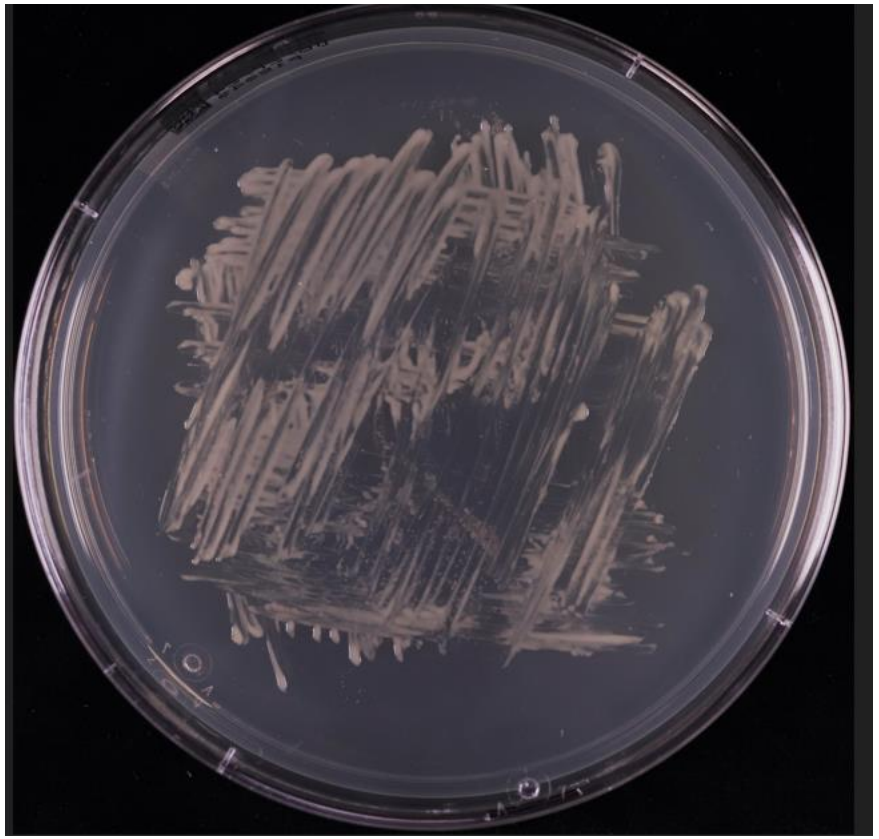
1. With a sterile loop – pick 1 colony from the “Master Plate”

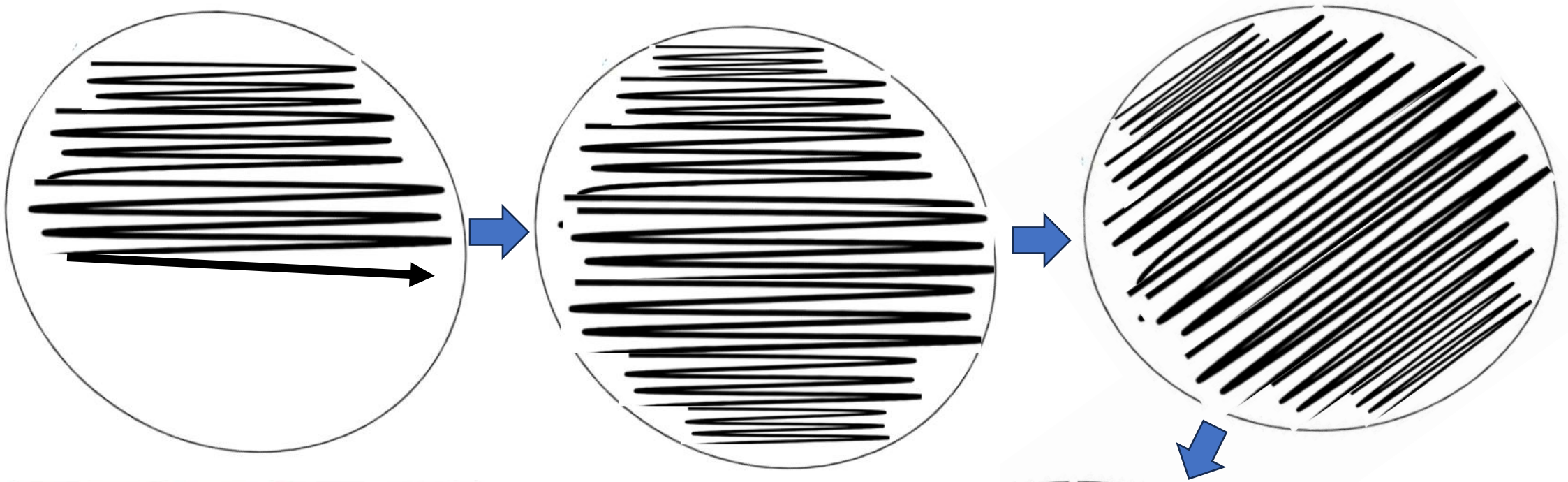
1. Make one long streak on a fresh plate.

2. Grow overnight at 27-29^{o}C

3. Streak a new working plate every day.

Image of a working plate ready for transformation





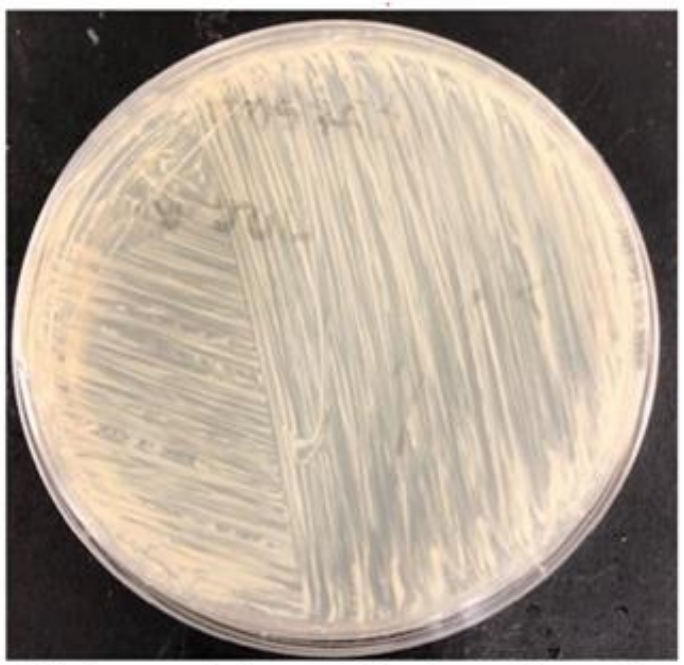


Plate ready to use for transformation

Repeat 1/5 Turn and Streak.

3x

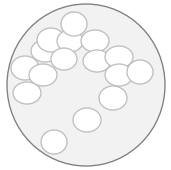
Streaking out a Working Plate for Leaf Transformation

Waking up/starting culture

- **Cultures should be happy, actively growing (log phase)**
- **Spun down, resuspended in proper medium to desired density**

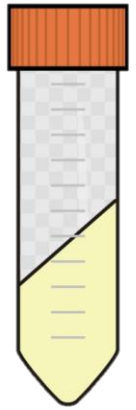
Wake up
Keep cold (one at a time QUICKLY, on ice, freezer block)

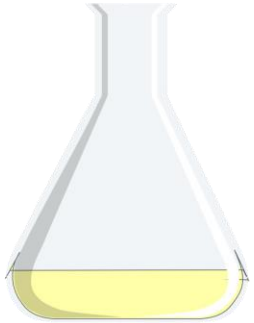
PCR confirm

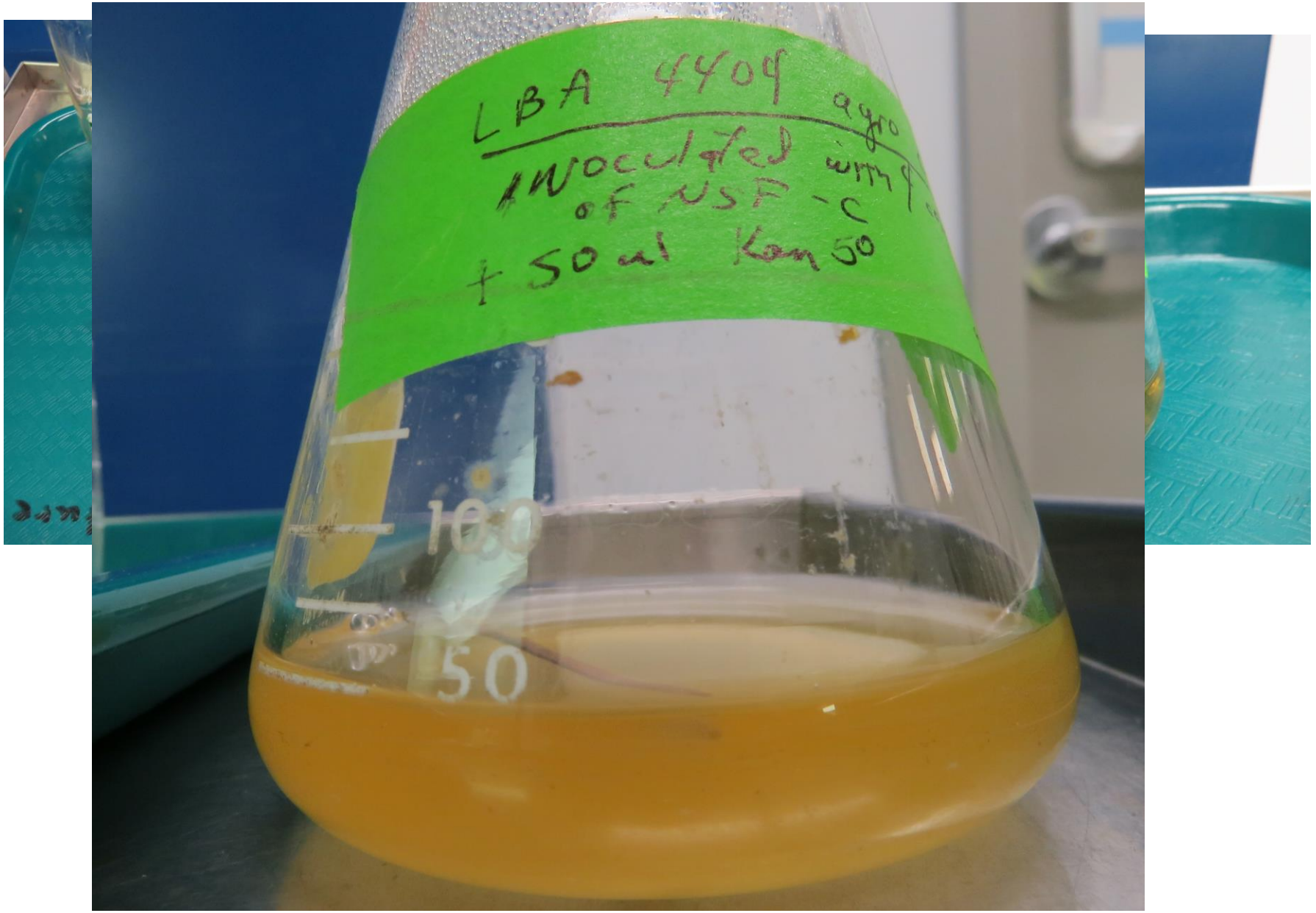


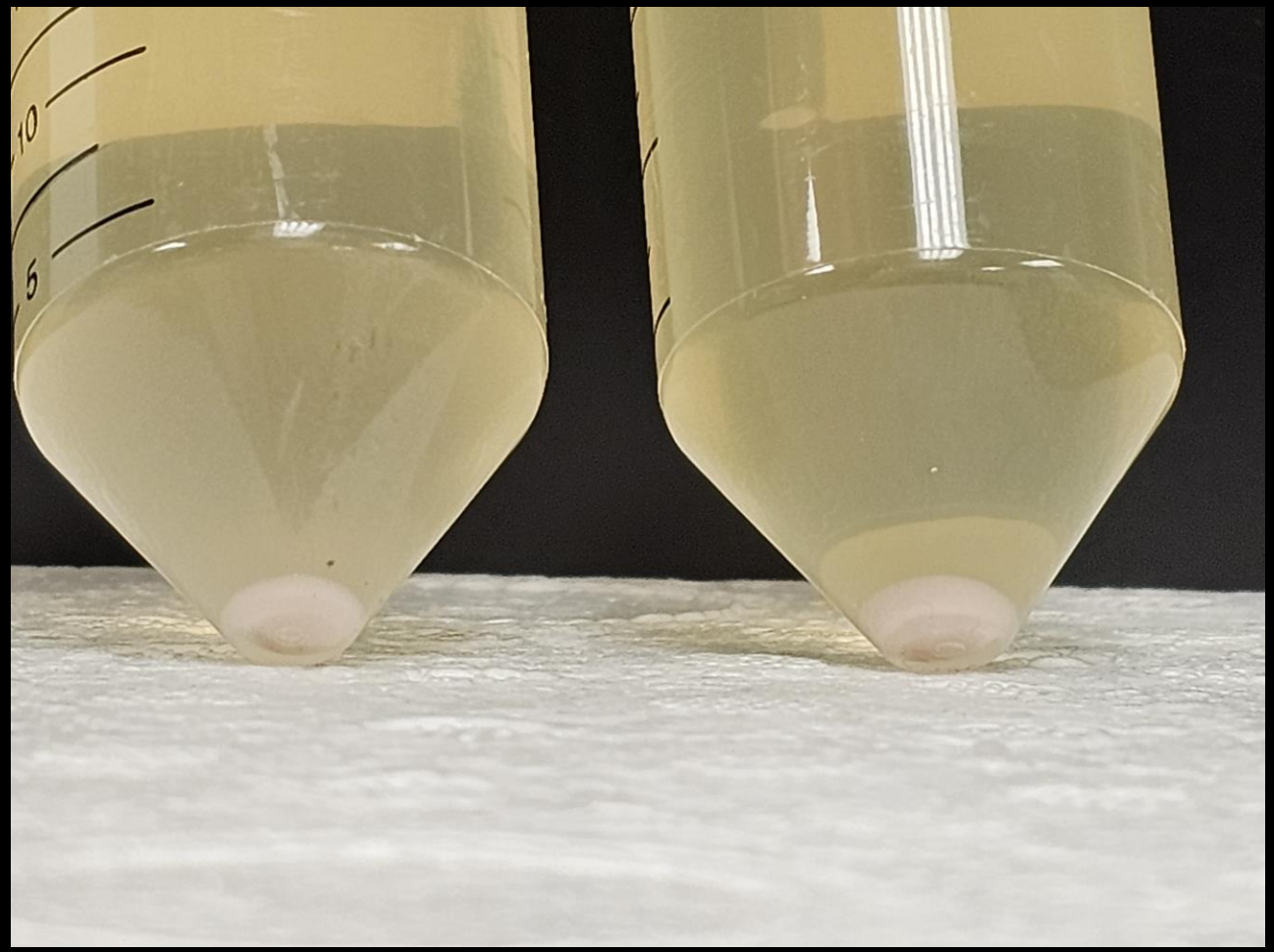
Log phase, active, start fresh culture day before!

Transfer to conical for centrifugation






LBA 4404 pgro
Inoculated with
of NSF-C
+ 50 ul Kan 50
100
50


10
5

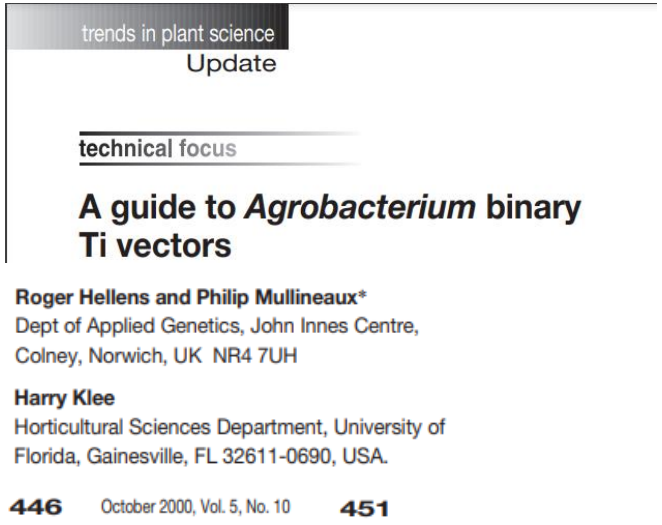
Growing *Agrobacterium*

- **Use of antibiotics**
  - Be aware some strains have chromosomal markers for antibiotic resistance
  - Plasmid-specific markers for antibiotic resistance

Antibiotics & their uses

Note: Timentin is a mixture of ticarcillin and clavulanic acid, a β-Lactamase inhibitor to confer stability to ticarcillin.

- To kill *Agrobacterium* after transformation
  - Cefoxitin, Cefotaxime, Meropenem, Ticarcillin, Timentin, Carbenicillin
- To select for engineered cells
  - Geneticin, Kanamycin, Hygromycin, Paramomycin, Spectinomycin
- To select for *Agrobacterium* & its plasmids
  - Topic of this talk

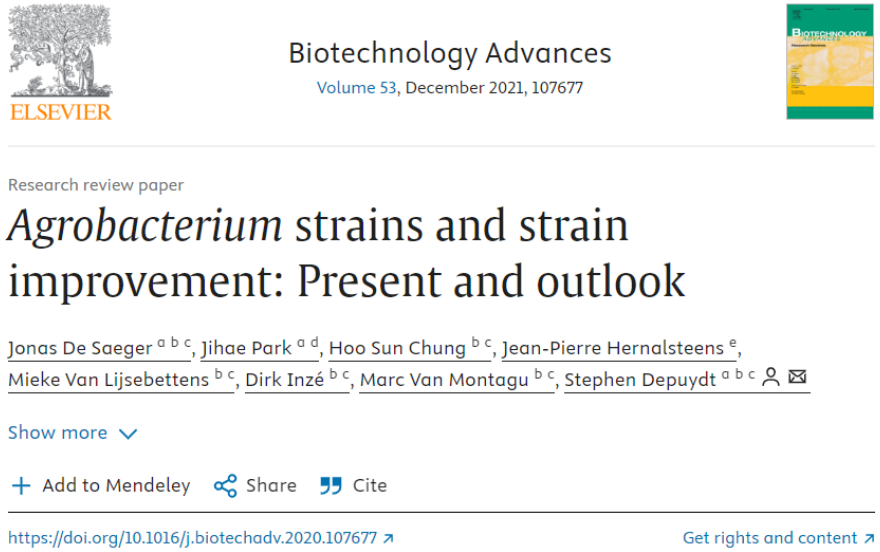

trends in plant science
Update

technical focus

A guide to *Agrobacterium* binary Ti vectors

Roger Hellens and Philip Mullineaux*
Dept of Applied Genetics, John Innes Centre, Colney, Norwich, UK NR4 7UH

Harry Klee
Horticultural Sciences Department, University of Florida, Gainesville, FL 32611-0690, USA.


ELSEVIER

Biotechnology Advances
Volume 53, December 2021, 107677

Research review paper

Agrobacterium strains and strain improvement: Present and outlook

Jonas De Saeger [a b c], Jihae Park [a d], Hoo Sun Chung [b c], Jean-Pierre Hernalsteens [e], Mieke Van Lijsebettens [b c], Dirk Inzé [b c], Marc Van Montagu [b c], Stephen Depuydt [a b c]

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https://doi.org/10.1016/j.biotechadv.2020.107677

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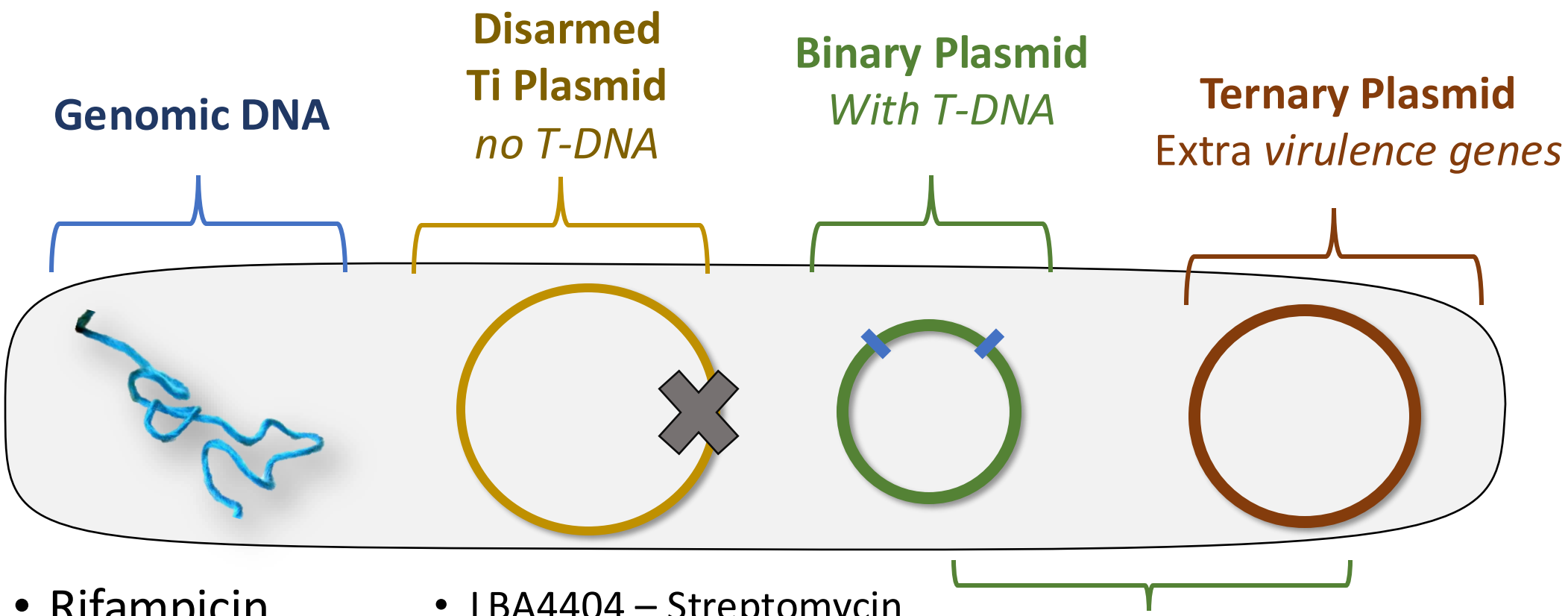
Agrobacterium & its plasmids



- Rifampicin
- Sometimes
  - Carbenicillin
  - Nalidixic acid

- LBA4404 – Streptomycin
- GV3101 – Gentamycin
- EHA101 – Kanamycin
- EHA105 –
- AGL1 –

- Ampicillin
- Carbenicillin
- Chloramphenicol
- Gentamycin

- Kanamycin
- Spectinomycin
- Streptomycin
- Tetracycline

Plasmid/strain inventory -> record antibiotics!

	A	B	C	D	E	F	G	
1	***Slot***	***Box***		***Binary***	**Resistance**	**Reference**	**Strain**	**Notes**
2								
3								
618	24	8		pOE-EcTGA5-F	Kan	EIB	EHA105met	GG pZmUbi:EcTGA5:OsCatT in pAG-K-PvU (full gene)
619	25	8		pOE-EcTGA5-T	Kan	EIB	EHA105met	GG pZmUbi:EcTGA5:OsCatT in pAG-K-PvU (Truncated gene)
621	27	8		pGG-CsRuby	Kan, thy, Strep, Rif	PRL	LBA4404 thyA- recA-	GG 2XCsVMVp:RUBY:StPinT in sPEN12
622	28	8		pGG-PcURuby	Kan, thy, Strep, Rif	PRL	LBA4404 thyA- recA-	GG PcUbi4-2p:RUBY:StPinT in sPEN12
623	29	8		pGG-RSK4	Kan, thy, Strep, Rif	PRL	LBA4404 thyA- recA-	GG Gmub3p:RUBY:AtHspT and StUbi3:Spec2:StUbiT in sPEN12
625	31	8		pOE-PvERD2	Kan, thy, Rif	PRL	PEN 202 (aka sPEN6)	GG pZmUbi:PvERD2:OsCatT in pAG-K-PvU
626	32	8		pOE-PvFRK2_VS16HAP1	Kan, thy, Rif	PRL	PEN 202 (aka sPEN6)	GG pZmUbi:PvFRK2_VS16:OsCatT in pAG-K-PvU
627	33	8		pOE-PvTGT1_VS16HAP1	Kan, thy, Rif	PRL	PEN 202 (aka sPEN6)	GG pZmUbi:PvTGT1_VS16:OsCatT in pAG-K-PvU
628	34	8		pOE-PvPDC2_VS16HAP1	Kan, thy, Rif	PRL	PEN 202 (aka sPEN6)	GG pZmUbi:PvPDC2_VS16:OsCatT in pAG-K-PvU
629	35	8		pOE-PvKNAT1$_{AP13}$	Kan, thy, Rif	PRL	PEN 202 (aka sPEN6)	GG PvKNAT1$_{pro}$: PvKNAT1$_{AP13}$:OsCatT in pAG-K-PvUH
630	36	8		pOE-PvKNAT1$_{VS16}$	Kan, thy, Rif	PRL	PEN 202 (aka sPEN6)	GG PvKNAT1$_{pro}$: PvKNAT1$_{VS16}$:OsCatT in pAG-K-PvUH
633	39	8		pOE_CIPK11	Kan, thy, Rif	PRL	sPEN6	
634	40	8		pGG_nos-RUBY	Kan, thy, Rif	SER	sPEN6	
635	41	8		pGG_ZmUbi-RUBY	Kan, thy, Rif	SER	sPEN6	
636	42	8		pGG_ZmHsp17.7-RUBY	Kan, thy, Rif	SER	sPEN6	
637	43	8		pGG_ZmHsp17.7-YFP	Kan, thy, Rif	SER	sPEN6	
638	44	8		pGG_PvUbi1-RUBY	Kan, thy, Rif	SER	sPEN6	
639	45	8		pGG_PvUbi2-RUBY	Kan, thy, Rif	SER	sPEN6	
640	46	8		pGG_PcUbi-RUBY	Kan, thy, Rif	SER PRL	sPEN6	
641	47	8		pGG_CsCVMV-RUBY	Kan, thy, Rif	SER PRL	sPEN6	
646	52	8		PEN102	Rif, strep	VJP	LBA4404 *thyA-* (Q151*)	LBA4404 *thyA-* (Q151*) produced through CRISPR-mediated base-editing of LBA4404
647	53	8		PEN104	Rif, strep	VJP	PEN102 recA- (Q178*)	LBA4404 *thyA-* (Q151*) *recA-* (Q178*) produced through CRISPR-mediated base-editing of PEN102
648	54	8		PEN202	Rif	VJP	EHA105 thyA- (Q151*)	EHA105 *thyA-* (Q151*) produced through CRISPR-mediated base-editing of At542 (EHA105)
649	55	8		PEN204	Rif	VJP	PEN202 *recA-* (Q178*)	EHA105 *thyA-* (Q151*) *recA-* (Q178*) produced through CRISPR-mediated base-editing of PEN202
650	56	8		PEN302	Rif, gent	VJP	V3101::pMP90 *thyA-* (Q151	GV3101::pMP90 *thyA-* (Q151*) produced through CRISPR-mediated base-editing of GV3101:pMP90
651	57	8		PEN304	Rif, gent	VJP	PEN302 recA- (Q178*)	GV3101::pMP90 *thyA-* (Q151*) recA- (Q178*) produced through CRISPR-mediated base-editing of PEN302

Tips

- Tetracycline is light-sensitive
  - Keep in the dark
- Always use the same brand/supplier

From Addgene:

Antibiotic	Recommended Stock Concentration	Recommended Working Concentration
Ampicillin	100 mg/mL	100 µg/mL
Carbenicillin*	100 mg/mL	100 µg/mL
Chloramphenicol	25 mg/mL (dissolve in EtOH)	25 µg/mL
Hygromycin B	200 mg/mL	200 µg/mL
Kanamycin	50 mg/mL	50 µg/mL
Spectinomycin	50 mg/mL	50 µg/mL
Tetracycline	10 mg/mL	10 µg/mL

- Make stock solutions
- Filter sterilize
- Freeze aliquots at -20C
- Add after autoclaving medium

Effective working concentration can be different for *E. coli* and *Agrobacterium*. For instance, effective Gentamicin concentration is 5-20 mg/L for *E. coli* and 50-100 mg/L for *Agrobacterium*.

https://www.protocolsonline.com/molecular-biology/antibiotics-used-in-molecular-biology/
https://www.addgene.org/mol-bio-reference/

Please fill out this survey!

Four categories of topics were presented today. Depending on community feedback, topics may be covered in more detail in future interactive sessions. Therefore, please rank the topics in the order of most interest to least interest to you.

https://s.zoom.us/m/bZlgtkGpk