


Title: : Infiltration of <i>Nicotiana tabacum</i> plants with <i>Agrobacterium</i> spp.	
Reference No: TSL029	Version No: 1.0

THE SAINSBURY LABORATORY STANDARD OPERATING PROCEDURE		<small>TheSainsburyLaboratory</small> 
TITLE: Infiltration of <i>Nicotiana tabacum</i> plants with <i>Agrobacterium</i> spp. cultures (TSL summer school Activity)		
APPLIES TO STAFF IN: The Sainsbury Laboratory		
HEALTH & SAFETY INFORMATION INCLUDED: YES		
REFERENCE No: TSL029	VERSION No: 1.0	
DATE EFFECTIVE: 20/08/2015	REVIEW DATE: August 2017	
AUTHOR: Oliver Furzer	APPROVED BY: Simon Foster	
QA AUTHORISATION:	DATE ADDED TO QA DATABASE:	

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1 PURPOSE OF PROCEDURE/METHOD AND ITS SCOPE

Co-express genes in the transient *Agrobacterium*/tobacco system to test for hypersensitive response.

2 EQUIPMENT NEEDED

N. tabacum plants

Syringe 1 ml (no needle)

Vials containing *Agrobacterium* spp. being used, resuspended in infiltration buffer.

Blue box/autoclave bag for disposal of biological waste

Kill Box for disposal of liquid biological waste (if using glass beakers)

Infiltration buffer (10mM MES, 10 mM $MgCl_2$, 150 mM acetosyringone all at pH5.6)

3 STEPS IN PROCEDURE

- Adjust the bacterial concentration to $OD_{600}=0.02$ (10.8 cfu) in infiltration buffer (includes 150mM acetosyringone) (done by supervisors in advance in TSL).
- Infiltrate tobacco leaves using a 1 ml syringe (without needle).
- Dispose of biologically contaminated disposable material in blue boxes.
- Dispose of unused bacterial culture in glass beakers in Kill Box.

4 RISK STATEMENT

Low Risk

Lab coats and gloves must be worn at all times. Safety glasses must be worn when infiltrating the plants.

SOP HEALTH RISK ASSESSMENT

[1] Activity:	Transient expression hypersensitive response assay		
[2] Location of activity:	JIC training suite		
[3] Who is involved:	TSL staff and students, summer school students		
[4] Frequency of activity:	Once		
[5] Duration of Activity:	1 hr		
[6] Chemical Hazard Name:	R Phrases and Definitions	Quantity Used	
Acetosyringone	H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation. R36/37/38: Irritating to eyes, respiratory system and skin	< 100 mL	
[7] Details of biological agents of risk to human health:		GMRA Number	
[8] Other Hazards:	Please <input type="checkbox"/> as necessary		
Hot or Cold Burns	<input type="checkbox"/> Ionising Radiation*	<input type="checkbox"/> Ultra Violet or Infra Red	<input type="checkbox"/>
Dust	<input type="checkbox"/> Noise	<input type="checkbox"/> Pollen Sensitizer	<input type="checkbox"/>
Repetitive Action	<input type="checkbox"/> Extreme Cold Environment (< 0°C)	<input type="checkbox"/> Lifting / Manual Handling	<input type="checkbox"/>
Asphyxiation	<input type="checkbox"/> Cuts	<input type="checkbox"/> Electrical	<input type="checkbox"/>
Slips / trips / falls	<input type="checkbox"/> Display Screen Equipment	<input type="checkbox"/> Other (give details)	<input type="checkbox"/>
[9] Control Measures:	Please <input type="checkbox"/> as necessary		
Fume Cupboard	<input type="checkbox"/> Microbiological Safety Cabinet	<input type="checkbox"/> Total Containment Cabinet	<input type="checkbox"/>
Ventilated Bench	<input type="checkbox"/> Spill Tray	<input type="checkbox"/> Trained personnel only	<input type="checkbox"/>

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Signs	<input type="checkbox"/>	Reduce frequency/alternate activity	<input type="checkbox"/>	Reduce duration of activity	<input type="checkbox"/>
Sub divide a load	<input type="checkbox"/>	2 man lift of equipment	<input type="checkbox"/>	Not for more than 1 hour	<input type="checkbox"/>
Regular, short breaks	<input type="checkbox"/>	Alternate activities	<input type="checkbox"/>	Other (give details)	<input type="checkbox"/>

[10] Personal Protection: Please <input type="checkbox"/> as necessary					
Lab coat	<input checked="" type="checkbox"/>	Safety Glasses	<input checked="" type="checkbox"/>	Face Shield	<input type="checkbox"/>
Goggles	<input type="checkbox"/>	Gloves	<input checked="" type="checkbox"/>	Thermal Protective Gloves	<input type="checkbox"/>
Ear defenders	<input type="checkbox"/>	Other (give details)	<input type="checkbox"/>		

[11] Is personal monitoring and/or health surveillance required? Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>					
Details: Students will all time be mentored by TSL staff/students					

[12] Restrictions: Please <input type="checkbox"/> as necessary					
No lone working	<input type="checkbox"/>	Not to be left unattended	<input type="checkbox"/>	Named persons only	<input type="checkbox"/>
In restricted area	<input type="checkbox"/>	Not by new or expectant mothers	<input type="checkbox"/>	Under constant supervision	<input checked="" type="checkbox"/>
Not by under 18's	<input type="checkbox"/>	Other (give details)	<input type="checkbox"/>		

[13] Level of Residual Risk: Please <input type="checkbox"/> as necessary					
Low	<input checked="" type="checkbox"/>	Medium	<input type="checkbox"/>	High	<input type="checkbox"/>

Name of Assessor:	Date: 20/08/2015
Simon Foster	

* A special risk assessment for ionising radiation is required. For more information or advice see Radiation Safety Information on the intranet (<http://intranet/infoserv/support/Safety/Radiation/index.htm>), or refer to your local Radiation Protection Supervisor (RPS).

A special risk assessment may be required for specific hazards where there is significant risk e.g. manual handling of heavy / large items.

Activities involving new or expectant mothers and young persons also require additional risk assessment.

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For specific risk assessment information and R and S phrases information please refer to Risk Assessment on the intranet:
(<http://intranet/infoserv/support/Safety/Risk/index.htm>).

5 DOCUMENTATION

Links to relevant H&S information on intranet or internet

Reference any relevant manuals

Link to: [JIC Chemical Tables](#)

Link to: [Good Laboratory Practice in the Use of Chemicals:](#)

Link to: [Guidance on Good Microbiological Practice](#)

6 RELATED PROCEDURES

7 NOTES

8 APPENDICES

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