

**Title:** Agroinfiltration of *Nicotiana benthamiana* leaves

Reference No: TSL027

Version No: 1.0

**THE SAINSBURY LABORATORY  
STANDARD OPERATING PROCEDURE**



**TITLE:** Agroinfiltration of *Nicotiana benthamiana* leaves

**APPLIES TO STAFF IN:** Sainsbury Laboratory

**HEALTH & SAFETY INFORMATION INCLUDED:** YES

**REFERENCE No:** TSL027

**VERSION No:** 1.0

**DATE EFFECTIVE:** 19/08/2015

**REVIEW DATE:** August 2017

**AUTHOR:** Ben Petre

**APPROVED BY:** Simon Foster

**QA AUTHORISATION:**

**DATE ADDED TO QA DATABASE:**

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## 1 PURPOSE OF PROCEDURE/METHOD AND ITS SCOPE

This procedure details the Agroinfiltration of *Nicotiana benthamiana* leaves.

## 2 EQUIPMENT NEEDED

Syringe (1ml, no needle)

*Nicotiana benthamiana* plants

Solution of *Agrobacterium tumefaciens* carrying an appropriate binary vector for leaf cell transformation (in bottle or centrifuge tube, resuspended in infiltration buffer)

### Infiltration buffer:

10 mM MES

10 mM MgCl<sub>2</sub>

150 mM acetosyringone

pH5.6

Note: Add 7.5 mL 1M acetosyringone into 50 mL MES-MgCl<sub>2</sub> buffer before use.

## 3 STEPS IN PROCEDURE

- 1) Wear safety spectacles, lab coats and gloves for following steps.
- 2) Draw the solution of *A. tumefaciens* up into 1ml syringe.
- 3) Press syringe firmly against underside of leaf but without puncturing the leaf.
- 4) Depress plunger on syringe and force the solution into plant leaf.
- 5) Dispose of all biologically contaminated waste according to local safety rules via the blue box route.

## 4 RISK STATEMENT

This activity is low risk and does not involve handling any hazardous chemicals. Safety glasses must be worn when infiltrating the plants to prevent bacterial solutions from entering eyes.

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## SOP HEALTH RISK ASSESSMENT

<b>[1] Activity:</b>	<b>Agroinfiltration of <i>Nicotiana benthamiana</i> leaves</b>		
<b>[2] Location of activity:</b>	John Innes Centre, Training laboratory		
<b>[3] Who is involved:</b>	Science staff in The Sainsbury Laboratory and participants to the TSL Summer School (August 2015)		
<b>[4] Frequency of activity:</b>	Variable		
<b>[5] Duration of Activity:</b>	Approximately 10 minutes		
<b>[6] Chemical Hazard Name:</b>	<b>R Phrases and Definitions</b>	<b>Quantity Used</b>	
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	
<b>[7] Details of biological agents of risk to human health:</b>			<b>GMRA Number</b>
<b>[8] Other Hazards:</b>	Please <input type="checkbox"/> as necessary		
Hot or Cold Burns	<input type="checkbox"/>	Ionising Radiation*	<input type="checkbox"/>
Dust	<input type="checkbox"/>	Noise	<input type="checkbox"/>
Repetitive Action	<input type="checkbox"/>	Extreme Cold Environment (< 0°C)	<input type="checkbox"/>
Asphyxiation	<input type="checkbox"/>	Cuts	<input type="checkbox"/>
Slips / trips / falls	<input type="checkbox"/>	Display Screen Equipment	<input type="checkbox"/>
		Ultra Violet or Infra Red	<input type="checkbox"/>
		Pollen Sensitizer	<input type="checkbox"/>
		Lifting / Manual Handling	<input type="checkbox"/>
		Electrical	<input type="checkbox"/>
		Other (give details)	<input type="checkbox"/>
<b>[9] Control Measures:</b>	Please <input type="checkbox"/> as necessary		
Fume Cupboard	<input type="checkbox"/>	Microbiological Safety Cabinet	<input type="checkbox"/>
Ventilated Bench	<input type="checkbox"/>	Spill Tray	<input type="checkbox"/>
		Total Containment Cabinet	<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 ☒ 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 ☐ No ☒  
Details: Participants will all time be mentored by TSL Science staff  
  
**[12] Restrictions:** Please ☒ 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 type="checkbox"/>
Not by under 18's	<input type="checkbox"/>	Other (give details)	<input type="checkbox"/>		

  
**[13] Level of Residual Risk:** Please ☒ as necessary  

Low	<input checked="" type="checkbox"/>	Medium	<input type="checkbox"/>	High	<input type="checkbox"/>
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Name of Assessor: Simon Foster  
Date: 19/08/2015

\* 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**

## **6 RELATED PROCEDURES**

## **7 NOTES**

## **8 APPENDICES**

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