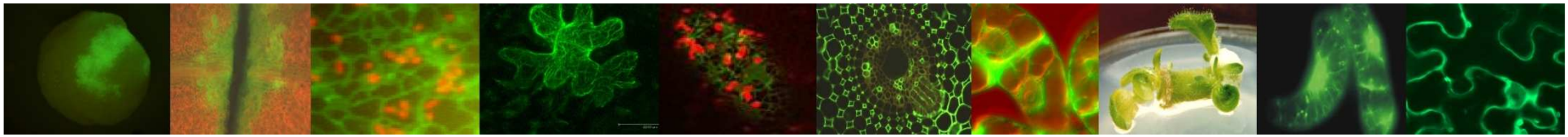
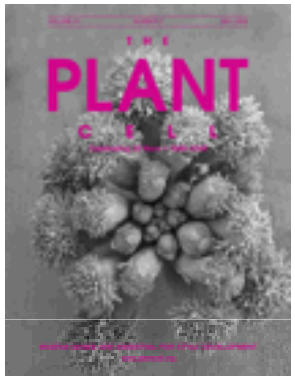


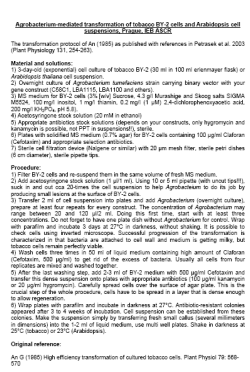
# Praktické základy vědecké práce MB130P16





## zimní semestr, 0/2, Z, 2 kredity

## Přednášející: RNDr. Jan Petrášek, Ph.D.


Agrobacterium-mediated transformation of tobacco BY-2 cells and Arabidopsis cell suspensions, Prague, IEB ASCR

The transformation protocol of An (1985) as published with references in Petrasek et al. 2003 (Plant Physiology 131: 254-263).

**Material and solutions:**

1) 3-day-old (exponential) cell culture of tobacco BY-2 (30 ml in 100 ml erlenmayer flask) or *Arabidopsis thaliana* cell suspension.
2) Overnight culture of *Agrobacterium tumefaciens* strain carrying binary vector with your gene construct (C58C1, LBA1115, LBA1100 and others).
3) MS medium for BY-2 cells (3% [w/v] Sucrose, 4.3 g/l Murashige and Skoog salts SIGMA M5524, 100 mg/l inositol, 1 mg/l thiamin, 0.2 mg/l (1 μM) 2,4-dichlorophenoxyacetic acid, 200 mg/l $KH_2PO_4$, pH 5.8).
4) Acetosyringone stock solution (20 mM in ethanol)
5) Appropriate antibiotics stock solutions (depends on your constructs, only hygromycin and kanamycin is possible, not PPT in suspension!), sterile.
6) Plates with solidified MS medium (0.7% agar) for BY-2 cells containing 100 μg/ml Claforan (Cefotaxim) and appropriate selection antibiotics.
7) Sterile cell filtration device (Rotipore or similar) with 20 μm mesh filter, sterile petri dishes (9 cm diameter), sterile pipette tips.

**Procedure:**

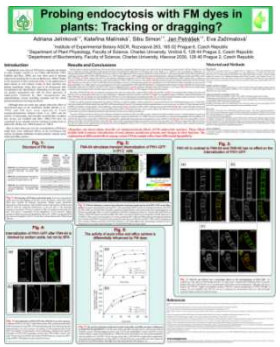
1) Filter BY-2 cells and re-suspend them in the same volume of fresh MS medium.
2) Add acetosyringone stock solution (1 μl/1 ml). Using 10 or 5 ml pipette (with uncut tipsIf), suck in and out cca 20-times the cell suspension to help Agrobacterium to do its job by producing small lesions at the surface of BY-2 cells.
3) Transfer 2 ml of cell suspension into plates and add Agrobacterium (overnight culture), prepare at least four repeats for every construct. The concentration of Agrobacterium may range between 20 and 120 μl/2 ml. Doing this first time, start with at least three concentrations. Do not forget to have one plate dish without Agrobacterium for control. Wrap with parafilm and incubate 3 days at 27°C in darkness, without shaking. It is possible to check cells using inverse microscope. Successful progression of the transformation is characterized in that bacteria are attached to cell wall and medium is getting milky, but bacteria do not form colonies yet.
4) Wash cells three times in 50 ml of liquid medium containing high amount of Claforan (Cefotaxim, 600 μg/ml) to get rid of the excess of bacteria. Usually all cells from four replicates are mixed and washed together.
5) After the last washing step, add 2-3 ml of BY-2 medium with 500 μg/ml Cefotaxim and transfer this dense suspension onto plates with appropriate antibiotics (100 μg/ml kanamycin or 20 μg/ml hygromycin). Carefully spread cells over the surface of agar plate. This is the crucial step of the whole procedure, cells have to be spread in a layer that is dense enough to allow regeneration.
6) Wrap plates with parafilm and incubate in darkness at 27°C. Antibiotic-resistant colonies appeared after 3 to 4 weeks of incubation. Cell suspension can be established from these colonies. Make the suspension simply by transferring fresh small callos (several millimeters in dimensions) into the 1-2 ml of liquid medium, use multi well plates. Shake in darkness at 25°C (tobacco) or 23°C (Arabidopsis).

**Original reference:**

An G (1985) High efficiency transformation of cultured tobacco cells. Plant Physiol 79: 568-570



Auxin transport routes in plant development


Probing endocytosis with FM dyes in plants: Tracking or dragging?

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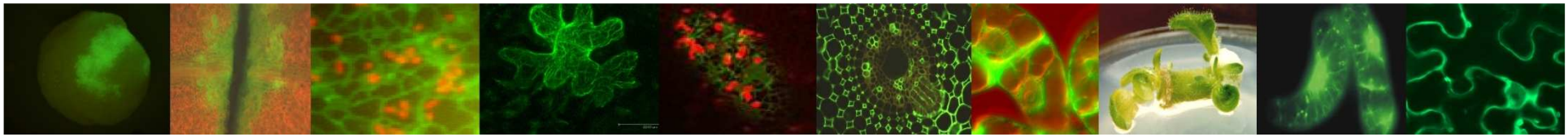
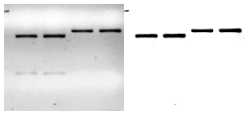
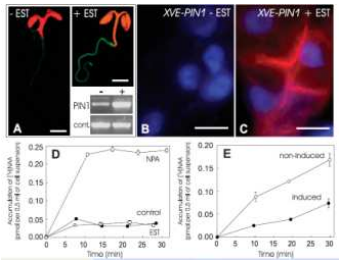


# *Co máte šanci se dozvědět a také si vyzkoušet:*

- Jak prakticky funguje vědecký tým.
- Jak navrhovat, vést a zpracovávat výsledky experimentů.
- Jak vyhledávat a třídit odbornou literaturu.
- Jak účelně využívat databáze sekvencí.
- Jak napsat bakalářskou a diplomovou práci.
- Jak vyrobit efektní plakát s Vašimi výsledky.
- Jak napsat vědeckou publikaci.






**Přednáška/cvičení je určena především pro bakalářské studenty experimentálních oborů, více informací na:**

**http://lhr.ueb.cas.cz/petrasek**

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