Author: Kasia Lipinska
created: 06.04.2021 21:22
updated: 02.06.2021 20:20

Entry 1/11: Seed germination and glass preparation for tobacco chloroplast transforamtion
In Project: In vivo chloroplast transformation
With tags: 06.04.21, transformation, in vivo, chloroplast, Jonas, Kasia, tabacco, clean bench, seed sterilization, glass sterilization

MMB_editing2011.pdf

**Glass preparation for plant cultures (performed on 6-7.04.21)**

25 Weck glasses were washed in a dishwasher at 80$^{o}$C and rinsed twice with distilled water. The next day, the glasses were secured from contamination with aluminum foil and baked at 200$^{o}$C for 4 h.

**Medium for seed germination**

MS medium for aseptic growth of tobacco plants was prepared on 6.04.21 and followed the protocol 2.2.1 by Ruf and Bock *In Vivo* Analysis of RNA Editing in Plastids:

In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)

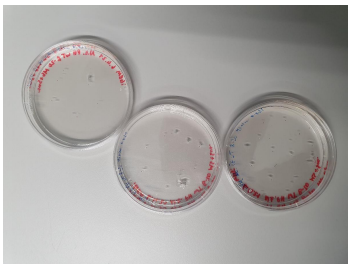
1. Murashige & Skoog Medium Basal Salt Mixture (Prod. No. M0221, Duchefa Biochemie) 4.3 g/L
2. Sucrose (Ja!, Rewe) 30 g/L
3. Phytagel 2.3 g/L

The MS salts and sucrose were dissolved with Milli-Q water. Next, the pH was adjusted with KOH to 5.6, and phytagel was added. The sterilization was performed by autoclaving. The medium was poured on Petri dishes for plant tissue culture on a clean bench.

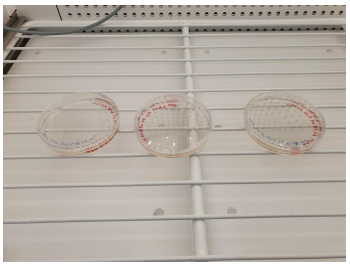
**Seed washing and plating (performed on 6.04.21) based on 3.1. Plant Material and Growth Conditions in In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)**

We used the seeds of *Nicotiana tabacum* (N.t. PH wildtype B -20; 5687-001; 838992 AG Bock). Around 25 seeds were surface-sterilized with 400 ul 70% ethanol and 400 ul 6% bleach (sodium hypochlorite solution). The solution with seeds was shaken for 2-3 minutes. Subsequently, the liquid is removed and the seeds are washed six times with 1.5 ml of sterile water. Next, the seeds were transferred to sterile Petri dishes and germinated on the MS medium.

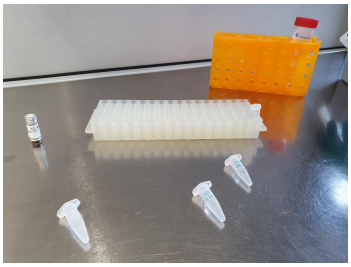
20210406_planted seeds.jpg



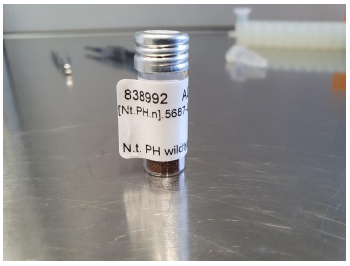
20210406_seeds in a chamber.jpg



20210406_seed washing.jpg



20210406_seeds of N.t..jpg



Author: Kasia Lipinska
created: 18.04.2021 23:31
updated: 27.06.2021 16:03
Entry 2/11: Medium preparattion for plant cultures and planting
In Project: In vivo chloroplast transformation
With tags: 21.04.2021, 23.04.2021, in vivo, Kasia, Jonas, seed sterilization, transformation, glass sterilization, medium, ms salts, clean bench, plant culture, sterile wrok, bock, tobacco

## Medium for plant cultures (from seedling)

MS medium for aseptic growth of tobacco plants was prepared on 21.04.21 and followed the protocol 2.2.1 by Ruf and Bock *In Vivo* Analysis of RNA Editing in Plastids:

In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in the project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)

1. Murashige & Skoog Medium Including Modified Vitamins (Prod. No. M0245, Duchefa Biochemie) 4.3 g/L
2. Sucrose (Rewe) 30 g/L
3. Micro Agar (Duchefa Biochemie) 7.4 g/L

The MS salts and sucrose were dissolved with Milli-Q water. Next, the pH was adjusted with 0.1 M KOH to 5.6. The Micro Agar was weighted and put in the 1 L bottle. The bottle was filled in with medium liquid to 800 ml. The sterilization was performed by autoclaving. A cooled-down medium was poured into the previously sterilized 20 Weck glasses on a clean bench and left there to solidify.

## Seedling transfer

The transfer of seedling was done on 23.04.21. The procedure was performed on a clean bench. The tweezers were sterilized with ethanol and Bunsen burner between handling each plant. 20 individual plants in Weck glasses were then put in a growth chamber at 25$^{o}$C under a 16h/8h dark cycle (light intensity ?).

One "too small" seedling and 3 seedlings from a contaminated plate were not transferred to a new medium.

Author: Kasia Lipinska
created: 06.05.2021 16:42
updated: 06.05.2021 16:43

Entry 3/11: S30 extract preparation 23.04.2021 - Tobacco, Percoll, leaves harvested the day before vs freshly cut (isolation from 20.04.2021)
In Project: In vivo chloroplast transformation
With tags: CFE, chloroplast, gradient, gradients, isolation, dialysis, 20.04.2021, 23.04.2021, S30, extract, tobacco, percoll, cut day before, fresh cut, dark, dark for 24h, Jessi, Kasia

The S30 extract preparation was done on 23.04.2021 and followed the protocol:

Tobacco Chloroplast harvest & S30 preparation protocol - entry #5 in project 'Chloroplast isolation & S30 extracts' (iGEM Marburg Admin, 27.01.2021)

The extracts were prepared from Tobacco chloroplasts isolated on 20.04.2021:

chloroplast isolation 20.04.2021, tobacco - entry #32 in project 'Chloroplast isolation & S30 extracts' (Jessica Baumann, 21.04.2021)

The treatments tested were the following:

treatment 1. Leaves harvested the day before isolation

treatment 2. Leaves harvested right before isolation

Remarks:

Dialysis was omitted due to insufficient volume of the samples (protocol steps 4-5).

Samples (each around 200 ul):

12T_1 - Leaves harvested the day before isolation

13T_1 - Leaves harvested the day before isolation

14T_2 - Leaves harvested right before isolation

15T_2 - Leaves harvested right before isolation

Author: Kasia Lipinska
created: 07.05.2021 14:36
updated: 18.06.2021 15:19

Entry 4/11: In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock
In Project: In vivo chloroplast transformation
With tags: in vivo analysis, in vivo, bock, ruf, transformation#, transformation, medium, plant culture, protocol

# *In Vivo* Analysis of RNA Editing in Plastids

Stephanie Ruf and Ralph Bock

Chapter 8

MMB_editing2011.pdf

Author: Kasia Lipinska
created: 02.06.2021 20:01
updated: 27.06.2021 16:03
Entry 5/11: Seed sterilization, glass preparation and media 02.06.21
In Project: In vivo chloroplast transformation
With tags: 02.06.21, in vivo, transformation, tabacco, Jessi, Jonas, Kasia, Clemens, sterile wrok, clean bench, seed sterilization, glass sterilization

Seed sterilization and glass preparation for *in vivo* tobacco transformation was performed by repeating the following protocol:

Seed germination and glass preparation for tabacco chloroplast transforamtion - entry #1 in project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)

The protocol is based on:

In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)

The following medium prepared on 21.04.21 was used again (dissolved in autoclave) for the 5 available glasses and 5 plates:

Medium for seed germination

MS medium for aseptic growth of tobacco plants was prepared on 21.04.21 and followed the protocol 2.2.1 by Ruf and Bock *In Vivo* Analysis of RNA Editing in Plastids:

In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in the project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)

1. Murashige & Skoog Medium Including Modified Vitamins (Prod. No. M0245, Duchefa Biochemie) 4.3 g/L
2. Sucrose (Rewe) 30 g/L
3. Micro Agar (Duchefa Biochemie) 7.4 g/L

The MS salts and sucrose were dissolved with Milli-Q water. Next, the pH was adjusted with 0.1 M KOH to 5.6. The Micro Agar was weighted and put in the 1 L bottle. The bottle was filled in with medium liquid to 800 ml. The sterilization was performed by autoclaving. A cooled-down medium was poured into the previously sterilized 20 Weck glasses on a clean bench and left there to solidify.

Seed washing and plating based on 3.1. Plant Material and Growth Conditions in **In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)**

We used the seeds of *Nicotiana tabacum* (N.t. PH wildtype B -20; 5687-001; 838992 AG Bock). Around 30 seeds were surface-sterilized with 400 ul 70% ethanol and 400 ul 6% bleach (sodium hypochlorite solution). The solution with seeds was shaken for 2-3 minutes. Subsequently, the liquid was removed and the seeds were washed six times with 1 ml of sterile water. Next, over 25 seeds were transferred to sterile Petri dishes with the MS medium and put in a growth chamber.

Glass preparation for plant cultures

20 Weck glasses were washed in a dishwasher at 80$^{o}$C and rinsed with distilled water.

Remarks:

*The MS medium took quite a long time to solidify enough

*The rejected tobacco from previous plant culture was planted in soil and moved to the greenhouse

Author: Michael Burgis
Entry 6/11: No entry title yet
In Project: In vivo chloroplast transformation
No tags associated

created: 03.06.2021 21:30
updated: 03.06.2021 21:33

Author: Jessica Baumann
created: 29.06.2021 11:17
updated: 07.10.2021 17:13

Entry 7/11: seedling transfer 28.06.2021
In Project: In vivo chloroplast transformation
With tags: seedlings, transfer, medium, agar, cleanbench, sterile

**Seedling transfer**

The transfer of seedling was done on 28.06.21. The procedure was performed on a clean bench. The tweezers were sterilized with ethanol and Bunsen burner between handling each plant. After they were taken put of the agar Plates, the plants were cleaned in sterile water in an extra Plate. 20 individual plants in Weck glasses were then put in a growth chamber at 25$^{o}$C under a 16h/8h dark cycle (light intensity ?).

15 of those seedlings were placed in contaminated agar plates. 5 seedlings came  from a not-contaminated plate. All the seedlings were transfered.

Author: Jonas Freudigmann
created: 19.07.2021 12:02
updated: 19.07.2021 12:39
Entry 8/11: Tobacco seed sterilisation & transfer for sterile culture
In Project: In vivo chloroplast transformation
No tags associated

**Friday, July 2**

Media preparation

0.5L of fresh MS Medium was prepared according to the protocol 2.2.1 by Ruf and Bock In Vivo Analysis of RNA Editing in Plastids:

In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in project 'In vivo chloroplast transformation' (Kasia Lipinska, 18.06.2021)

Material:

1. Murashige & Skoog Medium Including Modified Vitamins (Prod. No. M0245, Duchefa Biochemie) 4.3 g/L
2. Sucrose (Rewe) 30 g/L
3. Micro Agar (Duchefa Biochemie) 7.4 g/L

To this end, the MS medium and sucrose was weighed and dissolved in Milli-Q water. pH was adjusted with 0.1M KOH to 5.7. Medium was filled to 0.5L with Milli-Q and the Micro Agar was added. Medium was autoclaved and poured into petri dishes after cooling.

Seed sterilisation and plating

Sterilisation of *Nicotiana tabacum* wildtype B-20 seeds was performed following section 3.1 in the protocol linked above.

Around 75 seeds were surface sterilised with 400 µL 70% EtOH and 400 µL 6% sodium hypochlorite solution. The solution with seeds was shaken vigorously for 3 minutes. Hereafter, all liquid was removed with a P-1000 and seeds were washed 8 times in 1 mL sterile water.

About 15 seeds each were carefully placed on 5 different petri dishes (with the MS Medium prepared above) with a pipette. Petri dishes were wrapped in 3M Micropore tape to prevent contamination.

Petri dishes were placed in a growth chamber at 25ºC under a 16h/8h light-dark cycle with a light intensity of ~100 µmol photons $m^{-2}$ $s^{-1}$.

**Monday, July 5**

Glass jar preparation
15 of the plants transferred the last time (seedling transfer 28.06.2021 - entry #7 in project 'In vivo chloroplast transformation' (Jessica Baumann, 29.06.2021)) were contaminated.

Jars were autoclaved with the plants and the contamination inside, in order to prevent the fungus to potentially spread in the research group.

The autoclaved material inside the jars was discarded.
Autoclaved jars were hereafter washed in the dishwasher and subsequently sterilised in an oven at 200°C for 6 hours.

**Monday, July 12**

3 bottles of 800 mL fresh MS medium were prepared as on July 2.
The medium was poured into 15 sterilised glass jars under sterile conditions, using about 150 mL / jar. Jars were left under the clean bench for the medium to harden and hereafter stored in the growth chamber for further use.

**Tuesday, July 13**

Seedling transfer

The sterile seeds planted on July 2 have germinated until now and small seedlings have emerged.

15 seedlings were now transferred into the sterilised glass jars with MS Medium prepared the day before.

To this end, the sterilised jars, tweezers, a beaker with 70% EtOH and a bunsen burner were UV-sterilised under a clean bench.
After UV sterilisation, two of the plates with germinated seeds were put under the clean bench.

Tweezers were sterilised with EtOH and the bunsen burner between each round of transfer.
For the transfer, seedlings were carefully picked out of the agar plates and gently put on top of the agar in a glass jar. All seedlings were handled with care to make sure not to harm any roots or leaves.
After transfer of 15 seedlings to the 15 glass jars, all jars were put in a growth chamber at 25°C under a 16h/8h light-dark cycle with a light intensity of ~100 µmol photons $m^{-2}$ $s^{-1}$.

Remaining plates and seedlings were wrapped in micropore tape again and put back into the growth chamber to keep as a backup.

Author: Kasia Lipinska
Entry 9/11: Medium and plates preparation
In Project: In vivo chloroplast transformation
With tags: in vivo, plates, sterile, transformation, tobacco, Kasia, clean bench, Clemens

created: 04.10.2021 13:30
updated: 05.10.2021 12:10

RMOP medium was prepared on 1.10.2021 according to the following protocol:

In Vivo Analysis of RNA Editing in Plastids protocol by S. Ruf and R. Bock - entry #4 in project 'In vivo chloroplast transformation' (Kasia Lipinska, 07.05.2021)

Materials:

1. Murashige & Skoog Medium Including Modified Vitamins (Prod. No. M0245, Duchefa Biochemie) 4.4 g/L
2. Sucrose (Rewe) 30 g/L
3. Micro Agar (Duchefa Biochemie) 7.4 g/L
4. NAA 1mg/ml
5. BAP 1mg/ml

The pH was adjusted with 0.2 M KOH to 5.8. Then, the agar was added and the media was autoclaved. At the end, we added filter-sterilized antibiotics: spectinomycin 500 mg/L (marked with one stripe) or spectinomycin and streptomycin 500 mg/L (marked with two stripes).

The calli chosen for transfer (green or greenish-brownish) were marked.

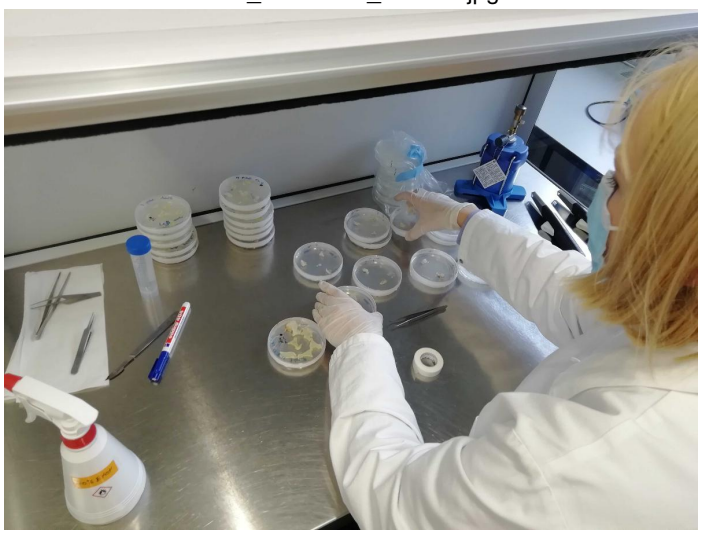
Author: Jessica Baumann
Entry 10/11: Transfer of Calli 04.10.2021
In Project: In vivo chloroplast transformation
No tags associated
created: 04.10.2021 20:05
updated: 04.10.2021 20:34

The transfer of diverse calli onto plates containing spectinomycin, as well as on plates that contain spectino and streptomycin was done at the 4th of October 2021.

14 calli were transfered in total. One callus was very green and all the other calli had a brownish, green colour.

While taking the calli out of the phytochamber, one contamination was noticed. The plates were transported to the cleanbench by sterilising a sealable box and carefully placing the plates that are secured with micropore tape into that box. The cleanbench, as well as all tools needed were sterilized under UV light for half an hour. The new plates were labeled according to the calli. The micropore tape of the plates containing the calli samples was cut carefully with a razorblade to open the plates. The calli were each grabbed carefully wth a tweezer and cut in half with a scalpel. One half of the callus was then transfered to a plate only containing spectinomycin and one was transfered on a plate containing spectinomycin and streptomycin as well. Onto each new plate, a maximum of 3 Calli-halves were placed. After the finished transfer,all plates were sealed with micropore tape again. The plates were transported to the phytochamber again by placing them in the sterile box. Inside the phytochamber, the transfered plates were put far away from all the other plates in case a contamination occurs.

IMG_20211004_153638.jpg



Author: Clemens Böhm
created: 07.10.2021 15:38
updated: 07.10.2021 15:46

Entry 11/11: Chloroplast isolation 07.10.21 oak Percoll Kasia, Jessi, Clemens
In Project: In vivo chloroplast transformation
No tags associated

Chloroplast isolation from oakleaves was performed on 07.10.21 following this protocol:

Tobacco Chloroplast harvest & S30 preparation protocol - entry #5 in project 'Chloroplast isolation & S30 extracts' (iGEM Marburg Admin, 27.01.2021)

Plant: ~100g oak from the campus, incubated for 7 days.

Gradients: 8x 11 ml 80%/ 12 ml 40%/ 7 ml of 20% Percoll

Remarks: Considering the short (for oak) incubation time, there was suprisingly little starch in the sample.

Two out of eight gradients had only a very faint lower band.