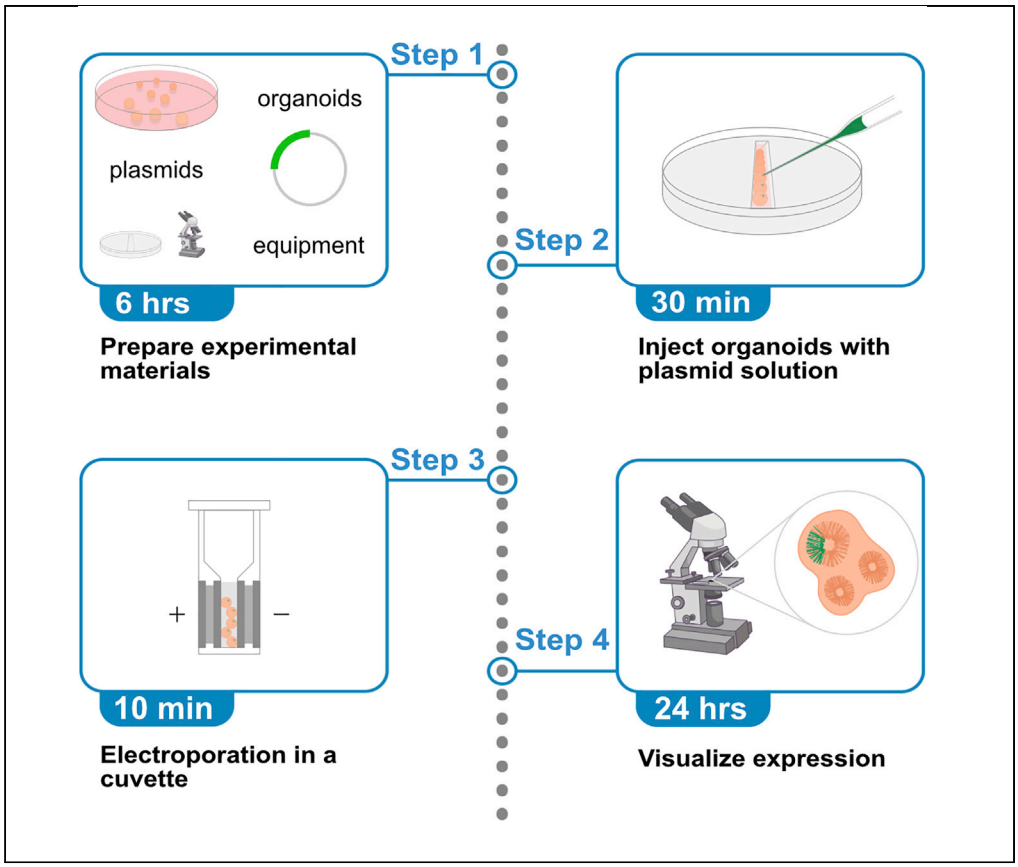
STAR Protocols

CellPress
OPEN ACCESS

Protocol

Injection and electroporation of plasmid DNA into human cortical organoids



Pluripotent stem cell-derived human cortical organoids allow for the analysis of stem cell behavior and neurogenesis in neural tissues. Delivery of plasmid DNA into organoids permits visualization of individual cells, characterization of cellular components, and manipulation of gene expression. We describe a protocol to transfect cells inside organoids with plasmid DNA using micro-injection and electroporation, allowing for DNA delivery to cells within cortical units. This protocol was optimized for cortical organoids; however, it may be adapted to other organoid models.

Annina Denoth-Lippuner, Lars N. Royall, Daniel Gonzalez-Bohorquez, Diana Machado, Sebastian Jessberger

denoth@hifo.uzh.ch (A.D.-L.)
jessberger@hifo.uzh.ch (S.J.)

Highlights

Plasmid injection of pluripotent stem cell-derived human cortical organoids

Electroporation of human cortical organoids

Genetic manipulation of progenitors and their progeny in cortical organoids

Protocol enables robust DNA transfection of human cortical organoids

Denoth-Lippuner et al., STAR Protocols 3, 101129
March 18, 2022
https://doi.org/10.1016/j.xpro.2022.101129

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Injection and electroporation of plasmid DNA into human cortical organoids

Annina Denoth-Lippuner,[1,2,3,*] Lars N. Royall,[1,2] Daniel Gonzalez-Bohorquez,[1,2] Diana Machado,[1] and Sebastian Jessberger[1,4,*]

[1]Laboratory of Neural Plasticity, Faculties of Medicine and Science, Brain Research Institute, University of Zurich, 8057 Zurich, Switzerland

[2]These authors contributed equally

[3]Technical contact

[4]Lead contact

*Correspondence: denoth@hifo.uzh.ch (A.D.-L.), jessberger@hifo.uzh.ch (S.J.)
https://doi.org/10.1016/j.xpro.2022.101129

SUMMARY

Pluripotent stem cell-derived human cortical organoids allow for the analysis of stem cell behavior and neurogenesis in neural tissues. Delivery of plasmid DNA into organoids permits visualization of individual cells, characterization of cellular components, and manipulation of gene expression. We describe a protocol to transfect cells inside organoids with plasmid DNA using micro-injection and electroporation, allowing for DNA delivery to cells within cortical units. This protocol was optimized for cortical organoids; however, it may be adapted to other organoid models.

For complete details on the use and execution of this protocol, please refer to Denoth-Lippuner et al. (2021)

BEFORE YOU BEGIN

Brain organoids are derived from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) and represent a novel tool to investigate early steps of human brain development and neurological diseases (Di Lullo and Kriegstein, 2017). Cortical organoids consist of many cortical units, each one consisting of a ventricle surrounded by neural stem cells (NSCs) (Qian et al., 2016). Dividing NSCs generate progenitor cells and newborn neurons that migrate away from the ventricular zone to the surface of the cortical unit (Lancaster et al., 2013; Kadoshima et al., 2013). However, visualizing single NSCs and their progeny, proteins within them, or manipulating them, often requires addition of genetic material. Several techniques have been previously used to deliver DNA into organoids, including viral treatment and plasmid transfection (Fischer et al., 2019). Plasmid DNA is generally introduced using cuvette electroporation at very early stages (embryoid bodies; Bian et al., 2018) or using a combination of micro-injection followed by electroporation using petri dish electrodes or electrode paddles (Lancaster et al., 2013; Yoon et al., 2017). Here, we provide a detailed protocol of how plasmid DNA is injected into cortical organoids using micro-injection followed by electroporation using cuvettes, as previously described (Denoth-Lippuner et al., 2021). This protocol reports the introduction of plasmid DNA into cortical organoids generated in a SpinΩ spinning bioreactor (Qian et al., 2016, 2018); however, the protocol could be adjusted to other types of brain organoids and organoids mimicking other organs.

Grow cortical organoids

Timing: 20–40 days

1. Choose your starting cell line. Here, we used H9 or H9-derived H3.1-miCOUNT hESCs (Denoth-Lippuner et al., 2021; Thomson et al., 1998).
2. Generate organoids using the previously published protocol (Qian et al., 2018). Some alterations were used, such as using AggreWell plates to generate embryoid bodies from a fixed number of cells (Denoth-Lippuner et al., 2021).

⚠ CRITICAL: If your starting cell line and plasmid to be introduced both express fluorescent proteins make sure the emission spectra are compatible.

Note: Some pluripotent stem cell lines are better at producing cortical organoids than others, make sure that your cell line of choice is able to generate cortical organoids containing several cortical units comprised of NSCs lining a ventricle-like structure and surrounded by their neuronal progeny. This protocol was optimized for cortical organoids of an average radius of 500 μm with a range of approximately 250 μm to 2 mm. In the protocol described here, we used H9 hESCs as source (Thomson et al., 1998). Organoids were between 20 to 40 days in culture and a starting population of 4000 cells was used per embryoid body. The injection parameters might require adjustment for younger or older organoids, as well as organoids of different sizes.

Note: Organoids used here are not embedded in Matrigel anymore.

Prepare DNA solution for injection

Timing: 3 h

3. Generate plasmid DNA using the Qiagen EndoFree Plasmid Maxi Kit following the instructor's manual (See: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/plasmid-dna/endofree-plasmid-kits/; for alternative plasmid kits see materials and equipment). Elute the plasmid in ultrapure distilled water.
4. Measure DNA concentration.
5. Prepare an Eppendorf tube with 18 μL of plasmid DNA containing a total of 20 ug DNA (per plasmid and condition).
6. Add 2 μL of 10 × Fast Green solution and mix by pipetting (alternative dye see materials and equipment).
7. Prepare one Eppendorf tube with 18 μL of H_2O with 2 μL of 10 × Fast Green solution to adjust injection conditions (Fast Green test solution).

Note: For all injections a plasmid DNA concentration of 1 μg/μL was used. DNA concentrations that are lower or higher may be used but could affect targeting efficiency or cause DNA toxicity depending on the constructs used. The final volume of injected solution will vary depending on the number of organoids and the success of the injections. Back-up solutions should be readily available.

⚠ CRITICAL: Use an EndoFree Plasmid Kit to generate endotoxin free plasmid DNA. Endotoxin containing DNA might decrease survival of the injected and electroporated organoids.

Prepare organoid injection furrow

Timing: 1 h

8. Prepare a 1 % agarose solution in a flask/beaker.
9. Heat the solution in the microwave until it is completely dissolved.

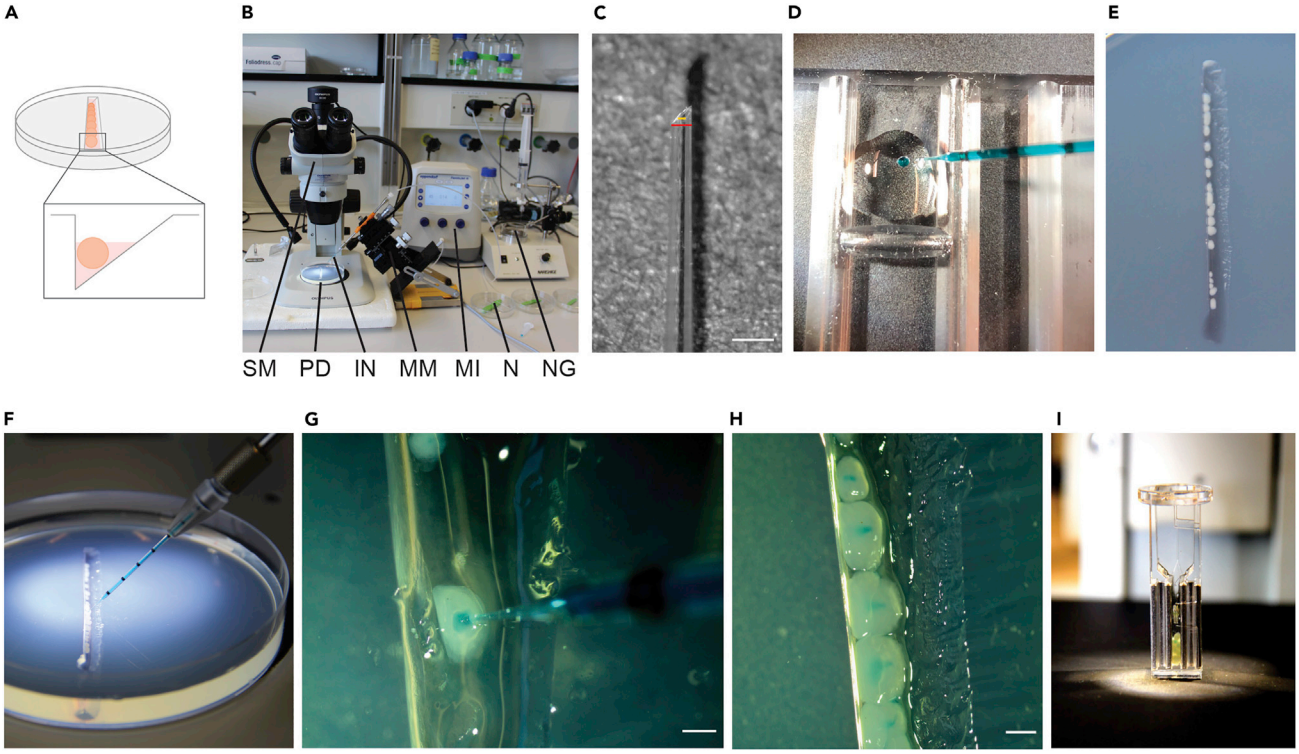


Figure 1. Microinjection and electroporation setup for cortical organoid transfection

(A) Scheme of how the organoids are embedded in the injection furrow.

(B) Overview of the used setup. SM = stereo microscope; PD = petri dish containing injection furrow; IN = injection needle; MM = manual micromanipulator; MI = micro injector; N = needles; NG = needle grinder.

(C) Image of a needle tip used for injections. Red line depicts needle diameter (55 μm) and yellow line depicts diameter of the needle opening (20 μm).

(D) Image of a drop of Fast Green test solution injected into an oil drop to estimate the injection volume.

(E) Image of organoids loaded onto the injection furrow.

(F) Image of how the needle is positioned to inject organoids located in the injection furrow.

(G) Image of the needle injecting plasmid DNA into the center of an organoid.

(H) Image of injected organoids in the injection furrow. Note how the addition of Fast Green facilitates the identification of successfully injected organoids.

(I) Injected organoids located in the cuvette for electroporation. All organoids were grown for 35 days (D35) prior to the experiment. Scale bars are 100 μm in (C); 1 mm in (G) and (H).

10. Swirl gently.
11. Cool down solution to 55°C–60°C.
12. Pour solution into Petri dish, ensuring 1–2 cm agarose layer thickness.
13. Let it solidify at 23°C or 4°C (faster).
14. Use a razor blade to cut out agarose furrow (approximately 1 cm width, 1 cm depth, 6 cm length) as shown in Figures 1A (scheme) and 1E.
15. Use immediately or keep the plate for later use by sealing with parafilm and storing at 4°C. Always ensure agarose is cooled down completely.

⚠ CRITICAL: Clean working space with ethanol and use new or ethanol cleaned razor blade to avoid contaminations.

⚠ CRITICAL: Carefully handle agarose solution after heating in microwave due to high risk of eruptive boiling. Wear goggles to protect eyes.

⚠ CRITICAL: The furrow should not be too deep, otherwise the needle might not reach the organoids. Several furrows can be cut into one agarose plate in order to get an ideal furrow.

Prepare injection needle

Timing: 1.5 h

16. Pull needles from glass capillaries using a needle puller, keeping the parameters of the needle puller consistent between experiments.
17. Cut needle with tweezer.
18. Grind the needle using a microgrinder, placing the needle with a 45° angle, until the needle has the desired tip size with smooth edges. Confirm thickness, shape and quality of the needle tip with a light-microscope.

Note: Needles were pulled using a Flaming/Brown Micropipette puller P-87 (Sutter Instrument) using Program 8 applying heat 850, velocity 50, and time 250 (note these are arbitrary units and only define pulling parameters on this instrument).

Note: The diameter of the needle tip should be approximately 55 μm with an opening of approximately 20 μm (see Figure 1C).

⚠ CRITICAL: To generate as little damage as possible to the organoid during injection, the needle tip should be small and sharp. However, if the needle tip is too small it can get clogged with tissue. If you experience frequent clogging during injections, grind the needle longer to increase the tip size. If the needle tip is too big, the liquid might leak out and too much damage will be done on the injected tissue.

Note: Needles should be washed with 70% Ethanol followed by DPBS washes before the first use and for reuse.

Prepare workspace

Timing: 10 min

19. Prepare labeled Eppendorf tubes to transport the organoids.
20. Turn on the microinjector, as it needs several minutes for calibration.
21. Prepare cuvettes and Nucleofector device on Program A-023 (for alternative electroporator see materials and equipment).
22. Cut off the tip of several P1000 pipet tips with a razorblade to transfer organoids.
23. Injections were done with the aid of a SZ61 Stereo Microscope (Olympus), but it can be done without optical aid.

⚠ CRITICAL: The tip size of the cut off P1000 depends on the size of the organoids. If the opening is too big, pipetting small volumes is difficult. If the opening is too small, organoids might be damaged. Always pre-wet the pipette tip before handling organoids as they might get stuck to the inside walls of the pipette tip.

Note: Microinjections and electroporations can be performed outside the cell culture hood. However, clean the workspace with 70% Ethanol before and after use to minimize contaminations.

Prepare media

Timing: 10 min

24. Prewarm (23°C) Eppendorf tubes containing 100 µL of Cell Line Nucleofector Solution V (for alternative transfection solutions see materials and equipment).
25. Prepare 3 mL fresh media to collect organoids and fresh media to feed organoids immediately after electroporation and warm up to 37°C.
26. Prepare 1 collection 6-well plate containing 3 mL media per well (37°C incubator).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat anti Sox2 (1:200)	Santa Cruz Biotechnology	Cat# sc17320 RRID: AB_2286684
AffiniPure Donkey Anti-Goat IgG (H+L) antibody coupled to different fluorophores (1:250)	Jackson ImmunoResearch Labs	Cat# 705-005-147 RRID: AB_2340385
Biological samples		
Human ESC line H9	Wicell	Cat# WA09-PCBC
Chemicals, peptides, and recombinant proteins		
Fast Green	Merck	Cat# F7252
UltraPure Agarose	Thermo Fisher Scientific	Cat# 16500500
DPBS	Thermo Fisher Scientific	Cat# 14190-094
Ethanol	Thommen-Furler AG	Cat# 02860
DMEM/F-12 GlutaMAX	Thermo Fisher Scientific	Cat# 31331028
N-2 Supplement	Thermo Fisher Scientific	Cat# 17502048
B-27 Supplement	Thermo Fisher Scientific	Cat# 17504044
Antibiotic-Antimycotic (Anti-Anti)	Thermo Fisher Scientific	Cat# 15240062
Insulin	Sigma-Aldrich	Cat# I9278
beta-Mercaptoethanol	Thermo Fisher Scientific	Cat# 31350-010
MEM Non-Essential Amino Acids Solution	Thermo Fisher Scientific	Cat# 11140050
Oil (Immersol 518 F for 30°C)	Zeiss	Cat# 444970-9000-000
UltraPure Distilled Water	Thermo Fisher Scientific	Cat# 10977-035
Cell Line Nucleofector Solution	Lonza	N/A
Critical commercial assays		
EndoFree Plasmid Maxi Kit	QIAGEN	Cat# 12391
Cell Line Nucleofector Kit V	Lonza	Cat#VVCA-1003
Experimental models: cell lines		
Human ESCs H3.1-miCOUNT	(Denoth-Lippuner et al., 2021)	N/A
Recombinant DNA		
pMax-GFP	Lonza	N/A
pCAG-Cre	Addgene	Cat# 13775
Other		
AggreWell 800	Stem Cell Technologies	Cat# 34821
NuncDish 100×15	Thermo Fisher Scientific	Cat# 150350
Nunc 6-well plate, Round	Thermo Fisher Scientific	Cat# 140675
Parafilm	Merck	Cat# P7793
Microwave	N/A	N/A
Glass PCR Pipet 1-10ul Plungers	Drummond Scientific	Cat# 5-000-1001-X10
Flaming/Brown Micropipette puller P-87	Sutter Instrument	Cat# 0299c (20081016)
Microgrinder	Narishige	Cat# EG-45
Manual Micromanipulator	World Precision Instruments	Cat# M3301-M3-R
Stereo Microscope	Olympus	Cat# SZ61
AMAXA Electroporation System	Lonza	N/A
FemtoJet 4i microinjector	Eppendorf	N/A
PIPETMAN Classic P1000	Gilson	Cat# F123602

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
1000 µL Universal Fit Filter Tips	Corning	Cat# TF-1000-R-S
200 µL Universal Fit Filter Tips	Corning	Cat# TF-200-R-S
Neubauer improved Cell-chamber/Hemocytometer	Brand	Cat# 717805
Microloader™ tips	Eppendorf	Cat# 5242956003
Eppendorf Safe-Lock Tubes, 1,5 mL	Eppendorf	Cat# 0030120086
FLoid Cell Imaging Station	Thermo Fisher Scientific	Cat# 4471136
Surgical Blade	Braun	Cat# BB510

MATERIALS AND EQUIPMENT

Eppendorf™ FemtoJet™ 4i Microinjector:

Here, we used the electronic microinjector "FemtoJet™ 4i" to inject plasmid DNA solution into organoids.

Alternatives: The manual injection device Eppendorf™ CellTram® 4r Oil was previously used successfully.

Amaxa Nucleofector™ device:

We used the Amaxa Nucleofector™ device from Lonza.

Alternatives: As an alternative electroporation device the GenePulser® Xcell Electroporation System from Bio-Rad might be used.

Cell Line Nucleofector™ Kit V:

We used the Cell Line Nucleofector™ Kit V containing the electroporation solution and cuvettes.

Alternatives: The Ingenio® Solution from Bio-Rad could be used.

Qiagen EndoFree Plasmid Maxi Kit:

To amplify and purify plasmid DNA the EndoFree Plasmid Maxi Kit from Qiagen was used.

Alternatives: Any endotoxin free plasmid DNA purification kit could be used, as for example the NucleoBond Xtra Maxi EF from Macherey-Nagel.

EG-44 Microgrinder from Narishige:

After pulling the needles the EG-44 Microgrinder from Narishige was used to grind the needle opening.

Manual Micromanipulator from World Precision Instruments:

In our setup the capillary holder is held by a manual micromanipulator allowing precise injections.

Alternatives: manual handling of the capillary holder was previously used successfully.

10 × Fast Green solution		
Reagent	Final concentration	Amount
Fast Green	1%	100 mg
ddH_2O	n/a	10 mL
Total		**10 mL**
Storage at 4°C for up to 18 months		

⚠ CRITICAL: Fast Green powder should be prepared under a chemical hood to prevent inhalation. Furthermore, wear gloves and lab coat to prevent green stains. Filter sterilize fast green solution before usage.

Alternatives: Brilliant blue or Phenol Red could be used as an alternative stain.

1% Agarose solution		
Reagent	Final concentration	Amount
UltraPure Agarose	1%	1 g
ddH_2O	n/a	100 mL
Total		**100 mL**
Use immediately.		

Organoid culturing media		
Reagent	Final concentration	Amount
DMEM:F12 GlutaMAX	n/a	474.5 mL
N-2 Supplement	1 ×	5 mL
B-27 Supplement	1 ×	10 mL
MEM Non-Essential AA	1 ×	5 mL
beta-Mercaptoethanol	0.1 ×	0.5 mL
Anti-Anti	1 ×	5 mL
Total		**500 mL**
Mix, filter-sterilize and keep at 4°C for up to 1 month. Add fresh Insulin (2.5 µg/mL) before use.		

STEP-BY-STEP METHOD DETAILS

Injection of plasmid DNA into cortical organoids

Timing: 30 min

Note: Injections can be performed outside the cell culture hood. However, the area and all tools used should be sterilized with 70% Ethanol prior to usage.

After placing the organoids into the organoid injection furrow (Figure 1A), plasmid DNA is injected into the center of single organoids using a sharp injection needle. Mixing the plasmid DNA with the Fast Green dye facilitates assessment of the injection site. For automated injection an Eppendorf™ FemtoJet™ 4i Microinjector is used (alternative microinjection device see materials and equipment) and injections are performed under a stereo microscope. For setup see Figure 1B.

1. Needle loading and adjustment of injection settings
   a. Take the needle (prepared in step 16–18).
   b. Fill the needle with 10 µL of the Fast Green test solution (prepared in step 7) using 20 µL Microloader pipet tips.

c. Insert needle into capillary holder.
d. Start with the following settings: 45 hPa injection pressure (p_i), 0.14 s injection time (t_i), 0 hPa compensation pressure (p_c).
e. Eject a drop onto a hemocytometer.
f. Adjust settings in order to generate a drop with approximately 250 µm radius which corresponds to approximately 65 nL.
g. Eject H_2O solution containing Fast Green onto paper tissue by pressing "clean" for several seconds.
h. Reload needle with plasmid DNA solution.

Alternatives: To estimate the volume of the injected DNA solution: a drop of oil is placed on a hemocytometer and the solution is injected into the oil (Figure 1D). The radius of the drop should be approximately 1/2 of the radius of the organoids, which corresponds to 1/8 of their volume. If the size of organoids of one batch varies substantially, the drop should be adjusted by the smaller ones. Bigger organoids can be either injected several times or at multiple locations.

Note: These calibration steps are essential for a successful injection session and should be done every time the glass needle is changed. To increase the size of the drop, increase p_i or t_i; however, p_i should be kept relatively low to prevent damaging the organoids. If the liquid dries inside the tip of the needle and blocks the needle p_c can be increased; however, high p_c will result in a constant flow of plasmid DNA.

⚠ CRITICAL: Before cleaning the needle make sure the needle is properly screwed into the capillary holder to prevent ejecting the needle.

2. Load organoids into injection furrow (Figure 1E)
   a. Collect organoids using a cut off P1000 tip (prepared in step 22).
   b. Distribute 10–20 organoids along the injection furrow.
   c. Remove some excess media using an uncut P1000 or P200.
   d. Place the plate containing organoids under the stereo microscope.

⚠ CRITICAL: The organoids should be half-covered and surrounded by media to prevent them from drying out but they should not be fully covered by media in order to keep them stable during injections (see Figure 1A). Avoid injecting more than 20 organoids at a time.

3. Inject organoids (Figures 1F–1G)
   a. Gently push the needle into the center of the organoid.
   b. Push "inject" key (problem 1).
   c. Gently pull out the needle.
   d. Repeat until the green DNA solution is clearly visible inside the organoid.
   e. Move to the next organoid in the row.

Note: Big organoids can be either injected several times (keep needle inserted and press the "inject" key several times) or at multiple locations. Especially elongated organoids might be injected at multiple areas.

⚠ CRITICAL: The organoid should be filled with green plasmid DNA solution (see Methods video S1). If plasmid DNA solution flows out of the organoid the needle was not placed correctly or the injection volume needs to be adjusted (lower p_i or t_i). If no DNA solution is ejected the needle might be clogged (problem 1) or dried out (problem 4).

⚠ CRITICAL: Avoid for the needle to be inserted into the agarose as it might break or clog. Equally avoid the needle to perforate through the organoid as this will cause leakiness of the plasmid solution and too much disruption on tissue integrity (see Methods video S2).

⚠ CRITICAL: The time organoids spend on the injection furrow should be kept at a minimum (max 15 min). Inexperienced experimenters should start with low numbers of organoids injected at once.

4. Collect organoids from injection furrow once all organoids are injected (Figure 1H and problem 2)
   a. Flush organoids out of the injection furrow with DPBS using the P1000 with cut off tip.
   b. Transfer organoids into Eppendorf tube.
   c. Remove as much DPBS as possible.
   d. Clean injection furrow with DPBS for immediate reuse.

Note: Carefully check by eye that all organoids are collected from the injection plate.

Note: injection furrow should be trashed after a full day of usage as cell culture media deposits might get contaminated and create issues during further injections.

Electroporation of injected organoids

Timing: 10 min

After injecting all organoids with plasmid DNA they are pooled together (max. 20 organoids) into one cuvette and electroporated using a nucleofector device.

5. Electroporate organoids (Figure 1I)
   a. Add 100 μL of prewarmed (23°C) nucleofector solution to the organoids.
   b. Transfer whole solution containing organoids from the Eppendorf tube to the cuvette (problem 3).
   c. Electroporate organoids using program A-023.
   d. Add 1 mL of prewarmed media into the cuvette.
   e. Flush out organoids by pouring cuvette into a prepared collection 6-well plate.
   f. Repeat flushing out organoids until all organoids are in the collection 6-well plate.
   g. Collect organoids and transfer them back to the original well. This step removes excess debris and dissociated tissue resulting from injection and electroporation.
   h. Add fresh media for further culturing.
   i. Assess transfection at selected time points using method of choice (problem 5).

⚠ CRITICAL: Do not overfill the electroporation cuvette with nucleofector solution (solution containing organoids should not exceed the limit of the paddle), as more than 100 uL will give an error message from the electroporator.

⚠ CRITICAL: Flush out organoids carefully from the cuvette to prevent damaging organoids that are fragile after electroporation. Try to keep the number of organoids inside the cuvette consistent between experiments to reduce variability.

⚠ CRITICAL: Electroporation should be executed as fast as possible in order to prevent diffusion of the plasmids out of the organoids and to minimize the time organoids are spent outside the incubator.

Note: Here, the transfection efficiency was assessed using fluorescence microscopy of the electroporated organoids 1–5 days post injection.

EXPECTED OUTCOMES

This protocol guides users through individual steps to successfully transfect cells within cortical organoids using plasmid DNA. The successful transfection will reveal one or several cortical units containing cells expressing the gene encoded by the plasmid DNA. Here, we show examples of H3.1 miCOUNT organoids (Denoth-Lippuner et al., 2021) expressing H3.1-tdTomato (red) that were injected and electroporated using Cre recombinase-expressing plasmid DNA. Cells expressing Cre recombinase recombine the miCOUNT color and thus express H3.1-mNeonGreen (green, see Figure 2A). Furthermore, we used plasmid DNA expressing GFP under the CMV promoter (pMax-GFP) to visualize the targeted cells using this transfection protocol (Figures 2B and 2C). To illustrate the benefit of injecting plasmid DNA into the ventricle prior to the electroporation we show a representative image of an organoid where GFP plasmid solution was added to the electroporation solution (Figure 2D) or that was injected with GFP plasmid solution (Figure 2E) prior to electroporation. Without DNA injection the plasmid DNA only transfects cells at the surface of the organoid (Figure 2D) whereas the injection leads to a substantial increase in numbers of targeted cells that are located within cortical units (Figure 2E).

Following injection and electroporation organoids can be cultured until desired time points (1 day in Figures 2B and 2C or 5 days in Figures 2A, 2D and 2E) and can be analyzed using methods of choice, such as for example by immunostaining of sectioned organoids, fluorescence activated cell sorting (FACS), or live-imaging (Bowers et al., 2020; Denoth-Lippuner et al., 2021; Gonzalez-Bohorquez et al., 2022).

LIMITATIONS

The presented protocol allows efficient transfection of cells residing inside a cortical organoid. However, we only observed a few cortical units targeted by the injection and electroporation in each organoid. Therefore, the number of transfected cells per organoid remains a fraction of the whole organoid. This might be increased by several injections per organoid; however, too many injections might damage the organoid and lead to its dissociation (maximally 3 injections unless extremely elongated organoid). Post-hoc analyses are also important to confirm the identity of the targeted cells as, even though in small proportion, cells outside of the cortical units might be targeted.

In general, successful transfection largely depends on the quality of the organoids. Organoids that are of firm structure and contain several ventricles are ideal. The protocol is optimized for organoids ranging from 20 to 40 days in culture. If the organoids are younger they might suffer more from the injection due to the small size. Adjusting the needle size might adjust for the size difference. Here, the protocol was optimized for organoids containing many ventricles. Therefore, the injected plasmid solution quickly fills the ventricles, thereby mostly targeting NSCs; however, this might depend on the overall structure of individual organoids and used protocols to generate organoids. In organoids kept longer in culture containing less ventricles other cell types might be targeted instead. It is also important to consider that this protocol is invasive and very careful quality assessment on the overall health of the organoid should be done after injection and electroporation. Generally, 1 or 2 days after electroporation weakened organoids will dissociate and should not be used for further experimentation. Injection and electroporation of plasmid DNA causes a certain amount of cell death; thus, it is important to use organoids injected and electroporated with control plasmids for quantitative comparisons.

TROUBLESHOOTING

Problem 1

Organoid tissue clogs the needle.

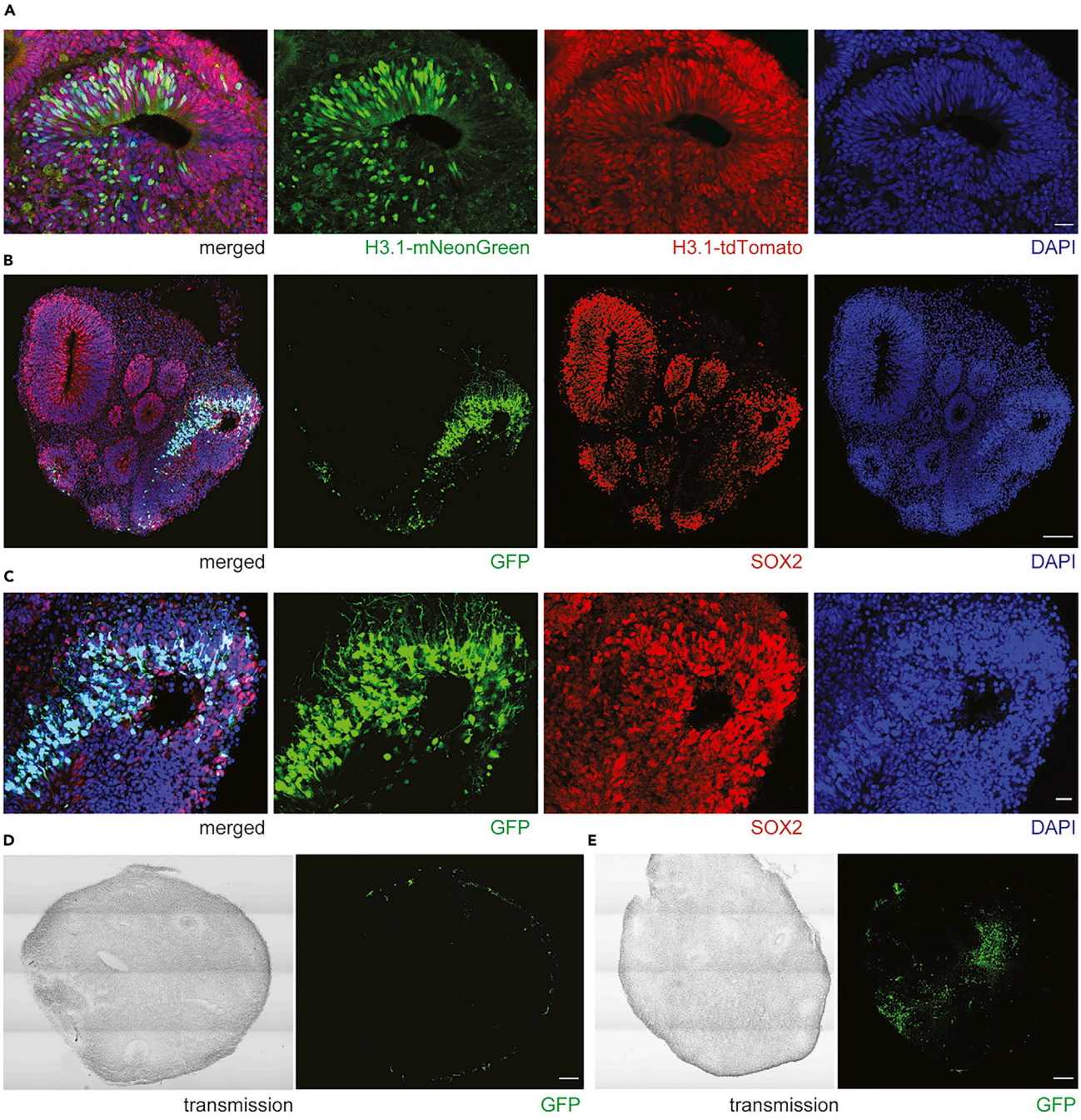


Figure 2. Outcome of plasmid DNA transfection into cortical organoids

(A) Fluorescence image of a H3.1-miCOUNT organoid injected with CRE expressing plasmid DNA to induce the color switch from tdTomato (red) to mNeonGreen (green).

(B) Micrograph of a wt organoid injected with GFP expressing plasmid DNA and stained for SOX2.

(C) Zoomed in view of the organoid shown in (B).

(D) Overview of an organoid electroporated with GFP expressing plasmid DNA.

(E) Overview of an organoid injected with GFP expressing plasmid DNA prior to electroporation. Note how only few cells at the surface of the organoid are targeted if plasmid DNA is not injected (D), whereas many cells within different cortical units express GFP if plasmid DNA is injected into the organoid prior to electroporation (E). All organoids were grown for 35 days (D35) prior to the experiment. Organoids were analyzed 5 days (A, D–E) or 1 day (B and C) after injections. Images were stitched. Scale bars are 20 μm in (A) and (C); 100 μm in (B), (D), (E). Nuclei were counterstained using DAPI.

Potential solution 1
The clog can be removed by pushing the "inject" key several times into surrounding media or carefully using paper tissue.

Potential solution 2
If the clog persists the needle has to be cleaned as described in 1.g.

Potential solution 3
If the clog persists the needle can be replaced but a new calibration should be performed.

Problem 2
Organoids remain stuck in the injection furrow.

Potential solution
Organoids start to stick to the agarose and between themselves if they are not surrounded by sufficient media. Add some DPBS to flush off organoids using a pipette to gently flush them out of the furrow. Use more volume of media for the next round of injections.

Problem 3
Collecting all organoids from the Eppendorf tube (5.b.) using only 100 µL of nucleofector solution can be difficult and some organoids might be left in the Eppendorf tube.

Potential solution
After collecting some organoids using all nucleofector solution, let the organoids sink in your P1000 cut off pipet tip and pipet only the organoid containing solution into the cuvette. The remaining nucleofector solution can be used to pick up residual organoids from the Eppendorf tube. Otherwise, one could split the organoids and electroporate them in several cuvettes, with 100 uL of nucleofector solution each.

Problem 4
The DNA solution dries inside the injection needle.

Potential solution 1
Load the needle with DNA solution immediately before injections are performed. Time spent for injections should be kept as low as possible.

Potential solution 2
Leave loaded needle touching media inside the injection furrow or DPBS in a separate well.

Potential solution 3
If dried DNA solution clogs the needle a new needle can be used; however, every new needle needs to be calibrated first.

Problem 5
No signal is detected.

Potential solution 1
Perform the experiment using a reliable control plasmid (e.g., pMax-GFP provided by Lonza).

Potential solution 2
Check your plasmid first on cells grown as monolayers.

Potential solution 3
Check plasmid sequence, concentration and purity.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Sebastian Jessberger, jessberger@hifo.uzh.ch

Materials availability

H3.1-miCOUNT human embryonic stem cells used in this protocol will be shared upon request upon execution of a Material Transfer Agreement and after consulting WiCell for permission.

Data and code availability

All images are shared upon request.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101129.

ACKNOWLEDGMENTS

We thank G.L. Ming and H.J. Song for training with culturing organoids and R. Bachmann-Gagescu for help and providing equipment for micro-injections. A.D.-L. was supported by a Forschungskredit of the University of Zurich (UZH) and the Novartis Foundation. L.N.R. was supported by a fellowship of the Boehringer Ingelheim Fonds. D.G.-B. was supported by a Candoc fellowship. Further support was provided by the European Research Council (STEMBAR to S.J.), the Swiss National Science Foundation (BSCGI0_157859 to S.J.), the University Research Priority Program (URPP) AdaBD of UZH, the Betty and David Koetser Foundation, and the Zurich Neuroscience Center (ZNZ).

AUTHOR CONTRIBUTIONS

A.D.-L. developed the concept, performed experiments, and cowrote the protocol. L.N.R. and D.G.-B developed the method, performed experiments, and cowrote the protocol. D.M. performed experiments. S.J. developed the concept, secured funding, and cowrote the protocol.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Bian, S., Repic, M., Guo, Z., Kavirayani, A., Burkard, T., Bagley, J.A., Krauditsch, C., and Knoblich, J.A. (2018). Genetically engineered cerebral organoids model brain tumor formation. Nat. Methods *15*, 631–639.

Bowers, M., Liang, T., Gonzalez-Bohorquez, D., Zocher, S., Jaeger, B.N., Kovacs, W.J., Rohrl, C., Cramb, K.M.L., Winterer, J., Kruse, M., et al. (2020). FASN-dependent lipid metabolism links neurogenic stem/progenitor cell activity to learning and memory deficits. Cell Stem Cell *27*, 98–109.e11.

Denoth-Lippuner, A., Jaeger, B.N., Liang, T., Royall, L.N., Chie, S.E., Buthey, K., Machado, D., Korobeynyk, V.I., Kruse, M., Munz, C.M., et al. (2021). Visualization of individual cell division history in complex tissues using iCOUNT. Cell Stem Cell *28*, 2020–2034.e12.

Di Lullo, E., and Kriegstein, A.R. (2017). The use of brain organoids to investigate neural development and disease. Nat. Rev. Neurosci. *18*, 573–584.

Fischer, J., Heide, M., and Huttner, W.B. (2019). Genetic modification of brain organoids. Front. Cell. Neurosci. *13*, 558.

Gonzalez-Bohorquez, D., Gallego López, I.M., Jaeger, B.N., Pfammatter, S., Bowers, M., Semenkovich, C.F., and Jessberger, S. (2022). FASN-dependent de novo lipogenesis is required for brain development. PNAS *119*, e2112040119.

Kadoshima, T., Sakaguchi, H., Nakano, T., Soen, M., Ando, S., Eiraku, M., and Sasai, Y. (2013). Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. Proc. Natl. Acad. Sci. U S A *110*, 20284–20289.

Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., and Knoblich, J.A. (2013). Cerebral organoids model human brain development and microcephaly. Nature *501*, 373–379.

Qian, X., Jacob, F., Song, M.M., Nguyen, H.N., Song, H., and Ming, G.L. (2018). Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. Nat. Protoc. *13*, 565–580.

Qian, X., Nguyen, H.N., Song, M.M., Hadiono, C., Ogden, S.C., Hammack, C., Yao, B., Hamersky, G.R., Jacob, F., Zhong, C., et al. (2016). Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell *165*, 1238–1254.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. Science *282*, 1145–1147.

Yoon, K.J., Song, G., Qian, X., Pan, J., Xu, D., Rho, H.S., Kim, N.S., Habela, C., Zheng, L., Jacob, F., et al. (2017). Zika-virus-encoded NS2A disrupts mammalian cortical neurogenesis by degrading adherens junction proteins. Cell Stem Cell *21*, 349–358 e6.