Tissue Optimisation Manual

Version 180925

SPATIAL TRANSCRIPTOMICS

For Research Use Only

Table of Contents

Revision History

Revised Part	Date	Revision
All parts	2016-10-17	Minor updates of instructions and reagent amounts/descriptions
All parts	2016-10-31	Minor updates and more specified description of some reagents.
Preparing Stock Reagents and Buffers	2016-10-31	More detailled description of SSC/SDS buffer
All parts	2016-11-14	Minor updates and more specified description of some reagents.
All parts	2017-02-10	A different slide module is suggested to be used during the protocol
Preparing Stock Reagents and Buffers	2017-07-11	Updated recipe for Tris / Acetic Acid Buffer
All Parts	2018-03-29	Updated throughout. Reverse transcriptase changed to Super-Script III
All parts	2018-05-15	Minor updates throughout
All parts	2018-05-29	Removed positive control. Added new slide modules
All parts	2018-09-25	Minor updates

Introduction

Tissue Optimisation

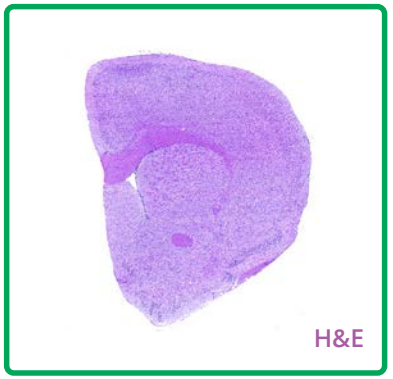
The Tissue Optimisation (**TO**) procedure is employed to test whether a tissue of interest is compatible with Spatial Transcriptomics (**ST**), and to optimise the experimental parameters throughout the ST protocol for a given tissue.

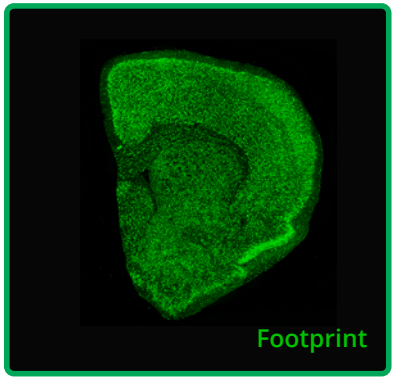
Tissue must be fresh frozen for this protocol to work.

TO slides have six square subregions that have been coated with surface probes that capture mRNA and serve as primers for reverse transcription (similar to those used in the full Library Preparation (**LP**) protocol). A single tissue section is positioned on each subregion. The tissue is then formaldeyhde fixed, stained with H&E (Hematoxylin & Eosin), and imaged. Next, permeabilisation agents are added to allow RNA from the tissue sections to hybridize to adjacent surface probes. Testing a series of different permeabilisation conditions allows the user to determine the optimal protocol for a tisssue of interest. cDNA synthesis incorporating a fluorescently labelled nucleotide is subsequently performed. Finally, in order to properly detect the fluorescent cDNA that is generated, the tissue must be removed. The tissue removal step is another key point of optimisation.

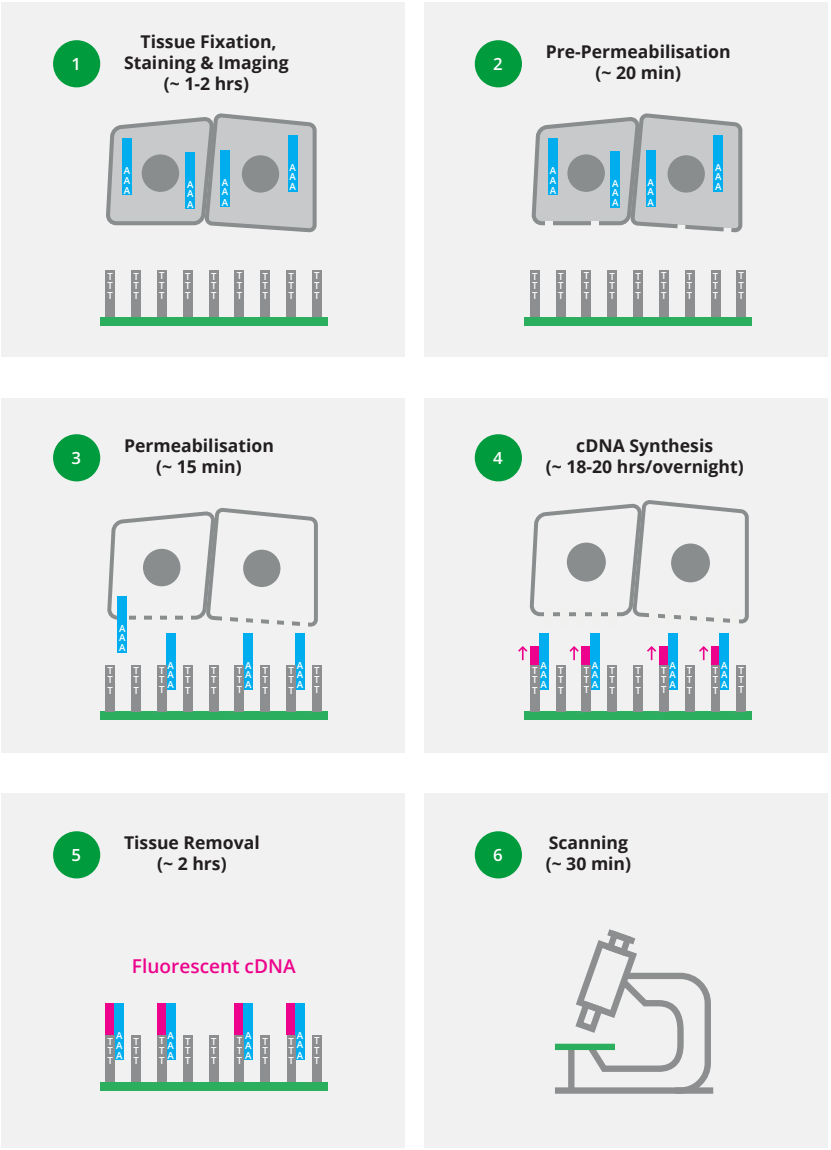
Optimal conditions result in a fluorescent cDNA footprint on the TO slide that mirrors the tissue morphology seen in the previous H&E stain (see below).

The TO protocol is very similiar to the first part of the longer LP protocol. Therefore the parameters determined in the TO experiment can be transferred to the LP protocol.





Workflow



General instructions

Before You Start

- **Read this manual thoroughly.**
- **When the TO label faces up the active surface of the slide faces up.**
- **Never touch the active surface of the slide!**
- **The TO slide is placed into the slide module (microarray hybridization cassette) for pre-permeabilisation -NOT BEFORE.**
- **Perform the protocol without interruption.**
- **All reagents should be vortexed and spun down before pipetting if not otherwise noted.**
- **Prepare stock solutions and buffers (pages 28-29) before beginning your TO experiment.**

The TO protocol has been used with many different tissue types. The Appendix contains a table with some example tissues and their successfully used parameters (page 26) that can be used as starting points for optimisation.

Parameters that can be changed for different tissue types are highlighted in orange throughout the text, as shown here: **7 minutes.**

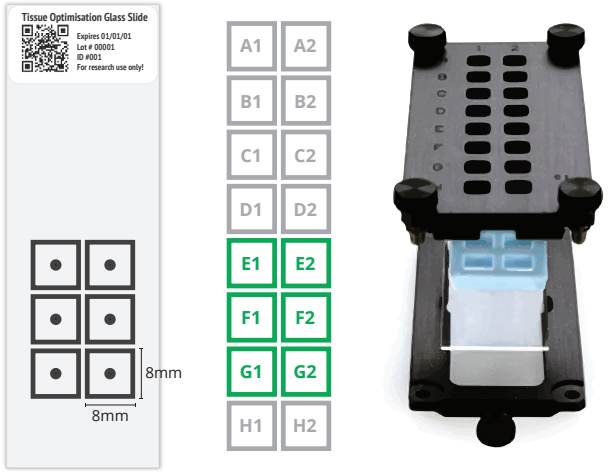
During some incubation steps, reagents for the subsequent step in the protocol need to be prepared. These preparatory steps are highlighted with **purple text**.

*Please read all **M**aterial **S**afety **D**ata **S**heets provided by the individual reagent suppliers carefully. By using this protocol you agree to the terms and conditions that can be found under* **https://www.spatialtranscriptomics.com/terms-and-conditions/**

Experimental Design

• The TO slides have 6 square, 8x8mm, subregions coated with surface probes that capture mRNA and serve as primers for reverse transcription. These subregions are demarcated by black frames printed on the back of the slide.

• The slide module that is used to create individual reaction wells generates 2 columns (1-2) of eight rows (A-H). The active regions on TO slides correspond to rows E, F, and G, as indicated in the picture below.



• **We recommend testing experimental conditions in each of five wells, and using the remaining well for a negative control.**

• **Experimental**
• **Negative Control**

Negative Control

The negative control well contains a tissue section like the experimental wells. However, the negative control is treated with 0.1x SSC instead of Pepsin/HCl during the permeabilisation step.

Partial permeabilisation occurs during the pre-permeabilisation step, and results in a weak signal from the tissue.

A well optimised permeabilisation step will yield a stronger signal than the negative control.

RNA Quality Requirements

Agilent Bioanalyzer RNA Pico Kit
RNA Extraction kit

• In order to ensure the best possible results we recommend using tissue with a **RIN (RNA Integrity Number) value of 7 or higher**.

• To evaluate tissue RIN we recommend collecting a sample containing at least 8 tissue sections (10 µm thick) for testing. We also recommend the use of a commercially available RNA extraction kit and 2100 Bioanalyzer analysis to determine the RIN value.

• To acheive a representative RIN please ensure that the tissue does not thaw prior to RNA extraction. One should, for example, pre-cool the extration tube into which the tissue sections are placed, and handle the extraction tube with forceps.

Tissue Handling & Sectioning

- Store fresh frozen tissue at -80°C and avoid thawing.

- If tissue was frozen without Optimal Cutting Temperature (**OCT**) embedding medium, it should be embedded in OCT prior to sectioning. Cool the OCT close to the point where it hardens before embedding.

- A tissue section size of 8mm x 8mm is compatible with TO slides (however, if planning to use the same tissue block for later LP experiments, one should remember that for LP the tissue sections should be no larger than 6.3 mm x 6.7 mm).

- Tissue + OCT should fit within a 10x10mm square (the OCT is dissolved and washed away during fixation and staining).

- Tissue should be sectioned with a cryostat.

A Video Demonstration of the Sectioning process can be found at: https://vimeo.com/154600991

- Pre-cool your TO slide in the cryostat.

- We recommend a tissue section thickness of 5-16 µm (see page 26 for tissue specific suggestions).

- Place one tissue section in each of the marked areas on the TO slide. **Make sure to place the sections on the active surface of the glass slide**.

IMPORTANT: Flatten the tissue section carefully. Make sure not to touch the tissue itself but rather touch the surrounding OCT. Place a finger on the backside of a square on the TO slide for several seconds in order to warm the glass. Once the targeted area of the slide is heated, the tissue should attach automatically when the slide is held close to it.

- **When a tissue section has been placed in each of the marked squares, the slide can either be stored at -80 °C for up to 7 days or the TO experiment can be started immediately.**

Brightfield Scanning

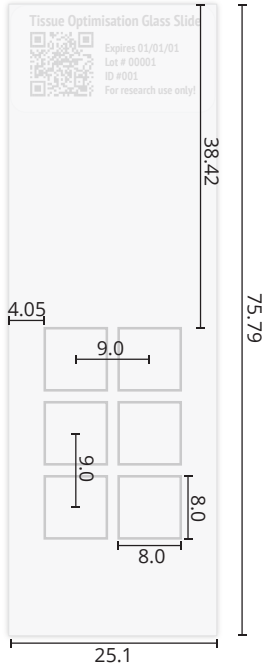
A brightfield imaging system is required to capture the H&E stainings.

In order to generate high resolution images of the stained tissue sections in a reasonable timeframe we recommend low magnification, high numerical aperture (NA), objectives e.g. 10x NA 0.45, 20x NA 0.5, 20x NA 0.75.

At these magnifications many captured images (tiles) will be needed to reconstruct your entire tissue section. These are usually captured with the aid of a motorized scanning microscope stage.

Captured image tiles must then be stitched together using computer software.

Below is a schematic of the TO slide. Six evenly spaced 8mm x 8mm squares with a distance of 9mm between centre points:



measurements in millimetres

Fluorescent Scanning

In order to evaluate the outcome of the TO experiment an image of the fluorescent cDNA produced on the slide surface must be captured.

Cyanine-3 has an excitation maximum of approximately 550nm, and an emission maximum of approximately 570nm.

In microarray scanners (e.g. Innoscan 710) Cyanine-3 can be excited efficiently with the 532nm laser. When using a microarray scanner we recommend a resolution of at least 10 µm/pixel.

The fluorescent signal generated by the cDNA footprint of a successful TO is relatively weak, but has an excellent signal to noise ratio.

It is possible to capture the signal with a standard fluorescent microscope. However, you will require a broad Cy3/TRITC filter set (e.g. Chroma 49004; Zeiss 43HE), a high NA objective (e.g. 20x NA 0.75), and long camera exposure times.

A positive result is a Cyanine-3 footprint that mimics the morphology seen in the H&E stain and is readily distinguishable from background.

Tissue Optimisation Protocol

1.Tissue Fixation, Staining & Imaging

Formaldehyde solution, 36.5-38%	Eosin Y (aqueous)
1XPBS	Tris/Acetic acid buffer
Isopropanol (2-Propanol)	85% Glycerol
Mayer's Hematoxylin Solution	HBSS buffer
Bluing Buffer	BSA, 20mg/ml

We recommended optimising the duration of the H&E stain for your tissue type of interest prior to starting TO (this can be performed on standard adhesive microscope slides e.g. Superfrost Plus).

- **Pre-heat a thermomixer for plates and a thermomixer for tubes to 37°C.**
- **Prepare fresh formaldehyde solution by adding 100 µl of formaldahyde to 900 µl of PBS.**
- **Mix 5 ml Eosin with 45 ml Tris/Acetic acid buffer in a 50 ml tube to prepare Eosin solution.**
- **Prepare Pre-permeabilisation buffer: 986 µl HBSS + 10 µl BSA. Prehaeat to 37°C.**
- **Prepare 50 ml tube of 80% ethanol.**
- **Prepare 50 ml tube of PBS.**
- **Prepare 50 ml tube of ultrapure (e.g. Milli-Q) water.**
- **Prepare three beakers containing 800 ml of ultrapure water each.**

1.Tissue Fixation, Staining & Imaging

1. Heat the TO slide for **1 minute** at 37°C in a thermomixer.

2. Immediately after heating, place the slide on a flat clean surface inside a laminar hood.

3. Apply 900 µl of formaldehyde solution to the active surface of the glass slide, and ensure that all tissue sections are covered.

4. Incubate at room temperature for **10 minutes**.

5. Decant the formaldehyde solution from the glass slide and immediately wash by slowly, and completely, dipping the slide into a 50 ml tube filled with PBS. Dip the slide in PBS **five times**.

6. Remove excess fluid from the slide by wiping the outer edge with tissue paper. **Do not to touch the active surface of the glass slide!**

7. Return the slide to a flat surface and pipette 500 µl isopropanol on to it, making sure that all squares are covered. Incubate for **1 minute**.

8. Remove excess fluid from the slide by wiping the outer edge with tissue paper. Let the slide **air-dry**.

9. When completely dry, pipette 900 µl of Mayer's hematoxylin on to the slide, making sure all squares are covered. Incubate for **7 minutes**.

10. Dip the slide in a 50 ml conical centrifuge tube containing ultrapure water. Repeat **5 times**.

11. Dip the slide in a beaker containing 800 ml ultrapure water. Repeat **15 times**.

12. Taking a fresh beaker, dip the slide in 800 ml ultrapure water. Repeat **15 times**. Let most of the excess water drip off and wipe the back of the slide with tissue paper.

13. Pipette 900 µl Bluing Buffer on to the slide so that it covers all 6 squares. Incubate at room temperature for **2 minutes**.

14. Dip the slide in a beaker of ultrapure water. Repeat **5 times**.

15. For counterstaining dip the glass slide for **1 min** in a 50 ml centrifuge tube containing the freshly prepared Eosion solution.

16. Dip the slide in a beaker of ultrapure water. Repeat **10-15 times**.

17. **Air dry** the glass slide until the tissue sections are completely dry.

18. Incubate the slide for **5 minutes** at 37°C.

19. Remove the black squares from the back of the slide with an ethanol soaked tissue.

20. Carefully pipette 200 µl of 85% glycerol on to the tissue sections and cover with a coverglass. Image the sections.

21. Remove the cover glass by holding the glass slide and dipping it in a beaker of ultrapure water. Gently move the glass slide up and down and let the cover glass detach under its own weight. Once the cover glass has detached, dip the slide in the water three times.

22. Dip the glass slide swiftly in 80% ethanol in order to remove any remaining glycerol.

23. Air dry at room temperature.

24. Warm the slide for **1 minute** at 37°C.

- **Proceed directly to Pre-Permeabilisation.**

2. Pre-Permeabilisation

Collagenase, 50U/µl
Pepsin (0.1%) in HCl aliquot

1. Prepare Pre-permeabilisation mix by adding 4 µl collagenase to the Pre-Permeabilisation buffer. Invert carefully, spin down quickly and put back at 37°C until use. Do not vortex!

2. **Place the slide in the slide module**. Make sure to tighten the screws.

3. Add 70 µl of the Collagenase mix to each of the wells containing a tissue section.

Whenever pipetting into wells containing tissue sections be careful not to pipette directly onto the tissue or to touch the tissue with the pipette tip. Rather pipette down slowly at the edge of the well.

4. Cover all sample wells with a plastic seal to avoid evaporation.

5. Incubate at 37°C for **20 minutes** (no shake) in a thermomixer.

During the incubation, prepare reagents needed for the cDNA synthesis (See page 18). But **do not** add SuperScript III or RNaseOUT at this point!

When 10 min of the Pre-Permabilization incubation remain, pre-heat one aliquot of Pepsin to 37°C.

6. When the incubation is finished: carefully remove the seal and Pre-Permeabilisation mix from all sample wells by slowly pipetting.

7. Wash each well carefully by slowly adding 100 µl 0.1x SSC.

3. Permeabilisation

Pepsin (0.1%) in HCl aliquot

In order to determine the optimal permeabilisation duration you can test a different duration in each experimental (non-control) well.

Please see the tissue conditions table in the appendix (page 26) for proven examples.

1. Exchange the SSC in each well for 70 µl of Pepsin in a staggered sequence starting with the well with the longest incubation time. **Remember not to add Pepsin to any negative contol well**.

2. When not pipetting, place the slide module back into the incubator so that it is held at a temperature as close to 37°C as possible.

3. Incubate at 37°C (no shake) in a thermomixer.

4. When the incubation is finished carefully remove the Pepsin mix by pipetting slowly.

5. Wash all experimental wells carefully by slowly adding 100µl 0.1x SSC.

- **Proceed directly to cDNA synthesis**

4. Fluorescent cDNA Synthesis

5X First-Srand Buffer	TO dNTP mix
DTT (100 mM)	Cyanine-3 dCTP (1mM) Avoid exposure to light!
Water (RNase & DNase free)	Superscript III Reverse Transcriptase
Actinomycin D (5 mg/ml in DMSO, as previously aliquoted)	RNaseOUT™ Recombinat Ribonuclease Inhibitor
BSA (20 mg/ml), molecular biology grade	

- Prepare the cDNA synthesis mix as follows:

5X First-Strand Buffer	96 µl
Water (RNase & DNase free)	252.3 µl
DTT	24 µl
Actinomycin D	4.8 µl
BSA (20 mg/ml)	4.5 µl
TO dNTP mix (prepared earlier)	24 µl
Cyanine-3 dCTP (avoid exposure to light!)	2.4 µl

Mix well by pipetting up and down several times and preheat to 42 °C.

1. Add 48 µl **Superscript III** and 24 µl **of RNaseOUT™** to the cDNA Synthesis mix. Mix by pipetting up and down carefully. This tube now contains the finished cDNA Synthesis master mix. Put the tube back at 42°C.

2. Slowly remove the 0.1x SSC buffer from each well.

3. Add 75 µl of cDNA Synthesis Master Mix to each well. Avoid bubbles in the wells by not releasing the last few microliters from the pipette tip.

4. Cover all wells with a plastic seal.

5. Incubate at 42°C for **18-20 hours** (no shake).

5. Tissue Removal

There is a choice between two tissue removal procedures:

Option 1: For less fibrous, fatty tissue like brain. **Proteinase K** treatment.

Option 2: For more fibrous tissue. **β-mercaptoethanol** followed by **Proteinase K**.

Please see the tissue conditions table in the appendix (page 26) for proven examples.

Option 1: Proteinase K tissue removal

Proteinase K
Buffer PKD

1. Mix 420 µl PKD buffer with 60 µl proteinase K, by pipetting (do not vortex) and pre-heat to 56°C.

2. Remove the seal from the slide module and carefully remove the cDNA Synthesis mixture from the wells by pipetting slowly.

3. Wash each well by adding 100 µl 0.1xSSC and then removing it slowly.

4. Add 70 µl proteinase K tissue removal mixture to each sample well. Seal all wells with a plastic seal.

5. Incubate the glass slide in a thermomixer at 56°C for **1hour** with interval mixing (e.g. 300 rpm, 15 second shake, 15 second rest).

6. Remove the plastic seal and remove the proteinase K from the wells.

7. Remove the glass slide from the slide module.

8. Pour 100 ml SSC/SDS buffer preheated to 50 °C into a wash pan.

9. Place the glass slide on the bottom of the wash pan with the active surface facing upwards so that the glass slide is covered by the buffer.

10. Incubate for **10 minutes** at 50°C while shaking (300 rpm).

11. During the incubation: Prepare a second wash pan with 100 ml 0.2x SSC and a third wash pan with 100 ml 0.1x SSC.

12. Move the glass slide to the second wash pan filled with 100 ml 0.2x SSC at room temperature and incubate for **1 minute** while shaking (300 rpm).

13. Move the glass slide to the third wash pan filled with 0.1x SSC at room temperature and incubate for **1 minute** while shaking (300 rpm).

14. Spin the glass slide in a centrifuge for glass slides for 5 seconds or until dry. In case no glass slide centrifuge is available, the slide can be dried at room temperature. Once the glass slide is dry, be careful not to expose it to strong light as this will bleach the fluorophore. Scan the slide.

5. Tissue Removal

Option 2: β-mercaptoethanol followed by Proteinase K

β-mercaptoethanol	Buffer RLT or RLT Plus
Proteinase K	Buffer PKD

In a fume hood:

Prepare 1x β-mercaptoethanol tissue removal reagent by mixing **990 µl** RLT buffer with **10 µl** β-mercaptoethanol in a 1.5ml eppendorf tube.

Pre-heat the β-mercaptoethanol mix to 56°C for at least 5 min.

1. Remove the seal from the hybridization cassette and carefully remove all cDNA Synthesis Reagent mixture from the wells by pipetting slowly.
2. Wash each well carefully with 100 µl 0.1xSSC.
3. Add around 70 µl of the β-mercaptoethanol mix to each active well; try to avoid bubbles in the wells. Cover wells with a plate sealer.
4. Incubate at 56°C with 300 rpm shake for the following time depending on tissue type:

Tissue Type	**β-mercaptoethanol incubation time**
less fibrous tissues	**1 h**
fibrous tissues such as heart tissue	**1 hour 30 minutes**

5. When ~5 min of the incubation remains, mix 420 µl PKD buffer with 60 µl proteinase K by pipetting (do not vortex) and pre-heat to 56°C.
6. Remove the β-mercaptoethanol mix from each well by pipetting.
7. Wash each well by adding 100 µl 0.1xSSC and then removing it slowly.
8. Add 70 µl proteinase K tissue removal mixture to each sample well. Seal all wells with a plastic seal.
9. Incubate the glass slide in a thermomixer at 56°C for **1 hour** with interval mixing (e.g. 300 rpm, 15 second shake, 15 second rest).
10. Remove the plastic seal and remove the proteinase K tissue removal mixture from the wells.
11. Remove the glass slide from the slide module.
12. Pour 100ml SSC/SDS buffer preheated to 50 °C into a wash pan.
13. Place the glass slide on the bottom of the wash pan with the active surface facing upwards so that the glass slide is covered by the buffer.
14. Incubate for **10 minutes** at 50°C while shaking (300 rpm).
15. During the incubation: Prepare a second wash pan with 100 ml 0.2x SSC and a third wash pan with 100 ml 0.1x SSC.
16. Move the glass slide to the second wash pan filled with 100 ml 0.2x SSC at room temperature and incubate for **1 minute** while shaking (300 rpm).
17. Move the glass slide to the third wash pan filled with 0.1x SSC at room temperature and incubate for **1 minute** while shaking (300 rpm).
18. Spin the glass slide in a centrifuge for glass slides for 5 seconds or until dry. In case no glass slide centrifuge is available, the slide can be dried at room temperature.

6. Scanning

If tissue removal has failed, and tissue remains on the slide, you should perform another TO experiment with added or stronger and/or longer tissue removal steps. If there are only parts of the tissue left you can still try to scan the slide. However, it is important to be able to distinguish the strong tissue autofluorescence from the relatively weak fluorescent cDNA footprint.

The sample **does not** need to be mounted when using a microarray scanner for imaging.

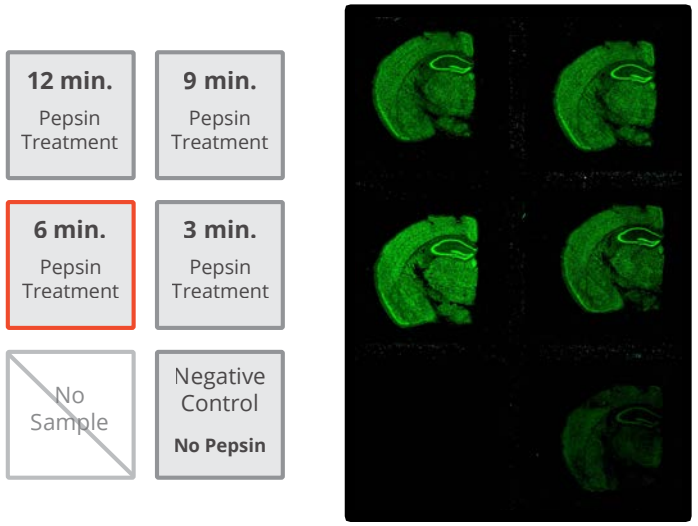
If imaging the fluorescent footprint with a microscope, mount the sample as appropriate for your system. e.g. carefully pipette 200 µl of 85% glycerol on to the sample and cover with a coverglass when using standard microscope objectives.

Analysis

The Picture below shows the results of a TO experiment testing permeabilisation conditions on consecutive 10µm mouse brain sections.

Note that Pepsin permeabilisation yields a significantly increased signal (indicating significantly more tissue RNA was captured by the slide) compared to the negative control.

For this tissue a 6 minute Pepsin treatment produced the strongest fluorescent cDNA footprint. Therefore 6 minutes would be chosen as the optimum duration of Pepsin permeabilisation.



Please be aware that any tissue left on the slide after tissue removal will lead to autofluorescence and give a very strong signal at the same wavelength as Cyanine-3. Therefore it is very important to completely remove the tissue.

Appendix

Tissue Recommendations

The following table gives recommendations on how to treat different tissue types.

Please note that these conditions can vary between samples and laboratories, thus they can only serve as a starting point for your tissue optimisation.

In general our experience is that more fibrous tissue requires longer tissue removal steps.

Tissue Type	Mouse Brain	Human Breast Cancer	Mouse Heart	Mouse Lung
Sectioning Thicness	10 µm	16 µm	10 µm	10 µm
Hematoxylin incubation	7 min.	7 min.	7 min.	7 min.
Eosin Y incubation	60 sec.	60 sec.	60 sec.	60 sec.
Permeabilisation (Pepsin/HCl)	6 min.	10 min.	8 min.	10 min.
Tissue Removal RLT/Beta-Mercaptoethoanol 1X (RLT 990 µl : 10 µl β-m.)	Skip		1 hour	1 hour
2X (RLT 980 µl : 20 µl β-m.)				
3X (RLT 970 µl : 30 µl β-m.)		1 hour		
4X (RLT 960 µl : 40 µl β-m.)				
Tissue Removal Proteinase K	1 hour	1 hour	1 hour	1 hour

Preparing Stock Reagents and Buffers

Pre-Permeabilisation Reagent (50 U/µl collagenase)

1. Add HBSS buffer to the collagenase powder to reach a final concentration of 50 U/µl. *Each collagenase powder bottle contains a bottle specific amount of U/mg.*
2. Filter the collagenase solution through a 0.45 µm low protein binding filter.
3. Make 10 µl aliquots and store them at -20 °C.

1x Permeabilisation Reagent (0.1 % pepsin in HCl)

Please note: Our pepsin stock is around 700U/mg, and we aim for a final concentration of approximately 0.7U/µl

1. Weigh 0.1 g pepsin
2. Dilute the pepsin to 0.1% as follows:
   - Add 1 ml H2O to the tube with pepsin and mix well by pipetting.
   - Take 60 µl of the diluted pepsin solution and add to 5940 µl 0.1 M HCl.
   - Make 500 µl aliquots and store them at -20 °C.

Actinomycin D (5mg/ml)

Work in a ventilated hood.

1. Add 400 µl of 100% filtered DMSO to the bottle containing 2 mg actinomycin D and vortex properly until all powder has dissolved.
2. Make 10 µl aliquots and store them at -20 °C.

TO dNTP mix

Prepare the cDNA synthesis dNTP mix as follows:

Water (RNase/DNase free)	690 µl
dATP (100 mM)	100 µl
dGTP (100 mM)	100 µl
dTTP (100 mM)	100 µl
dCTP (25mM)	10 µl

0.1X SSC

Prepare 0.1X SSC by mixing the following:

SSC (20x) buffer	10 ml
Water (RNase & DNase free)	1990 ml

0.2X SSC

Prepare 0.2X SSC by mixing the following:

SSC (20x) buffer	20 ml
Water (RNase & DNase free)	1980 ml

2x SSC/0.1 % SDS buffer

Prepare buffer by mixing the following

SSC (20x) buffer	200 ml
10% SDS in water	20 ml
Water (RNase/DNase free)	1780 ml

0.45 M Tris/Acetic Acid buffer

1. Weigh 55 g of Tris base powder and dissolve it in 500 ml RNase and DNase free water.
2. Adjust pH to 6.0 by carefully adding 100 % acetic acid.
3. Fill up with RNase and DNase free water to 1000 ml.

Required Consumables

The following list contains the reagents and consumables necessary to perform a Tissue Optimisation (TO) experiment. The list might not be complete, so please check the detailled TO Protocol when planning a TO experiment.

Consumable	Supplier
Optional: Lysing kit e.g. Precellys Lysing Kit Article # KT03961-1-1-003.2	Bertin Instruments
Optional: RNA extraction kit e.g. RNeasy Plus Mini Kit Article # 74134	Qiagen
Optional: Agilent RNA Pico kit Article # 5067-1513	Agilent Technologies
Optional: Qubit™ RNA BR Assay Kit Article # Q10210	Thermo Fisher Scientific
Formaldehyde solution, 36.5-38% in H_2O e.g. Article #F8775-25ML	General Laboratory Supplier Sigma Aldrich
Isopropanol (2-Propanol)	General Laboratory Supplier
Phosphate buffered saline (PBS)	General Laboratory Supplier
Mayer's Hematoxylin Solution e.g. Article # S3309	General Laboratory Supplier Dako
Bluing Buffer e.g. Article # CS702	General Laboratory Supplier Dako
Tris Base (Powder)	General Laboratory Supplier
Eosin Y (Aqueous), 0.5% in water e.g. Article # HT110216-500ML	General Laboratory Supplier Sigma Aldrich
Glycerol (85%)	General Laboratory Supplier
Ethanol 96-99%	General Laboratory Supplier
HBSS buffer Article # 14025-050	ThermoFisher Scientific
Collagenase Article # 17018-029	ThermoFisher Scientific
Low protein binding syringe filters e.g. Article # CLS431219-50EA	General Laboratory Supplier Sigma-Aldrich
BSA (20 mg/ml) Article # B9000S	New England Biolabs
Pepsin Article # P7000-25G	Sigma Aldrich
0.1 M HCl	General Laboratory Supplier
Actinomycin D Article # A1410-2MG	Sigma Aldrich
DMSO	General Laboratory Supplier
Chemical resistant syringe filters e.g. Article # CLS43122250EA	General Laboratory Supplier Sigma-Aldrich
dATP Article # R0141	ThemoFisher Scientific
dGTP Article # R0161	ThermoFisher Scientific
dTTP Article # R0171	ThermoFisher Scientific
dCTP Article # R0151	ThermoFisher Scientific
Cyanine 3-dCTP Article # NEL576001EA	PerkinElmer
SuperScript III Reverse Transcriptase Article # 18080044	ThermoFisher Scientific
5X First Strand Buffer (comes with SuperScript III)	ThermoFisher Scientific
DTT (100 mM) (comes with SuperScript III)	ThermoFisher Scientific
RNaseOUT™ Recombi- nat Ribonuclease Inhibitor Article # 10777019	ThemoFisher Scientific
Buffer RLT Article # 79216	Qiagen

β-mercaptoethanol	General Laboratory Supplier
Proteinase K Article # 19131	Qiagen
Buffer PKD Article # 1034963	Qiagen
SSC (20x) e.g. Article # S6639-1L	General Laboratory Supplier Sigma Aldrich
SDS e.g. SDS 10% Article # 71736-100ML	General Laboratory Supplier Sigma Aldrich

Required Equipment

The following table lists equipment required to perform a Tissue Optimisation (TO) experiment:

Instrument	**Supplier**
Hybridization Cassette* ("**Slide Module**") Article # AHC1X16 (as seen on page 7.)	Arrayit
Hybridization Cassette* ("**Slide Module**") ProChamber 16-well # 645508 (as seen on page 7.)	Grace Bio-Labs (contact Grace Bio-Labs sales support to order)
Hybridization Cassette* ("**Slide Module**") ProPlate Multi-Well Chamber # RD482441 (a lower cost alternative to the above chambers)	Grace Bio-Labs (contact Grace Bio-Labs sales support to order)
Optional: 2100 Bioanalyzer Desktop System Article # G2940CA	Agilent Technologies
Optional: Tissue Homogenizer e.g. Minilys Homogenizer Article # P000673-MLYSO-A	General Laboratory Supplier Bertin Instruments
Bright-field microscope / slide scanner	
Vortex	General Laboratory Supplier
Microcentrifuge for tubes of 1.5 mL	General Laboratory Supplier
Thermostat for 1.5 mL tubes	General Laboratory Supplier
Thermomixer fo plates e.g. ThermoMixer® C + smartblock for plates Article # 5382000015 + # 5363000039	General Laboratory Supplier Eppendorf
Optional: Microcentrifuge for glass slides with the dimensions 25 mm x 75 mm x 1 mm	General Laboratory Supplier
Fluorescent Slide Scanner / microscope	
Cryomicrotome	

*we list three alternative hyridization cassettes to choose from. You **do not** need to order all three.