CELLENION
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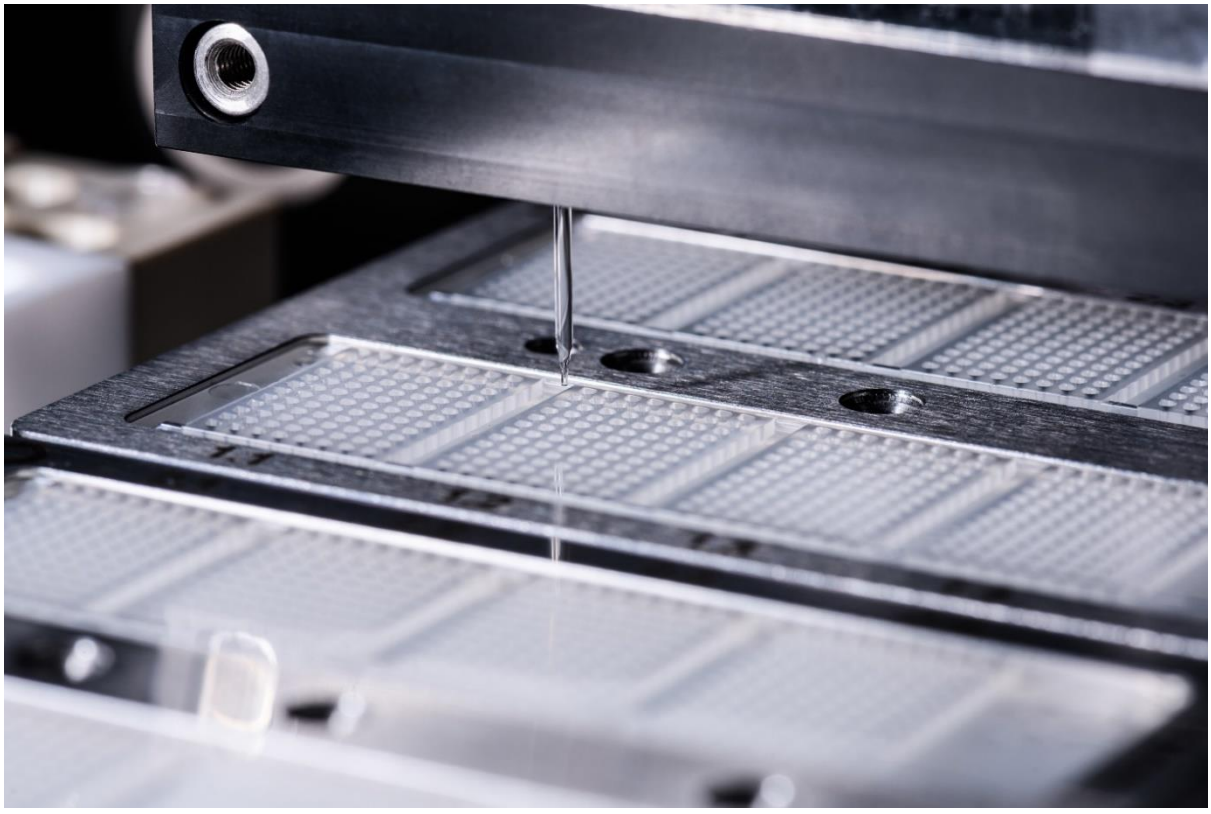
cellenCHIP 384 - 3'RNA-seq Kit
User guide



REF **CTR-5016- 1-4 CTR-5016-5 -8 CTR-5016-9-12 CTR-5016- 13-16**

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Safety and environmental regulations

Disposal of Products

Used cellenCHIPs and reagents should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

Introduction

This manual describes the usage of the “cellenCHIP 384 - 3'RNA-Seq Kit”, from the assembly of the cellenCHIP components, to the software settings for the experiments as well as the chemistry of the kit.

The “cellenCHIP 384 - 3'RNA-seq Kit” is designed to create high quality single cell 3´transcriptome libraries by efficiently capturing poly-adenylated mRNA from single cells dispensed with the cellenONE technology. Thanks to our nanowell cellenCHIP™ technology in combination with a specific chemistry, the kit allows the accurate analysis of the gene expression of individual cells in a highly efficient manner, perfectly tailored to investigate rare cells. Furthermore, all required reagents for cell lysis and reverse transcription, including 384 different barcoded oligodT primers, are already pre-dispensed into the chips, facilitating sample preparation and reducing precious hands-on lab time.

Once single cells are distributed into the cellenCHIP 384 RTready a unique buffer formulation allows cell lysis and reverse transcription of the mRNA into barcoded cDNA in a one-step reaction.

Afterwards, the cDNA from all single cells can easily be pooled via centrifugation using a standard plate centrifuge with the help of the special designed cellenCHIP 384 Funnel and the cellenCHIP 384 Centrifuge Adapter.

Subsequent amplification, full length cDNA purification and library construction are performed in single tube reactions. Sequencing library construction utilizes special barcoded cellenTAG Reagents allowing for a quick and reliable tagmentation and amplification, adding a unique i7 index to each library.

Each kit contains sufficient reagents to prepare Illumina-compatible libraries from up to 1536 cells on four cellenCHIP 384, thereby allowing multiple small and medium scale experiment designs. In addition, the Kit comes with 4 different sets of illumina index adapters allowing the multiplexing of up to 16 libraries (6144 cells) on one single sequencing run for large scale experiments.

“cellenCHIP 384 - 3'RNA-seq Kit” derived libraries are compatible with the Illumina iSeq, MiniSeq, MiSeq, NextSeq, HiSeq and NovaSeq systems.

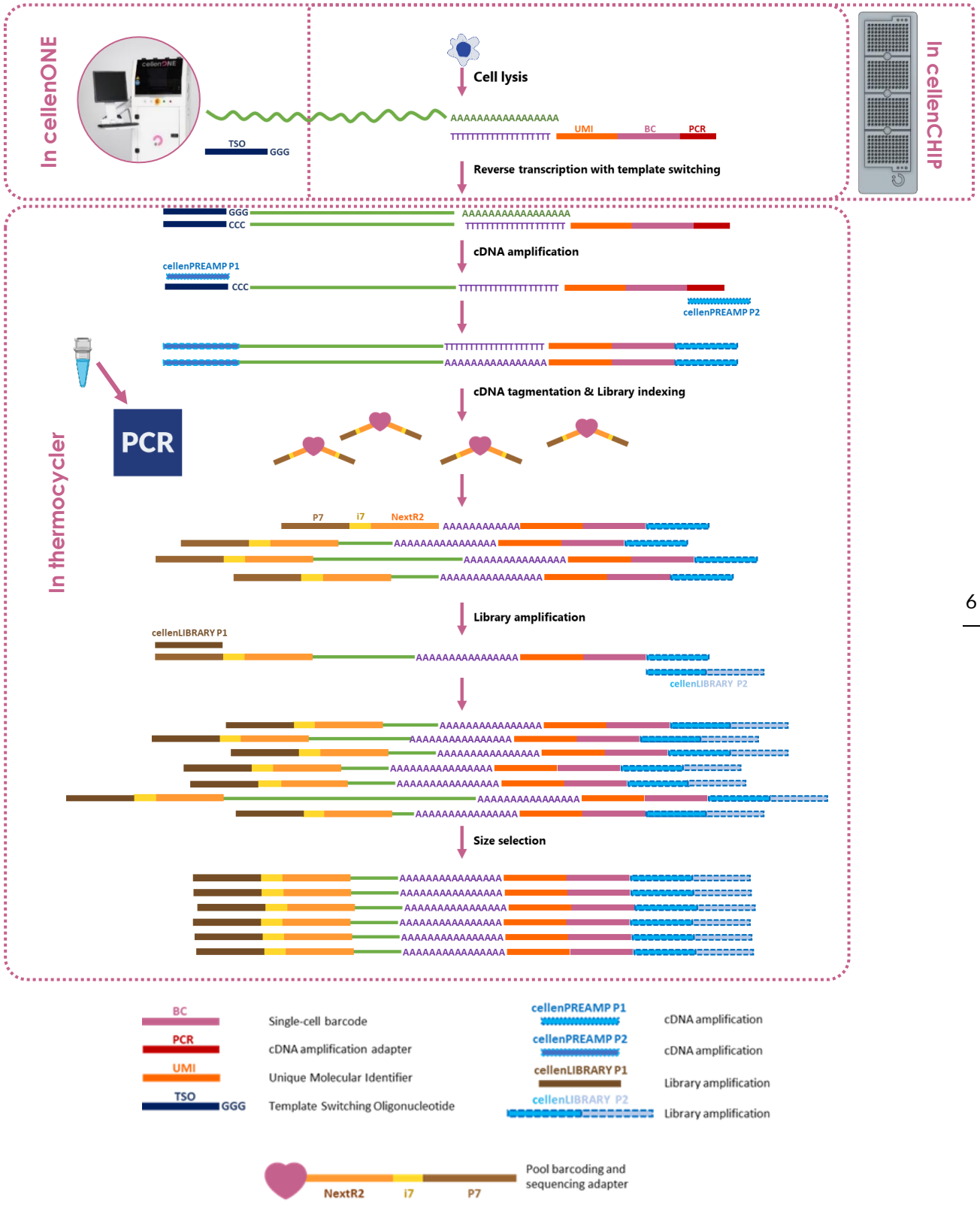

Fig 1. Schematic representation of the molecular workflow of the “cellenCHIP 384 - 3'RNA-seq Kit”.

Timetable: from the cell to sequencing

Prepare the cellenONE Total: 40 min		
Install PDC & align	-Degas and filter water. -Switch ON the cellenONE. -Switch ON the chiller. -Install and prime the PDC.	30 min
Set drop parameters	-Check drop stability. -Perform Nozzle-head camera wizard.	10 min
Single-cell isolation, lysis and cDNA generation Total: 2h 50 min		
Prepare cell suspension	-Cell suspension in degassed PBS 200 cell/µl.	Cell type dependent
Set detection and isolation parameters	-Run mapping & analysis. -Set detection and isolation parameters.	5 min
Install cellenCHIP 384	-Insert the cellenCHIP 384 RTready into the frame. -Centrifuge the cellenCHIP 384 RTready mounted into the cellenCHIP 384 Holder . -Place the cellenCHIP 384 RTready mounted into the cellenCHIP 384 Holder onto the cellenCHIP 384 Print Platform.	5 min
Single cell isolation	-Set probe: 384. -Set run: cellenCHIP_FTRP. -Set target: cellenCHIP. -Load specific field.	5 min
Cell collection	-Seal the cellenCHIP 384 RTready mounted into the cellenCHIP 384 Holder using a cellenCHIP Seal. -Install it in the cellenCHIP 384 Centrifuge Adapter. -Centrifuge the for 1 min 30 sec at 2000 x g.	5 min
RT	-Install the cellenCHIP 384 Thermal Adapter into the cycler. -Place cellenCHIP 384 RTready mounted into the cellenCHIP 384 Holder on top. -Run the RT program.	1h 35 min
cDNA recovery	-Place the cellenCHIP 384 Funnels into the cellenCHIP 384 Centrifuge Adapter. -Install the cellenCHIP 384 RTready mounted into the cellenCHIP 384 Holder upside down into the cellenCHIP 384 Centrifuge Adapter with cellenCHIP 384 Funnels. - Centrifuge the for 1 min 30 sec at 2000 x g.	5 min
Pooling	-Collect the RT product of each cellenCHIP 384 RTready in a tube.	10 min
	• SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.	
cDNA cleanUP	- Purify of the cDNA using the cellenCLEAN Beads.	40 min
	• SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.	
cDNA amplification Total: 3h 40 min		
cDNA amplification 1	- Prepare the cDNA Amplification Mix 1. - Run the cDNA Amplification Protocol 1.	1h 5 min
	• SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.	
Amplification cleanUP 1	- Purify of the amplified cDNA using the cellenCLEAN Beads.	30 min
	• SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.	

cDNA amplification 2	- Prepare the cDNA Amplification Mix 2. - Run the cDNA Amplification Protocol 2.	1h 5 min
Amplification cleanUP 2	- Purify of the amplified cDNA using the cellenCLEAN Beads.	30 min
cDNA quantification & QC	- cDNAQuantification with Qubit. - cDNAQuality control with the TapeStation.	30 min

- SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.

Library preparation **Total: 2h 55 min**		
Library barcoding	-Prepare the cellenTAG reaction. -Run the TAG program.	20 min
Library barcoding stop	-Add the cellenSTOP Solution. -Run the program STOP.	15 min
Library barcoding purification	-Perform library purification.	20 min

- SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.

Library amplification	-Prepare Library Amplification Mix -Run the Fill_Amp program.	1h

- SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.

Library purification and size selection	- Perform clean-up and size selection of the library.	30 min

- SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC

Library quantification & QC	-Library Quantification with Qubit. -Library Quality control with the TapeStation.	30 min

- SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC

Kit Components

Box	Component	Description	Storage	Qty
Box 1	cellenCHIP Seal	Plastic bag	room temperature	8
	1.5 mL Reaction Tube	Plastic bag	room temperature	50
	cellenCHIP 384 Funnel	Plastic bag	room temperature	4
	cellenCLEAN Beads	1.5 ml tube, purple cap, 780 µl	4°C	1
	Nuclease Free Water	1.5 ml tube, white cap, 1 ml	4°C	1
	80% Ethanol	15 ml bottle, white cap, 8.5 ml	4°C	1
	cellenTAG Buffer	0.5 ml tube, blue cap, 30 µl	4°C	1
	cellenSTOP Solution	0.5 ml tube, black cap, 42 µl	4°C	1
Box 2	cellenAMP Enzyme Mix	0.5 ml tube, yellow cap, 290 µl	-80°C	1
	cellenAMP Primer Mix	0.5 ml tube, green cap, 48 µl	-80°C	1
	cellenLIBRARY Primer Mix	0.5 ml tube, pink cap, 24 µl	-80°C	1
	cellenTAG Reagents*	0.5 ml tube, red cap, 10 µl	-80°C	4
	cellenCHIP 384 RTready		-80°C	4

*Different depending in the kit version (A, B C or D).

Additional Kits, Reagents & Equipment

Additional reagents, consumables and equipment is necessary to successfully perform a single cell 3'RNAseq experiment using the "cellenCHIP 384 - 3'RNA-seq Kit". In the following table we provide the information for the required items. While similar reagents and consumables might work as well it is highly recommended to use the exact products listed below.

Supplier	Component	Cat. No
Cellenion	cellenCHIP 384 Accessory Kit	CAK-5015
***	200 µl -20 µl -10 µl filter tips RNase/DNase free	***
***	DNA low binding PCR tubes or plates	***
Thermo Fisher Scientific	DynaMag™-2 Magnet	12321D
Roche	KAPA Library Quantification Kits	Specific to thermal cycler
Invitrogen	Qubit™ 1X dsDNA HS Kit	Q33230
Agilent	Agilent High Sensitivity D5000 Kit	5067-5592 / 5067-5593
Agilent	TapeStation system	G2964AA

Step 1: Single-cell isolation, lysis and cDNA generation

Reagents and equipment

Prepare the following reagents and consumables:

Equipment	Catalog N°	Stored at
cellenCHIP 384 RTready	CEC-5015-5	-80°C
cellenCHIP 384 Holder	CCH-5015	room temperature
cellenCHIP 384 Seal	CAS-5015-50	room temperature
cellenCHIP 384 Dummy	CCD -5015-5	room temperature
PDC cellenONE M	---	room temperature
cellenCHIP 384 Print Platform	CPP-5015	room temperature
sciSOURCEPLATE 384 PS	CPG-5501-1	room temperature
cellenCHIP 384 Thermal Adapter	CTA-5015	room temperature
cellenCHIP 384 Centrifuge Adapter	CCA-5015	room temperature
cellenCHIP 384 Compression Mat	CCM-5015	room temperature
cellenCHIP 384 Funnel	CEF-5015-8	room temperature
Slide Holder	F00C-2071	room temperature
sciCHIP CLEAN glass slide	CSC-5350-5	room temperature
Static Removal Kit*	F00C-222	room temperature
Thermal Cycler		room temperature
Reagents		**Stored at**
Filtered and degassed water		room temperature

**For cellenONE devices not equipped with antistatic solution.*

Before starting

a) **Degas filtered water** for the cellenONE system.
b) Switch ON the centrifuge and cool it down to 4°C.
c) Switch ON the cellenONE and set the temperature control to ***Dew point*** with a *Dew point correction* of -1.60.
d) Install the PDC, **align it, set voltage and pulse parameters**, place the filtered and degassed system water in the cellenONE and start the **"Prime".**
e) When the PDC is primed:
f) **Check drop stability** (make vary by +/- 3 voltage and pulse parameters)
g) Perform a **drop volume** and check that the % volume std deviation is below 0.5%
Useful tip: *perform sciCLEAN task (and airex task if needed) if those 2 settings are not optimual.*
h) Perform a "**Nozzle-Head camera wizard**": *(refer to Accessory Kit Manual p.21)*

Important! *Nozzle camera wizard calibration is necessary every time you change the nozzle or offset of the nozzle. It is recommended to perform this task twice before running the experiment.*

i) Remove the "Slide holder" and replace it in the target 1 by the **cellenCHIP Print Platform** (Fig 3).
j) Insert a **sciSOURCEPLATE 384 PS** as probe.

Prepare Run and Cell isolation parameters

a) In the cellenONE "**Main**" window, select your "**MTP 384**" as Probe, "cellenONE_**FTRP**_**cellenCHIP**" as Run, and "**cellenCHIP**" as Target.
b) Load your field to fill the cellenCHIP, set the number of arrays to dispense, set 1 drop to dispense in each well.
Important! *Edit the target field if needed. (Refer to cellenCHIP Accessory Kit User Guide p.17).*
c) Load **20-30 µl** of cell suspension in the probe **sciSOURCEPLATE 384.**
d) Take **10 µl** with the PDC and **check drop stability**.
e) **Perform mapping** and define detection and isolation parameters.
f) **Flush the PDC or activate the stand-by mode**.

Prepare the cellenCHIP for dispensing

a) Take a **cellenCHIP 384 RTready** (Fig 2A) stored at -80°C and place it in the **cellenCHIP 384 Holder** (Fig 2B).
b) Place the ensemble into the **cellenCHIP 384 Centrifuge Adapter** with the sealed side of the Chip **facing upwards** as showed in Fig 3.

***NOTE**: check compatibility of the cellenCHIP 384 Centrifuge Adaptors swing-bucket rotor of the centrifuge designed for this protocol. If compatibility problems are encountered with certain centrifuges, please contact support@cellenion.com indicating the brand and model of centrifuge, a solution will be provided*.

c) If only dispensing in one cellenCHIP use a **cellenCHIP 384 Dummy** to fill the cellenCHIP Holder and equilibrate both.

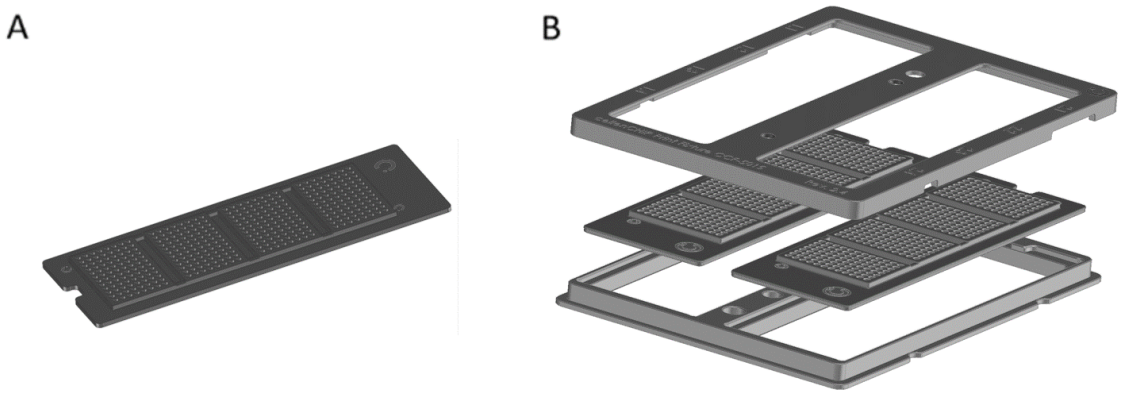

Fig 2. cellenCHIP 384 Holder and cellenCHIP 384 assembly. A) cellenCHIP 384. B) cellenCHIP 384 Holder assemblage with cellenCHIP 384.

d) **Centrifuge** the **cellenCHIP 384 RTready** for **1 min 30 sec** at **2000 x g** at **4°C**.
e) Once centrifuged, take the ensemble of the **cellenCHIP 384 RTready** and **cellenCHIP 384 Holder** and place them onto the **cellenCHIP 384 Printing Platform** as represented in Fig 4.

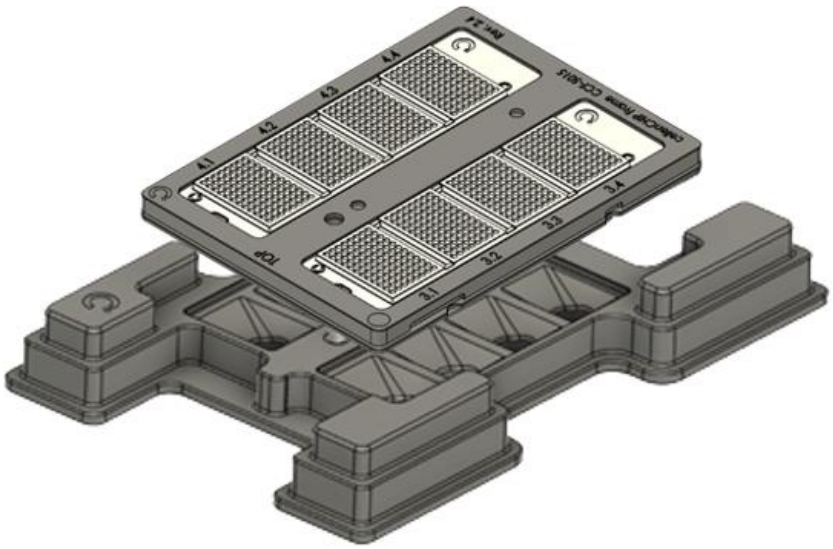

Fig 3. cellenCHIP 384 assembly for centrifugation. A) Representation of the cellenCHIP 384 placed facing upwards in the cellenCHIP 384 Centrifuge Adaptor.

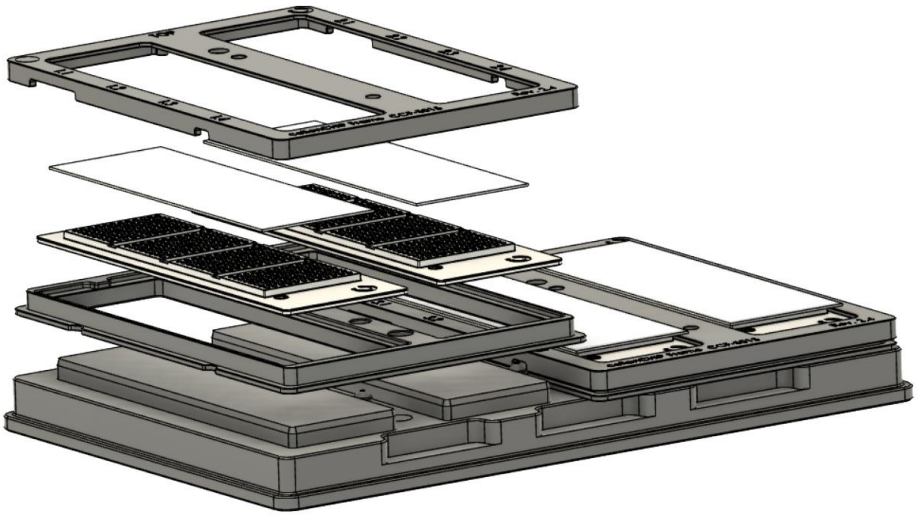

Fig 4. Assembly of cellenCHIP 384, cellenCHIP 384 Holder and cellenCHIP 384 Print Platform.

f) **Place** the ensemble **inside of the cellenONE** in the **top of the chilled holder**.
g) Carefully **remove** the **cellenCHIP 384 Seal** from the **cellenCHIP 384 RTready**.
h) Run a "**Destatic_targets task**" and check again that the statics of the **cellenCHIP 384 RTready** is 0V (±0.1-0.3) with a static meter tool.

Single-cell dispensing in the cellenCHIP

a) **Re-load the sample** in the sciSOURCEPLATE. Start the run.

Note: *"cellenONE_FTRP_cellenCHIP" can be performed with the Z movement OFF.*

b) Once the run is finished seal the **cellenCHIP 384 RTready** with a new **cellenCHIP 384 Seal.**

Important*: if the cellenCHIP 384 Seal has been misplaced at the first attempt and must be peeled off, do not reuse it, use instead a clean cellenCHIP 384 Seal.*

c) Place the **cellenCHIP 384 RTready** mounted into the **cellenCHIP 384 Holder** in the **cellenCHIP 384 Centrifuge Adapter facing upwards** (Fig 3); **Centrifuge** for **1 min 30 sec** at **2000 x g** at **4°C**.

d) Proceed directly with RT incubation.

Important! *Flush the PDC and run a sciCLEAN task. If not using the PDC afterwards launch a "Nozzle removal wash" task to remove the PDC from the cellenONE.*

RT incubation

a) Place the sealed **cellenCHIP 384 RTready** either:
   - mounted in the **cellenCHIP 384 Holder** onto the **cellenCHIP 384 Thermal Adaptor** in one of the compatible thermocycler (Fig 5), or
   - directly in the cellenCYCLER

b) Place the **cellenCHIP 384 Mat** on top and close the lid of the thermocycler.

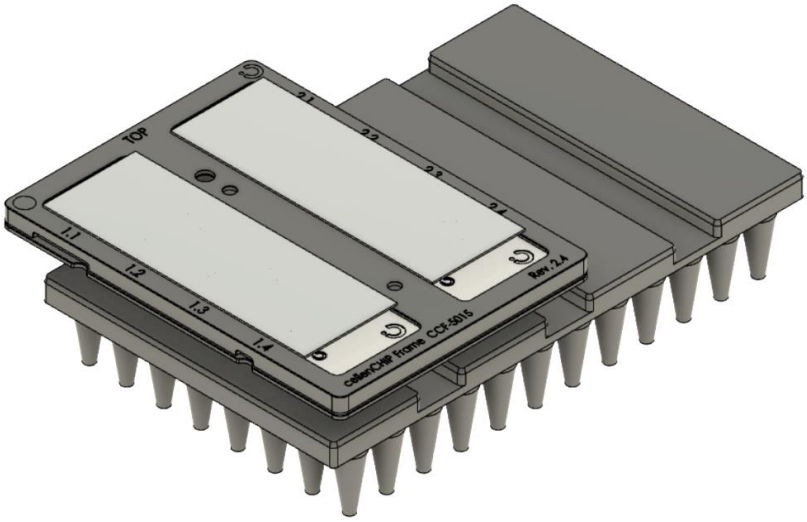

Fig 5. cellenCHIP 384 Thermal Adaptor assembly with the cellenCHIP 384 assembled in the cellenCHIP 384 Holder.

Important: the cellenCHIP 384 Thermal Adaptor is compatible with the thermocyclers listed in the table below. If you cannot access one of these, please contact your Scienion/Cellenion representative.

Brand	Model(s)
Biorad	T100, C1000, S1000, CFX96
Applied Biosystems	SimpliAmp, Veriti 96
G-storm	GS1
Analytik Jena	Biometra 96

c) Set the thermocycler program as below, indicating 50 or 100 µl reaction volume, depending on your cycler maximum specification for that parameter, and run the RT reaction.

cellenCHIP target temperature	Time	Temperature settings (°C)					
		cellenCHIP 384 Thermal Adaptor + Applied Biosystems SimpliAmp, Bio Rad C1000, S1000 or CFX96 OR **cellenCYCLER**		**cellenCHIP 384 Thermal Adaptor** + Bio Rad T100		**cellenCHIP 384 Thermal Adaptor** + Applied Biosystems Veriti 96	
		Block	**Lid**	**Block**	**Lid**	**Block**	**Lid**
42°C	1h30	41	45	41.5	45	40.4	50
4°C	Hold	0	OFF	4	OFF	0	OFF

d) At the end of RT reaction take the **cellenCHIP 384 RTready**, place it back in the **cellenCHIP 384 Holder** if the RT occurred in the cellenCYCLER and place into the **cellenCHIP 384 Centrifuge Adapter facing upwards** (Fig 3); **spin down the RT product at 2000x g for 1 min 30 sec at 4°C**.

Recovery of RT product

a) Take one **cellenCHIP 384 Funnel** per cellenCHIP 384 RTready used in the experiment. Remove the seal of the **cellenCHIP 384 Funnels** and place them in the **cellenCHIP 384 Centrifuge Adapter** (Fig 6).
b) **Remove** the **cellenCHIP 384 Seal** from the **cellenCHIP 384 RTready** carefully and place the assembly **facing downwards** on the top of the funnels (Fig 6).

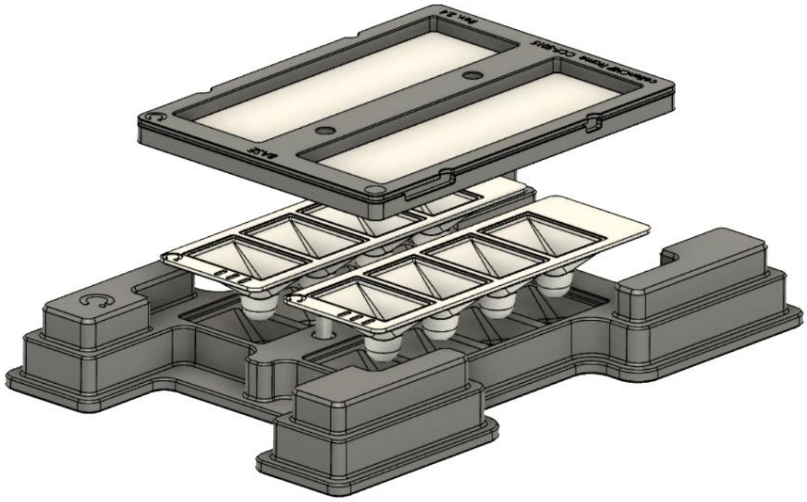

Fig 6. Recovering of the RT product. cellenCHIP 384 Funnels placed in the cellenCHIP 384 Centrifuge Adapter. Assembly of cellenCHIP 384 RTready in cellenCHIP 384 Holder placed upside-down on the top of the cellenCHIP 384 Funnels and the cellenCHIP 384 Centrifuge Adaptor.

c) **Centrifuge at 2000 x g for 1 min 30 sec at 4°C.**
d) Carefully **remove** the **cellenCHIP 384 Holder** with the **cellenCHIP 384 RTready** from the **cellenCHIP 384 Centrifuge Adapter**.
e) **Recover** the **RT product** from each of the **cellenCHIP 384 Funnels** in a supplied **1.5 mL Reaction Tube** using a 10 µl pipette.

Important! *do not pool together the RT products from different cellenCHIPs.*

- **SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC up to 72h.**

Step 2: cDNA amplification

Reagents and equipment

Prepare the following reagents and consumables:

Reagents	Stored at	keep at
Nuclease Free Water	room temperature	room temperature
cellenCLEAN Beads	4°C	room temperature
80% Ethanol	room temperature	room temperature
cellenAMP Enzyme Mix	-80°C	ice
cellenAMP Primer Mix	-80°C	ice
Equipment	**Stored at**	
Magnet stand	room temperature	
Thermo cycler	room temperature	
1.5 mL Reaction Tube	room temperature	
Qubit dsDNA HS Assay Kit	*See manufacturer*	
Agilent TapeStation ScreenTape and Reagents	*See manufacturer*	

RT product cleanup and cDNA amplifications

Cleanup

a) Allow **cellenCLEAN Beads** to warm up to **room temperature 30 min** before use.
b) Centrifuge briefly the RT product. **Measure the total volume**
Note: volumes normally change due to film-loss and evaporation during thermal cycling, so it is important to measure the volume prior to the cleanup steps below.
c) **Fill up** the RT product **to a final volume of 50 µl using Nuclease Free Water.**
d) **Vortex** (or vigorously pipette) the **cellenCLEAN Beads** to ensure they are completely resuspended.
e) **Add 50 µl of cellenCLEAN Beads** (1 volume equivalent) to the RT product. Mix thoroughly by pipetting up and down.
f) **Incubate** in a non-magnetic tube rack on the bench for **10 minutes** to allow the cDNA to bind.
g) Transfer the tube to a **magnetic stand** and let the beads settle completely before proceeding to the next step. (A bead pellet will form along one side of the tube and the supernatant should appear completely clear after ⩽ 5 minutes).
h) Carefully discard the supernatant.

Note: *this is a critical step, be sure to not disturbing the bead pellet while removing the supernatant. Use a large pipette tip to remove most of the supernatant and then, if necessary, use a smaller one to remove the remaining supernatant.*

i) With the tube still on the magnet, wash the beads twice with 200 µL of **80% Ethanol**.
- With the tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
- After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet, to complete the wash step.

j) Wash beads with **80% Ethanol** a second time.
- With the tube in the magnetic stand, **add 200 µl of 80% ethanol** without disturbing beads.
- After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet.
- **Cap tube, centrifuge briefly and return to magnet**, letting the beads settle (<30 seconds). Use a small pipet tip (⩽20 µl) to remove any residual ethanol at the bottom of the tube.

k) Incubate for **3 minutes** on the magnet to allow the beads to **air-dry.**

l) Remove the tube from the magnetic stand and add **16 µl of Nuclease Free Water**, resuspend vigorously through pipetting and incubate at room temperature for **10 minutes.**

m) Return tube to **magnetic stand** and allow a bead pellet to form on the inner wall of the tube (**~ 2 minutes**).

n) **When the supernatant has cleared completely**, carefully **transfer 15 µl of cDNA eluate** to a new **1.5 mL Reaction Tube** or directly into a PCR tube for the following step.

- **SAFE-STOPPING POINT Proceed immediately to cDNA amplification or store at -20°C.**

cDNA amplification 1

a) Prepare the cDNA Amplification Mix 1 on ice:

Amplification Mix 1	1rxn (µl)	2 rxns (µl)	4 rxns (µl)
cellenAMP Enzyme Mix	20	44	88
cellenAMP Primer Mix	5	11	22
Total	25	55	110

Volumes for 2 and 4 reactions calculated with 10% excess for proper dispensing, 1 rxn (reaction) corresponds to one cellenCHIP.

b) **Add 25 µL of the cDNA Amplification Mix 1** together with the **15 µl of the cleaned cDNA** in a PCR tube or well, pipette mix gently.
c) **Centrifuge briefly** and run the program "**sc_cDNA Amplification**", below, with lid heating on:

Lid temperature	Reaction volume	Run time	
105°C	40 µl	1h	
Step	**Temperature**	**Time**	
1	98°C	5 min	
2	98°C	30 sec	X10
3	65°C	1 min	
4	72°C	4 min	
5	4°C	Hold	

- **SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.**

cDNA cleanup 1

a) Allow **cellenCLEAN Beads** to warm up to **room temperature 30 min** before use.
b) **Centrifuge briefly** the PCR product.
c) **Transfer the PCR product to a** to a new **1.5 mL Reaction Tube**
d) **Vortex** (or vigorously pipette) room temperature **cellenCLEAN Beads** to ensure they are completely resuspended.
e) **Add 32 µl of cellenCLEAN Beads** to the PCR product. Mix thoroughly by pipetting up and down.
f) **Incubate** in a non-magnetic tube rack on the bench for **10 minutes** to allow the cDNA to bind.
g) Transfer the tube to a **magnetic stand** and let the beads settle completely before proceeding to the next step.
h) Carefully **discard the supernatant**.
i) With the tube still in the magnets wash the beads twice with 200 µL of **80% Ethanol**.
   - With the tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
   - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet, to complete the wash step.
j) Wash beads with **80% Ethanol** a second time.
   - With the tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
   - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet.
   - **Cap tube, centrifuge briefly and return to magnet**, letting beads settle (<30 seconds). Use a small pipet tip (⩽20 µl) to remove any residual ethanol at the bottom of the tube.
k) Incubate for **3 minutes** on the magnet to allow the beads to **air-dry.**

l) Remove the tube from the magnetic stand and add **17 µl of Nuclease Free Water**, resuspend vigorously through pipetting with a p10 pipette set to 10µl incubate at room temperature for **10 minutes**.
m) Return tube to **magnetic stand** and allow a bead pellet to form on the inner wall of the tube (**~ 2 minutes**).
n) **When the supernatant has cleared completely**, carefully **transfer 15 µl of DNA eluate** to a new **1.5 mL Reaction Tube** .

- **SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.**

cDNA amplification 2

d) Prepare the cDNA Amplification Mix 2 on ice:

Amplification Mix 2	1rxn (µl)	2 rxns (µl)	4 rxns (µl)
cellenAMP Enzyme Mix	20	44	88
cellenAMP Primer Mix	5	11	22
Total	25	55	110

Volumes for 2 and 4 reactions calculated with 10% excess for proper dispensing, 1 rxn (reaction) corresponds to one cellenCHIP.

e) **Add 25 µL of the cDNA Amplification Mix 2** together with the **15 µl of the cleaned amplified cDNA** in a PCR tube or well, pipette mix gently.
f) **Centrifuge briefly** and run the program "**sc_cDNA Amplification**", below, with lid heating on:

Lid temperature	Reaction volume	Run time	
105°C	40 µl	1h	
Step	**Temperature**	**Time**	
1	98°C	5 min	
2	98°C	30 sec	X10
3	65°C	1 min	
4	72°C	4 min	
5	4°C	Hold	

- **SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.**

cDNA cleanup 2

o) Allow **cellenCLEAN Beads** to warm up to **room temperature 30 min** before use.
p) **Centrifuge briefly** the PCR product.
q) **Transfer the PCR product to a** to a new **1.5 mL Reaction Tube**
r) **Vortex** (or vigorously pipette) room temperature **cellenCLEAN Beads** to ensure they are completely resuspended.
s) **Add 32 µl of cellenCLEAN Beads** to the PCR product. Mix thoroughly by pipetting up and down.

t) **Incubate** in a non-magnetic tube rack on the bench for **10 minutes** to allow the cDNA to bind.
u) Transfer the tube to a **magnetic stand** and let the beads settle completely before proceeding to the next step.
v) Carefully **discard the supernatant**.
w) With the tube still in the magnets wash the beads twice with 200 µL of **80% Ethanol**.
   - With the tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
   - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet, to complete the wash step.
x) Wash beads with **80% Ethanol** a second time.
   - With the tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
   - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet.
   - **Cap tube, centrifuge briefly and return to magnet**, letting beads settle (<30 seconds). Use a small pipet tip (⩽20 µl) to remove any residual ethanol at the bottom of the tube.
y) Incubate for **3 minutes** on the magnet to allow the beads to **air-dry.**
z) Remove the tube from the magnetic stand and add **17 µl of Nuclease Free Water**, resuspend vigorously through pipetting with a p10 pipette set to 10µl incubate at room temperature for **10 minutes**.
aa) Return tube to **magnetic stand** and allow a bead pellet to form on the inner wall of the tube (**~ 2 minutes**).
bb) **When the supernatant has cleared completely**, carefully **transfer 15 µl of DNA eluate** to a new **1.5 mL Reaction Tube**.

- **SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.**

cDNA quantification & Quality Control

cDNA quantification

Measure the amplified cDNA concentration using a fluorometric method such as Qubit (recommended) using the Qubit™ 1X dsDNA HS Kit, *see manufacturer's protocol*.

cDNA Quality Control

Control the size distribution of the amplified cDNA using on-chip capillary electrophoresis. We recommend running **2 µl of each** cDNA **sample at a concentration of 1 ng/µl** on the TapeStation system using the Agilent High Sensitivity D5000 Kit, *see manufacturer's protocol.*

If the majority of the cDNA fragments are comprised between 250-5000pb, continue to the step 3 "library preparation". Otherwise please see the FAQ (page 27).

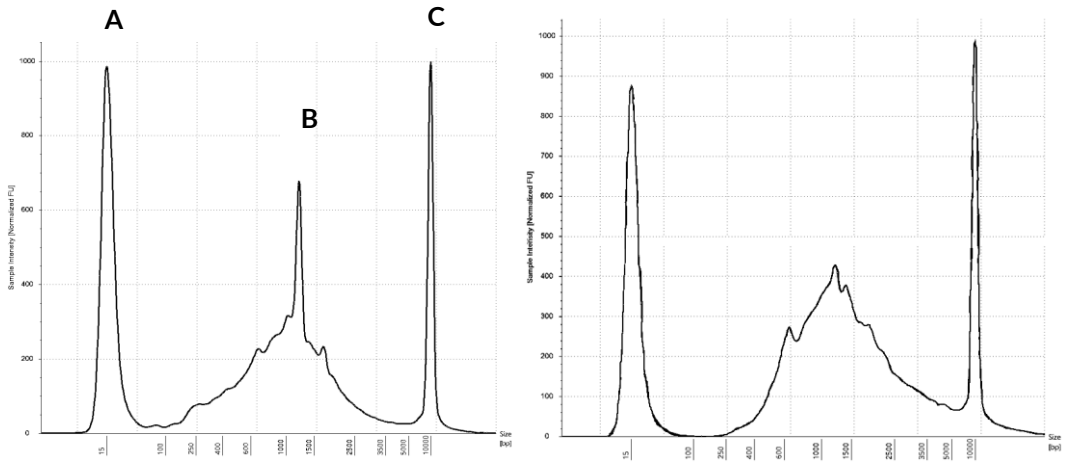

Fig 7. Representation of 2 examples for normal profile of amplified cDNA on the TapeStation. A) and C) markers, B) amplified cDNA.

Step 3: Library preparation for Illumina sequencing

Reagents and equipment

Prepare the following reagents and consumables:

Reagents	Stored at	keep at
Nuclease Free Water	room temperature	room temperature
cellenCLEAN Beads	4°C	room temperature
80% Ethanol	room temperature	room temperature
cellenTAG Buffer	room temperature	room temperature
cellenTAG Reagent	-80°C	ice
cellenSTOP Solution	room temperature	room temperature
cellenAMP Enzyme Mix	-80°C	ice
cellenLIBRARY Primers	-80°C	ice

Equipment	Stored at
Magnet	room temperature
Thermal Cycler	room temperature
1.5 mL Reaction Tube	room temperature
Qubit dsDNA HS Assay Kit	*See manufacturer*
Agilent TapeStation ScreenTape and Reagents	*See manufacturer*

Library Tagmentation

a) Based on the quantification prepare at least **4 µl** of a **0.25 ng/µl dilution** of the cDNA using **Nuclease Free Water.**
b) Allow **cellenCLEAN Beads** to warm up to room temperature 30 min before use.
c) **Centrifuge briefly** the **cellenTAG reagent** in a centrifuge.
d) Transfer **8 µl of the cellenTAG reagent** to a pre-labeled PCR 8-tube strip(s) or PCR plate .
Note: use a different cellenTAG reagent for each cellenCHIP of the experiment.
e) Add **4 µl of cDNA (0.25 ng/µl)** and mix the cDNA thoroughly with the cellenTAG Reagent by pipetting up and down (5 times at 4 µl), avoid introducing excessive bubbles.
f) Next, carefully pipette **6 µl of cellenTAG Buffer** to the tube. Mix thoroughly but slowly by pipetting up and down (10 times at 6 µl), avoid introducing excessive bubbles.
g) **Cap or seal the cellenTAG reaction**, centrifuge briefly, then transfer to a thermal cycler, and run the TAG program, below, with lid-heating on:

Lid temperature	Reaction volume	Run time
105°C	100 µl	15 min
Step	**Temperature**	**Time**
1	55°C	15 min
2	25°C	Hold

Library Tagmentation Stop

a) **Centrifuge briefly the cellenTAG reaction**, and then uncap/seal the reactions.
b) Add **9 µl of cellenSTOP Solution** to the reaction. Pipette slowly up and down (10 times at 9 µl) to mix, being careful not to introduce excessive bubbles.
c) Securely **re-cap or seal the cellenSTOP reaction**, centrifuge briefly, then transfer to a thermal cycler and run the STOP program, below, with lid-heating on:

Lid temperature	Reaction volume	Run time
105°C	100 µl	10 min
Step	**Temperature**	**Time**
1	68°C	10 min
2	25°C	Hold

Library Barcoding Purification

a) Allow **cellenCLEAN Beads** to warm up to **room temperature 30 min** before use.
b) After incubation**, centrifuge briefly** and **transfer** the cellenSTOP reaction to a new **1.5 mL Reaction Tube.**
c) Vortex (or vigorously pipette) the **cellenCLEAN Beads** to ensure that the beads are fully resuspended.
d) Add **27 µl of cellenCLEAN Beads and** mix thoroughly by pipetting.
e) Incubate in a non-magnetic tube rack on the bench for **5 minutes** to allow the DNA to bind.
f) Place tube on magnetic stand and let beads settle**, 5 minutes.** A pellet should form on one side of the tube and the supernatant should be visibly clear after 5 minutes.
g) **Remove and discard supernatant** with pipette. Be careful not to disturb the pellet.
Note: A large pipette tip may be used to remove most of the supernatant and then, if necessary, a smaller one to remove the remaining supernatant.
h) Wash beads with **80% Ethanol** .
    - With the tube in the magnetic stand, add **200 µl of 80% Ethanol** without disturbing beads.
    - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet, to complete the wash step.
i) Wash beads with **80% Ethanol** a second time.
    - With the tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
    - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet.
    - Cap tube, centrifuge briefly and return to magnet, letting the beads settle (<30 seconds). Use a small pipet tip (⩽20 µl) to remove any residual ethanol at the bottom of the tube.
j) Incubate for 3 minutes on the magnet to allow the beads to air-dry.
k) Remove the tube from the magnetic stand and add **21 µl** of **Nuclease Free Water** to the bead pellet and resuspend vigorously through pipetting with a p100 or p200 pipette set to 21 µl incubate at room temperature for **10 minutes**.
l) Return tube to **magnetic stand** and allow a bead pellet to reform on the inner wall of the tube (**~2 minutes**).
When the supernatant has cleared completely, carefully **transfer 20 µl** of DNA eluate to a new **1.5 mL Reaction Tube** or directly into a PCR tube for the following step. The transferred eluate contains the purified cellenLIBRARY.

Note: Presence of some beads in the purified cellenLIBRARY will not inhibit the subsequent CellenLIBRARY amplification.

- **SAFE-STOPPING POINT Proceed immediately to the next step, or store at -20ºC.**

Library Amplification

Prepare the Library Amplification Mix on ice:

Index PCR Mix	1rxn (µl)	2 rxns (µl)	4 rxns (µl)
cellenAMP Enzyme Mix	25	55	110
cellenLIBRARY Primers	5	11	22
Total	30	66	132

Volumes for 2 and 4 reactions calculated with 10% excess for proper dispensing, 1 rxn corresponds to one sample.

a) Add **30 µl of the Library Amplification Mix** to the purified cellenLIBRARY and mix well by pipetting.
b) Cap the PCR tube, centrifuge briefly and run the FILL_AMP program, below, with lid heating on:

Lid temperature	Reaction volume	Run time	
105°C	50 µl	36 min	
Step	**Temperature**	**Time**	
1	72°C	10 min	
2	95°C	3 min	
3	98°C	30 sec	X12
4	64°C	15 sec	
5	72°C	30 sec	
6	72°C	3 min	
5	4°C	Hold	

- **SAFE-STOPPING POINT Proceed immediately to the next step, or at -20°C.**

Library Purification

Following library amplification, it is necessary to remove residual primers and short library fragments.

a) Allow **cellenCLEAN Beads** to warm up to **room temperature 30 min** before use.
b) After PCR, **centrifuge briefly and transfer** the amplified cellenLIBRARY to a new **1.5 mL Reaction Tube. Measure the total volume using a pipettor.**
Note: volumes normally change due to film-loss and evaporation during thermal cycling so it is important to measure the volume prior to the size selection steps below.
c) **Fill up** the amplified cellenLIBRARY **to a final volume of 50 µl with Nuclease Free Water.**
d) **Vortex** (or vigorously pipette) room temperature **cellenCLEAN Beads** to ensure they are completely resuspended.
e) **Add 25 µl of cellenCLEAN Beads** to the diluted library. Mix thoroughly by pipetting up and down.

f) **Incubate** in a non-magnetic tube rack on the bench for **5 minutes** to allow the DNA to bind.
g) Transfer the tube to a **magnetic stand** and let the beads settle completely before proceeding to the next step. (A bead pellet will form along one side of the tube and the supernatant should appear completely clear after ⩽ 5 minutes).
h) Transfer the supernatant (75 µl) to a new **1.5 mL Reaction Tube.**
i) **Add 15 µl the cellenCLEAN Beads**. Mix thoroughly by pipetting up and down.
j) **Incubate** in a non-magnetic tube rack on the bench for **5 minutes** to allow the DNA to bind.
k) Transfer the tube to a **magnetic stand** and let the beads settle completely before proceeding to the next step. (A bead pellet will form along one side of the tube and the supernatant should appear completely clear after ⩽ 5 minutes).
l) Slowly **remove the supernatant** with a pipettor and discard. Be careful not to disturb the bead pellet.
m) Wash beads with **80% Ethanol**.
    - With tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
    - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet, to complete the wash step.
n) Wash beads with **80% Ethanol** a second time.
    - With tube in the magnetic stand, **add 200 µl of 80% Ethanol** without disturbing beads.
    - After ⩾30 seconds, **remove and discard supernatant**, being careful not to disturb pellet.
    - **Cap tube,** centrifuge briefly **and return to magnet**, letting beads settle (<30 seconds). Use a small pipet tip (⩽20 µl) to remove any residual ethanol at the bottom of the tube.
b) Remove the tube from the magnetic stand and add **17 µl** of **Nuclease Free Water** to the bead pellet and resuspend vigorously through pipetting with a p20 pipette set to 17 µl incubate at room temperature for **10 minutes**.
o) **Incubate for 5 minutes** in a tube rack (non-magnetic) on the bench to elute the size selected cellenLIBRARY from the magnetic beads.
p) Return tubes to **magnetic stand** and allow bead pellet to form on the inner wall of the tube (**~ 2 minutes**).
q) **When the supernatant has cleared completely**, carefully **transfer 15 µl of DNA eluate** to a new to a new **1.5 mL Reaction Tube**. The transferred eluate contains the purified, size selected cellenLIBRARY.

- **SAFE-STOPPING POINT Store the purified, size-selected library at -20ºC, or proceed directly to library QC and quantification.**

Library Quantification & Quality Control

Library quantification

Measure the cellenLIBRARY concentration using a fluorometric method such as Qubit (recommended) using the Qubit™ 1X dsDNA HS Kit, *see manufacturer's protocol*.

Library Quality Control

Control the size distribution of the cellenLIBRARY using on-chip electrophoresis. We recommend running **2 µl of each** library **sample at a concentration of 0.5 ng/µl** on the TapeStation system using the Agilent High Sensitivity D5000 Kit, *see manufacturer's protocol.*

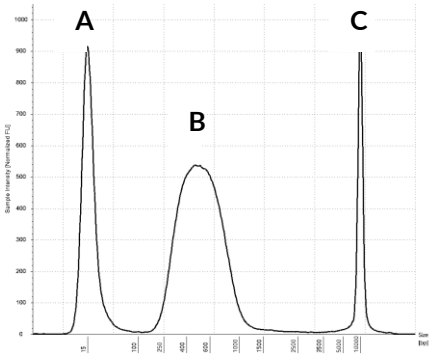

Fig 8. Representation of a normal library profile. A and C) markers, B) library

qPCR quantification

- Quantification of cellenLIBRARY is further performed using a quantitative PCR method such as the Kapa Library Quant Kit (recommended).
- Libraries are diluted 1/1000 and 1/10,000 in 2 different replicates.
- Per dilution replicate, 2-3 qPCR replicates are performed.
- Standard samples (1-6) are as well performed in duplicate or triplicates.

To determine the concentration of the cellenLIBRARY for sequencing, refer to the Illumina sequencing manual.

Appendix

The i7 sequences:

Kit version	CellenTAG reagent	i7 sequence for demultiplexing
A	1	TTGGAATG
	2	TTAATGCG
	3	AGCTACGT
	4	GCCTCCTG
B	5	GGGACAAC
	6	TATCCCAC
	7	CAACTGTG
	8	ATGACTAG
C	9	CCATATCC
	10	GTAGTCAC
	11	CGGAGATA
	12	CACTCTCA
D	13	GAGTTCTC
	14	TCGCCAGC
	15	TGTGACTA
	16	TTGACGTC

FAQs

Experimental Design

1. I need to analyse as many cells as possible per run

For each run of cell isolation using the CellenONE, it is possible to use 4 cellenCHIPs in parallel containing 4 arrays of 96 wells each. It allows to sort 1536 wells in one run. However, for RT incubation it is possible to use 2 cellenCHIPs in parallel per run. If samples from several cellenCHIPs need to be pooled for sequencing, please use compatible indexes (cellenTAG reagents).

2. Sorting my cells is taking a long time, is it a problem?

Please make sure that the cellenCHIP is cooled to DP-1.6 °C. We cannot recommend sorting for longer than 4 hours.

3. I have cells from different species, can I pool everything?

It is possible since all cells acquire a specific barcode sequence during reverse transcription, which can be used to assign each sequencing read to a specific cell. However, concerning the sequencing depth: if some cells with high mRNA amount (specie 1) are pooled with cells containing low mRNA amount (specie 2), the sequencing depth will be bias towards specie 1. Thus, limiting the resolution/sensitivity of the analysis for specie 1.

4. Can I use frozen or fixed samples for RNA-seq?

scRNA-seq directly from frozen cells is commonly used. We highly recommend however to perform thawing using the thawing protocol of *Mereu et al., 2020* (https://doi.org/10.1038/s41587-020-0469-4).
Fixation of cells makes scRNA-seq very difficult. Except for methanol fixed cells, we cannot guarantee success with fixed cells.

5. Can ERCC spike-ins be used for normalization?

Yes. ERCC spike ins can be used by adding them into the single cell solution before dispensing. Calculate the concentration required for the appropriate number of molecules using the drop volume function in the cellenONE.

Cell isolation and cDNA synthesis

6. My run FTRP did not start.

Causes	Recommendation
Fiducial not recognized	• Perform a **"Nozzle-Head camera wizard"** task. • Contact support@cellenion.com.

7. How can I know that my cells were properly dispensed? Can I check in the microscope the cell dispensing before launching the RT?

Although in theory possible, we would not recommend postponing the RT step after cell dispensing for an evaluation using a microscope. This will have a negative effect on the RT efficiency.

8. Can I run the RT over night?

Yes, reverse transcription can be run over night when stored at 4°C within the cycler after reverse transcription.

9. Can I seal the cellenCHIP after cell dispensing and launch the RT after x days?

No, we do not recommend to postpone RT incubation after cell dispensing.

10. Why are there 2 cDNA amplification steps?

cDNA amplification is a very important and sensitive step. Due to the low amounts of input in combination with the multi template character of the cDNA several known PCR artefacts can arise, e.g. the creation of primer dimers or the creation of chimeric PCR products. The 2 step amplification highly reduces these artefacts as it keeps the reaction always within the best conditions possible (Primer abundance, Polymerase state).

11. Can I perform cDNA amplification for each array individually?

No, the "cellenCHIP384 - 3'RNA-seq Kit" is designed for pooling all 4 cellenCHIPs directly after reverse transcription. The kit does not contain enough downstream reagents for individual reactions. However, if required you can choose whether to include a specific cellenCHIPs or not, e.g. because one cellenCHIPs did have a low recovery volume after RT (See FAQ 1).

12. My volume recovery after RT is very low.

Causes	Recommendation
Evaporation due to target plate not being cooled during cell dispensing	Repeat experiment with a new cellenCHIP384 RTready and adjusted target plate cooling.
Incorrect sealing of the cellenCHIPs before RT incubation	Repeat experiment with a new cellenCHIP384 RTready and correct sealing.
Wrong centrifugation parameters	• If centrifugation parameters were below the recommended speed and time, repeat the centrifugation step • If centrifugation temperature was above 4°C and/or time was set to >1min, continue with PreAmplification.

13. My Volume recovery after RT is very high

Causes	Recommendation
Condensation due to target plate being too cooled during cell dispensing	Continue with PreAmplification

14. I got no yield after cDNA amplification.

Causes	Recommendation
Cell population contains a large number of apoptotic cells.	Use Live/dead staining during sorting or remove dead/dying cells before cell dispensing.
Cells were not correctly dispensed into the wells	Verify correct Setup of the CellenOne system in concordance with the cellenCHIP target. See cellenCHIP manual.
Incorrect Storage of Kit components	Verify that all Kit components are stored at the outlined storing conditions in the manual.
Pipetting error/Missing reagent	Verify that all reactions were set up with the correct amount of components.

15. My Bioanalyzer/Tapestation trace of the cDNA shows a lower fragment size than expected

Causes	Recommendation
Cell population contains a large number of apoptotic cells.	Use Live/dead staining during sorting or remove dead/dying cells before cell dispensing.
RNase contamination in the lab	Clean with appropriate cleaning solutions to inactivate RNases on benches, pipettes and gloves. Use only RNase free filter tips!
Incorrect RT temperature	Verify correct cycler settings. Increased temperatures during reverse transcription can lead to RNA degradation.
RNA degradation due to target plate not being cooled during cell dispensing	Verify Target cooling to DP-1.6°C

16. I see a peak around 100 bp in the Bioanalyzer/TapeStation trace of my cDNA

A peak around 100 bp hints towards low levels of primer carryover (fragments <100bp). This is normal and does not affect sequencing or application performance. It is commonly observed for samples with a low RNA content. If the primer peaks correspond to more than 10% of the total cDNA concentration, we recommend to correct the concentration of the actual cDNA using the region function of the Bioanalyzer/Tapestation analysis software.

17. My cDNA Bioanalyzer/TapeStation trace shows high peaks within the cDNA distribution.

Some cell types express high amounts of specific transcripts accounting for a large proportion of total cellular mRNA. This may lead to additional peaks on the Bioanalyzer trace.

18. My cDNA Bioanalyzer/TapeStation trace shows another abnormality

Causes	Recommendation
Contamination with external DNA/RNA sources	External contamination e.g. by pollen can lead to various artefacts. Please ensure that all surfaces and pipettes are cleaned before use and only PCR grade filter tips are used.
cellenCLEAN Beads carryover can lead to elevated baseline	Incubate the cleaned cDNA on a magnetic rack and incubate 5 mins. Transfer the supernatant to a fresh tube and repeat quality control of cDNA.
Bioanalyzer/TapeStation specific artefacts	Please consult the corresponding manual

Library preparation

19. Is there a bias during Fragmentation?

We have not observed evidence of sequence preferences in the cut sites of the cellenTAG reagents although it as to be confirmed for other species with extreme GC or AT contents.

20. How is the sequencing library enriched for the 3´end?

Only the 3' portion of the transcript is retained during the sequencing library preparation, as the fragmented molecules are amplified with primers that only recognize fragments that contain the correct sequence introduced via the oligodT primer during reverse transcription.

Sequencing

21. Do I need to perform any other QC before sequencing?

We highly recommend performing a quantification of the sequencing library using the Kapa library quantification Kits (Roche). While TapeStation and Bioanalyzer Kits also provide a molarity measurement, a qPCR quantification is much more accurate and reliable, reducing the risk of over or under clustering on the flowcell as well as wrong read distribution over samples.

22. Where can I obtain the i7 index sequences for demultiplexing. Do I need to use the reverse complement depending on the sequencing workflow?

The i7 sequences can be found under Appendix IV of the Kit manual. You do not need to change the sequence orientation. It is the correct sequence for both, Illumina`s forward strand and reverse complement workflow.

23. Is it possible to uniquely dual index the libraries to inhibit index hopping?

Although in principal possible, the “cellenCHIP384 - 3'RNA-seq Kit” utilizes a sequencing library generation method which highly decreases index hopping. We do not see any requirement to use unique dual indexing. However, if the cellenLIBRARY is pooled with other samples, it might get affected by index hopping due to free primers from the other libraries. We highly encourage all users of patterned flowcell Illumina instruments to minimize index hopping by following Illumina’s recommendations (“*Effects of Index*

Misassignment on Multiplexing and Downstream Analysis", Illumina 2018, 770-2017-004-D).

24. Can I sequence several cellenLIBRARY on the same flowcell?

Yes. Each cellenCHIP will be tagged with a unique index sequence during sequencing library preparation using the cellenTAG reagents. For each Chip, there is one specific cellenTAG reagent. When sequencing Chips from different Kits, ensure that each cellenTAG reagent is only present for one cellenCHIP.

25. Do I need special sequencing primers or sequencing reagents?

No. The cellenSeq 3' RNA-seq libraries utilize standard Nextera (Read2 and i7) and TruSeq (Read 1) sequencing primers.

26. Can I de- or increase the sequencing length of Read 1 or 2?

The sequencing length of read 1 should not be altered, neither increased nor decreased. A shorter sequencing read leads to the loss of UMI bases and a longer sequencing read could potentially reduce the quality of the whole run as it would start to read the PolyA tail. Further information can be found at your sequencing facility or by contacting Illumina tech support.
Sequencing read 2 can be increased to up to 100 base pairs if required e.g. with inefficiently annotated genomes. Sequencing read 2 should not be decreased below 50 bp for accurate mapping, even with human samples.
In general, we recommend sequencing 20 bases for read 1 and 50 bp for read 2, with an additional 8 bp read to obtain the i7.

27. My libraries will be on a dual index sequencing run, which i5 sequence do I need to provide?

In a dual sequencing run the second index read will read the first 8 bases of the Read 1 Sequence when using the forward strand workflow or the last 8 bases of the P5 Adapter in the reverse Complement workflow. For the first please utilize "TCTTTCCC" as the i5 index sequence, for the latter please utilize "AGATCTCG".

General

28. During Elution, the cellenCLEAN Beads stick together and do not resuspend.

Causes	Recommendation
Overdrying of beads	Overdrying of beads can lead to clumping of beads and low purification yields. Increase elution time to up 30 mins and, if necessary, incubate the tube during elution at 37°C.

29. When removing the supernatant from the cellenCLEAN Beads , the beads do not stick to the wall.

Causes	Recommendation
Incubation too short	Incubate for another 5 mins on magnetic rack to ensure strong binding of the beads to the tube wall.
Too fast pipetting	When removing the supernatant form the beads always pipette slowly! Incubate for another 5 mins on magnetic rack and slowly remove the supernatant.

30. How long can I store purified cDNA/sequencing libraries?

We have not systematically tested for how long amplified cDNA and or sequencing libraries can be stored at -20°C. In general, we recommend proceeding to sequencing as soon as possible and to avoid any unnecessary Freeze/Thaw cycle.

31. Can I store the reagents at -20°C instead of -80°C?

No, we cannot guarantee functionality or shelf-life accuracy if the reagents are not stored at -80°C as indicated.

32. Do you recommend specific lab equipment and consumables to be used?

We do not recommend a specific type of lab equipment or brands as long as the supplier guarantees the following parameters:

Type of Equipment	Parameters
Centrifuge for cellenCHIP	Centrifuge with swing rotor for 96/384 plates. Cooling to 4°C possible. >= 2000x g
Table centrifuge for tubes	>= 2000 x g
Micropipettes	Micropipettes capable of pipetting down to 1 µl and up to 200 µl Calibrated regularly

	Resistant against cleaning with RNase removing agents
Pipette tips	Certified RNase/DNase, RNA/DNA free Filter tips
Tubes	For cellenBEAD clean ups please use the provided 1.5 ml tubes For PCR tubes, we recommend using PCR grade tubes or 8 strips which are certified for your specific Thermal Cycler.
Magnetic Rack	In principal all magentic racks for 1.5 ml should work with the cellenCLEAN Beads. If required, we propose the following 2: Invitrogen™ DynaMag™-2 Magnet (12321D) Invitrogen™ DynaMag™- Spin Magnet (12320D)

33. What are your recommendations concerning best practice while performing scRNA-seq?

Generally, we recommend working in a as clean as possible environment. Before each experiment, we recommend to clean Benches, micropipettes and gloves using appropriate cleaning solutions to remove RNase/DNase as well as Nucleic acid contaminations. In addition, proper clean protective clothing should be used.
Furthermore, we do not recommend performing single cell reverse transcription reactions in the same environment as amplified DNA (post PCR).