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Maturation exploration of kidney organoids from single-cell RNA-seq data analysis

Solène Brohard

Bio-informatician

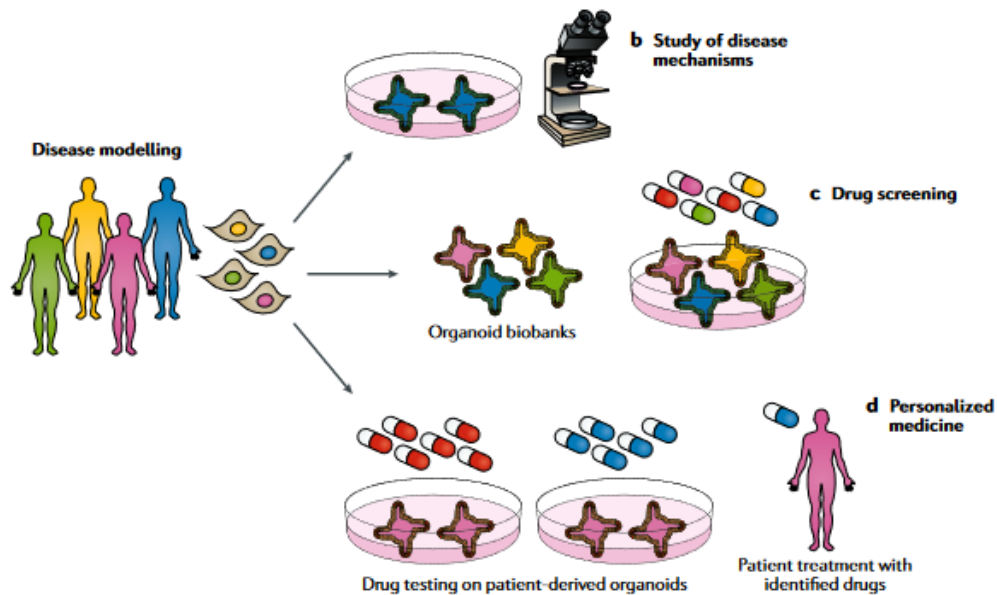


Organoids model organs development

Definition

Organoids are **3D structures** derived from adult (ASCs) or pluripotent (**PSCs**) stem-cells. They are cultured *in vitro* and display architectures and functionalities similar to organs *in vivo*. At a much smaller scale, the multicellular repertoire of target organs is expected to validate organoid **maturation**.

Therapeutic Application



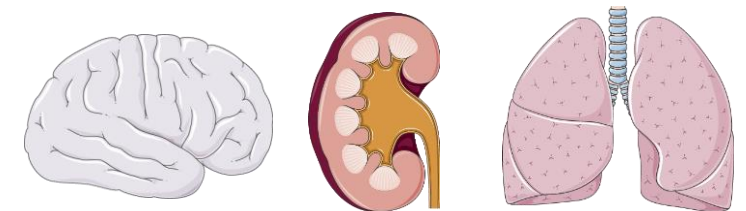
Rossi *et al.*, 2018, Nature Review.

Ethical implication

Animal experimentations



Tissue collection



De Kanter *et al.*, 2023, Tissue Engineering.

Organoid maturation limits/challenges

Limits

Cell differentiation in organoids is closer to **fetal** than adult target organ.

- Restricted lifespan limits ability to reach latter stages.
- In vitro environment lacks specific in vivo factors to diffuse nutrients.

Challenges

- Improve organoid **specificity** to the target organ.
- Define **organ specific cells** vs off target cells for quality control.
- Vascularization by bioengineering or transplantation.

→ The single-cell RNA-seq is widely used to control the cell composition of organoids.

Rossi *et al.*, 2018, Nature Review.
Piccollet-D'hahan *et al.*, 2017, Trends in Biotechnology.
The Organoid Cell Atlas, 2021, Nature Biotechnology.

Available resources on kidney organoids

- Kidney cell fate, targeted and off target cell types in kidney tissues.
- Single-cell RNA-seq data available and analyzed.

Cell Stem Cell
Resource

High-Throughput Screening Enhances Kidney Organoid Differentiation from Human Pluripotent Stem Cells and Enables Automated Multidimensional Phenotyping

Stefan M. Czerniacki,^{1,2,3,9} Nelly M. Cruz,^{1,2,3,9} Jennifer L. Harder,^{4,9} Rajasree Menon,⁵ James Annis,³ Edgar A. Otto,⁴ Ramila E. Gulieva,^{1,2,3} Laura V. Islas,^{1,2,3} Yong Kyun Kim,^{1,2,3} Linh M. Tran,^{1,2,3,6} Timothy J. Martins,³ Jeffrey W. Pippin,¹ Hongxia Fu,^{3,6} Matthias Kretzler,^{4,5} Stuart J. Shankland,¹ Jonathan Himmelfarb,^{1,2} Randall T. Moon,^{3,7} Neal Paragas,¹ and Benjamin S. Freedman^{1,2,3,8,10,*}

Cell Stem Cell 22, 929–940, June 1, 2018

CellPress
OPEN ACCESS

Cell Stem Cell

Resource

Spatial dynamic metabolomics identifies metabolic cell fate trajectories in human kidney differentiation

Gangqi Wang,^{1,2} Bram Heijs,^{2,3} Sarantos Kostidis,³ Rosalie G.J. Rietjens,¹ Marije Koning,¹ Lushun Yuan,¹ Gesa L. Tiemeier,¹ Ahmed Mahfouz,^{4,5} Sébastien J. Dumas,^{1,2,6} Martin Giera,^{2,3} Jesper Kers,⁷ Susana M. Chuva de Sousa Lopes,^{2,8} Cathelijne W. van den Berg,^{1,2} Bernard M. van den Berg,¹ and Ton J. Rabelink^{1,2,9,*}

Cell Stem Cell 29, 1580–1593, November 3, 2022

npj | Regenerative Medicine

www.nature.com/npjregenmed

REVIEW ARTICLE OPEN

Check for updates

Defining the variety of cell types in developing and adult human kidneys by single-cell RNA sequencing

A. Schumacher¹, M. B. Rookmaaker², J. A. Joles², R. Kramann^{3,4}, T. Q. Nguyen⁵, M. van Griensven⁶ and V. L. S. LaPointe^{1,8*}

npj Regenerative Medicine (2021) 45

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npj Regenerative Medicine (2021) 45

PROTOCOL

Generation of kidney organoids from human pluripotent stem cells

Minoru Takasato^{1,2}, Pei X Er^{1,2}, Han S Chiu² & Melissa H Little^{1–3}

¹Murdoch Childrens Research Institute, Parkville, Melbourne, Victoria, Australia. ²Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, Australia. ³Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia. Correspondence should be addressed to M.T. (minoru.takasato@mcri.edu.au) or M.H.L. (melissa.little@mcri.edu.au).

Published online 18 August 2016; doi:10.1038/nprot.2016.098

NATURE PROTOCOLS | VOL.11 NO.9 | 2016



ARTICLE

<https://doi.org/10.1038/s41467-019-13382-0> OPEN

Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation

Ayshwarya Subramanian^{1,6}, Eriene-Heidi Sidhom^{1,2,6}, Maheswarareddy Emani^{1,6}, Katherine Vernon^{1,2}, Nareh Sahakian¹, Yiming Zhou^{1,2}, Maria Kost-Alimova³, Michal Slyper¹, Julia Waldman¹, Danielle Dionne¹, Lan T. Nguyen¹, Astrid Weins⁴, Jamie L. Marshall¹, Orit Rosenblatt-Rosen¹, Aviv Regev^{1,5} & Anna Greka^{1,2*}

NATURE COMMUNICATIONS | (2019)10:5462 |

Combes et al. *Genome Medicine* (2019) 11:3
<https://doi.org/10.1186/s13073-019-0615-0>

Genome Medicine

RESEARCH

Open Access

Single-cell analysis reveals congruence between kidney organoids and human fetal kidney

Alexander N. Combes^{1,2†}, Luke Zappia^{2,3†}, Pei Xuan Er², Alicia Oshlack^{2,3} and Melissa H. Little^{1,2,3,4*}

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- Kidney cell fate, targeted and off target cell types in kidney tissues.
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REVIEW ARTICLE OPEN

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Check for updates

Can we assess the tissue specificity and the variation of cell composition of kidney organoids from single-cell RNA-seq data?

Generation of kidney organoids from human pluripotent stem cells

Minoru Takasato^{1,2}, Pei X Er^{1,2}, Han S Chiu² & Melissa H Little^{1–3}

¹Murdoch Childrens Research Institute, Parkville, Melbourne, Victoria, Australia. ²Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, Australia. ³Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia. Correspondence should be addressed to M.T. (minoru.takasato@mcri.edu.au) or M.H.L. (melissa.little@mcri.edu.au).

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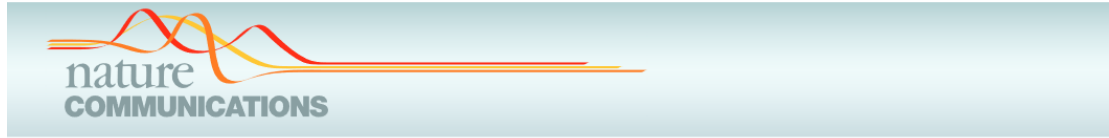
RESEARCH

Open Access

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Alexander N. Combes^{1,2†}, Luke Zappia^{2,3†}, Pei Xuan Er², Alicia Oshlack^{2,3} and Melissa H. Little^{1,2,3,4*}

Public datasets used of kidney organoids



ARTICLE

<https://doi.org/10.1038/s41467-019-13382-0>

OPEN

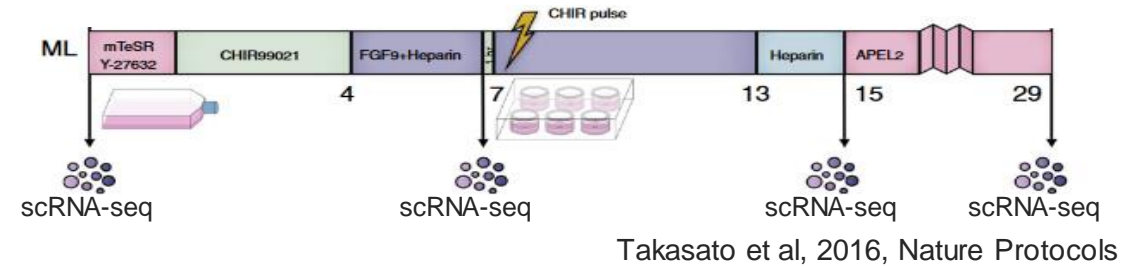
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NATURE COMMUNICATIONS | (2019)10:5462 |

- Freely available datasets.
- Alstem(AS) human induced pluripotent stem cell line.
- 4 time points of maturation (D0, D7, D15 and D29).

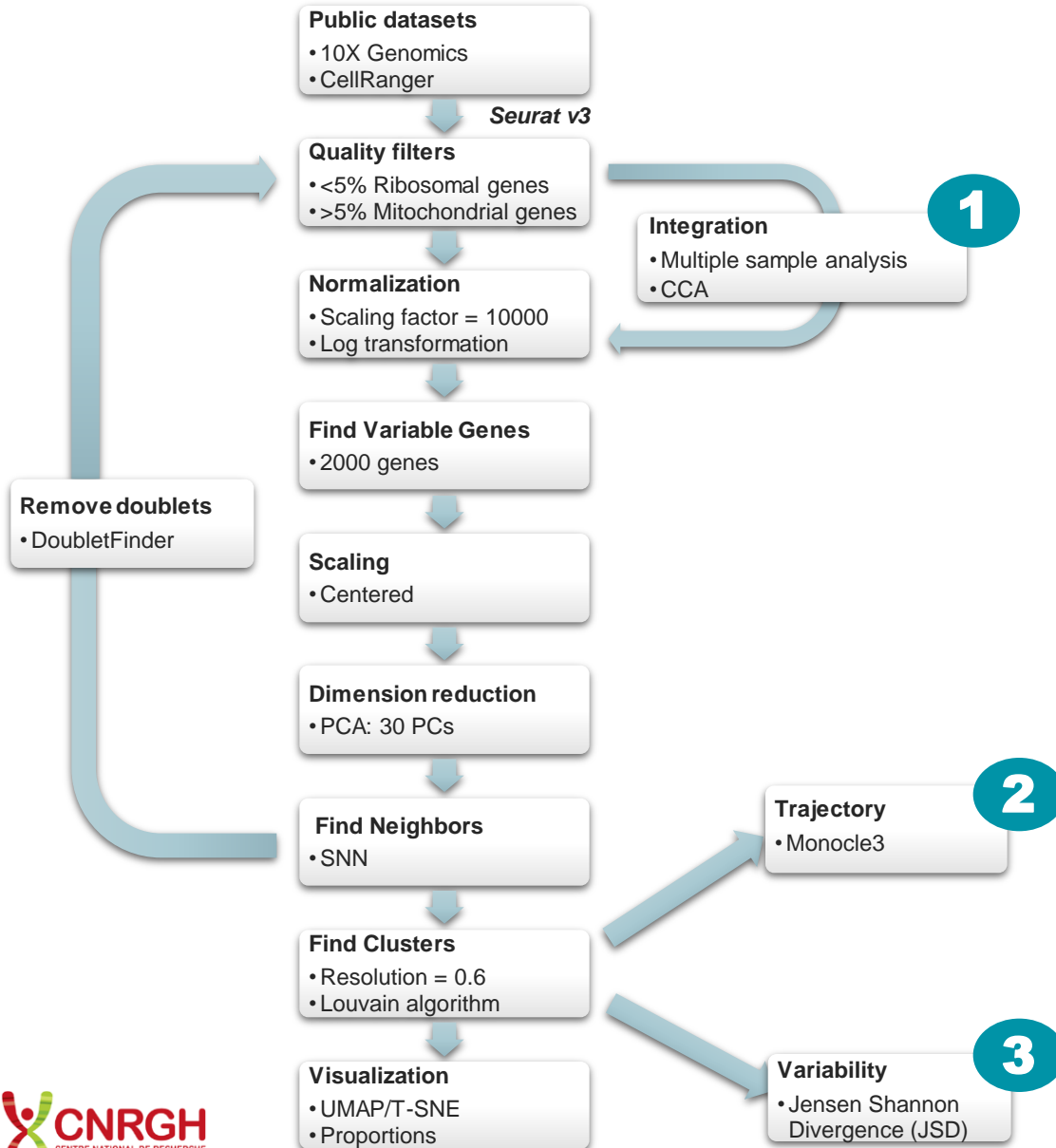
Protocol



Single-cell RNA-seq datasets

Day of maturation	Number of cells
D0	16786
D7	16072
D15	50976
D29	47799

Single-cell RNA-seq workflow adapted for organoids



cea **Maturation study of organoids from single-cell RNA-seq data analysis** **CNRGH**
 Solène Brohard¹, Camille Lemerrier, Alexandre Hubert, Jean-François Deleuze and Eric Bonnef
 Université Paris-Saclay, CEA, Institut Jussieu, Centre National de Recherche en Génétique Humaine (CNRGH), 91000, Evry-Courcouronnes, France

Introduction
 Organoids are stem-cell derived complex 3D structures that display architectures and functionalities similar to *in vivo* organs¹. Organ-specific studies were able to confirm the structure and the composition of mature organoids by comparing cell populations found in organoids with the ones found in the target organ. In this context, we wanted to study the organoid maturation by exploring descriptive and statistical methods from single-cell RNA-seq data of organoids at different maturation stages. We investigated single-cell RNA-seq public datasets of kidney organoids at different times of maturation. We integrated the datasets to highlight cell specificities for each stage of maturation. We also studied cell trajectories to observe if there is a correlation between the cell differentiation and the date of maturation. We finally used statistical methods to explore the variability of cell proportions between stages of maturation.

Organoids

1 Integration

Definition
 Organoids are 3D structures derived from adult (ASCs) or pluripotent (PSCs) stem-cells¹. They are cultured *in vitro* and display architectures and functionalities similar to *in vivo* organs. At a much smaller scale, the multicellular repertoire of targeted organs is expected to validate the maturation of organoids. This concept is based on the self-organization process of cell differentiation that results from autonomous mechanisms and local intercellular communication. Cell source and culture protocols depend on the targeted organ and the expected level of maturation.

Kidney organoid dataset
 Public datasets from Subramanian et al² allowing organoid maturation study are freely available. The protocol (ML) used in this paper has been defined to target kidney organoids. Single-cell RNA-seq (scRNA-seq) are done on organoids at different stages of maturation (D0, D7, D15 and D29). Two commercial human iPSC (hiPSC) lines (Alstem-AS and Thermo Fisher-THF) are tested (Fig. 1).

Figure 1. Differentiation protocol (ML) for kidney organoids derived from hiPSCs
 Modified from Subramanian et al².

scRNA-seq analysis

The workflow (Fig.2) applied on Subramanian et al² count matrices is based on R (Seurat package v3).

1 Filters to remove low quality cells:

- % of reads covering ribosomal genes < 5%
- % of reads covering mitochondrial genes > 5%
- Doublets: identification of droplets containing multiple cells (estimation by 10X Genomics technology of ~5% of droplets for 10 000 cells targeted).

2 The criteria to choose the number of principal components (PCs) used for principal component analysis (PCA) is to keep the fewer number of PCs that cover the maximum of variability (30 PCs).

3 The workflow and the parameters chosen follow the guidelines of Seurat tutorials.

Figure 2. Single-cell RNA-seq workflow

Figure 3. Dataset integration at different maturation levels
 Datasets of kidney organoids cultured from AS hiPSC line at the different days of maturation were integrated to compare the cell populations present in the samples. The method used to integrate datasets was the Canonical Correlation Analysis (CCA) (Fig.3).
 > The CCA seemed to correctly integrate datasets at the different maturation levels.
 > Final clusters were defined from integrated datasets.

Figure 4. Proportion of cells of maturation levels
 From this integration, we can distinguish clusters that are common to all the maturation days from those specific to one level with the percentage of cells belonging to each maturation day for every cluster (Fig.4).
 > A gradient of maturation can be observed (D0, intermediate (all levels), late maturation level (D15, D29)).
 > The clusters 4, 7, 12, 15 and 17 contain more than 75% of D0 cells.
 > From gene markers associated to each cluster, these specific clusters seem to be composed of stromal and proximal cells.

Figure 5. Trajectory visualization
 From integrated datasets of AS cell line, we use Monocle3 to visualize cell trajectories (Fig.5).
 > The tendency observed in Fig. 4 is visualized in cell trajectory.
 > The D02 specific clusters are well separated.
 > We also visualize the maturation and the cell differentiation from D0 to D29.

3 Variability

The Jensen Shannon Divergence (JSD) statistical approach computes a score comprised between 0 and 1, 0 corresponds to identical classification and 1 to a high level of divergence between the 2 classifications.

The JSD was applied on all integrated datasets (AS + THF) of all the days of maturation (D0, D7, D15 and D29). It compared the proportion of cells in each cluster between 2 samples.

> The JSD score between the AS and THF cell lines at the same age (Fig.6 A) indicated that the maturation was not affected by the initial cell line.

> For AS (Fig.6 B) or THF (Fig.6 C) only and between THF and AS (Fig.6 D) at different maturation levels, we observed a maturation gradient of variability.

> As observed in Fig. 4, D0x07 and D15x029 showed more similar classifications that was coherent with an early and late stages of maturation.

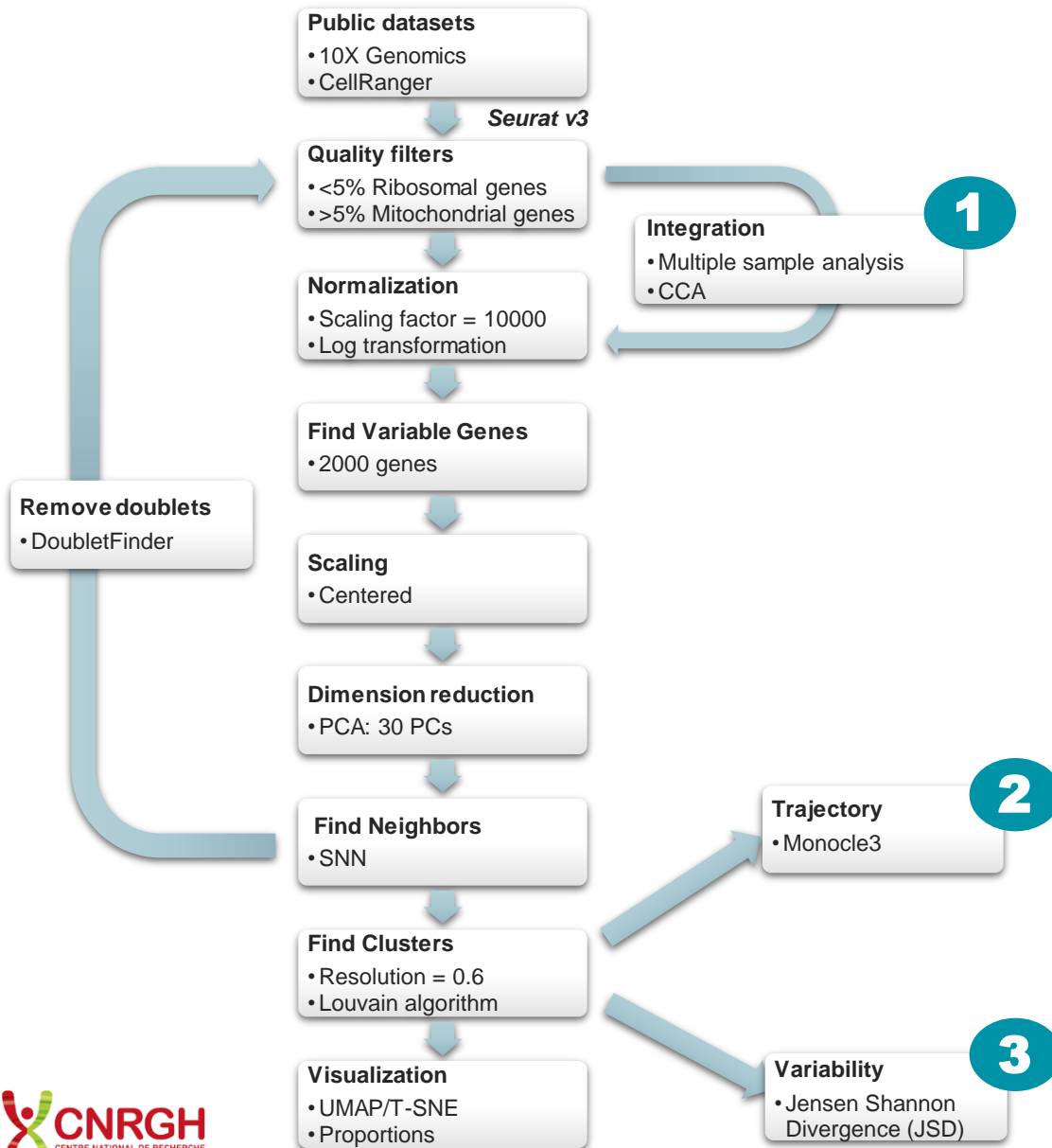
Figure 6. JSD of cell proportion between maturation levels.

Discussion
 In this study, we explored the maturation of organoids across public data of kidney organoids sequenced by scRNA-seq at different days of maturation. We applied commonly used workflow and methods that we adapted to specifically highlight the level of organoid maturation. The results obtained from datasets of one commercial hiPSC line (AS) showed that it is possible to explore the different maturation levels and to observe, from scRNA-seq analysis only, the cell differentiations along the maturation time. We could distinguish 2 distinct early and late maturation level. We also confirmed that the hiPSC line used for the organoid culture do not impact the organoid maturation timing. In perspective of this preliminary study is to explore and compare organoids of same targeted tissue from different maturation ages, cell lines and protocols. We also planned to use machine learning approaches to develop a method that could try to estimate the maturation level of organoids.

1. Rossi, Marfin, & Lutolf. Progress and potential in organoid research. *Nat Rev Genet* 19, 671-687 (2018).
 2. Subramanian, Sidhom, Eshani et al. Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation. *Nat Commun* 10, 5482 (2019).

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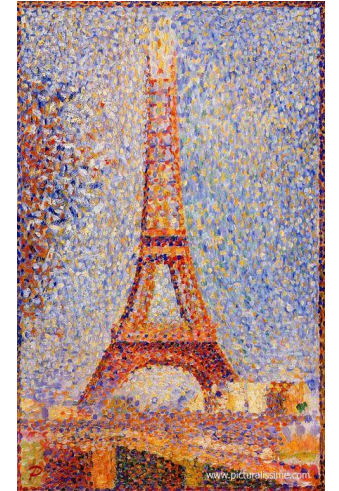
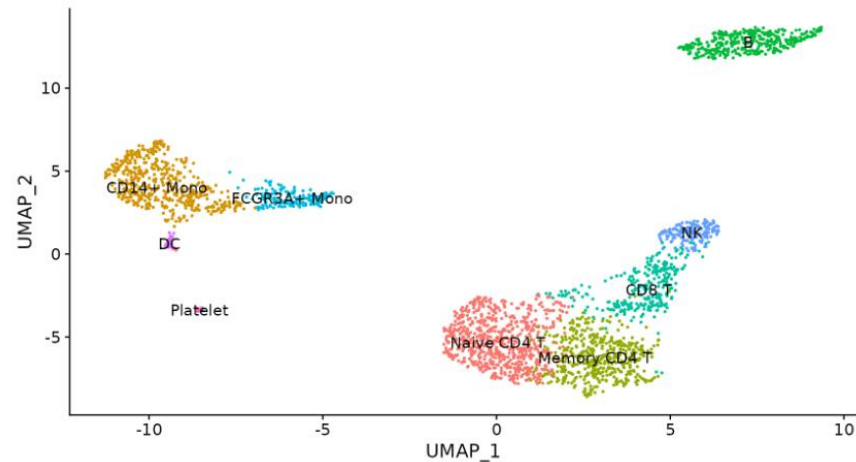
Single-cell RNA-seq workflow adapted for organoids



Seurat

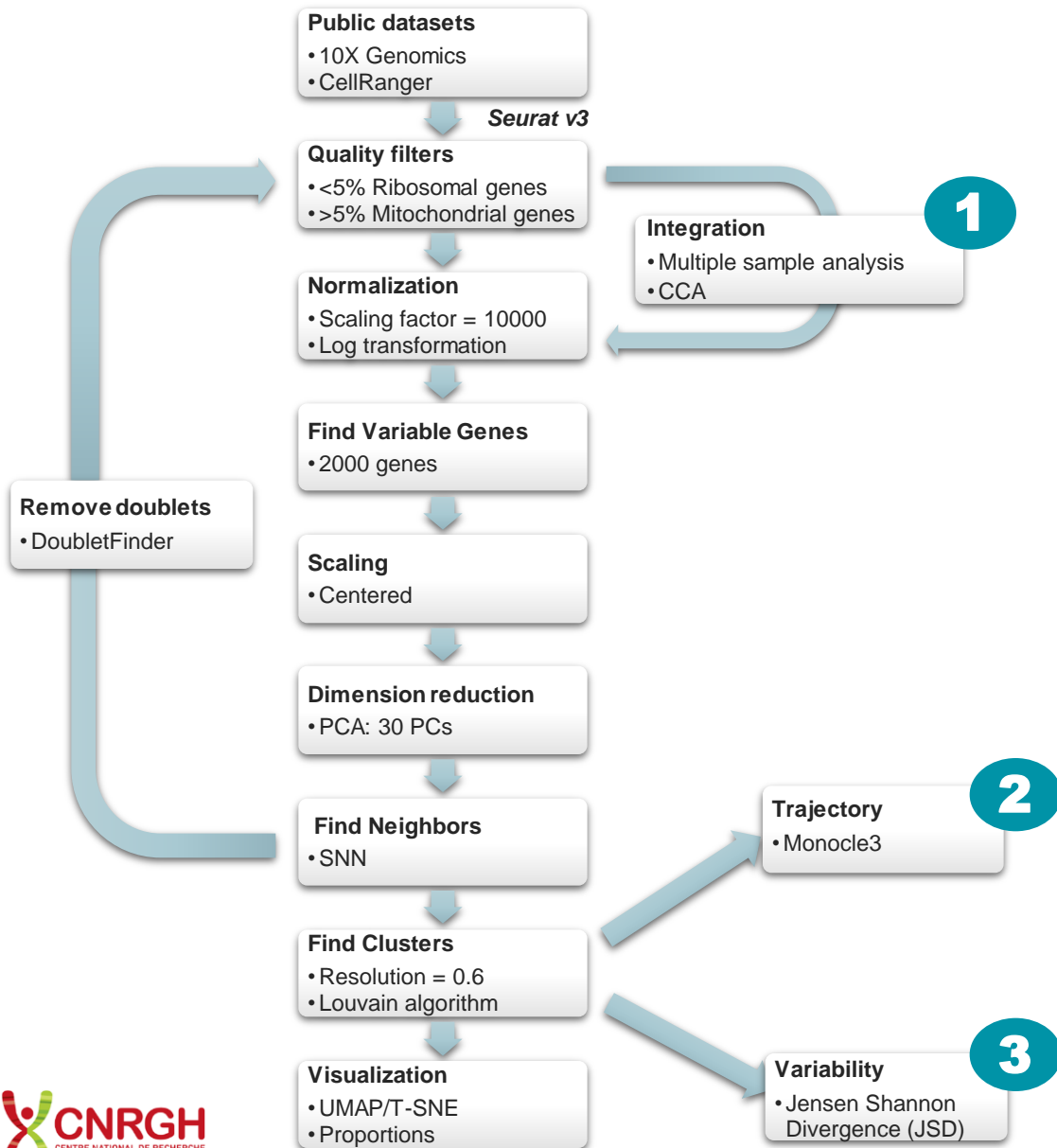
Stuart and Butler et al. Comprehensive Integration of Single-Cell Data. Cell (2019) [Seurat V3]

- Transcriptome of each cell.
- 1 point = 1 cell



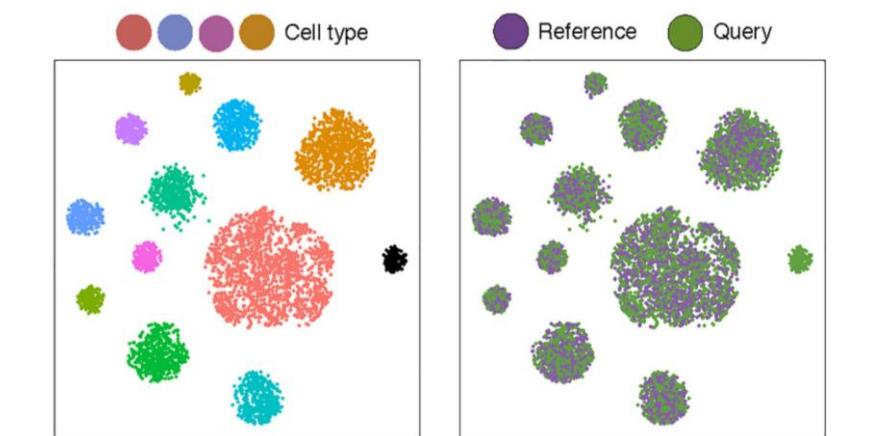
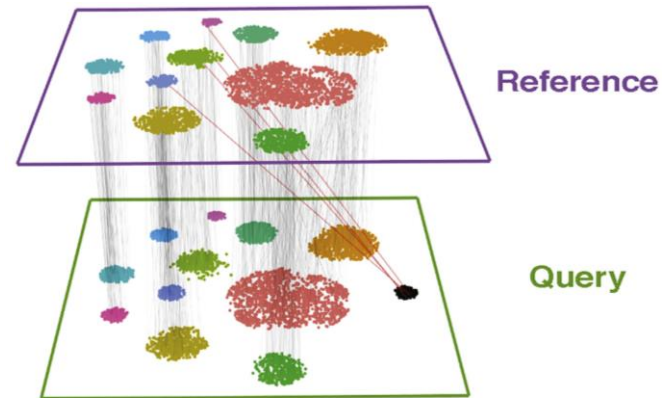
Georges Seurat, 1889

Single-cell RNA-seq workflow adapted for organoids



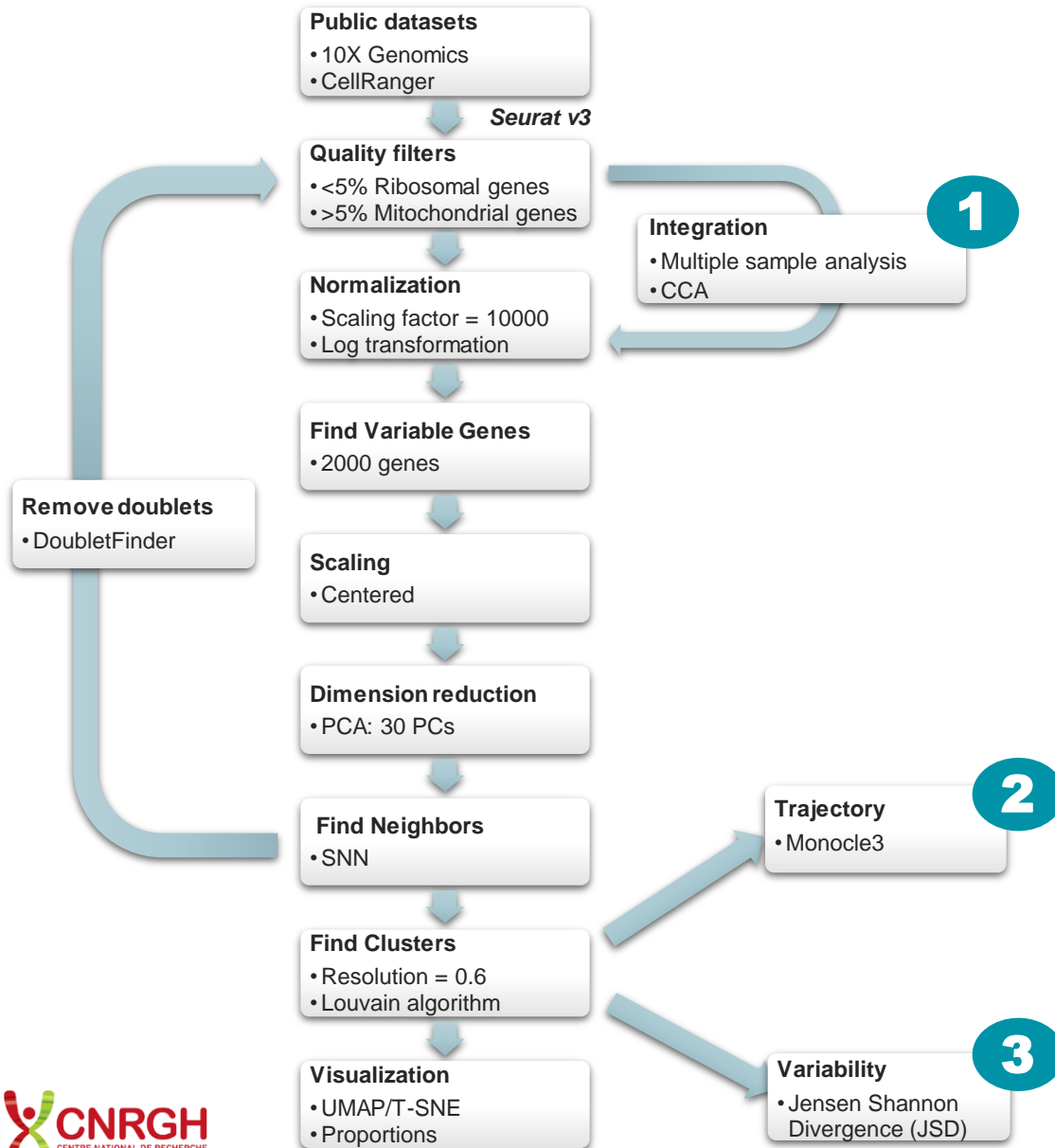
1 Integration

- Canonical Correlation Analysis (CCA)



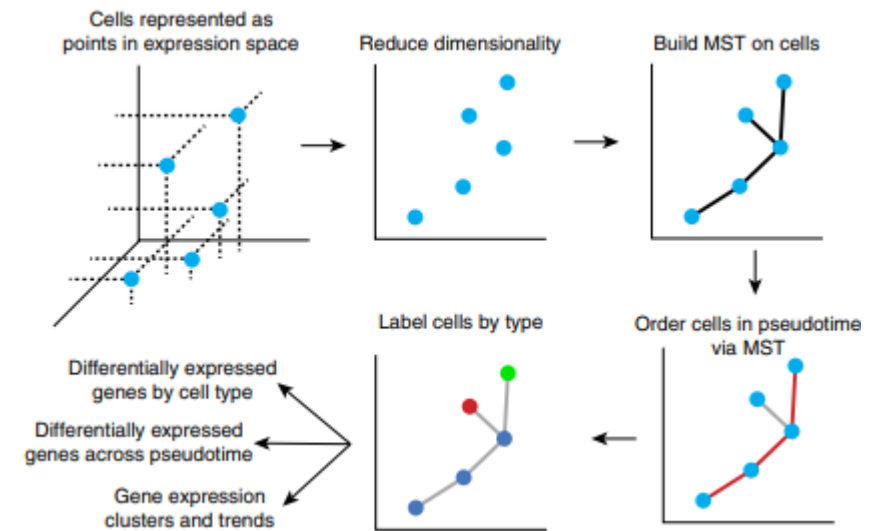
Stuart and Butler *et al*, 2019. Cell

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2 Trajectory

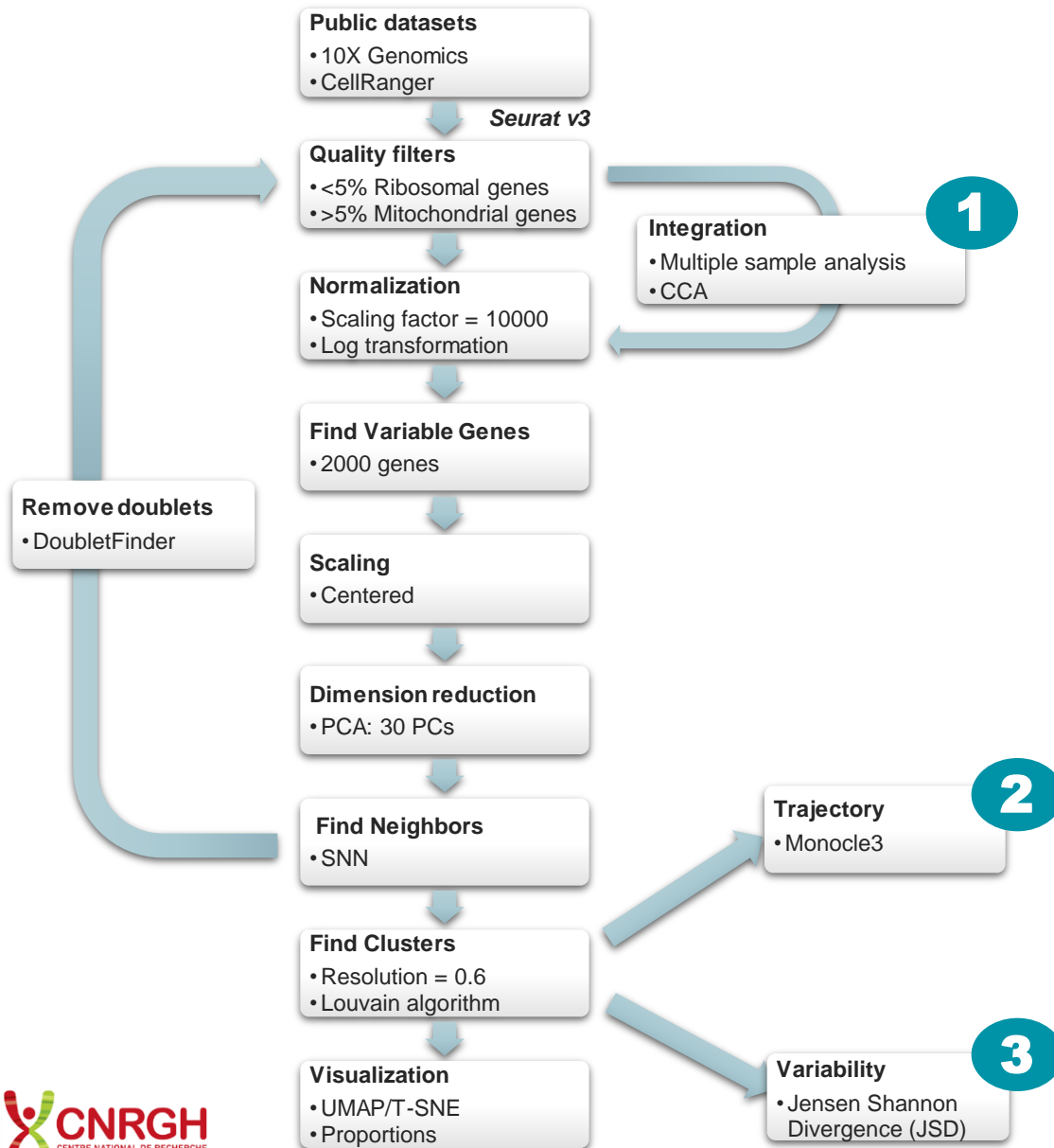
- Monocle3
- Order cell by pseudo-time from graph approach depending on cell distances.
- Visualization of cell differentiation



Trapnell *et al.*, 2014. Nature Biotechnology.

20/09/2023

Single-cell RNA-seq workflow adapted for organoids



3 Jensen Shannon Divergence

The Jensen Shannon Divergence (JSD) statistical approach that compare object frequencies in groups.

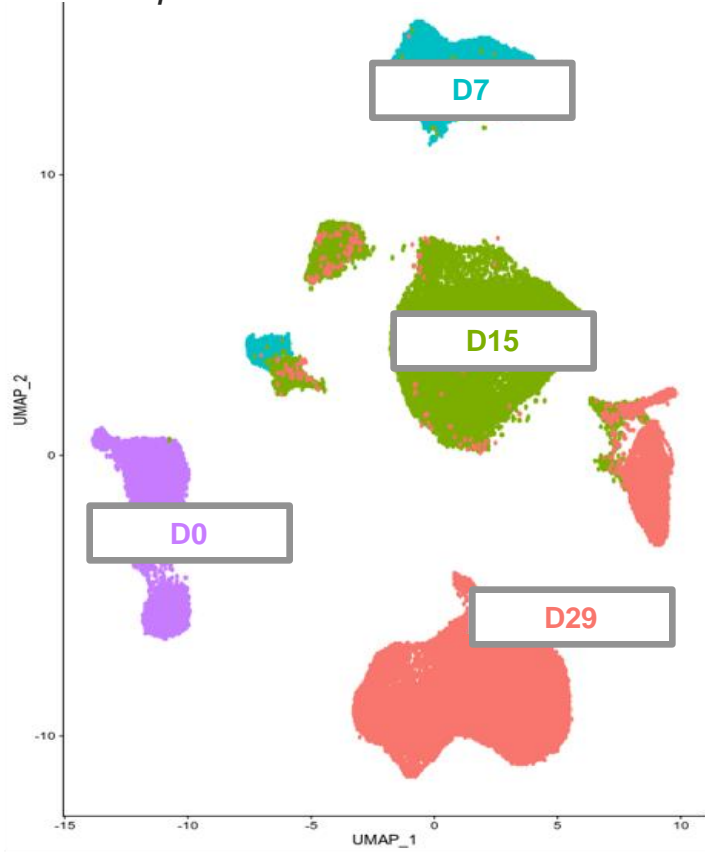
Score comprised between 0 and 1:

- 0: identical classifications.
- 1: high level of divergence between the 2 classifications.

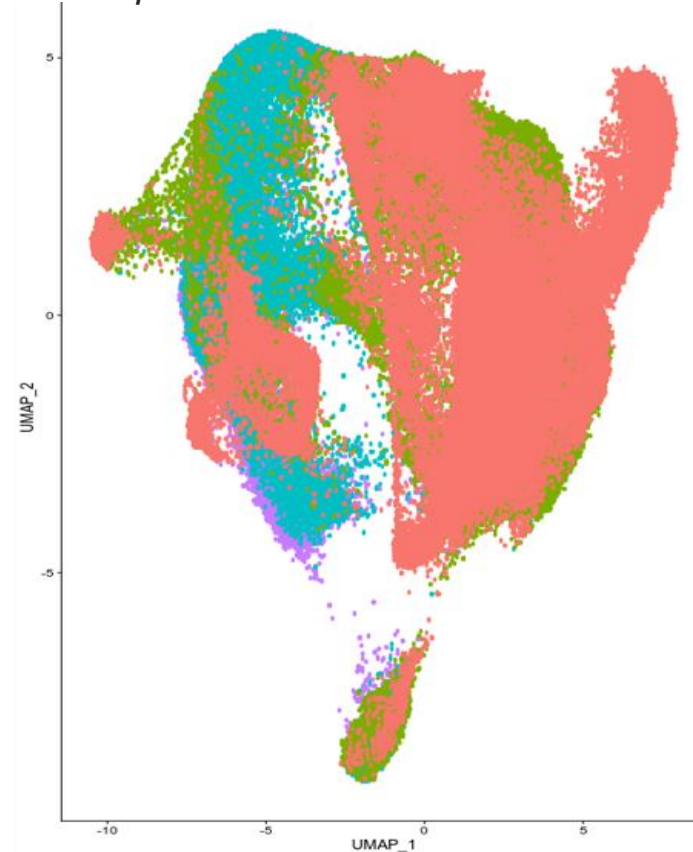
Integration and cluster identification



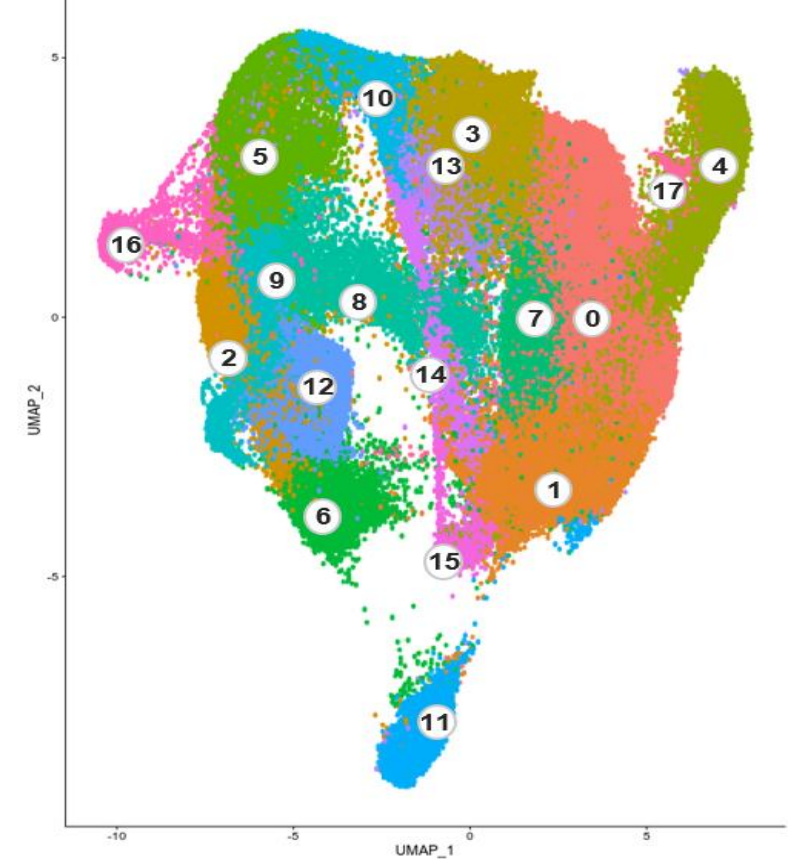
Samples before CCA



Samples after CCA

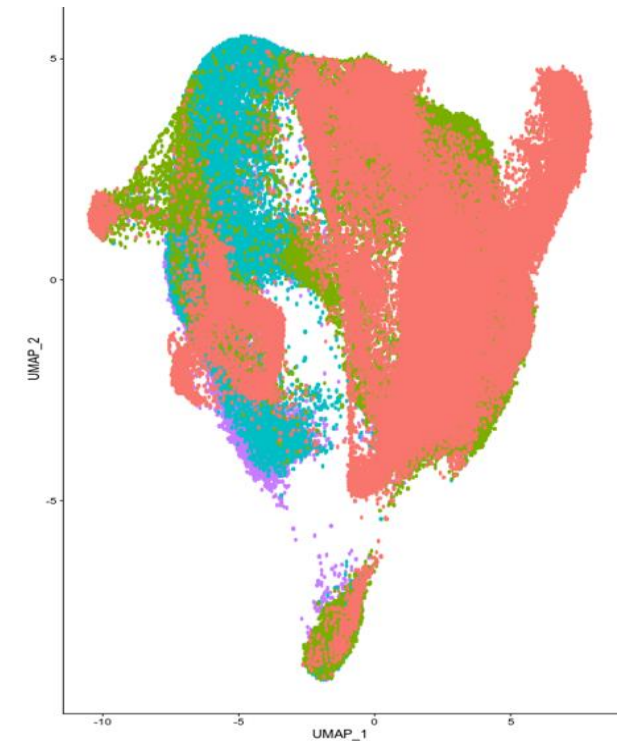
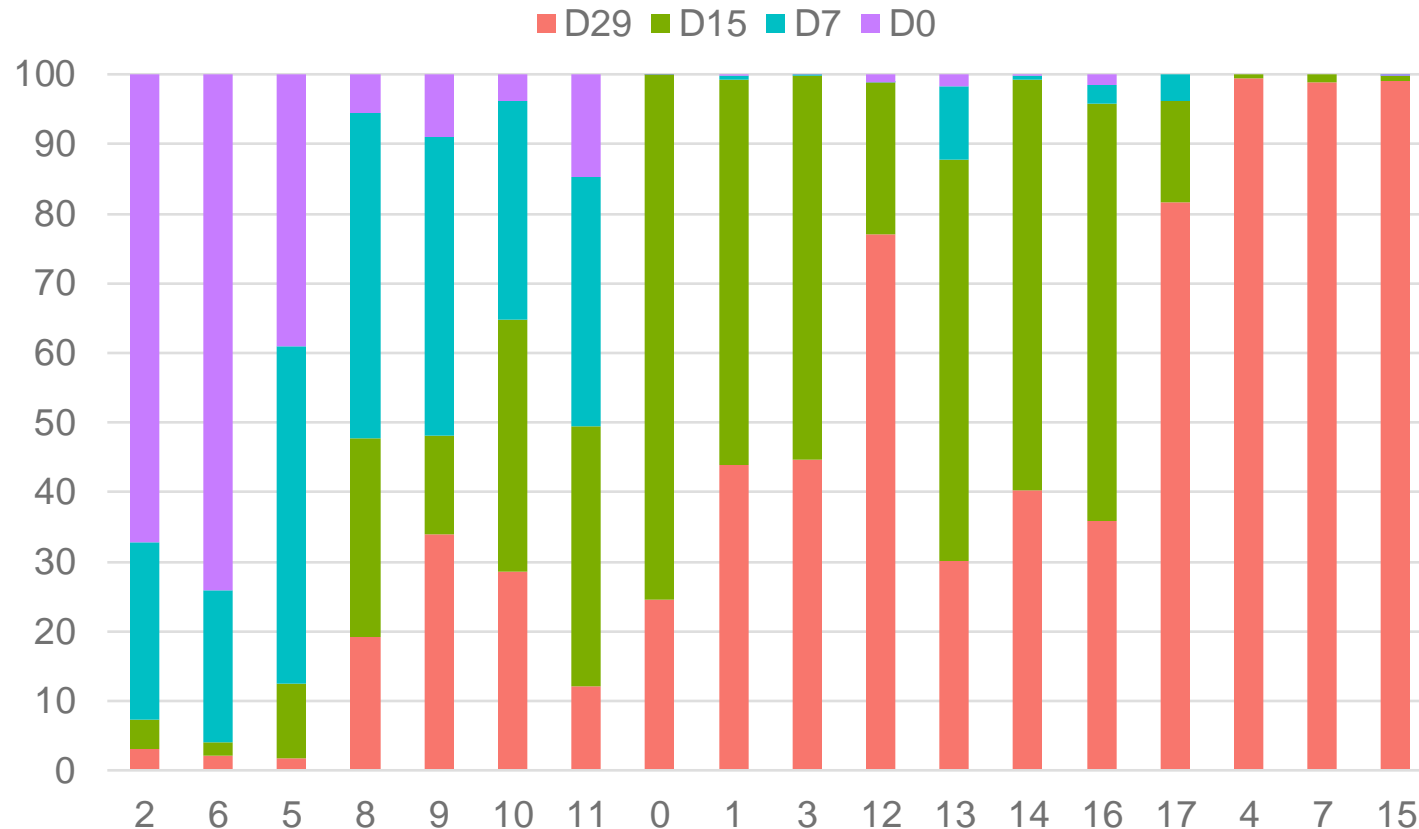


Cluster identification



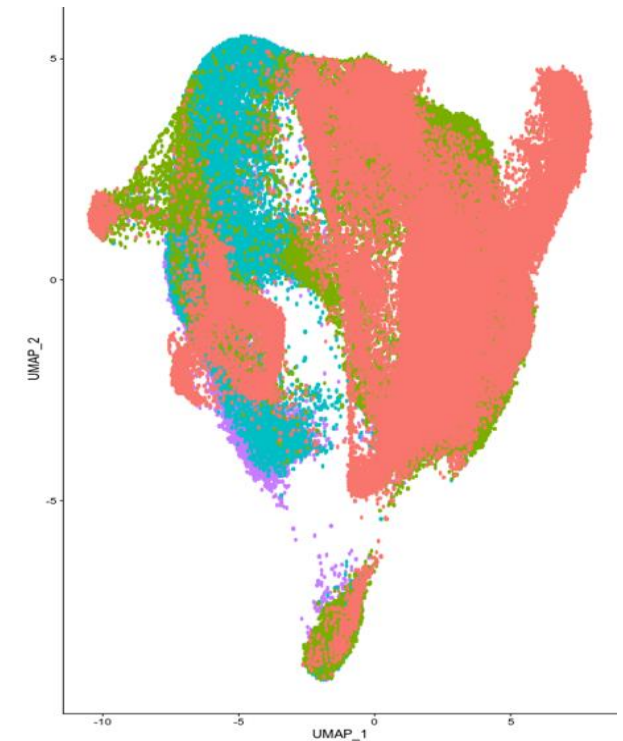
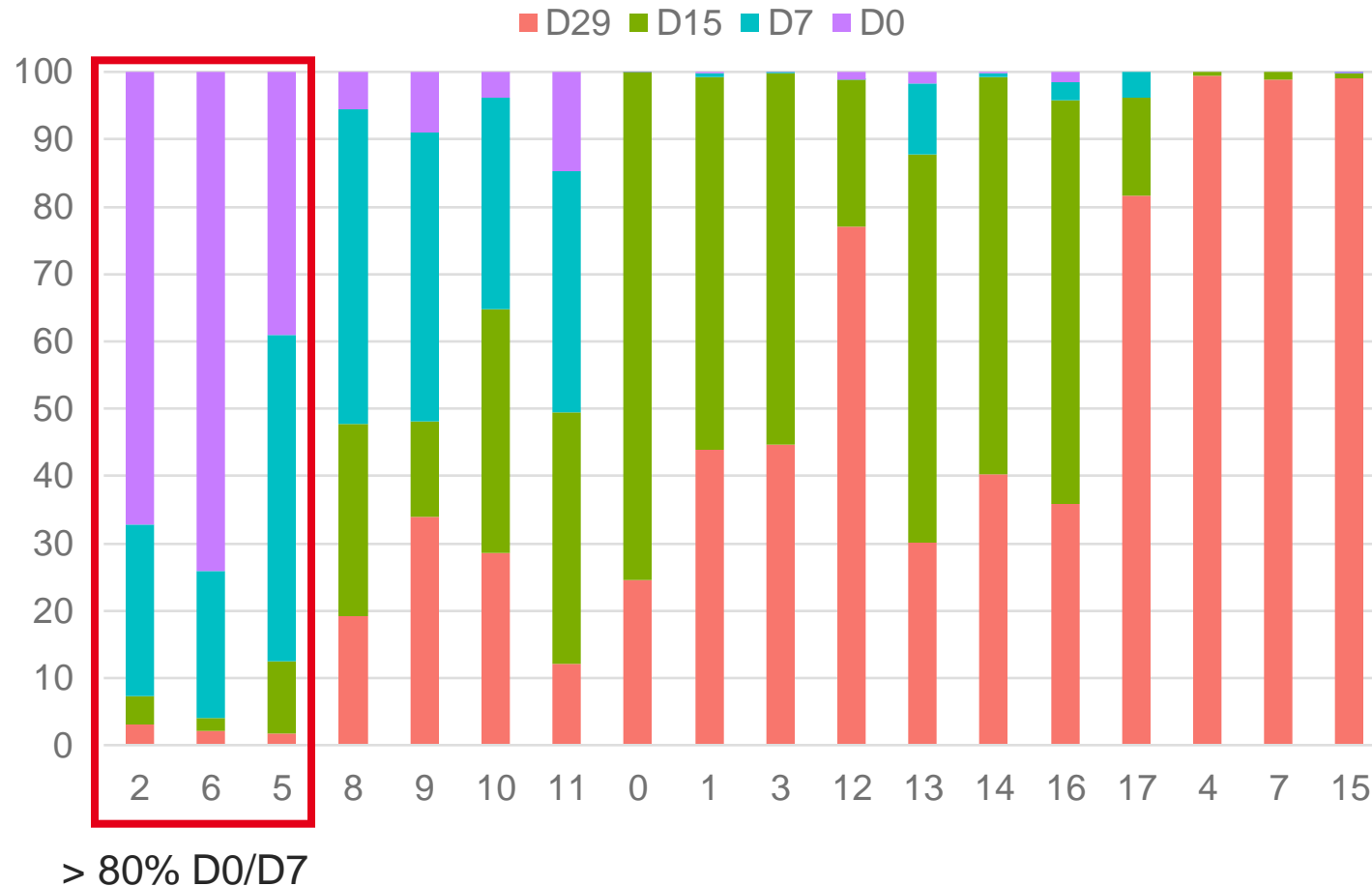
Heterogeneity of cell proportions in clusters

- Distinguish clusters that are common to all the maturation days from those specific to one level with the percentage of cells belonging to each maturation day for every cluster.



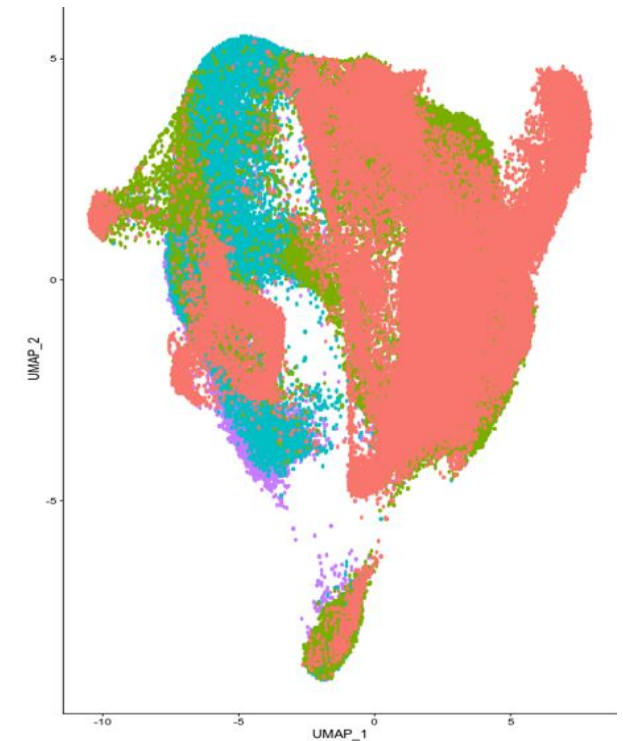
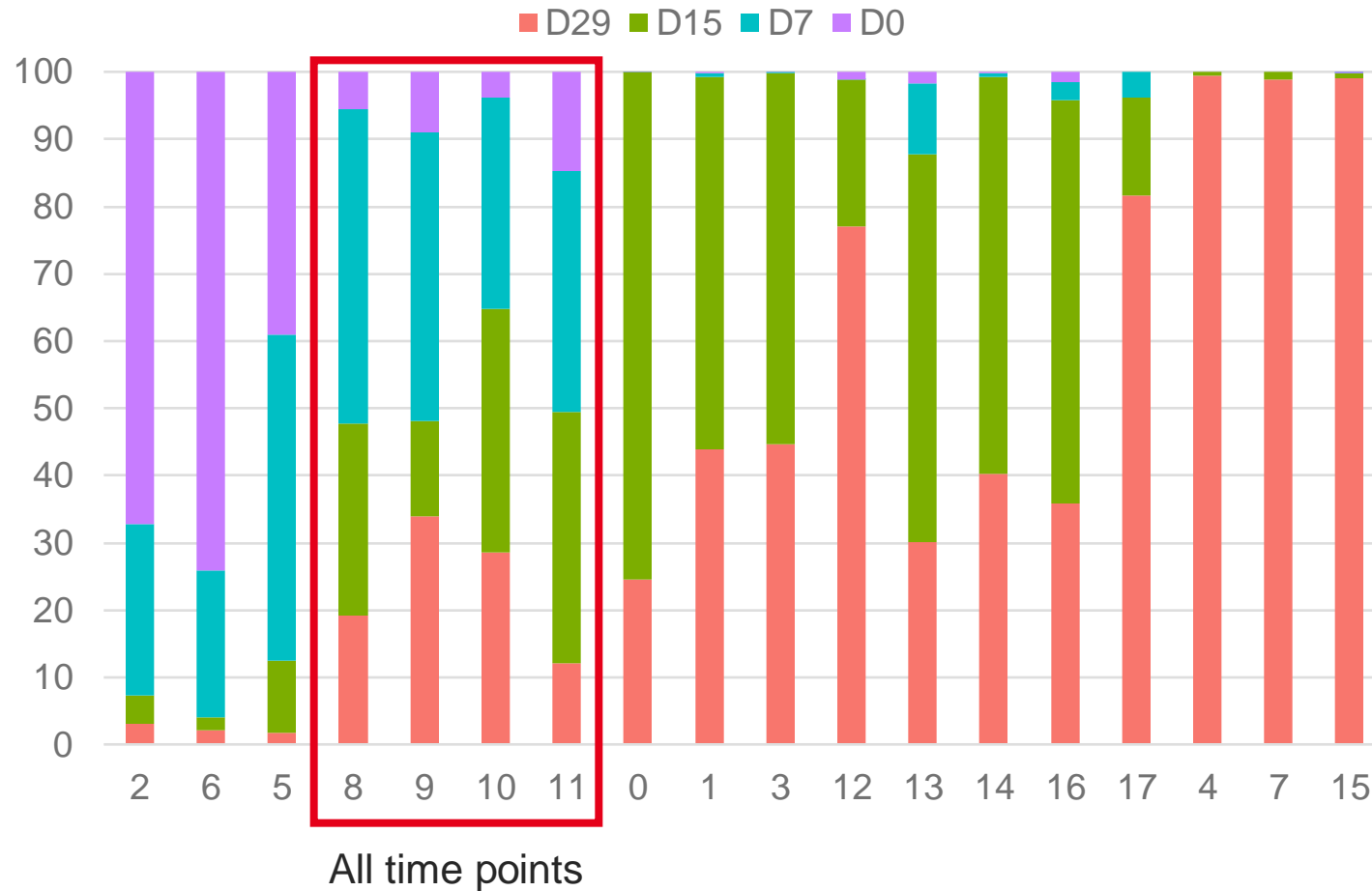
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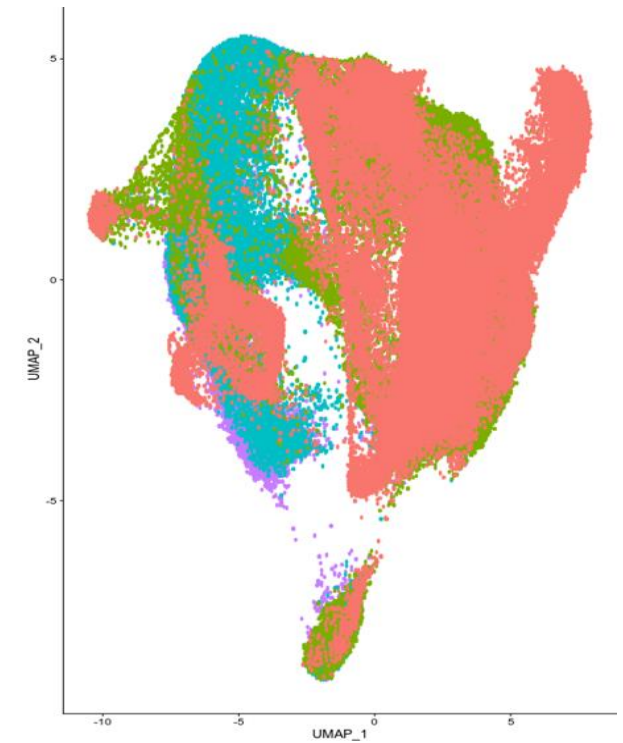
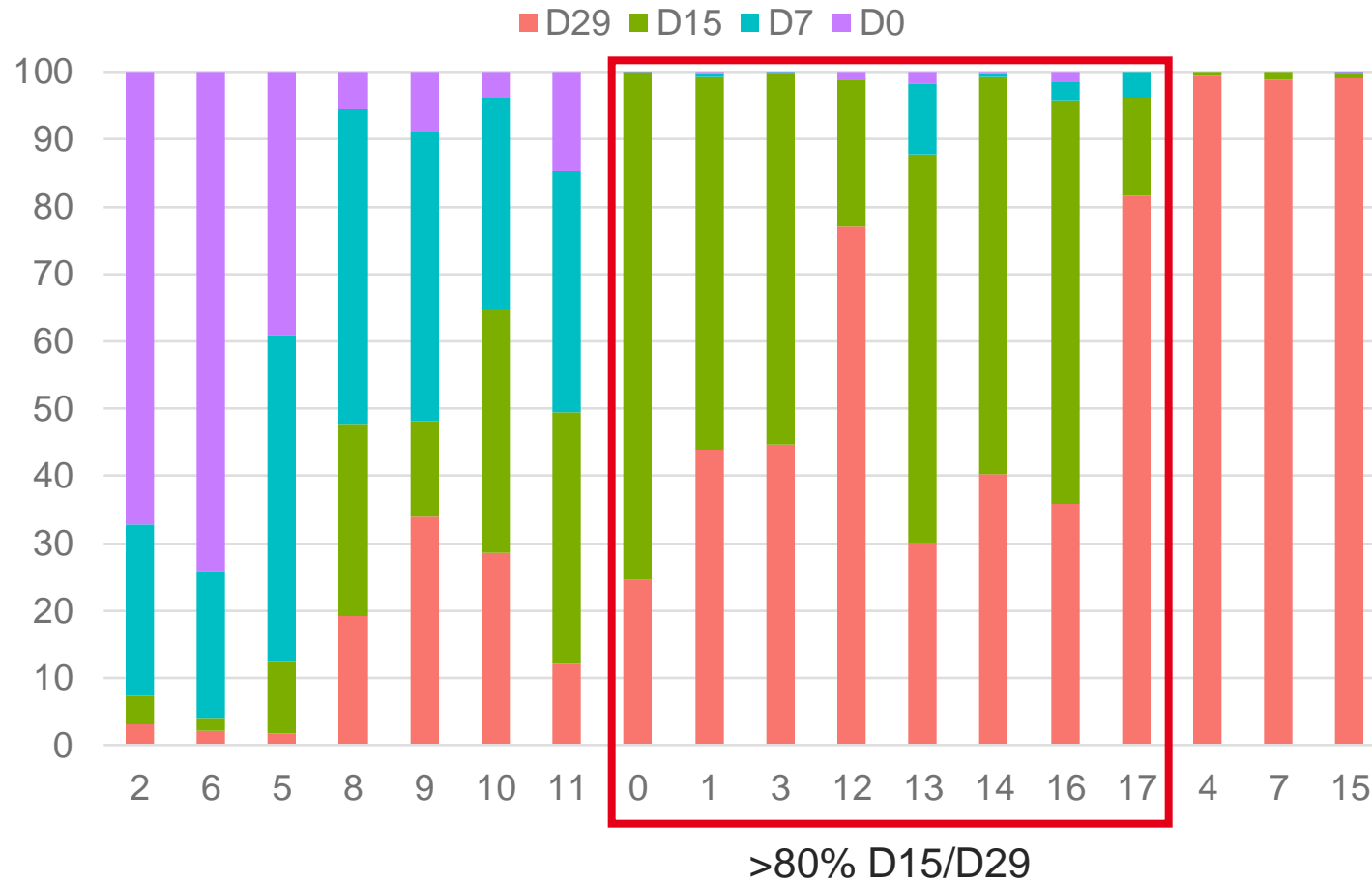
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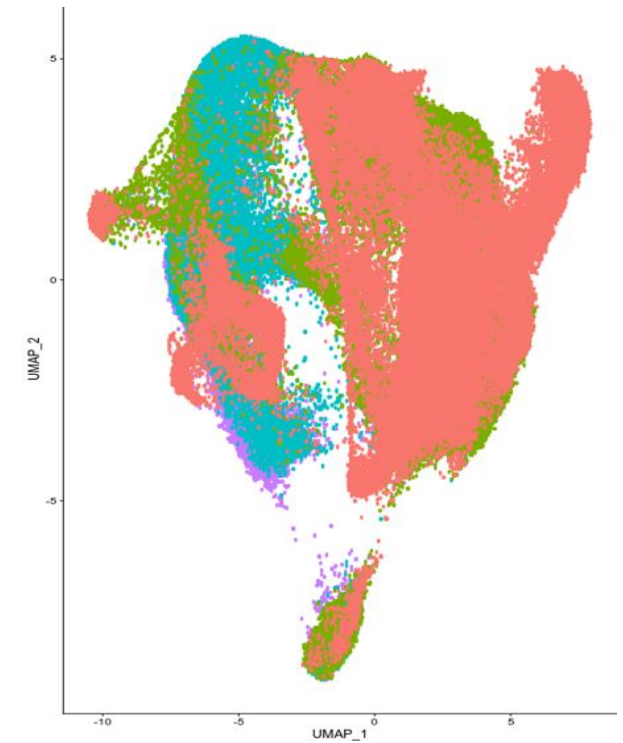
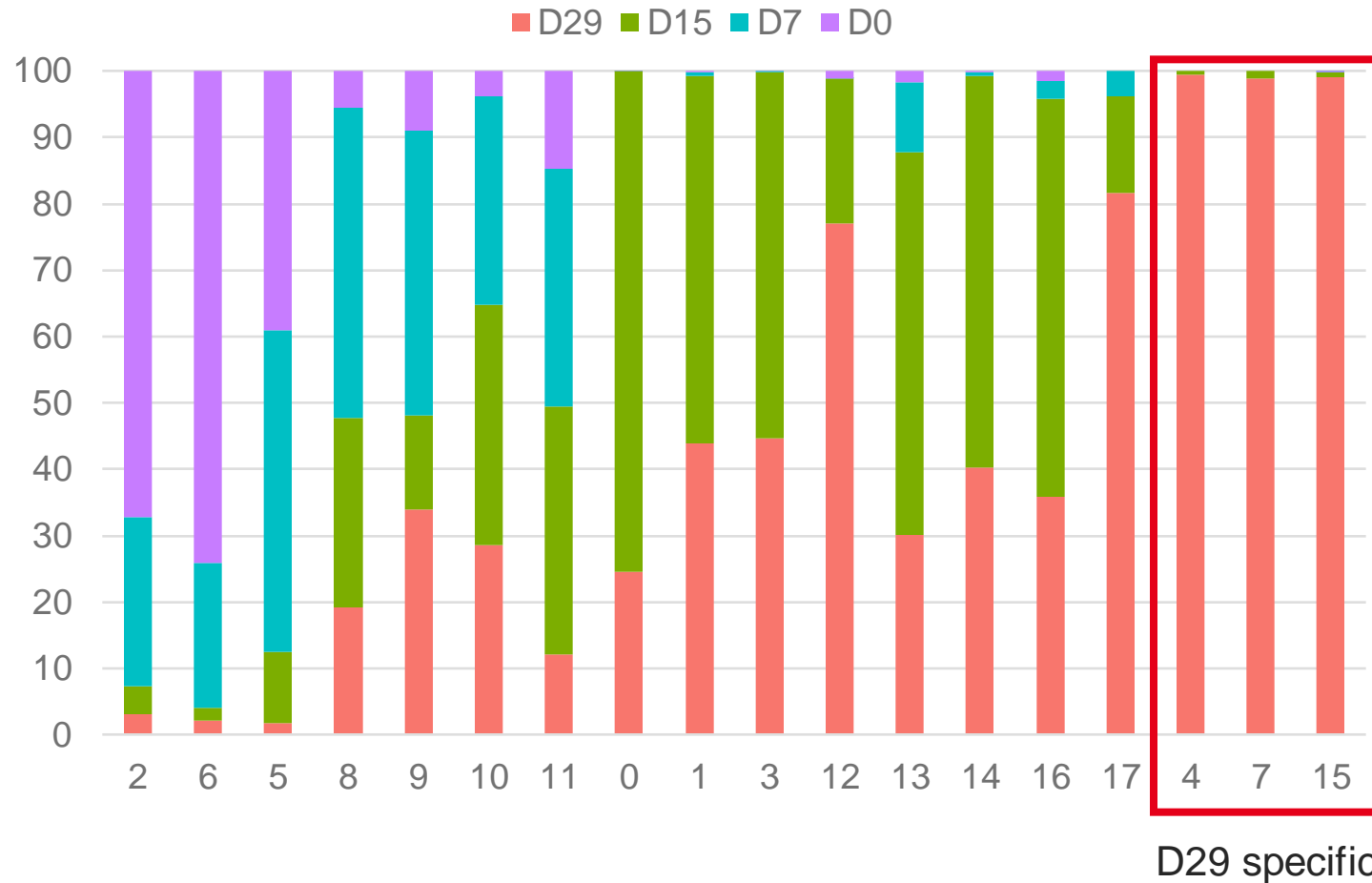
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Heterogeneity of cell proportion in clusters

- Distinguish clusters that are common to all the maturation days from those specific to one level with the percentage of cells belonging to each maturation day for every cluster.



➤ Observation of early and more advanced maturation stages.

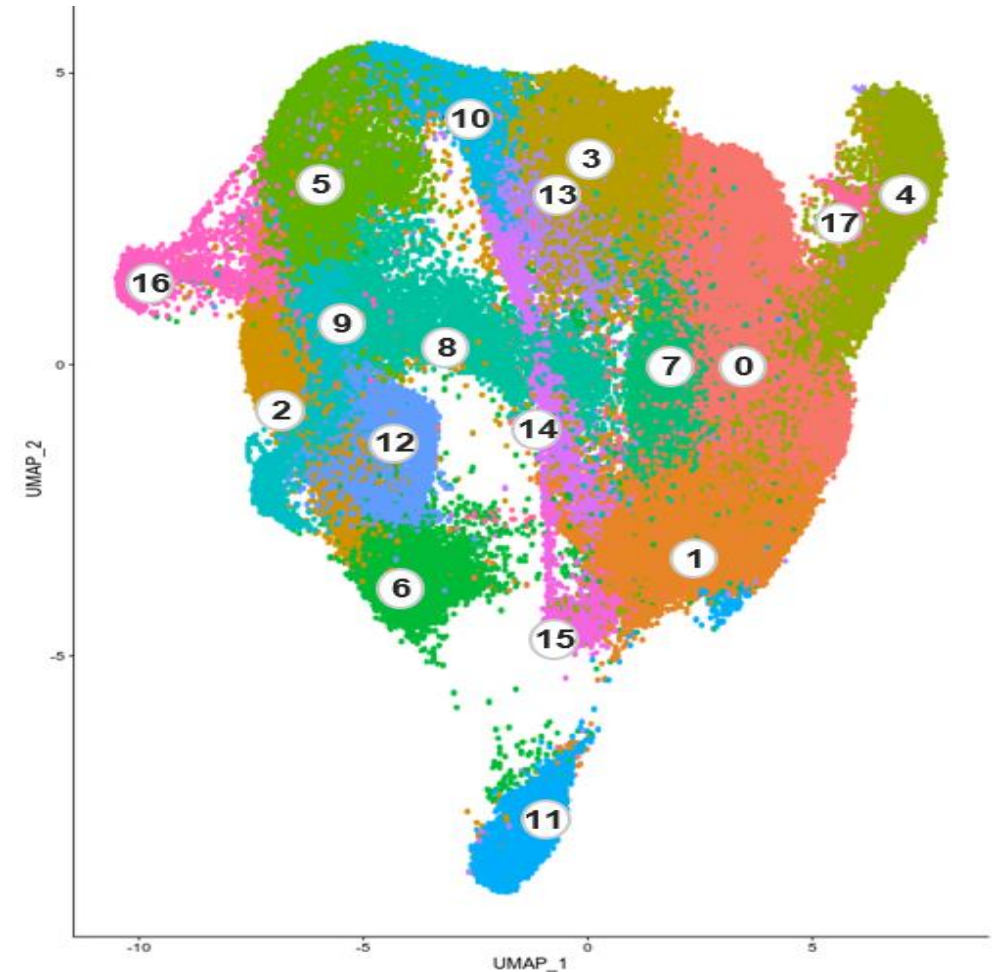
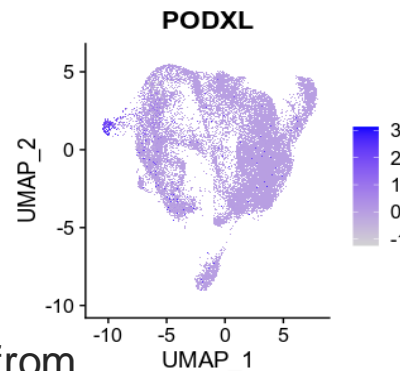
Cell labeling of clusters

Definition

- Assigning cell types to clusters identified by Seurat.
- Based on knowledge of the transcriptome specificity of cell populations.

Different methods used and aggregated

- Marker genes differentially expressed in clusters:
 - Known marker genes of cell populations (Schumacher *et al.*, 2021).
 - Human Protein Atlas.
 - marker gene expression in cell/clusters.
- Automated labeling with Seurat:
 - Comparison of a labelled reference with the query dataset.
 - References: D29 and adult kidney datasets from Subramanian *et al.* paper.



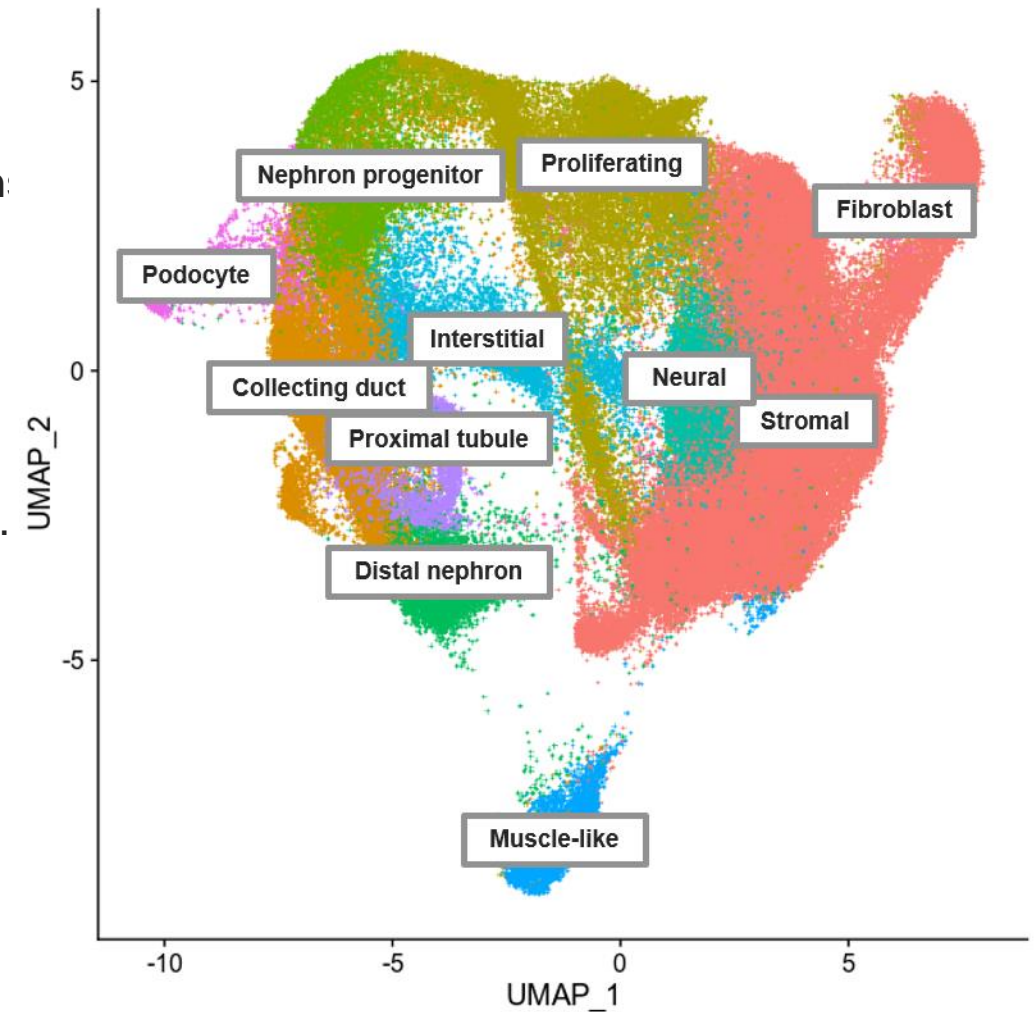
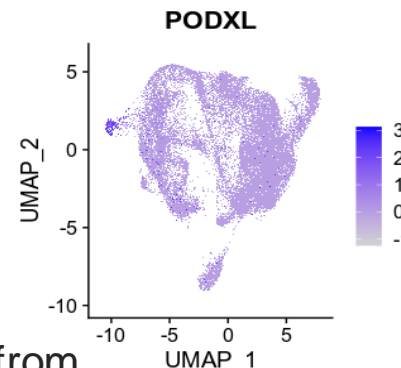
Cell labeling of clusters

Definition

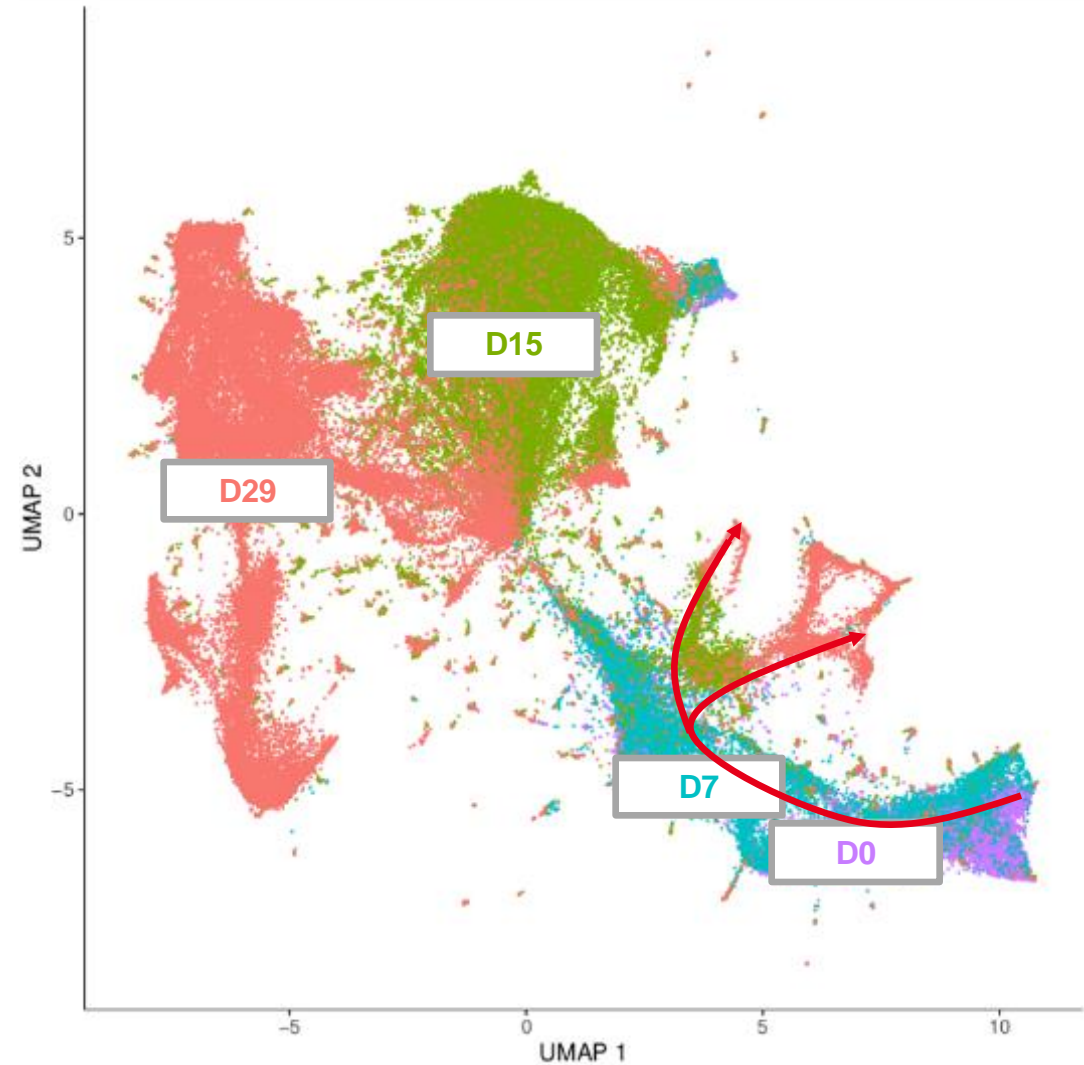
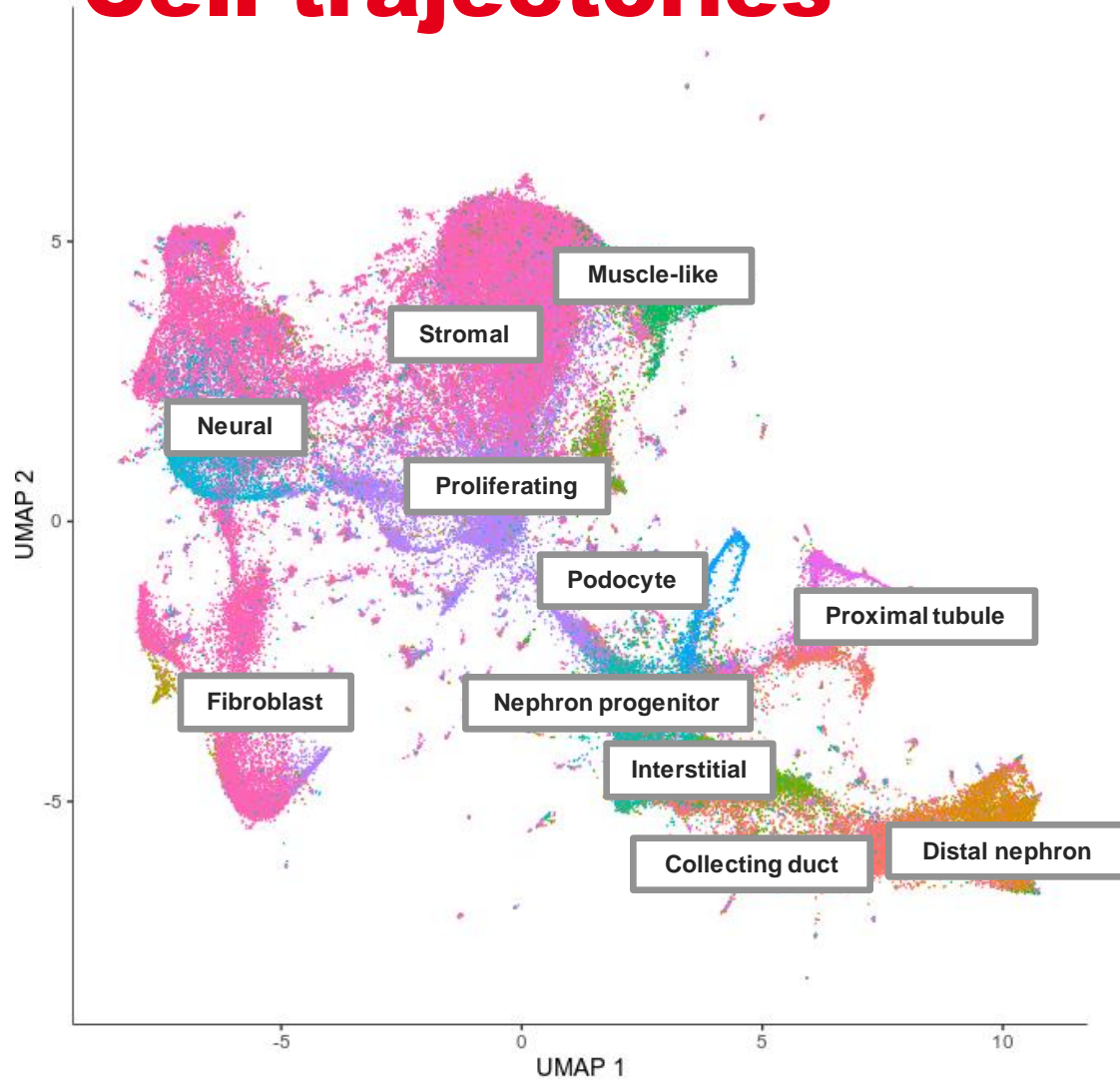
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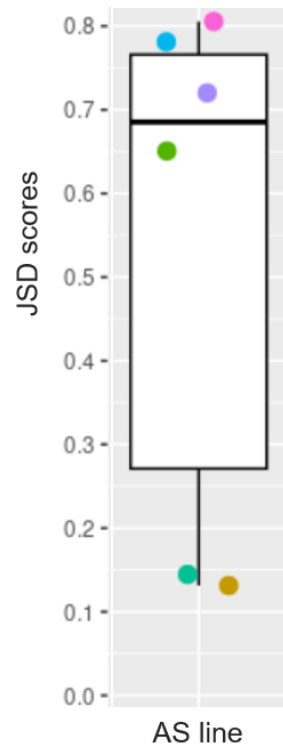
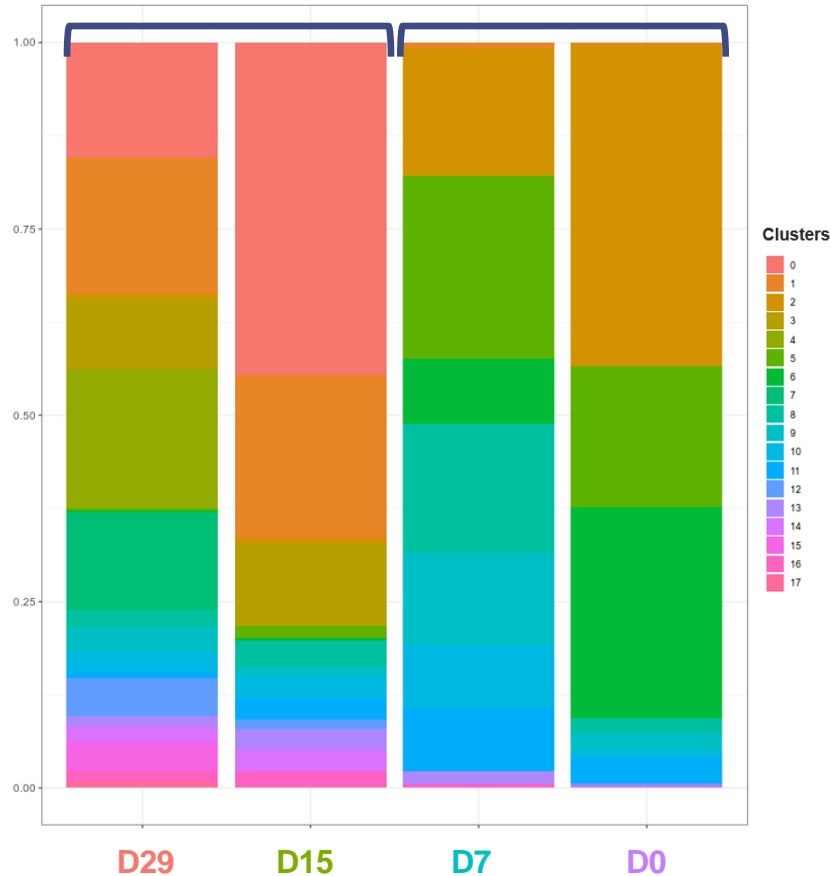
Cell trajectories



- The **D29** specific clusters are well separated.
- We also visualize the maturation and the cell differentiation from **D0** to **D29**.

Variability score between maturation ages

- The JSD (Jensen Shannon Divergence) was applied on AS integrated datasets of all the days of maturation (D0, D7, D15 and D29).
- It compared the proportion of cells in each cluster between 2 samples.



Maturation date (nb days)

- D0 vs D7
- D7 vs D15
- D15 vs D29
- D0 vs D15
- D7 vs D29
- D0 vs D29

➤ As observed previously D0vsD7 and D15vsD29 showed more similar classifications.

Validation of the method with a new dataset

A new organoid dataset

Combes et al. *Genome Medicine* (2019) 11:3
<https://doi.org/10.1186/s13073-019-0615-0>

Genome Medicine

RESEARCH

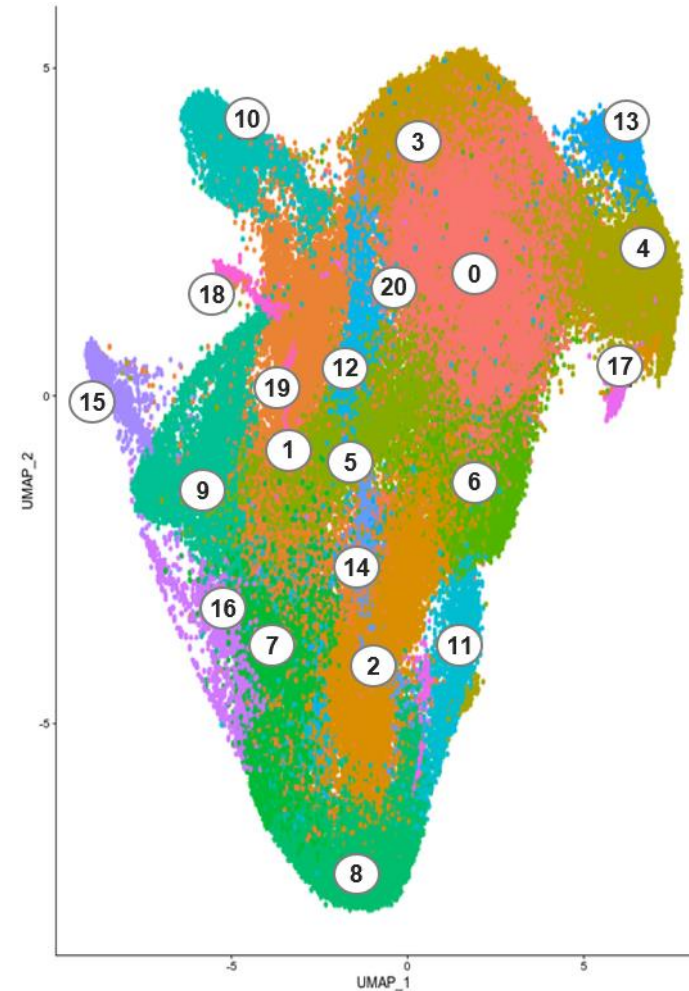
Open Access

Single-cell analysis reveals congruence between kidney organoids and human fetal kidney



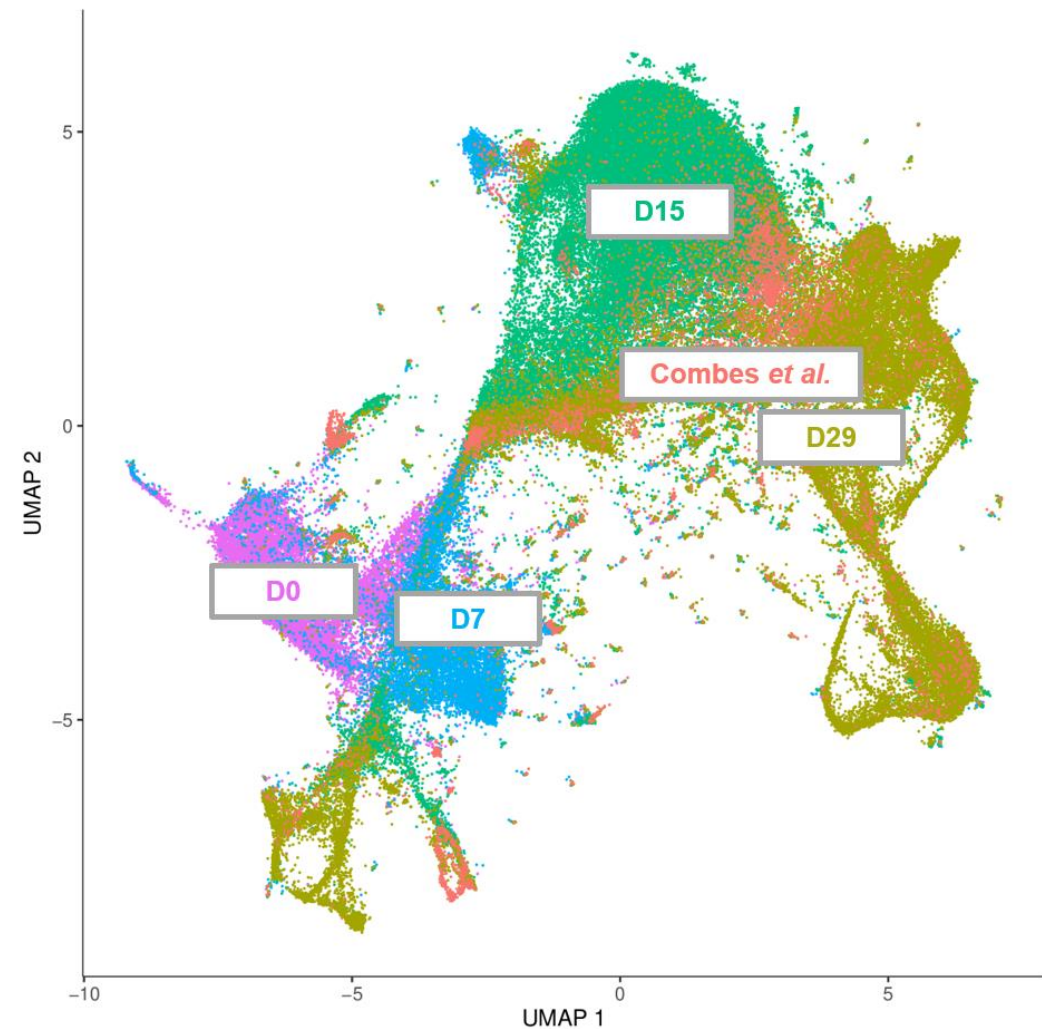
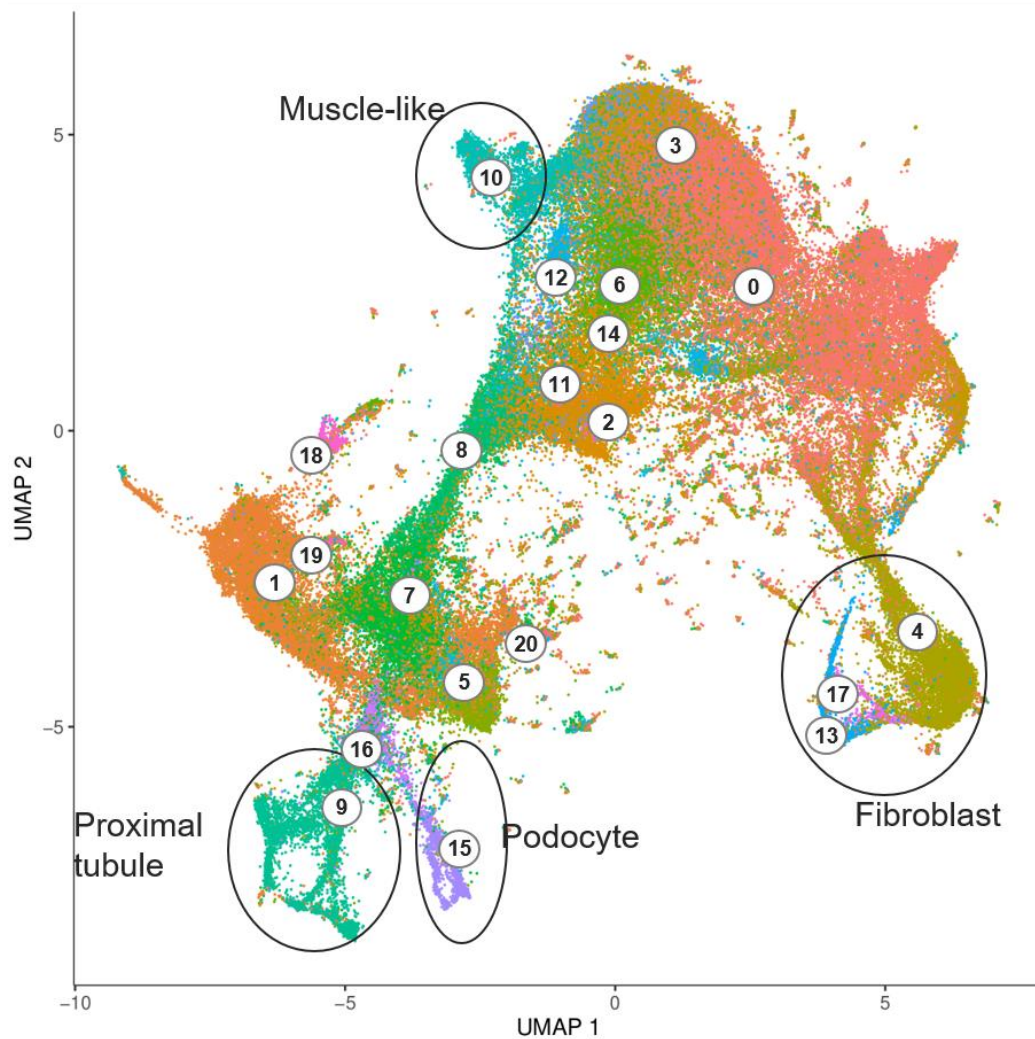
Alexander N. Combes^{1,2*†}, Luke Zappia^{2,3†}, Pei Xuan Er², Alicia Oshlack^{2,3} and Melissa H. Little^{1,2,3,4*}

- 3 single-cell RNA-seq datasets from the same organoid.
- 24 days of maturation.
- Same protocol (M. Little)



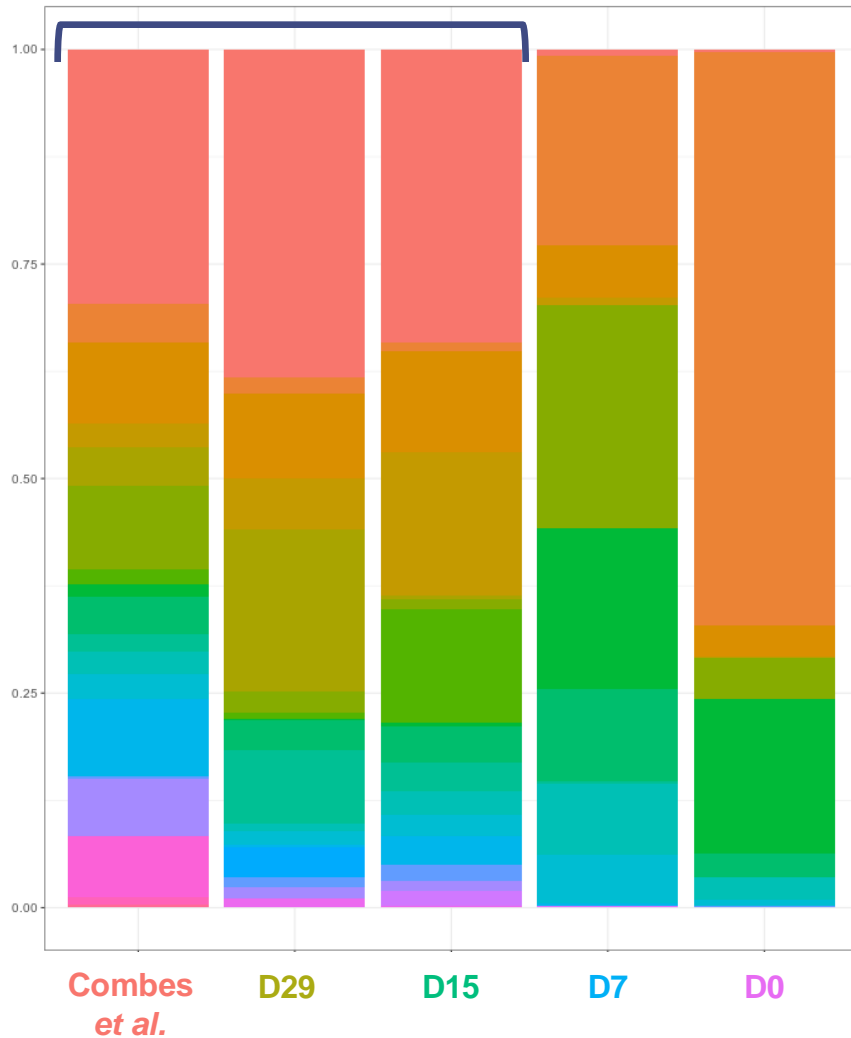
Validation of the method with a new dataset

- Comparison of clusters of Combes *et al.* with previous AS line organoids.



Validation of the method with a new dataset

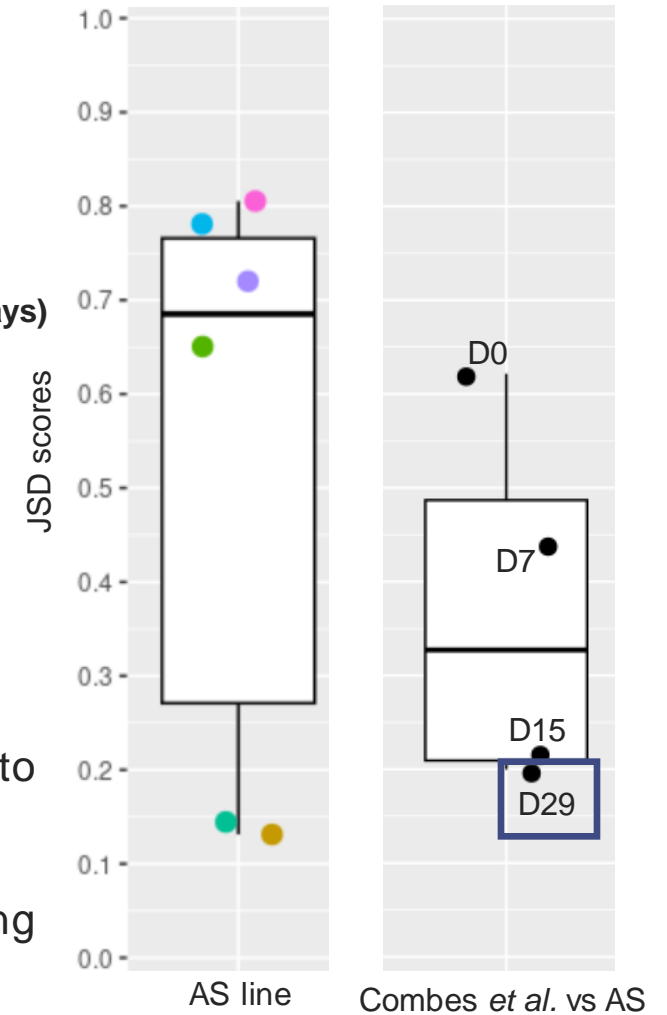
- Comparison of clusters of Combes *et al.* with previous AS line organoids.



Maturation date (nb days)

- D0 vs D7
- D7 vs D15
- D15 vs D29
- D0 vs D15
- D7 vs D29
- D0 vs D29

- Combes *et al.* organoid profile closer to D15 and D29 organoids of AS line.
- Consistent with the truth corresponding to a maturation at D24.



Main messages

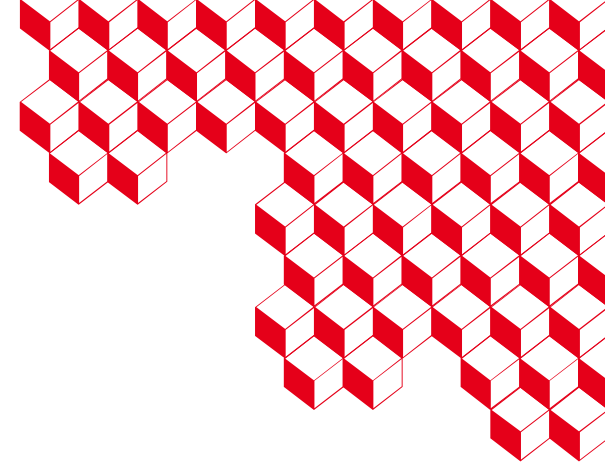
- Kidney organoids could be characterized at different maturation stages.
- The observation of 2 early and later maturation levels (D0-D7 and D15-D29) is consistent with Subramanian *et al.* future protocol improvements focused on this interval.
- The integration of another dataset validated the method with a correct estimation of the number of maturation days.

Perspectives...

- Apply the same method on organoids of different protocols and source cells to study the reproducibility.
- Test the method on organoids of another targeted organ (example: skin – Lee *et al.*, 2020, Nature)
- Use machine learning based approach to estimate the age of the organoid.



jacob



Acknowledgements

LBA:

Eric Bonnet

All the team!

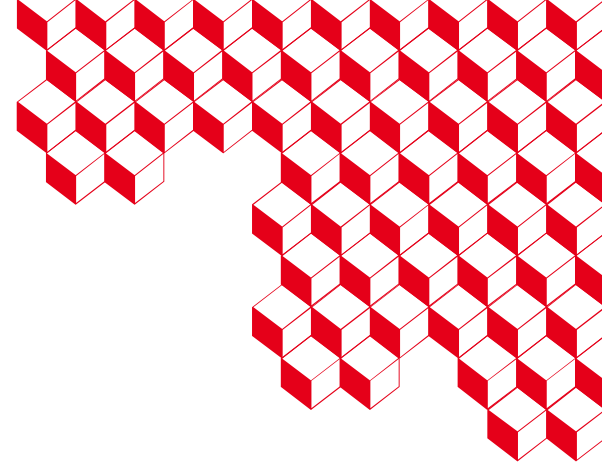
Alumni

Alexandre Hubert

Camille Lemerancier

Jean-François Deleuze





Thank you for your attention

Questions?