Technical and Translational SCRM Research Programs – October 8, 2021, 11:00 – 13:30, Zoom

Present:

- Amiq Gazdhar – lead
- Andreina Schoebberlein - lead
- Eliane J Müller - lead
- Volker Enzmann - lead
- Deborah Stroka - lead
- Pascal Escher
- Michele Bernasconi
- Marianna Kruithof-de Julio
- William Hariton (Eliane Müller)
- Geneviève Escher
- Niran Maharian (Smitha Saxena)
- Rao Tata Nageswara
- Patrizia Sauta (Eliane Müller)
- Siavash Rahmini (Eliane Müller)
- Andreas Croft (Ben Gantenbein)

Excused:

- Gabriela Baerlocher
- Carsten Riether (presenting next meeting)
- Benjamin Gantenbein - lead
- Olivier Guenat
- Tosso Leeb
- Stephen Leib
- Zeerleder Sacha
- Allam Ramanjaneyulu
- Thomas Marti
- Roberta Balansin Rigon (Kässmeyer Sabine)

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Please book **November 12 2021 13:00 – 18:00 SCRM Annual Meeting**, *Haus der Universität SCRM Research Program Launch (program attached)*

1. **Introduction**
   Recapitulation of the programs and goals – see Introduction, Program kick off meeting August 19 2021.

   Presentations will be shared on SCRM homepage (stemcellsbern.ch)

   Invitation is extended to attend the SCRM Annual Meeting, November 12, 2021, 13:00 – 18:00 (Haus der Universität) and contribute to discussion of future needs and to refine the program.

2. **Input in Cell storage**: Freezing, thawing, long-term storage & freezers
   Inputs (suggestion) and discussion – input presentations attached

   - **Volker Enzmann**
     - Precious cells can/should be stored in the liquid Biobank Inselspital (N²liq) (Appendix 1.1, Flyer)
     - Freezing media is defined by customer
     - Freezing is done by Biobank on a Askion Freezing Bench Freezing robot during working hours (until 16:00)
     - Retrieval is announced 24h in advance (in emergency cases 1h in advance) on working days.
     - Storage Price: 8 CHF / aliquot / year (very reasonable).
     - Contact: Tanja.Froehlich@insel.ch or biobank@insel.ch
     - Please fill out form in attachment (Appendix 1.2, Form)
William Hariton
Improved cryopreservation of primary human stem and progenitor cells from epidermis (keratinocytes) (Appendix 2):
  o Improved viability and proliferation of cells cryopreserved in commercial, fully defined freezing media (e.g. CELLnTEC or Stem Cell Technologies; see disclaimer)
  o Freezing: pre-cool and mix all components on ice
  o Thawing: No centrifugation, dilute sample 10x + medium change after 5 hrs
  o Long-term storage: at -150 °C or liqN2 not -80 °C

Benjamin Gantenbein / Adrian Croft
Short-term freezing of progenitor cells from human intervertebral disc (for one week) (Appendix 3)
  o Comparable viability and tri-lineage differentiation in conventional (20% FCS) and commercial freezing media

Marianna Kruithof-de Julio
Cryopreservation of tissue and cells applicable to derive organoids or establish PDX (Patient-derived Tumor Xenograft) models
  o Slow freezing ev in hydrogel
  o Protocol in attachment (Appendix 4)
Cryopreservation of Organoids
  o Viability drops by ~80% when entire organoids are frozen
  o Recommended to freeze single cells from dissociated organoids at P1 in N2liq
  o Freezing medium pre-warmed at 37°C: 40% Advanced F12/DMEM Basis Medium, 50% FCS, 10% DMSO freshly prepared

Deborah Stroka-Keogh
“Ressourcenausschuss” application 2021 via DBMR: VIA Freeze Uno Desktop (Appendix 5)
  o Portable freezing device, N2liq-free
  o According to supplier: enables controlled cooling, consistent quality
  o Pre-tests in the lab showed superior freezing, specifically when using the manual nucleation program (premature crystal formation)
  o Freezing medium used: Cryostor (StemCell Technologies) or 2% Trehalose (sugar from insect cocoons), 10% DMSO, 88% FCS.
  o Funding decision: around February 2022; costs ~40’000 CHF.

Call for instrument applications at the “Ressourcenausschuss” of the Medical Faculty goes to PI’s. Deadline for submission is usually in September of each year.

3. New input presentations from the list of 37 responders
 (~ 3 ppt slides: Aims Research, Technical Expertise, Needs)

A&B. Engineered cells & Tissue stem and specialised cells
Niran Maharjan (group Smitha Saxena)
(ALS: Amyotrophic Lateral Sclerosis (90% sporadic); SCA1: Spinocerebellar Ataxia type 1 (hereditary))

Tools and expertise in lab

**In vivo**

- Mouse model of ALS and SCA1
- Behavioral readouts
- Stereotaxic injections of AAV/compounds
- Biochemical studies

**In vitro**

- Immortal cell lines
- Slice culture of Spinal cord and Cerebellum (ex-vivo)
- Primary neuronal culture (Cortical neurons and Purkinje cells)
- Patient's derived iPSCs (ALS and SCA1)
- Differentiation of patient's derived iPSCs into Cortical neurons, Motor neurons and Cerebellar neurons
- Lentivirus
- Calcium imaging
- NMJ model
- Proximity ligation based proteomics (TurboID)

Needs:

- Common equipment (Ultracentrifuge in BSL2 lab)
- Bioinformatic support
- Core facility: they should be more affordable with bare minimum fees
- Common e-mailing list (spread news about bionews to group members)

Common equipment: Apply to the “Ressourcen Ausschuss” of the Medical Faculty (every year around September)

E-mailing list: all members of a group should be advised to join the bionews Network at UniBE (Network to ask for consumables and tools)

Subscribe here: [https://listserv.unibe.ch/mailman/listinfo/bionews](https://listserv.unibe.ch/mailman/listinfo/bionews)

Ensuing Collaborations:
Our Expertise

- Flow cytometry (FACS) of hematopoietic lineages
- Mouse and human HSC isolation culture, expansion and differentiation
- Bone marrow transplantations in mice
- Metabolic assays: Mitochondrial activity ex vivo, mitochondria quantification, glycolysis measurements, nutrient uptake, metabolic tracing
- RNA sequencing (bulk and single cell) and ATAC Seq analysis

Needs:

What we need and What we are looking for..

- More funding: Foundations and Industry
- Pre-clinical to Clinical
- Team up/Network -> Learn -> Enjoy applying

Ensuing collaborations:

- D. Stroka
- E. Müller: Expansion of HSC; mitochondrial activity etc
Aims of Research

- Transcriptional regulation of retinal development (photoreceptors)
- Disease mechanisms in inherited retinal diseases

Needs

What about metabolism? Translate from mouse to human?

Human Retinal Organoids

Ensuing collaborations:
➢ Rao Tata Nageswara?
4. General Needs - Conclusion

1. The opportunity to exchange and discuss (also in person) technical and translational know-how is considered a need of added value, summarized in the scheme by Prof G. Baerlocher

Our Needs in a Nutshell

Scientific Exchange
- e.g. topics based round tables

Teaching
- e.g. cellular therapy based advanced programs

Access to platforms/instruments
- e.g. website with contact details

Networking/Collaborations
- creation of complementary research/ project collaborations

2. Current specific technical needs identified / discussed (SCRM Research Program meetings August 19 and October 08 2021):

2.1 Services:
- Establishing primary cells from tissue is highly complex and requires long-standing experience which cannot be acquired in a simple work-shop. This expertise is present amongst our members (liver, skin, cornea, lung, prostate etc.). The need to provide cell culture as a service was identified, requires further discussions including regulatory considerations.
- Accordingly, access to a GMP room (A/B) is necessary (for cell and Extracellular Vesicle). To be discussed: establish a small GMP unity at sitem-insel.

2.2 Technical support:
- Lead scientists of SCRM Research Programs are available for direct inquiries from the network in their fields of expertise on a collaborative, non-fee basis.
- SCRM is affiliated and connects to facilities providing fee-based expertise and services (NGS platform, Organoid core, mass cytometry platform and organ-on-a-chip facility). Special conditions will be negotiated for SCRM platform members.
- Various techniques to freeze, thaw and cryopreserve primary cells in the long-term in a standardized manner is of primordial importance and was already successfully discussed (SCRM Research Programs Meeting October 08 2021). **Amongst others, SCRM supports their members in using the biobank of the Insel group. This topic will need continuous follow-up.**
- **Biostatistics** support is required.

2.3. Tissue samples:

Obtain **control and patients tissue samples** from Insel departments, key to our bio-medical research success. Hence, the connection to Insel departments is necessary, which is actively pursued by the SCRM lead.

2.4. Acquire costly Instruments:

- The SCRM research programs provide an optimal platform to identify and **collectively apply for costly instruments of common need** (e.g. devices for cryopreservation, ultracentrifuge under BSL2 conditions etc.) at the “Resourcenausschuss” of the Medical Faculty or the SNSF (R’equip or Infrastructure use).

2.4. **More Publications:**
Collaborations increase the number of publications per group (see DermFocus in Boris).

2.5 **More Funding:**
Joint publications prove collaborations allowing to apply for larger collaborative research grants from both Foundations and Industry.

3. Current specific **translational needs** identified / discussed (SCRM Research Programs Meeting August 19 and October 08 2021)

3.1 Collaborations with clinical departments for **clinical studies**:
- Connections to and collaborations with defined clinical departments are sought to pursue clinical trials for specific applications.

3.2. Collaboration with Industry
- **Clinical trials require Industry connections.** The SCRM platform has long-standing partner-ships with cell culture and biotech companies sponsoring our activities. Partner-ships may be extended towards co-sponsoring clinical trials.

3.3 Support for ethics applications:
- see 3.1. Clinical departments have vast experience in obtaining ethics approval which might be shared with SCRM Platform **members in exchange for research know-how.**

3.4 **Funding:**
- Collaborations with clinical departments are prerequisite to acquire funding for translation (e.g. SNSF: Longitudinal studies, Investigator-initiated clinical trials; Industry etc.).
Appendices

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SC&RM technology platforms

Volker Enzmann
Breites Dienstleistungsangebot
Zusätzlich zu Röhrchen und kontrollierter Lagerung umfassen die BBS eine Vielzahl von weiteren Dienstleistungen. Zum Beispiel stehen zwei mit Flüssigstickstoff gekühlte Workbenches von Askion zur Verfügung, welche kontrolliertes Einfrieren von Proben und ein Umsortieren von bestehenden Probensammlungen bei \(-100\,^\circ\text{C}\) ermöglichen.

Weitere Informationen
zum Angebot (z.B. Kostenvergleich und Dienstleistungen) finden Sie auf unserer Homepage:
zlm.insel.ch

Leitung: Prof. Dr. Carlo Largiadèr, PhD
Administration: Dr. Tanja Fröhlich, PhD
Tel.: 031 632 22 01
Fax.: 031 632 48 62
E-Mail: biobank@insel.ch
BioBank Services (BBS)

Pros

• Standardization
  ➢ Controlled environment
  ➢ Backup systems

• Automatization
  ➢ Entry of cells manually
  ➢ Automatic processing

• Own freezing medium

Cons

• Administration
  ➢ Pre-order necessary
  ➢ Access limited (Mon – Fri)

• Costs
  ➢ Fee for handling
  ➢ Monthly storage fee
Die **BBS** stellen Infrastruktur und Dienstleistungen rund um die Verarbeitung und Lagerung von flüssigen Bioproben (z.B. Blut, Serum, Blutzellen) zur Verfügung, um eine Humanforschungsgesetz (HFG)-konforme Aufbewahrung von Studienproben* am Universitäts- spital Bern zu gewährleisten.

**Probenlagerung nach höchsten Qualitätsstandards**

Für die Einlagerung von flüssigen Bioproben bietet die **BBS** zwei für bis zu –196 °C geeignete Röhrchen der Firma FluidX mit unterschiedlichen Füllvolumen an. Die Röhrchen entsprechen in ihren Eigenschaften den höchsten Qualitätsstandards von Kryoröhrchen.


Proben können **voll-automatisiert** bei zwei unterschiedlichen Temperaturen zeitlich unbeschränkt eingelagert werden.


Die **BBS** sind auch für externe Forschungsgruppen zugänglich und die Leistungen können über den gleichen Prozess wie andere Studienleistungen angefordert werden. Nähere Informationen zum Angebot sind auf der Homepage des ZLMs (zlm.insel.ch) zu finden.

**Welche Vorteile hat der Forschende?**

Die **BBS** bieten eine wesentlich kostengünstigere Möglichkeit zur Lagerung von klinischen Proben im Vergleich zu einer konventionellen Lagerung in einzelnen Gefrierschränken. Ausserdem garantieren sie eine HFG-konforme Lagerung und Verwaltung der Proben (HFG, Art. 43). Letztlich bietet die innovative Dienstleistung der **BBS** eine zukunftsweisende Lagerqualität und -sicherheit:

- Automatisierte Ein- und Auslagerung von Proben bei Lagertemperatur
- Gewährleistung der lückenlosen Wiederfindung aller Proben
- Lagerung bei kontrollierten Bedingungen
- Kein Verdunsten und Vereisen der Proben
- Dokumentierte Probenqualität (Lagerungstemperatur wird für jede Probe aufgezeichnet)
- Ausfallsicheres Lagersystem

**Füllmengen:** 525 µl und 270 µl

**Primary human keratinocytes**

William Hariton – Eliane J Müller; Mol Derm and Stem Cell Research, DBMR, Insel

**Disclaimer:** Prof Eliane J Müller is a founder and President of the Board of CELLnTEC, advanced cell systems AG
Primary human keratinocytes
William Hariton – Eliane J Müller; Mol Derm and Stem Cell Research, DBMR, Insel

Disclaimer: Prof Eliane J Müller is a founder and President of the Board of CELLnTEC, advanced cell systems AG
Effect of different Cryopreservation Media on Human Nucleus Pulposus Cells’ Viability and Trilineage Potential

Andreas S. Croft¹, Julien Guerrero¹, Katharina A. C. Oswald², Sonja Häckel², Christoph E. Albers², Benjamin Gantenbein¹,²

¹ Bone & Joint Program, Tissue Engineering for Orthopaedic & Mechno-Biology, Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland.
² Department of Orthopaedic Surgery & Traumatology, Inselspital, Bern University Hospital, University of Bern, Bern Switzerland.

INTRODUCTION

Over the past decade, progenitor cells capable of trilineage differentiation have been discovered within the nucleus pulposus (NP) of human intervertebral discs (IVDs).¹,²,³ These cells could have the potential to regenerate the IVD. Therefore, once isolated, a cost efficient method to store and preserve them has to be found.

However, in the context of cell therapy, little is known about the effect of cryopreservation and expansion on here called „heterogenic“ human NP cells (hNPCs).

STUDY AIMS

Find an optimal approach to cryopreserve hNPCs.

Expand hNPCs and to investigate their trilineage differentiation potential before and after cryopreservation.

MATERIALS AND METHODS

Cartilaginous and plate Nucleus pulposus Annulus fibrosus

Intervertebral disc

hNPC isolation and expansion

Two „commonly used“ vs. three commercially available cryopreservation media

Cryo-preservation for one week at -150°C

CELL VIABILITY AFTER CRYOPRESERVATION

Over the past decade, progenitor cells capable of trilineage differentiation have been discovered within the nucleus pulposus (NP) of human intervertebral discs (IVDs).¹,²,³ These cells could have the potential to regenerate the IVD. Therefore, once isolated, a cost efficient method to store and preserve them has to be found.

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CELL VIABILITY AFTER CRYOPRESERVATION

ADIPOGENESIS

Lipid droplets produced by hNPCs and stained with Oil Red O

CHONDROGENESIS

7 µm section of a hNPC pellet and stained with Alcian Blue

OSTEOGENESIS

Mineralized matrix of hNPCs and stained with Alizarin Red

LEGENDS

Fresh cells: Previously unfrozen cells
CMPC: Commonly applied cryo-medium for progenitor cells
CMDC: Commonly applied cryo-medium for differentiated cells
CnT: CellnTec Cryo-Defined Freezing Medium
CS10: cGMP-manufactured CryoStor® CS10
MesenCult: MesenCult™ -ACF Freezing Medium

REFERENCES


This project is funded by the Swiss National Science Foundation #310030E_192674/1 and by the Horizon2020 Project „iPSpine“ #825925.

We want to thank our lab technician Andrea Oberli for technical support.

ACKNOWLEDGEMENTS

Contact: andreas.croft@dbmr.unibe.ch
S.O.P. Tissue Cryopreservation

**Reagents:**

- 10 x 50mL tubes with 30mL medium or 10 x 15mL tubes with 7mL medium and label appropriately (e.g. for biopsy/tissue collection)
- Medium: DMEM+Glutamax +4.5g/L glucose, no Pyruvate (Gibco, 61965-026) containing 10% FBS and primocin 1x (InvivoGen)
- Cryo solution DMSO free (#CPL-A1, Lubio Science) or 90%FBS/10% DMSO

**Procedure:**

1. Prepare cryovials, label and add 1ml freshly prepared cryopreservation media 90%FBS-10%DMSO solution
2. Transfer tissue in Petri dish containing 5 ml of media. Quickly transfer the piece of tissue to be cut on top of the lid of the Petri. Cut tissue in approx. 100um size as equally as possible using scalpel/blade or scissors (clean cuts, minimize disruption of tissue architecture). Transfer back into the dish with media to keep tissue in contact with media at all times.
3. Include 3 small tissue pieces/cryo vial. Make sure they are submerged in media.
4. Store at -80°C in Mr. frosty box for at least 48-72 hours
5. For long term storage transfer vials to liquid N₂
Freezing Organoids / single cells

Notes:
Typically, split 1 well of a 24-well plate into 2-4 cryovials.

Prepare:
Cryovials
15 mL Falcon tube
P1000 and P200 pipettman
Freezing medium: 40% Advanced F12/DMEM Basis Medium, 50% FCS, 10%MgSO freshly prepared, prewarmed at 37C (aliquots are at min20C, C808drawer)
Cell freezing chamber with isopropanol

Procedure:
1. Follow the steps for harvesting organoids above and dissociate
2. If planning to passage and freeze organoids, divide resuspended organoids into separate 15 mL Falcon tubes, one containing organoids to be split and one containing organoids to be frozen.
3. Spin organoids at 1100 rpm (300g) for 3-5 minutes at RT.
4. Carefully aspirate off as much media as possible.
Get as much media as possible with aspirator and then carefully remove the rest with a P200 Pipetman.
5. Resuspend organoids in 1ml Freezing Medium per intended cryovial, and pipette organoids into cryovials.
6. Move cryovials into isopropanol cell freezing chamber and screw lid onto top.
7. Incubate freezing chamber with cells at -80oC for at least 24 hours before transferring cryovials to liquid nitrogen storage.

Thawing Organoids

Notes:
It is important to have Rho Kinase Inhibitor in the Media when organoids are thawed, to help cells cope with the stress of thawing.

You will need:
24- or 48- well low attachment culture plate
37oC water bath
15 mL Falcon tube on at 37C with Rho Kinase Inhibitor
15 mL Falcon tube on at 37C with Basis medium
P1000 and P200 Pipetman

Procedure:
1. Place Media at room temperature or in a 37oC water bath.
3. Prepare a 15 mL Falcon Tube with 10 mL Splitting Media and place on ice.
4. Remove cryovial containing organoids from liquid nitrogen freezer and thaw quickly in a 37oC water bath.
5. Pour thawed cells into 15 mL Falcon Tube with Splitting Media.
6. Spin organoids at 1100 rpm for 3 minutes at RT.
7. Carefully aspirate off as much media as possible.
Get as much media as possible with aspirator and then carefully remove the rest with a P200 Pipetman.
8. Resuspend organoid pellet in media as indicated above.
9. Depending on how quickly the organoids grow, change media 2-4 days after thawing. Once organoids have formed and begun to grow, Rho Kinase Inhibitor is no longer needed.
CyTOF

Mass cytometry by time-of-flight is a single cell proteomics technology. It empowers researchers to reveal the expression of up to 50 protein targets on each individual cell. Cells are stained with antibodies bound to metal probes and run in suspension, much like with a flow cytometer.

Primary Human Hepatocytes

50 g piece of tissue
>500M hepatocytes
gold standard for drug testing and metabolic studies
VIA freeze-Duo

STERLING ENGINE DRIVEN CONTROLLED RATE FREEZING

- Extremely precise control over freezing
- Liquid nitrogen free
- Portable, bench top device
- 96 x 2ml vials
- Manual nucleation of samples possible (-8 to -9°C)

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Primary Human Hepatocytes

frozen 3 hours post isolation in DMEM- 10% FCS, 10% DMSO - 0.2M Trehalose

Hepatocytes
72% cell viability

2 days post recovery
Neurodegenerative disease
Laboratory
(Saxena Lab)

Prof. Smita Saxena
Niran Maharjan
Aim of Lab (research focus)

Amyotrophic Lateral Sclerosis (ALS)

- More than 20 genes has been linked with ALS
- 10% Familial while 90% Sporadic
- SOD1: 15% of fALS cases, 1% of sALS
- C9ORF72: 33% of fALS, 5% of sALS

Spinocerebellar Ataxia type 1 (SCA1)

- Expansion of CAG repeat in ataxin1 gene resulting in polyQ repeat expansion
- Hereditary

- Understanding the pathomechanisms that drive neurodegeneration.
- Selective vulnerability
- Seeding and spreading of misfolded proteins
Tools and expertise in lab

**In vivo**
- Mouse model of ALS and SCA1
- Behavioral readouts
- Stereotaxic injections of AAV/ compounds
- Biochemical studies
- Expertise in working with Endoplasmic reticulum Stress (ER stress), misfolded proteins and its proteome
- Neuronal circuits and Electrophysiological recordings
- Chemogenetics and optogenetics
- Imaging and image analysis

**In vitro**
- Immortal cell lines
- Slice culture of Spinal cord and Cerebellum (ex-vivo)
- Primary neuronal culture (Cortical neurons and Purkinje cells)
- Patient’s derived iPSCs (ALS and SCA1)
- Differentiation of patient’s derived iPSCs into Cortical neurons, Motor neurons and Cerebellar neurons
- Lentivirus
- Calcium imaging
- NMJ model
- Proximity ligation based proteomics (TurboID)
Needs

• Common equipment (Ultracentrifuge in BSL2 lab)
• Bioinformatic support
• Core facility
• Common e-mailing list (spread news about bionews to group members)
Stem Cells, Leukemia, Aging and Metabolism (SLAM) Laboratory

Targeting Leukemic Stem Cells

Dr. Tata Nageswara Rao, PhD

https://www.dbmr.unibe.ch/research/research_programs/rao_tata_lab/index_eng.html
Elucidating Fundamental Properties of Healthy and Leukemic Stem Cells
Elucidating and Leveraging Specificities and Dependencies of Healthy and Leukemic Stem Cells

👉 Current therapies are NOT curative and NOT specific !!

👉 Target dependencies that are private to leukemic stem cells
Leveraging Metabolic Alterations to Identify Potential Therapeutic Targets and Biomarkers of Leukemia

WT  JAK2-V617F  JAK2-Ex12

n= 8  8  8

MPN Cohort

normal  PV  ET  PMF

n=24  30  5  16  9  24  12

Mass Spectrometry

Metabolite quantification
Biomarker identification
Functional validation

Driver mutation
Our Expertise

- Flow cytometry (FACS) of hematopoietic lineages
- Mouse and human HSC isolation culture, expansion and differentiation
- Bone marrow transplantations in mice
- Metabolic assays: Mitochondrial activity ex vivo, mitochondria quantification, glycolysis measurements, nutrient uptake, metabolic tracing
- RNA sequencing (bulk and single cell) and ATAC Seq analysis

- Umbilical cord blood
- Adult
- FACS
- HSC isolation and culture
- HSC differentiation (CFU-C)
- Bone marrow transplantation
- FACS sorting
- Mitotracker green
- LC-MS
- metabolites metabolic tracing
- differential gene expression and mutant allele burden
- HiSeq
- RNA sequencing
- ATAC-Seq on single cells
What we need and What we are looking for..

👉 More funding: Foundations and Industry
👉 Pre-clinical to Clinical

Team up/Network -> Learn -> Enjoy applying
Aims of Research
- Transcriptional regulation of retinal development (photoreceptors)
- Disease mechanisms in inherited retinal diseases

IHC 5 µm paraffin
in situ hybridization cryosections
retinal flatmounts

Fundus photography

H&E paraffin
cryosections

electroretinography

Gene expression analysis
Needs

What about metabolism? Translate from mouse to human?

Eiraku et al, 2011, Nature