Fedor Kryukov

Sample pre-processing for high-throughput “omics”

Brokerage Event 2016
Regenerative Medicine and Bioinformatics

www.fno.cz
R&D launch pad

BioBanking Ref Lab → Sample Pre-Processing Ref Lab → Data Processing Ref Lab

Hospital → Lab → Lab → Lab → Lab → Hospital

Researcher → Lab → Hospital

DEPARTMENT OF HEMATOONCOLOGY
What is Sample Pre-Processing?

- material preparation for high-throughput “omics”

What do we do?

- target cell sorting + DNA/RNA isolation

What is the purpose?

- essential for all downstream application in clinical studies as well as in basic research
Cell sorting

Our laboratory specializing in **magnetic** separation and **fluorescence-activated** cell sorting

Sample → DNA/RNA

Sample → Cells #1 → DNA/RNA #1

Sample → Cells #2 → DNA/RNA #2
Magnetic Activated Cell Sorting (MACS)

- Cell suspension
- Negative fraction
- Positive fraction
Magnetic Activated Cell Sorting (MACS)

AutoMACS Pro
(Miltenyi Biotec)

• 1 marker only
• Easy-to-use
• Cheap & Fast
• Purity limitation
Fluorescence-Activated Cell Sorting (FACS)

- development of flow cytometry
- enables cell-by-cell sorting into two or more fractions
- utilizing the scatter and fluorescence signals of each cell
Fluorescence-Activated Cell Sorting (FACS)

FACS Aria III
(Becton Dickinson)

- up to 8 markers
- aseptic sorting
- single-cell sorting
- up to 4 cell populations at once
- sorting on the tubes, slides or plates
- Expensive & User-Unfriendly
Nucleic acid isolation

<table>
<thead>
<tr>
<th>PCR</th>
<th>aCGH</th>
<th>WES</th>
<th>WGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 ng</td>
<td>200 ng</td>
<td>&gt; 25 ng*</td>
<td>&gt; 25 ng*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>qRT-PCR</th>
<th>GEP</th>
<th>WTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ng</td>
<td>0.5 ng</td>
<td>&gt; 25 ng*</td>
</tr>
</tbody>
</table>

* the lowest required amount of input material for NGS if using NeoPrep system for library preparation. Amount of input material may be higher for other library preparation protocols.
Tested kits

**DNA isolation kits:**
- AllPrep DNA/RNA Micro Kit (Qiagen)
- QIAamp DNA Mini Kit (Qiagen)

**RNA isolation kits:**
- AllPrep DNA/RNA Micro Kit (Qiagen)
- RNeasy Micro Kit (Qiagen)
- MagMAX Total RNA Isolation Kit (Life Technologies)
- μMACS mRNA Isolation Kit (MiltenyiBiotec)

**Nucleic acid amplification kits:**
- REPLI-g Mini Kit (Qiagen)
- REPLI-g Single Cell Kit (Qiagen)
- REPLI-g Cell WGA & WTA Kit (Qiagen)
Scheme of pre-processing of samples with small amount of input material

- AllPrep DNA/RNA Micro Kit (Qiagen)
- ≥10⁵ cells
- nucleic acids
  - 200 ng DNA
    - PCR
    - aCGH
    - WES
  - 50 ng RNA
    - qRT-PCR
    - GEP
    - WTS

DEPARTMENT OF HEMATOONCOLOGY
Scheme of pre-processing of samples with small amount of input material

**AllPrep DNA/RNA Micro Kit (Qiagen)**
- 2.2-20 ng DNA
- 0.5-2.5 ng RNA
- amplification
  - qRT-PCR
  - GEP

**REPLI-g Single Cell Kit (Qiagen)**
- ~3500 ng aDNA
- amplification
  - PCR
  - aCGH
  - NGS

**10^3 – 10^4 cells**
- nucleic acids
- cells

DEPARTMENT OF HEMATOONCOLOGY
The proposed lab guideline for sample processing was prepared to present the optimal way of cells collection and proper handling in order to maximize sample quality for further use.

Isolated nucleic acids are intended for use in NGS, aCGH, GEP or PCR.

This guideline can be used for standardized research in the field of molecular biology in the wide range of haematological malignancies.
Central lab for academic clinical trials

- AC – 004 – EU – AL amyloidosis, new diagnosis
- ECWM-1 – M. Waldenström, new diagnosis
- EMN02/HOVON – Multiple myeloma, new diagnosis
- EMN11/HO114 – Multiple myeloma, relapse
# Acknowledgement

**Team for MDS and MPN**
- prof. MUDr. Elena Tóthová, CSc.
- MUDr. Lukáš Stejskal
- MUDr. Cecília Bodzássová
- MUDr. Zuzana Kučerová
- MUDr. Petra Richterová

**Team for trombosis and haemostasia**
- prim. MUDr. Jaromír Gumulec
- prim. MUDr. Milan Matuška, Ph.D.

**Research team**
- MUDr. Fedor Kryukov, Ph.D.
- MUDr. Elena Kryukova, Ph.D.
- Mgr. Tereza Ševčiková, Ph.D.
- Mgr. Zuzana Kufová
- Mgr. Jana Filipová
- Mgr. Katerina Grovkova

**Laboratory team**
- Mgr. Jana Smejkalová, Ph.D.
- Mgr. Lucie Adamusová
- Mgr. Lukáš Grebeníček
- Bc. Petra Vrublová
- Mgr. Hana Svěchová

**ALA and WM team**
- prof. MUDr. Roman Hájek, CSc.
- MUDr. Lenka Zahradová, Ph.D.
- MUDr. Hana Plonková
- MUDr. Tomáš Jelínek
- MUDr. Michal Kaščák

**Myeloma team**
- prof. MUDr. Roman Hájek, CSc.
- MUDr. Lenka Zahradová, Ph.D.
- MUDr. Hana Plonková
- MUDr. Tomáš Jelínek

**CLL team**
- prof. MUDr. Roman Hájek, CSc.
- MUDr. Jana Zuchnická

**Team for ALL/AML**
- MUDr. Zdeněk Kořístek, Ph.D.
- MUDr. Petra Richterová

**Lymphoma team**
- MUDr. Juraj Ďuraš
- MUDr. Milan Navrátil
- MUDr. Michal Kaščák

**Team of nurses leaded by:**
- Mgr. Kateřiny Hašové
- Kateřiny Horákové
- Kateřina Výběrová
- Petra Ramikové

**Study nurses leaded by:**
- Mgr. Martiny Januškové

**Co-operative team:**
- Mgr. Jiri Jarkovsky, Ph.D.
- Mgr. Lucie Brozova

---

DEPARTMENT OF HEMATOONCOLOGY
Thank you for your attention