

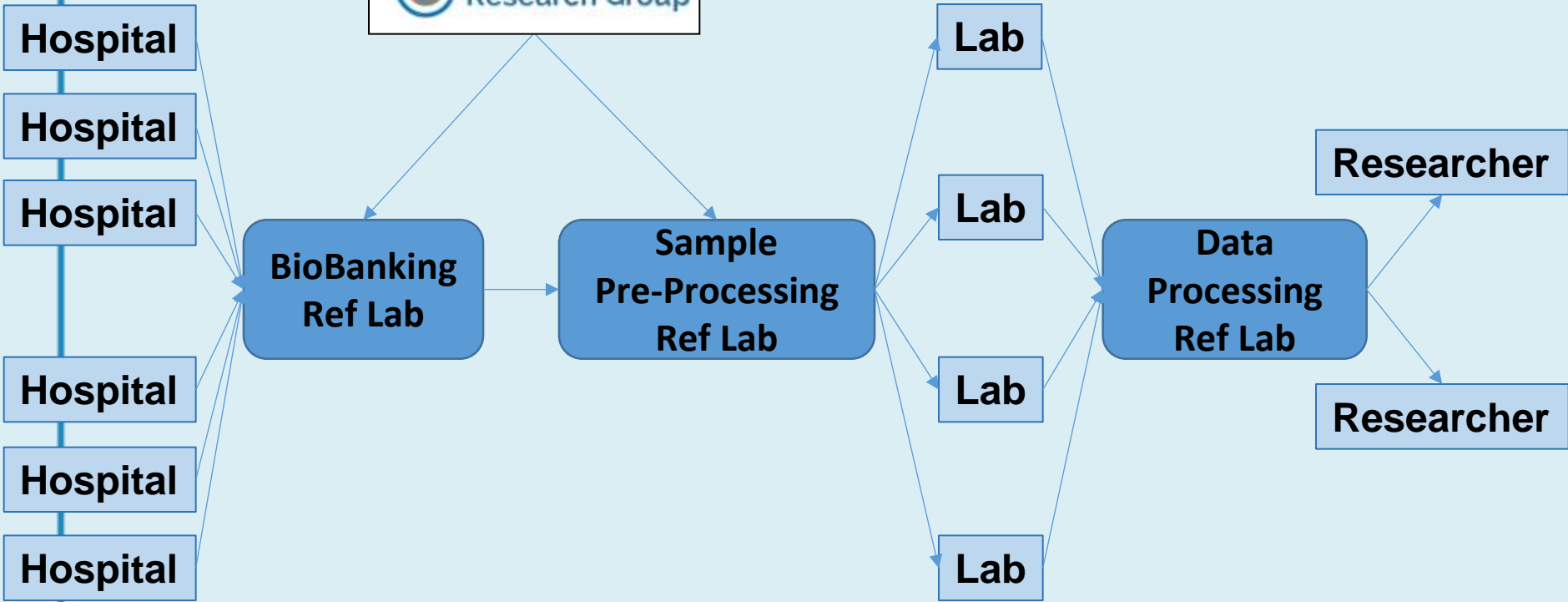


Sample pre-processing for high-throughput “omics”



Brokerage Event 2016
Regenerative
Medicine and
Bioinformatics

R&D launch pad



What is Sample Pre-Processing?



material preparation for high-throughput “omics”



What do we do?



target cell sorting + DNA/RNA isolation



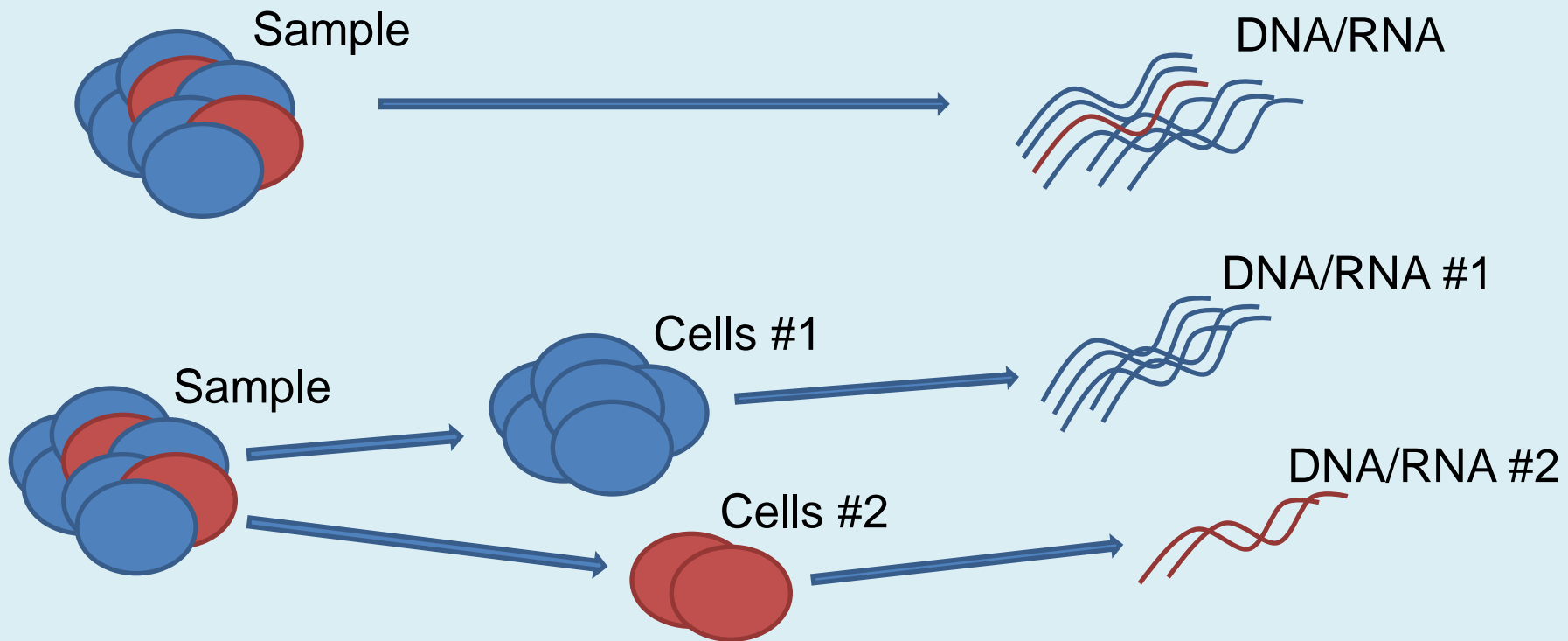
What is the purpose?



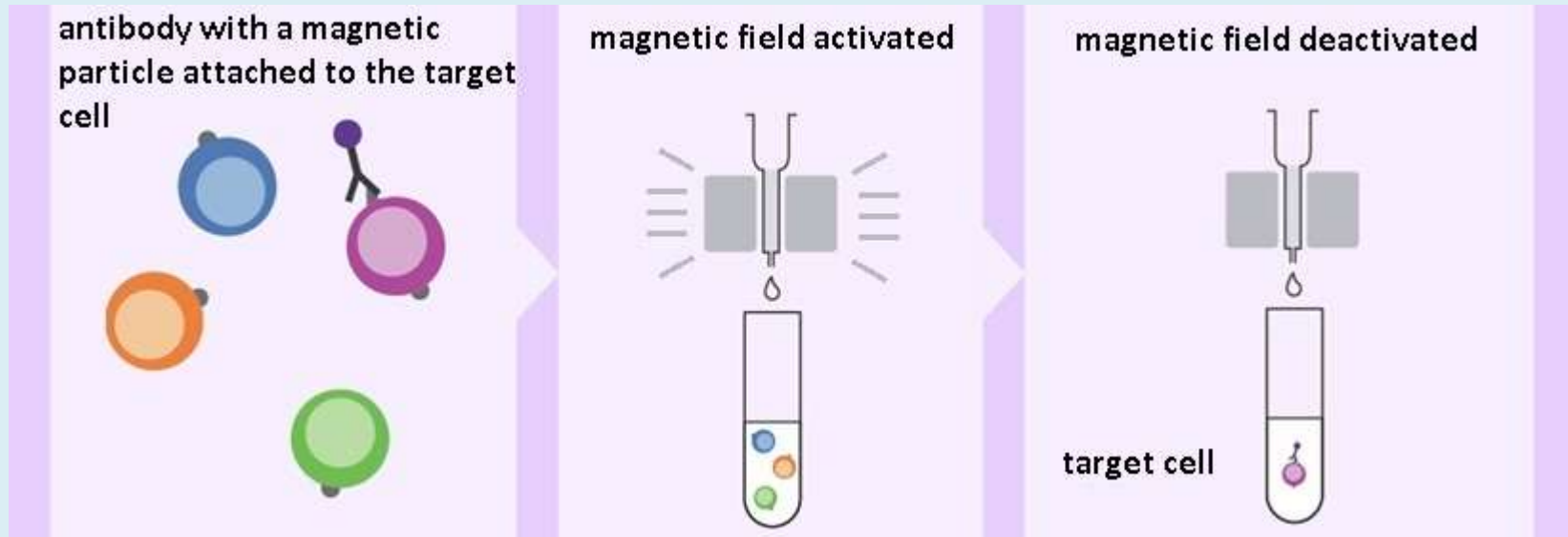
essential for all downstream application in clinical studies as well as in basic research

Cell sorting

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



Magnetic Activated Cell Sorting (MACS)



Cell suspension

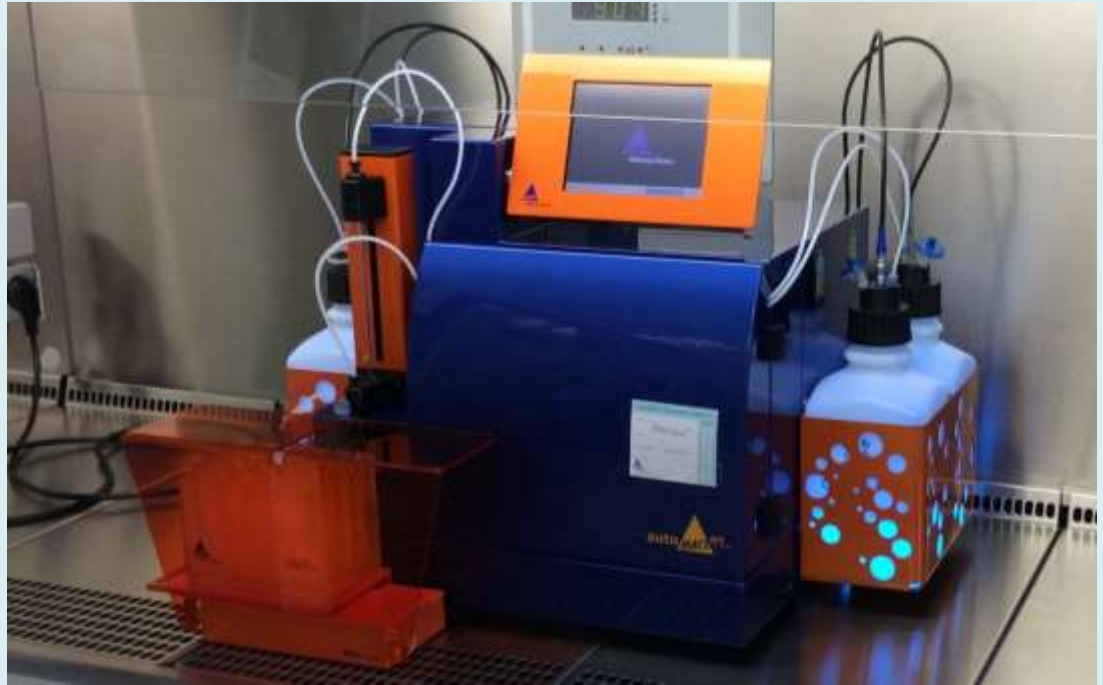
Negative fraction

Positive fraction

Magnetic Activated Cell Sorting (MACS)

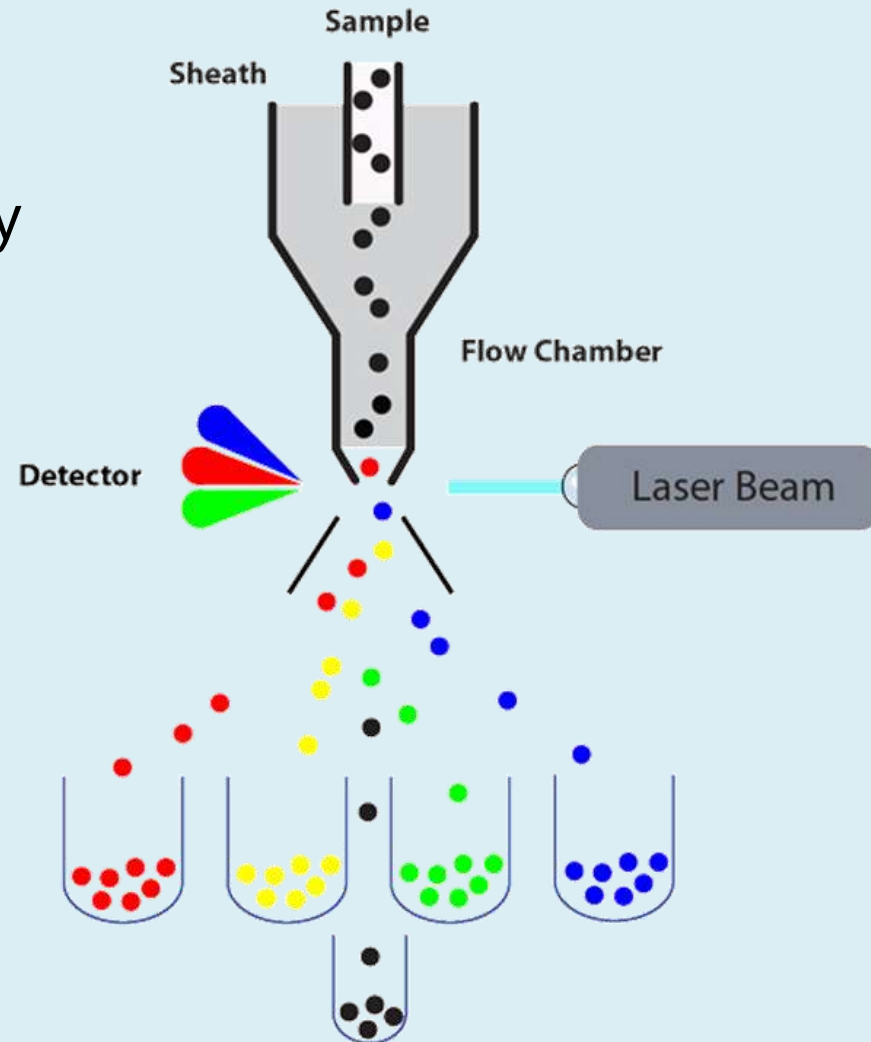
AutoMACS Pro
(Miltenyi Biotec)

- **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

DNA



PCR	aCGH	WES	WGS
20 ng	200 ng	> 25 ng*	> 25 ng*

RNA



qRT-PCR	GEP	WTS
0.1 ng	0.5 ng	> 25ng*

* 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)

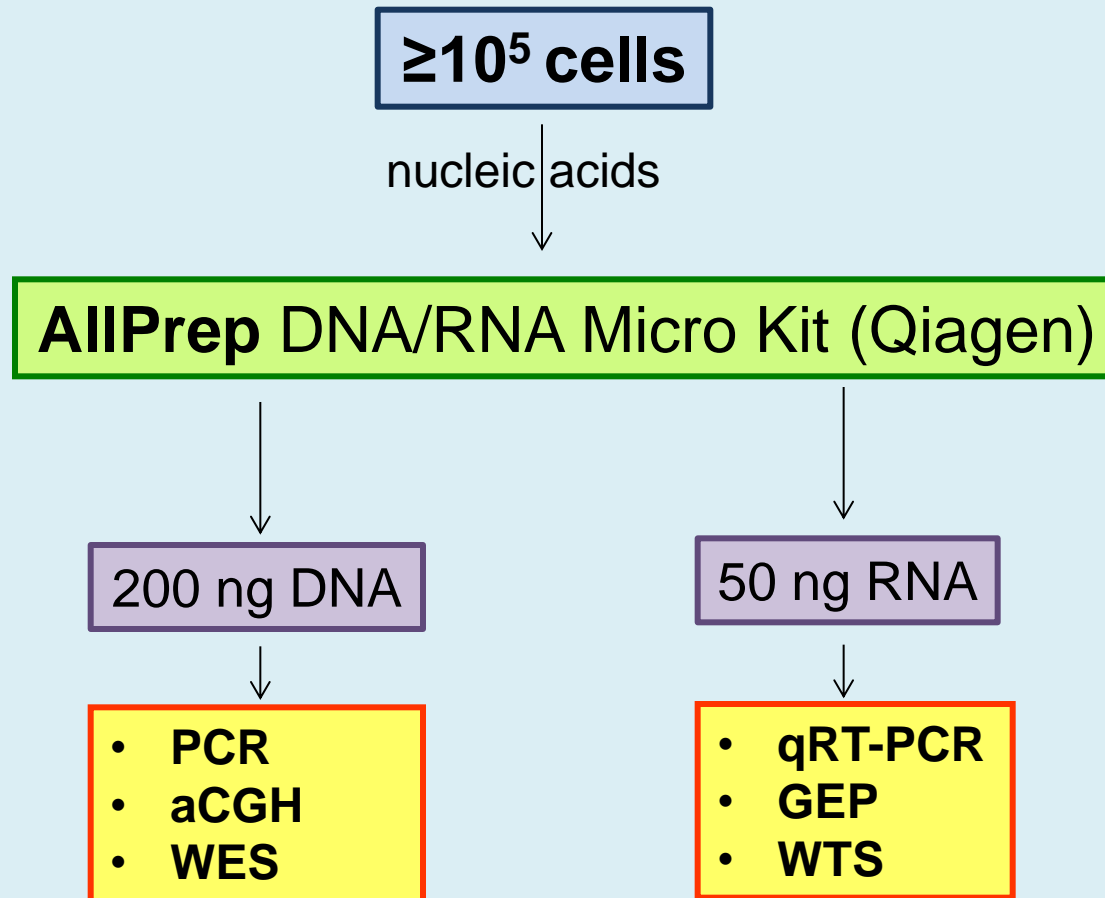
RNA isolation kits:

- AllPrep DNA/RNA Micro Kit (Qiagen)
- RNeasy Micro Kit (Qiagen)
- MagMAX Total RNA Isolation Kit (Life Technologies)
- μ MACS mRNA Isolation Kit (MiltenyiBiotec)

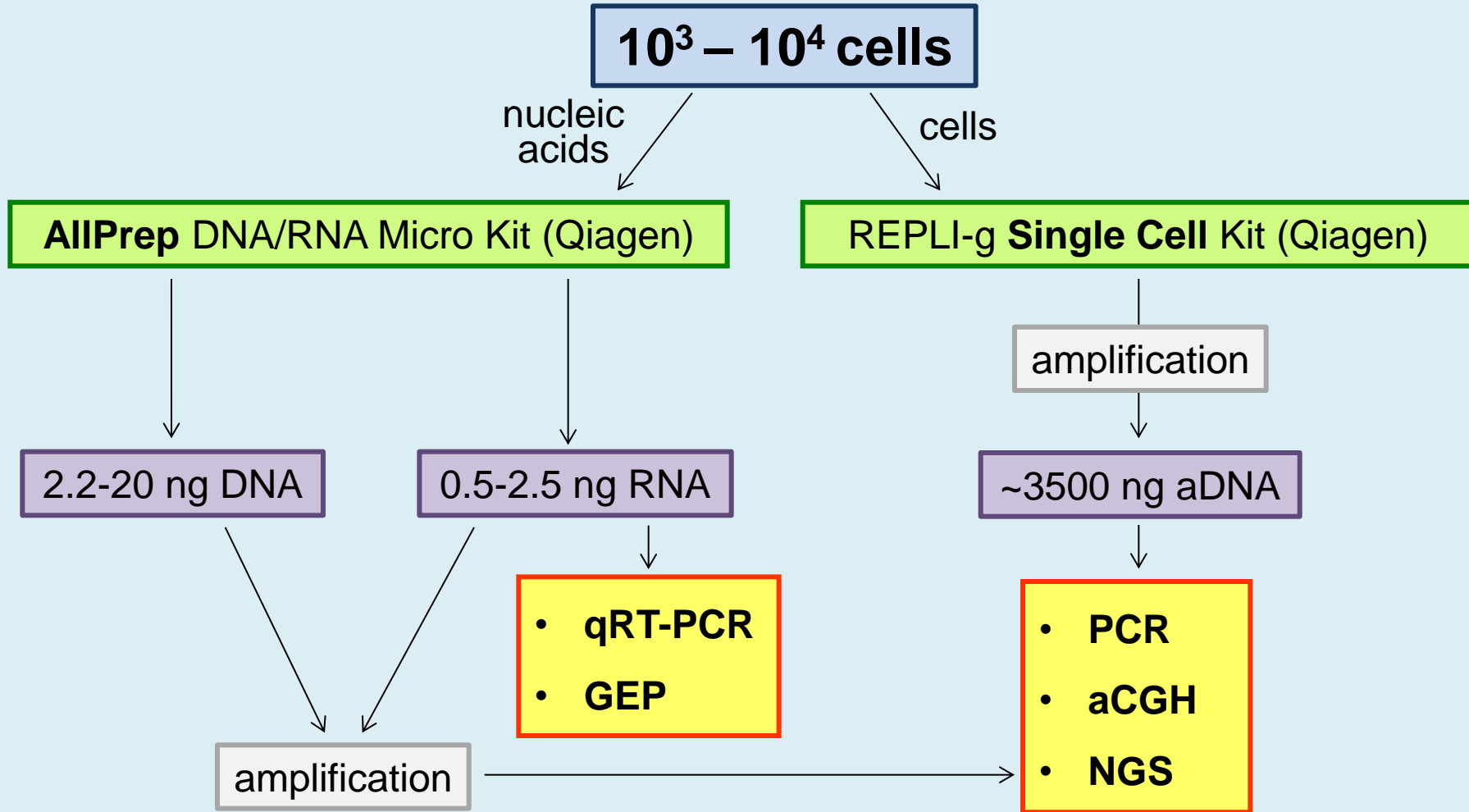
Nucleic acid amplification kits:

- REPLI-g Mini Kit (Qiagen)
- REPLI-g Single Cell Kit (Qiagen)
- REPLI-g Cell WGA & WTA Kit (Qiagen)

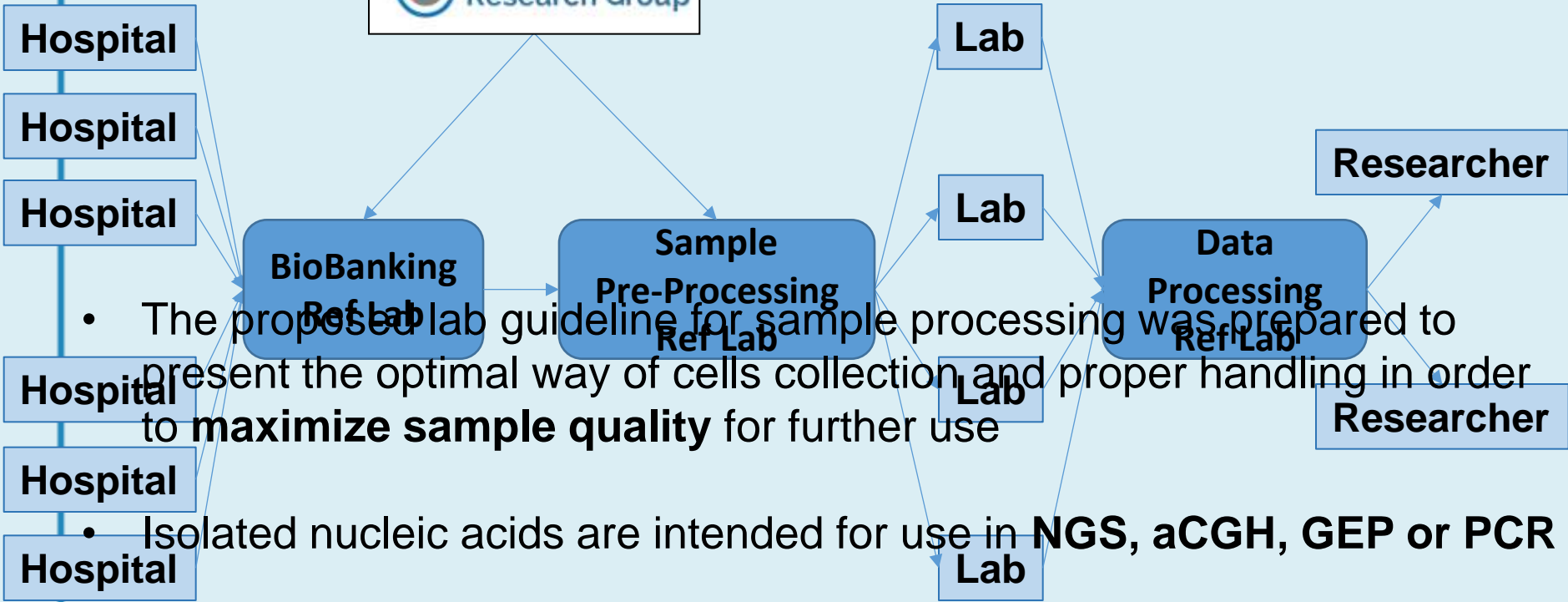
Scheme of pre-processing of samples with small amount of input material



Scheme of pre-processing of samples with small amount of input material



R&D launch pad



- 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

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**Thank you for your
attention**