Capacities/Research Potential
FP7-REGPOT-2012-2013-1

Project No. 316254

BASTION

"From Basic to Translational Research in Oncology"

Deliverable D4.1

Report on purchase, instalment and use of research equipment

Project start date:	1.09.2012
Project duration:	42 M
Due date of deliverable:	28.02.2014
Actual submission date:	26.02.2014
Dissemination level:	PUBLIC

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Introduction

Deliverable D4.1 corresponds to the tasks T4.1 – T4.6 in WP4, that were delivered in time. This deliverable contains report on acquisition and deployment of new research equipment. It should be emphasized that the cutting-edge research equipment purchased within these tasks has filled a significant gap in the research armamentarium of our teams. It now allows us to develop and expand the research methodologies and undertake research topics that were beyond our reach. Sharing of the purchased research equipment between our groups has already increased the level of cooperation and will allow us to undertake and carry out research projects at the highest European level.

Table 1 - Summary of the purchased equipment

No	Task	Equipment	Agreement no:	Date of signing the agreement	Date of Installment	Date of Payment	Estimated Net Price/* (EUR)
1	T4.1	Fluidigm Access Array 2AX + FC1 System	AEZ/365/S-321/12/249/2013	June 06th , 2013	July 02nd, 2013	July 30th, 2013	73 842,66
2	T4.2.1	Beckman Coulter ultracentrifuge Optima L100XPN	AEZ/365/S-321/12/127/2013	March 18th, 2013	April 26th, 2013	June 10th , 2013	84 707,84
3	T4.2.2	Miltenyi Biotec gentleMACS Dissociator	AEZ/365/S-321/12/128/2013	March 18th, 2013	March 20th, 2013	April 04th , 2013	7 904,32
4	T4.2.3	Hielscher Ultrasonics UP200ht handheld ultrasonic homogenizer	AEZ/365/S-321/12/129/2013	March 18th, 2013	April 17th, 2013	May 05th, 2013	6 589,99
5	T4.2.4	GE Healthcare preparative chromatography system AKTA avant 25	AEZ/365/S-321/21/130/2013	March 18th, 2013	May 14th, 2013	June 20th, 2013	121 899,82
6	T4.2.5	Andreas Hettich laboratory centrifuge ROTINA 420R	AEZ/365/S-321/12/131/2013	March 18th, 2013	April 15th, 2013	May15th, 2013	6 707,39
7	T4.3.1	Perkin Elmer Janus Integrator automated workstation	AEZ/365/S-208/564/2013	November 25th, 2013	February 04th, 2014	February 26th, 2014	194 126,64
8	T4.3.2	Perkin Elmer Delfia platewash					
9	T4.3.3	Perkin Elmer multilabel microplate reader EnVision 2104					
10	T4.4.1	Roche MagNA Pure 96 System	AEZ/365/S-023/250/2013	June 04th, 2013	June 11th, 2013	July 24th, 2013	172 311,35
11	T4.4.2	Roche LightCycler 96 System					
12	T4.4.3	Roche LightCycler 480 II System					
13	T4.4.4	Eppendorf Centrifuge 5430R					
14	T5.5	PALM Laser Microdissector	AEZ/365/S-023/251/2013	May 20th, 2013	September 5-9th, 2013	October 18th, 2013	166 102,18
15	T4.6	Life Technologies Ion Proton System	AEZ/365/S-321/12/132/2013	March 5th, 2013	March 28th, 2013	May 20th, 2013	169 958,29
						TOTAL	**1 004 150,48**

/ - exact costs will be presented in the I[st] Period Report and Form C (April 2014)*

1. Task 4.1 - Acquisition and deployment of a microfluidic chip Access Array

Task Leader: Dr. Rafal Ploski

The system includes 2 IFC Controllers AX (Access Array 48.48) and a single stand-alone FC1 Thermal Cycler. One controller is used in the pre PCR stage and the other at the post PCR stage of analysis.

Technical specification:
A system based on microfluidic chip that allows amplifying 48 specific amplicons from 48 unique samples, in effect preparing 48 libraries in a few hours. Each sample can be differentially barcoded and tagged at the amplification step, allowing for multiplexing at the sequencing step and completely eliminating the need for traditional library preparation which takes few days.

Date of the tender announcement:	March 19th, 2013
Date of the tender announcement decision (outcome):	April 29th, 2013
Date of signing the agreement no	June 06th , 2013
Remaining offers:	there were no other offers
Date of installation:	July 02nd, 2013
Date of user training:	July 03rd, 2013

Trained persons:

No	Name	Department
1	Konrad Szymański	Department of Medical Genetics MUW
2	Prof. Rafał Płoski	Department of Medical Genetics MUW

Equipment use:
The instrument is currently being used in several projects on aimed at identification of genetic variants of various genes on the course of human diseases. These studies are funded from Polish National Research Centre (NCN) to Konrad Szymański, Department of Medical Genetics, MUW. Moreover, other BASTION groups have started to use microfluidic chip Access Array in their studies on the role of genetic variants of tumor-progression associated genes in the development of susceptibility or resistance to anticancer therapies.

Access Array 2AX + FC1 System used by Konrad Szymański at Department of Medical Genetics MUW. **Photo 1**: (on the left): Pre-PCR controller unit, **Photo 2:** (on the top): Post-PCR controller unit and amplification unit

2. Task 4.2 - Acquisition of a protein purification work station

Task Leader: Dr. Dominika Nowis

Protein purification station consists of the following pieces of research equipment:

- ultracentrifuge
- instrument for automated tissue dissociation and homogenization
- ultrasonic homogenizer for fluids sonication
- preparative high-pressure liquid chromatography system
- laboratory centrifuge

The major purpose of the protein purification work station is to allow purification as well as biochemical and functional analyses of recombinant proteins. Recombinant proteins are indispensable to study protein function, interactions of proteins, and proteins with drugs and other small molecule ligands. The multi-component purification work station also serves to functionally characterize proteins, protein complexes, and protein-ligand interactions analysis. Ultracentrifuge will also be applied for applications other than lysate clarification in the protein purification process, like viral particle concentration, sub-cellular compartment isolation and protein interaction analysis. The flexibility of the system guarantees for high-quality, reproducible results and enables expanding our research to mechanistic and biochemical studies.

2.1. Acquisition of an ultracentrifuge

Equipment name: Beckman Coulter **ultracentrifuge Optima L100XPN** with the set of rotors and centrifuge tubes and bottles purchased by MUW in Comesa Ltd. company

Technical specification:

Parameter	**Requirement(s)**
max g force	≥ 800 000 x g
max speed	≥ 100 000 rpm
temperature control	from 0^0C to +40^0C with ±1^0C accuracy
controlled vaccum	required
swing – out rotor	made of titanium, g force min 160 000 x g, speed min 30 000 rpm, capacity 6 x 30 ml
swing – out rotor	made of titanium, g force min 400 000 x g, speed min 56 000 rpm, capacity min 6 x 3 ml
fixed angle rotor	made of titanium, g force min 400 000 x g, speed min 60 000 rpm, capacity 8 x 30 ml

Date of the tender announcement:	December 22nd, 2012
Date of the tender announcement decision (outcome):	February 6th, 2013
Date of signing the agreement:	March 18th, 2013
Remaining offers:	there were no other offers
Date of installation:	April 26th, 2013
Date of user training:	April 26th, 2013

Trained persons:

No	Name	Department
1	Dr. Dominika Nowis	Department of Immunology, MUW
2	Dr. Malgorzata Firczuk	Department of Immunology, MUW
3	Dr. Malgorzata Bajor	Department of Immunology, MUW
4	Dr. Malgorzata Czystowska	Department of Immunology, MUW
5	Dr. Beata Pyrzynska	Department of Immunology, MUW
6	Zofia Pilch	Department of Immunology, MUW
7	Katarzyna Roszczenko	Department of Immunology, MUW

Equipment use:
The instrument is being extensively used by many research groups in numerous projects implemented in the Department of Immunology, MUW aimed at the discovery of novel targets and their modulators for antitumor therapy. In particular, ultracentrifuge is extensively used for isolation of exosomes (microvesicles) from the following sources:
1. supernatants of tumor cell lines
2. serum and ascites of ovarian cancer patients.
The pre-filtered supernatants or body fluids are first centrifuged at 10 000 x g to remove larger vesicles (e.g. apoptotic bodies) and then the exosomes are pelleted at 110 000 x g. The ultracentrifuge is also used to prepare exosome-free fraction which is needed for the exosome experiments.
Moreover microcentrifuge is used for the pelleting of fine particulate cellular fractions, including cellular organelles such as mitochondria, microsomes, ribosomes as well as viruses used for transduction of tumor cells. Also gradient separations are used for the isolation of cellular subfractions.

Photo 3: Beckman Coulter ultracentrifuge Optima L100XPN installed in the Department of Immunology, MUW

2.2. Acquisition of an instrument for automated tissue dissociation and homogenization

Equipment name: Miltenyi Biotec **gentleMACS Dissociator** with the set of dissection tubes purchased by MUW from MEDianus Ltd. company

Technical specification:

Parameter	Requirement(s)
homogenization method	mild, guaranteeing obtaining of a single cell suspension of living cells, highly reproducible
speed	from 200 rpm to 4000 rpm
tubes	containing build-in rotors for sample dissociation in a closed, sterile system

Date of the tender announcement: December 22nd, 2012
Date of the tender announcement decision (outcome): February 6th, 2013
Date of signing the agreement: March 18th, 2013
Remaining offers: there were no other offers
Date of installation: March 20th, 2013
Date of user training: March 20th, 2013

Trained persons:

No	Name	Department
1	Dr. Tomasz Rygiel	Department of Immunology, MUW
2	Zofia Pilch	Department of Immunology, MUW
3	Katarzyna Roszczenko	Department of Immunology, MUW

Equipment use:

The instrument is being extensively used in numerous projects implemented in the Department of Immunology, MUW aimed at discovering novel targets and their modulators for antitumor therapy. In particular, for:

- dissociation of mouse organs (spleen and lungs) with the aim to obtain single cell suspensions of the viable cells for subsequent culture, adoptive transfer and/or flow cytometry analyses
- dissociation of the mouse syngeneic tumors (EMT6, CT26, B16F10, B78) with the aim to obtain single cell suspensions of the viable cells for subsequent culture and/or flow cytometry analysis.

Photo 4: Miltenyi Biotec gentleMACS Dissociator installed in the Department of Immunology, MUW being used by Dr. Tomasz Rygiel

2.3. Acquisition of an instrument for automated tissue dissociation and homogenization

Equipment name: **Hielscher Ultrasonics UP200ht handheld ultrasonic homogenizer** purchased by MUW in Labindex Ltd. company

Technical specification:

Parameter	**Requirement(s)**
weight	≤ 2000 g
power	≥ 200 W
generator frequency	≥ 25 kHz
regulated amplitude, pressure, time and temperature	required
touch screen display	≥ 2,5" in diameter
sonotrodes	1. 12 – 15 mm of dimeter 2. 6 – 8 mm of diameter
sound-proof cover	required

Date of the tender announcement: December 22nd, 2012
Date of the tender announcement decision (outcome): February 6th, 2013
Date of signing the agreement: March 18th, 2013
Remaining offers: there were no other offers
Date of installation: April 17th, 2013
Date of user training: April 17th, 2013

Trained persons:

No	**Name**	**Department**
1	Dr. Malgorzata Firczuk	Department of Immunology, MUW
2	Dr. Dominika Nowis	Department of Immunology, MUW

Equipment use

The instrument is being extensively used in numerous projects implemented in the Department of Immunology, MUW aimed at discovering novel targets and their modulators for antitumor therapy. Typical applications for this powerful ultrasonic device are:

1. tissue homogenization and disintegration,
2. dispersing of biological material,
3. emulsification,
4. cell disruption,
5. fluid degassing or
6. removing of air bubbles from buffers intended for liquid chromatography
7. preparation of bacterial cell lysates.

Photo 5: Hielscher Ultrasonics UP200ht handheld ultrasonic homogenizer installed in the Department of Immunology, MUW

2.4. Acquisition of a preparative chromatography system

Equipment name: GE Healthcare preparative chromatography system **AKTA avant 25** with the set of columns for HPLC purchased by MUW in LKB Biotech Violetta Kochmanska i Marek Welnicki Ltd. company

Technical specification:

Parameter	**Requirement(s)**
multiple sample injection	required
multiple wavelength detection	required
tubing i.d. [flow path]	0.75 mm
max operating pressure	≥ 20 MPa
flow cell path length	2 mm
UV wavelength	190-700 nm
buffer selection	required
flow rate	0.001 ml/min-25 ml/min
high salt, acid and alkaline proof	required
conductivity, pressure and pH detector	required

Date of the tender announcement: December 22nd, 2012
Date of the tender announcement decision (outcome): February 6th, 2013
Date of signing the agreement: March 18th, 2013
Remaining offers: there were no other offers
Date of installation: May 14th, 2013
Date of user training: May 14th, 2013

Trained persons:

No	**Name**	**Department**
1	Dr. Malgorzata Firczuk	Department of Immunology, MUW
2	Dr. Dominika Nowis	Department of Immunology, MUW
3	Prof. Jakub Golab	Department of Immunology, MUW
4	Agnieszka Zagozdzon	Department of Immunology, MUW
5	Dr. Malgorzata Bajor	Department of Immunology, MUW
6	Dr. Beata Pyrzynska	Department of Immunology, MUW
7	Katarzyna Roszczenko	Department of Immunology, MUW
8	Dr. Joanna Drzewinska	Department of Immunology, MUW
9	Dr. Malgorzata Czystowska	Department of Immunology, MUW

Equipment use:
The instrument is being extensively used in numerous projects implemented in the Department of Immunology, MUW aimed at discovering novel targets and their modulators for antitumor therapy. In particular, the equipment was used for the following applications:

1. purification of extracellular domain of human CD38 containing 6-histidine tag on HisTrap FF crude and HL Superdex 75 chromatography columns,
2. purification of 6 histidine-tagged human peroxiredoxin-1 on HisTrap FF crude and HL Superdex 75 chromatography columns,

3. purification of human protein disulfide isomerase on HL Superdex 200 chromatography column,
4. purification of Ig-Fc-conjugated CD200R on HiTrap Protein G column,
5. practionation of human lymphoma cell line (Raji) lysates on Superdex 200 gel filtration column,
6. purification of the CD200R-Fc and CD200-Fc recombinant proteins on HiTrap Protein G column
7. purification of rat monoclonal antibody (OX-110) on HiTrap Protein A column.

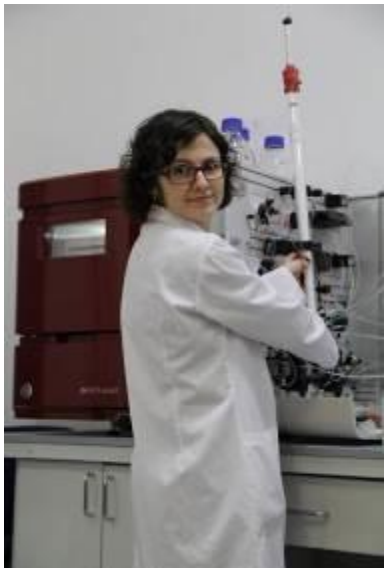

Photo 6: GE Healthcare preparative chromatography system AKTA avant 25 installed in the Department of Immunology, MUW being used by Dr. Malgorzata Firczuk

Photo 7: GE Healthcare preparative chromatography system AKTA avant 25 installed in the Department of Immunology, MUW

2.5. Acquisition of a laboratory centrifuge

Equipment name: Andreas Hettich **laboratory centrifuge ROTINA 420R** purchased by MUW in Merazet Ltd. company

Technical specification:

Parameter	**Requirement(s)**
max capacity	≥ 4 x 750ml
max speed	≥ 15 000 rpm
max g force	≥ 25 000 g
temperature regulation	from -20°C to + 40°C
constant work (over 10 hours)	required

Date of the tender announcement: December 22nd, 2012
Date of the tender announcement decision (outcome): February 6th, 2013
Date of signing the agreement: March 18th, 2013
Remaining offers:
Centrifuge offered by the Polygen Ltd. company was rejected due to the higher price than the price of the instrument offered by the Merazet Ltd. company.
Date of installation: April 15th, 2013
Date of user training: April 15th, 2013

Trained persons:

No	Name	Department
1	Dr. Dominika Nowis	Department of Immunology, MUW
2	Prof. Jakub Golab	Department of Immunology, MUW

All other researchers working at the Department of Immunology have been trained how to use this centrifuge by Dr. Nowis and Prof. Golab.

The equipment is extensively being used in all the routine everyday laboratory applications.

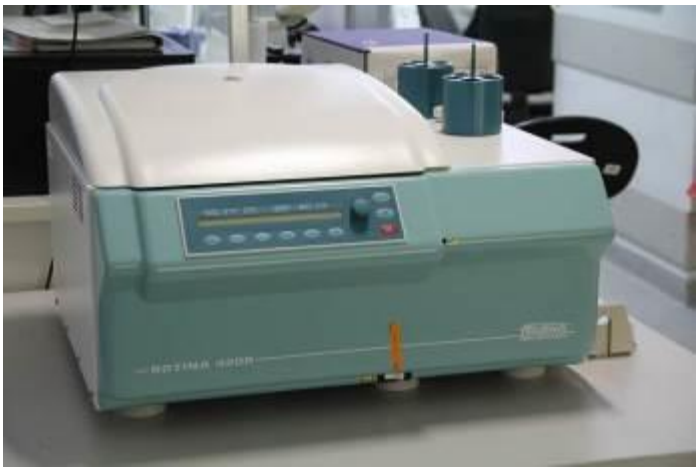

Photo 8: Andreas Hettich laboratory centrifuge ROTINA 420R installed in the Department of Immunology, MUW

3. Task 4.3 - Acquisition of an automatic platform for multispectral detection of fluorescence, absorbance and luminescence on microtiter plates

Task Leader: Dr. Dominika Nowis

An automatic platform for multispectral detection of fluorescence, absorbance and luminescence on microtiter plates consists of the following research equipment:

1. automatic system for preparation of enzymatic reactions, ELISA, PCR, RT-PCR and nucleic acid isolation by pipetting (biorobot)
2. microplate wash
3. multilabel microplate reader

All the above mentioned instruments were purchased by MUW in PerkinElmer Polska Ltd. company. There were no other offers in the tender process.

Date of the tender announcement: September 18th, 2013
Date of the tender announcement decision (outcome): November 18th, 2013
Date of signing the agreement: November 25th, 2013

3.1. Acquisition of an automated workstation

Equipment name: **Janus Integrator automated workstation**

Technical specification:

Parameter	**Requirement(s)**
pipetting arm	8-tip dispense arm
pipetting volume	from 1 µl up to 1000 µl
liquid detection	in each pipetting tip, enabling liquid detection in a microplate/tube
plate format	from 6- up to 384-well plate
plate temperature control	from +4°C up to +60°C
orbital shaker	required
magnetic separation unit	required

Date of installation: December 30th, 2013
Date of user training: January 14th, 2014 and January 24th, 2014

Trained persons:

No	**Name**	**Department**
1	Dr. Dominika Nowis	Department of Immunology, MUW
2	Slawomir Gruca	Department of Immunology, MUW
3	Dr. Pawel Gaj	Department of Immunology, MUW
4	Dr. Joanna Drzewinska	Department of Immunology, MUW
5	Dr. Beata Pyrzynska	Department of Immunology, MUW
6	Michal Machnicki	Department of Immunology, MUW
7	Dr. Tomasz Rygiel	Department of Immunology, MUW
8	Nikodem Latocha	Department of Immunology, MUW
9	Radoslaw Sadowski	Department of Immunology, MUW

Equipment use

The equipment has just been installed and we are currently intensively working on its implementation into the research projects taking place at the Department of Immunology, MUW. The first project will involve setting up of the enzymatic assay to measure inhibitory activity of chemical compounds targeting enzymes involved in tumor progression and development of inflammatory responses. Three research grants have been submitted to government agencies in Poland to support these studies and we expect to start a full-blown project in mid-March 2014. Of great importance is that the automated workstation will greatly reduce labor in the laboratory that could be spent on more effective research in other areas.

3.2. Acquisition of a platewash

Equipment name: **Delfia platewash**

Technical specification:

Parameter	Requirement(s)
automated	required
plate format	96-well
head	8-needle head

Date of installation: December 30th, 2013
Date of user training: December 30th, 2013

Trained persons:

No	Name	Department
1	Dr. Dominika Nowis	Department of Immunology, MUW
2	Prof. Jakub Golab	Department of Immunology, MUW

All the scientists working at the Department of Immunology have been next trained how to use this platewash by Dr. Nowis and Prof. Golab.

Equipment use:
The platewash has already been used for numerous ELISA experiments done at the Department of Immunology, MUW.

3.3. Acquisition of a multilabel microplate reader

Equipment name: **multilabel microplate reader EnVision 2104**

Technical specification:

Parameter	Requirement(s)
detection techniques	– absorbance (230 nm do 850 nm with dual monochromators) – fluorescence intensity (230 nm do 850 nm with dual monochromators) – glow and flash luminescence – dual luminescence – fluorescence from the top – fluorescence from the bottom – time-resolved fluorescence (TRF) – time-resolved fluorescence resonance energy transfer (TR-FRET) – fluorescence polarization – singlet oxygen transfer
plate format	1- to 1536-well microplates
sensitivity	- for fluorescence intensity not worse than 1 fmol of fluorescein /well of a 384-well plate - for singlet oxygen transfer not worse than 100 amol of phospohrized peptide/well of a 384-well plate - for luminescence not worse than 12 amol of ATP/well of a 384-well plate - for fluorescence polarization not worse than 2 mP of 1 nM fluorescein/well of a 384-well plate

	- for TRF not worse than 40 fM of europium standard/well of a 384-well plate
dispenser	dual, dead volume less than 500 µl
temperature control	from +4°C up to +42°C

Date of installation: December 30th, 2013
Date of user training: December 30th, 2013

Trained persons:

No	Name	Department
1	Dr. Dominika Nowis	Department of Immunology, MUW
2	Prof. Jakub Golab	Department of Immunology, MUW

All the scientists working at the Department of Immunology have been next trained how to use this plate reader by Dr. Nowis and Prof. Golab.

Equipment use:
The equipment has just been installed and we currently intensively work on its implementation into the scientific projects taking place at the Department of Immunology, MUW.

Photo 9: Delphia platewash and EnVision multilaber plate reader installed in the Department of Immunology, MUW

Photo 10: Janus Integrator automated workstation installed in the Department of Immunology, MUW

4. Task 4.4 - Acquisition of a system for DNA isolation

Task Leader – Dr. Krystian Jazdzewski

Equipment name: A system for DNA isolation together with additional equipment

A system for DNA isolation consists of the following research equipment:

1. System for automatic, high-throughput isolation of nucleic acids
2. System for amplification of nucleic acids in a real-time mode
3. System for amplification of nucleic acids with a gradient mode
4. Laboratory centrifuge

All the abovementioned instruments were purchased by Genomic Medicine, MUW from the Roche Diagnostic Poland S.A.

Technical specification:

4.1. Acquisition of a system for automatic, high-throughput isolation of nucleic acids

Technical specification:

Parameter	Requirement(s)
system allowing for isolation of nucleic acids from the following material:	blood, stabilized blood, serum, tissue (fresh or frozen), FFPE tissue specimens, cell cultures, urine, saliva, feces, CSF, BAL.
system equipped with	- UV lamp for decontamination of working surface - system for detection of micropipette tips - system for detection of micropipette tips blockages - separate containers for solid and liquid waste
system allowing for:	- simultaneous isolation of DNA and RNA - simultaneous isolation of nucleic acids from different kinds of biological material - isolation of nucleic acids from 50-1000 µl of material
quality requirements	CE-IVD certificate

Equipment name: **Roche MagNA Pure 96 System**

4.2. Acquisition of a system for amplification of nucleic acids in a real-time mode

Technical specification:

Parameter	Requirement(s)
system allowing for analysis of nucleic acids using	- use of SYBR Green I dye - use of intercalating dyes - use of Taqman probes - use of hybridization probes
system equipped with	- CCD camera - computer with screen

	- software allowing for quantitative and qualitative analysis of samples - 5 excitation channels - 6 detection channels
system allowing for:	- simultaneous analysis of min. 96 samples
quality requirements	certificate of thermal homogeneity of a 96-well block
heating block temperature range	37^0C to 95^0C

Equipment name: **Roche LightCycler 480 II System**

4.3. Acquisition of a system for amplification of nucleic acids with gradient

Technical specification:

Parameter	**Requirement(s)**
system allowing for analysis of nucleic acids	- in a gradient mode - with a gradient range between 1^0C and 20^0C
system allowing for amplification of material in:	- 96-wel plates - 0.2 ml reaction tubes - 0.5 ml reaction tubes
heating cover temperature range	37^0C to 110^0C

Equipment name: **Roche LightCycler 96 System**

4.4. Acquisition of a laboratory centrifuge

Technical specification:

Parameter	**Requirement(s)**
centrifuge allowing for:	- centrifugation of reaction tubes - centrifugation of reaction plates - centrifugation at up to 17 000 rpm
system allowing for:	- cooling - automatic rotor recognition

Equipment name: **Eppendorf Centrifuge 5430R**

Date of the tender announcement: March 19th, 2013
Date of the tender announcement decision (outcome): May 20th, 2013

Remaining offers: Comesa S.A. The price of the offered equipment was significantly higher than proposed by Roche Diagnostics Poland S.A.. Since the offered price was the only criterion used in tender evaluation, Roche S.A received 100 points, while Comesa S.A – 60.31 points
Date of signing the agreement: June 04th, 2013
Date of installation: June 11th, 2013
Date of user training: June 25-26th, 2013

Trained persons:

No	Name	Department
1	Marta Kotlarek, M.Sc.	Genomic Medicine, Medical University of Warsaw
2	Anna Kubiak, M.Sc.	Genomic Medicine, Medical University of Warsaw
3	Kinga Dymecka, M.Sc.	Genomic Medicine, Medical University of Warsaw
4	Monika Kolanowska, M.Sc	Genomic Medicine, Medical University of Warsaw
5	Monika Maciąg, Ph.D.	Genomic Medicine, Medical University of Warsaw
6	Wojciech Gierlikowski, Student	Genomic Medicine, Medical University of Warsaw

Equipment use:
The instrument is being extensively used in numerous projects implemented in the Department. In particular the following projects benefit from the purchase:

1. In search of new pathways of tumorigenesis - genome-wide functional analysis of microRNAs deregulated in human cancers (funded from Foundation for Polish Science)
2. The role of microRNA in thyroid cancer (funded from Foundation for Polish Science)
3. Analysis of newly discovered microRNA gene deregulated in papillary thyroid carcinoma (funded from National Science Centre)
4. Functional analysis of *RARB* gene and its regulatory microRNAs in liver cancer (funded from National Science Centre)
5. Impact of polymorphic microRNA-146a on *NTRK2* function in papillary thyroid carcinoma (funded from National Science Centre)
6. The role of microRNAs in regulation of *SLC5A8*, a tumor suppressor gene, in thyroid cancer (funded from Ministry of Science and Higher Education)
7. MicroRNA-dependent regulation of iodide transporters: NIS, AIT and Pendrin and aberrations of this process in papillary thyroid carcinoma (funded from National Science Centre)

The newly purchased equipment is also available to researchers from other scientific teams in MUW and other Universities, including University of Warsaw.

Photo 11:
DNA isolation system by Roche S.A

5. Task 4.5 Acquisition of a laser microdissector

Task Leader: Dr. Pawel Wlodarski

Equipment name**: PALM Laser Microdissector**

Technical specification:

Major required parameters
Dissection with impulse laser UV-A
Ability to dissect with objectives: 5x, 10x, 20x, 40x, 100x.
Ability to dissect tissue sections embedded in paraffin, kryosections and tissue culture (in vitro)
Ability to inspect morphology of dissected tissue fragments.
Contamination-free, contact-free method of tissue/cell transfer to test tube Transfer induced by laser catapult.
Motorized revolver for 6 objectives in a bright field Nomarski phase contrast
Optics corrected to infinity
Motorized focusing with 10 nm increments
Two camera ports
Automated slide table.
Abby condenser with magnifications 4x do 100x.
Planar objectives, focus 45mm, magnification/aperture: 5x/0,25, LD 20x/0,40, LD 40x/0,60
Halogen light source 120 W life time min. 2000 h
3 filters: FITC, Texas Red and DAPI
Monochromatic digital camera Resolution: 1388 x 1038 pixels Pixel size: 4.65 µm x 4.65 µm Chip size: 1/2" Transmission speed at full resolution: 15 frames/sec Integration time: 1 ms - 4 s Interface: IEEE1394 (FireWire)
Color digital camera Resolution: 1388 x 1038 pixels Pixel size: 4.65 µm x 4.65 µm Chip size: 1/2" Transmission speed at full resolution: 15 frames/sec Integration time: 1 ms - 4 s Interface: IEEE1394 (FireWire)
PC Computer with OS software: CPU class Xeon 6-Core or better, RAM 6 GB or more, graphic card 1GB RAM or more, monitor LCD 21” or more, Windows XP or newer
Additional LCD screen at least. 21” with touch screen pen
Software compatible with the hardware
Ability to export images in major graphic formats (bmp, tif, jpg, img, etc.),
Ability to save data form measurements on slides as well as microscope settings

Date of the tender announcement: March 19th, 2013
Date of the tender announcement decision (outcome): May 20th, 2013
Remaining offers: no other offers were submitted

Date of installation: September 5-9th, 2013
Date of user training: October 16-17th, 2013

Trained persons:

No	Name	Department
1	Paweł Włodarski	Histology and Embryology
2	Ryszard Galus	Histology and Embryology
3	MagdalenaBanach-Orłowska	Histology and Embryology
4	Wiktor Paskal	Histology and Embryology - student
5	Magdalena Bałkowiec	Histology and Embryology - student
6	Maria Różańska	Histology and Embryology - student
7	Kacper Pełka	Histology and Embryology - student

Equipment use:
The instrument is being used in several projects implemented in the Department. In particular in the projects related to endometriosis, where pathologic tissue is carefully separated from the healthy one for the molecular analysis. Thanks to this purchase Dr. Wlodarski team successfully applied for the grant founded by the National Science Center:
- Exome-wide search for somatic mutations in pathogenesis of endometriosis (No. 2013/09/B/NZ5/00790) - 992 405 PLN

Besides, other BASTION groups are encouraged to use the equipment. The member of the team have already demonstrated the abilities of microdissector to other researchers of MUW.

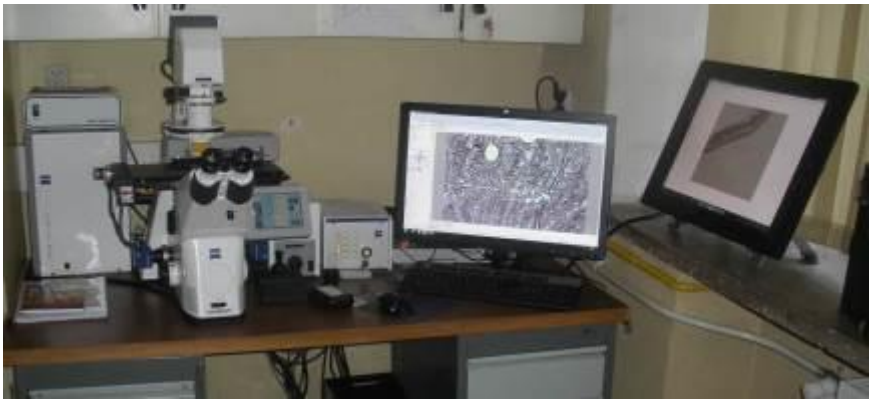

Photo 12: (on the top): PALM beam microdissector situated in the old Anatomicum Bldg (this is a temporary location; within few month the equipment will be relocated to the newly constructed CEpT Bldg in Ochota Campus.

Photo 13: (on the left) Doctors Makstym and Wlodarski at work using microdissector.

6. Task 4.6 Acquisition of a medium-throughput genomic sequencing system based on semiconductor technology

Task Leader: Prof. Zbigniew Gaciong

Equipment name:
Ion Proton System, Life Technologies.

Technical specification:
The method of sequencing is based on the detection of pH changes with the use of semiconductor technology, but without the use of optics. Modified nucleotides are not required in the process of sequencing. The sequencer enables scalable sequencing in the range of outputs greater than 8.5 Gb, with the use of chips with different throughput. The sequencer runs are complete in 6 hours or less for all reads on a chip of 10 Gb throughput or greater. The sequencing systems contains a module for emulsion PCR. The sequencer produces in a single run accurate reads with more than 300bp raw read length (single or paired end reads).The sequencer produces reads with more than 99.5% raw read accuracy.

Date of the tender announcement: December 27th, 2012
Date of the tender announcement decision (outcome): March 5th, 2013
Remaining offers: none
Date of installation: March 27th, 2013
Date of user training: April 8th, 2013

Trained persons:

No	Name	Department
1	Helena Kossowska, M.Sc.	Department of Internal Medicine, Hypertension and Vascular Diseases.
2	Jarosław Góra, M.D, Ph.D	Department of Internal Medicine, Hypertension and Vascular Diseases.
3	Grzegorz Placha, M.D, Ph.D.	Department of Internal Medicine, Hypertension and Vascular Diseases.

Equipment use
The instrument is being extensively used in numerous projects implemented in the Department. In particular the following projects benefit from the purchase:

- Search for genetic variants, novel genes and biological pathways influencing cardiac hypertrophy - an integrative genomics approach. Department of Internal Medicine, Hypertension and Vascular Diseases.
- Search for Diagnostically Useful Gene-Expression Profiles and Potential Genetic Markers in Pheochromocytoma. Department of Internal Medicine, Hypertension and Vascular Diseases.

Photo: 14: Ion Proton System, Life Technologies at the Department of Internal Medicine, Hypertension and Vascular Diseases.

Conclusions

The purchased equipment will be extensively used in all other WPs and form the basis for collaborative initiatives of the BASTION research teams. It is important to emphasize the complementarity of the purchases equipment. The automated nucleic acid extractor together with laser microdissection platform provide the means of acquisition of DNA/RNA, i.e. substances which are the main carriers of information in each cell and therefore represent subject of analyses in virtually every field of biological research. The machines complement each other as the former one can be used for majority of macroscopic samples whereas the latter is dedicated for extractions at the microscopic scale (even from single cells). The Fluidigm platform together with the DNA sequencer provide the means to carry out sophisticated analyses of DNA/RNA isolated with the first two machines. In particular the Fluidigm platform allows to efficiently prepare middle scale libraries from isolated nucleic acids whereas the DNA sequencer complements the previously acquired low throughput machine (Junior) allowing to sequence those libraries. The results obtained with the nucleic acid sequencing techniques can be next verified on the protein level with the use of the protein purification work station. The ability to express and obtain highly purified recombinant proteins creates the unique opportunity to study the detailed function and interactions of previously determined gene products. Moreover, acquisition of the pipetting biorobot and multispectral plate reader allows for further evaluation of the target gene(s) function in numerous cell-based in vitro assays implementing e.g. absorbance, luminescence, fluorescence and/or singlet oxygen transfer measurements. Thus, the abovementioned pieces of equipment allow to carry out a streamlined comprehensive pipeline of experiments consisting of purifying, analyzing nucleic acids and verify the role of their protein products in vitro which is expected to useful both within BASTION and by external researchers.

Corresponding estimated/*budget

PERSONNEL, EQUIPMENT, OTHER MAJOR DIRECT COST ITEMS FOR BENEFICIARY "1" FOR 18M			
	Item description	Amount [EUR]	Explanations
Work Package 4	Personnel costs	64 783,00	Salaries of the WP4 Task leaders T4.1-T4.6 (17,7 PM)
	Equipment	1 004 150,48	Acquisition of modern research equipment within Task T4.1- T4.6
	Other direct costs	0,00	Equipment Deployment
TOTAL DIRECT WP4 COST		1 068 933,49	

/ - exact costs for M1-M18 will be presented in the Ist Period Report and Form C (April 2014)*

All reports are available on BASTION Webpage: www.bastion.wum.edu.pl

Prof. Rafal Ploski
WP4 Leader
Dr Dominika Nowis
WP4 Co-leader

Prof. Jakub Golab
BASTION Project Coordinator

Warsaw, February 2014