

# **NGS Based Applications – Future of Genomics**

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**04.04.2019**



# NGS Applications – Basic Science

- Ancient DNA
- DNA mixtures from diverse ecosystems, metagenomics
- Resequencing previously published reference strains
- Identification of all mutations in an organism
- Errors in published literature
- Expand the number of available genomes
- Comparative studies



# NGS Applications – Basic Science

- Deciphering cell's transcripts at sequence level without knowledge of the genome sequence
- Sequencing extremely large genomes, crop plants
- Detection of cancer specific alleles avoiding traditional cloning
- Chip-seq: interactions protein-DNA
- Epigenomics
- Detecting ncRNA
- Genetic human variation : SNP, CNV (deseases)



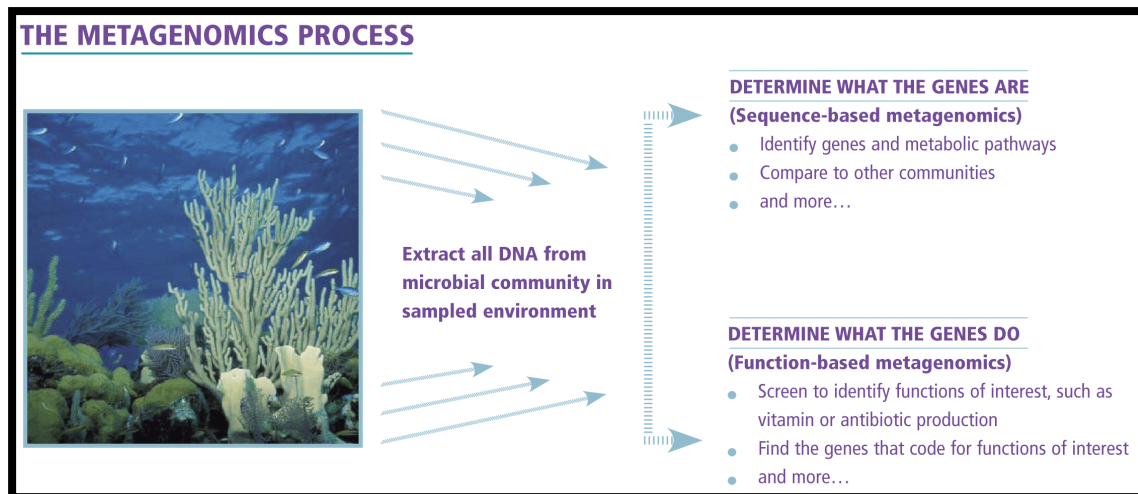
# NGS applications – Microbial Genomics

- rapidly detect and treat pathogens (disease-causing microbes) in clinical practice
- develop new energy sources (bio fuels)
- monitor environments to detect pollutants
- protect citizenry from biological and chemical warfare
- clean up toxic waste safely and efficiently

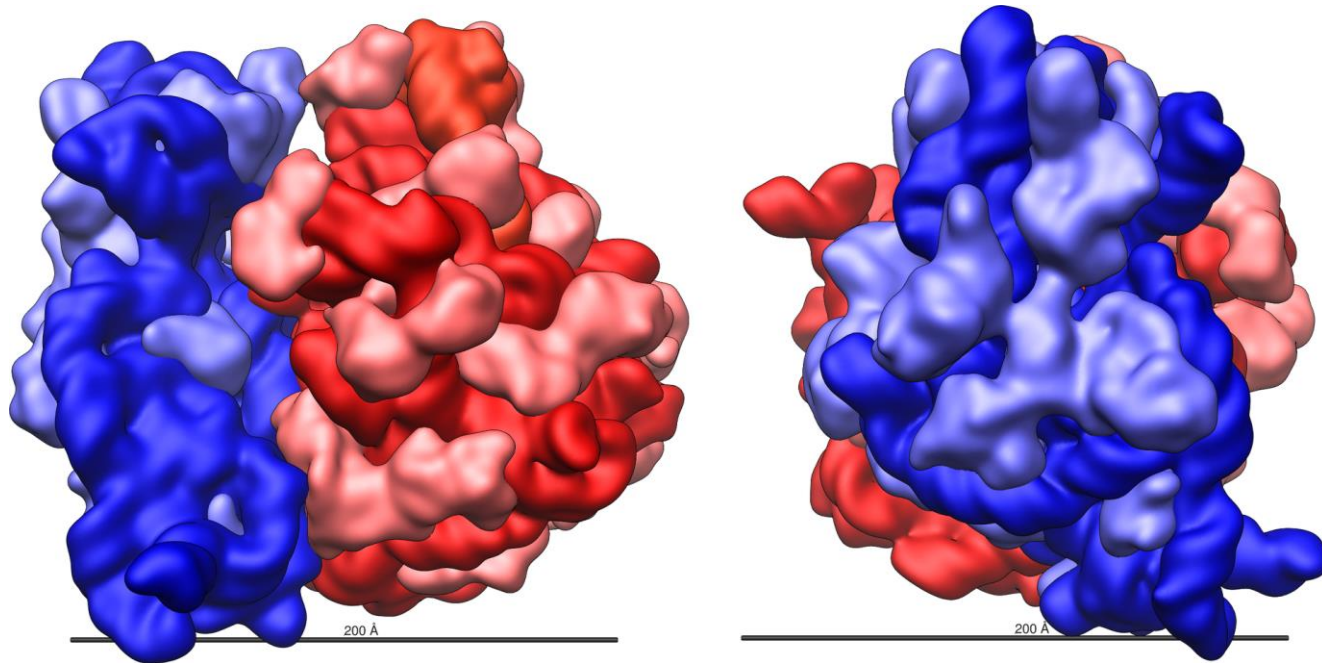


# NGS applications – Metagenomics

- Characterizing the biodiversity found on Earth
- The growing number of sequenced genomes enables us to interpret partial sequences obtained by direct sampling of specific environmental niches
- Examples: ocean, acid mine site, soil, coral reefs, human microbiome which may vary according to the health status of the individual



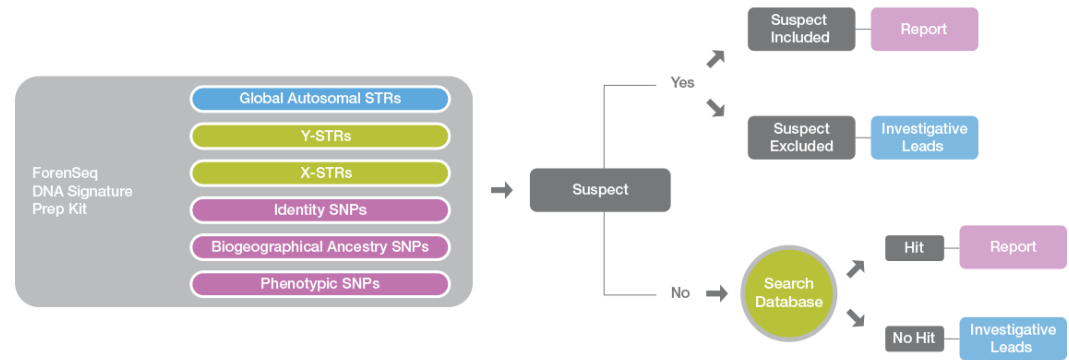
# NGS applications – 16S RNA Metagenomics



ribosomal ribonucleic acid (rRNA) is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms

Three-dimensional views of the ribosome, showing rRNA in dark blue (small subunit) and dark red (large subunit). Lighter colors represent ribosomal proteins.

# NGS applications – Forensics - MiSeq FGx



NGS can provide more information than traditional methods. Using the MiSeq FGx System DNA database laboratories can:

Simultaneously analyze every locus now in use by crime laboratories around the world

Future-proof local, national, and international databases

Easily expand sequencing runs to include more markers

Benefit from minimal sample preparation time

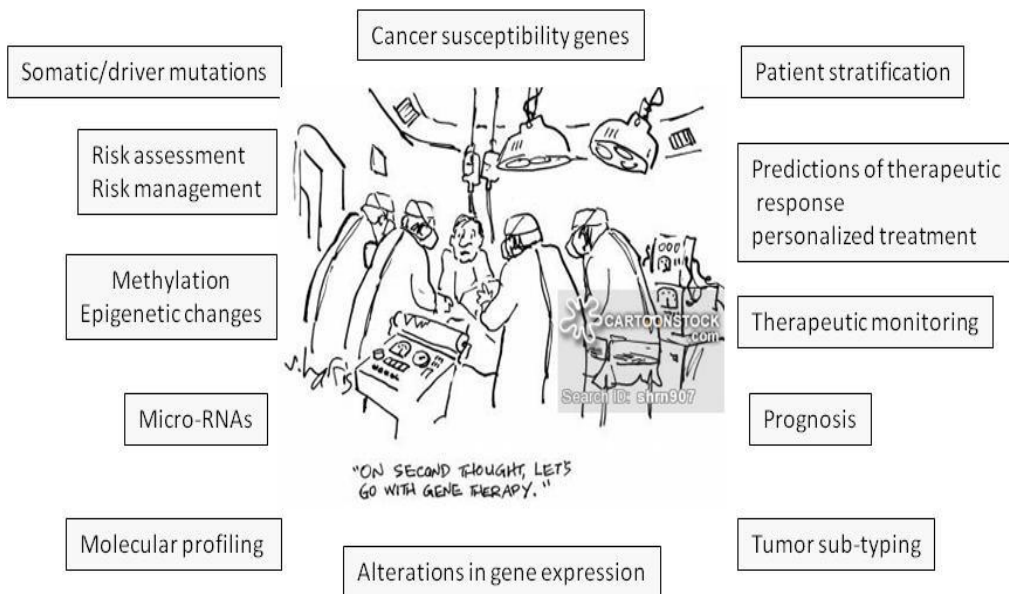
# NGS applications – Molecular Medicine

- improve diagnosis of disease
- detect genetic predispositions to disease
- create drugs based on molecular information
- use gene therapy and control systems as drugs
- design “custom drugs” (pharmacogenomics) based on individual genetic profiles





# NGS applications – Clinical – MiSeq Dx



The MiSeqDx System, designed specifically for clinical laboratories, delivers a broad range of sequencing applications right at your fingertips. The flexible, scalable system offers tunable read lengths and output options to support diagnostics and research needs.



# Groups of Genome - wide NGS Tests

- 1) **Diagnostic tests**: defining the cause of a rare disease or for identifying high-risk susceptibility for a specific disease
- 2) **Tests for Mechanisms of diseases**: e.g. examination of the genetic changes in a malignant tumor, Hematological Disorders, Neurology and Psychiatry, Vision and hearing, Cardiology as well as many undiagnosed Disease.
- 3) **Pharmacogenomics**: refers to the relation between the human genetic profile and individual differences in clinical drug response. The aim of pharmacogenomics is to optimize treatment outcome and individualize therapy.
- 4) **Predicting susceptibility**: genetic risk profiling for common diseases



# Groups of Genome - wide NGS Tests

## ❖ Analysis of Structural chromosomal variants using NGS

- structural variants in the Human Genomes
- structural variants and Human Diseases

## ❖ NGS in Oncology

- Familial Genetic Testing
- Molecular calcifications of Diseases
- Circulating cell free tumor DNA and circulating tumor cells
- Treatment in last resort patients
- Treatment monitoring
- Biomarkers in Clinical decision-making

## ❖ Next Generation Sequencing in hematological Disorders

- Acute Myeloid Leukemia
- CSF3R mutation in chronic Neutrophilic Leukemia
- Non-Hodgkin Lymphomas
- Thrombocytopenia Absent Radius Syndrome



# Groups of Genome - wide NGS Tests

## ❖ Next Generation Sequencing in Neurology and Psychiatry

- Epilepsy
- Ataxia
- Autism
- Neuromuscular Disorders
- Movement Disorders
- Neurodegenerative Disorders with Dementia

## ❖ Next Generation Sequencing in Dysmorphology

- Congenital defects syndromes
- Genetic testing in Dismorphology
- Screening of Rare Disorders in Newborn

## ❖ Next Generation Sequencing in Vision and Hearing Impariment

- Vision and hearing Disorders
- Digenic and Oligogenic Inheritance



# Groups of Genome - wide NGS Tests

## ❖ Next Generation Sequencing as a tool for Noninvasive prenatal tests

- NGS of Fetal DNA
- properties of cell free fetal DNA
- Maternal – Fetal DNA comparative analysis

## ❖ Next Generation Sequencing in Cardiology

- Cardiomyopathies
- Hypertrophic Cardiomyopathies
- Arrhythmias
- Brugada Syndrome
- Atrial Fibrillation

## ❖ Next Generation Sequencing in Pharmacogenomics

- NGS in noncancerous pharmacokinetics
- NGS in Transplantology
- Multigene Pharmacogenetics

## ❖ Next Generation Sequencing in Undiagnosed Diseases

- NGS testing for Rare Disease
- pathogenetics



# SOPHiA Genetics – Overview

SOPHiA DDM

WORKSPACE Requests VDB Variant Database Browser ANAL... ADNstand... #11030-0011

Demo Sophia Genetics

PROJECT Interpretation 2 SAMPLE #180672 ADNstandard < 1/22 RUN 19/07/2018 Solid Tumor Solution by SOPHiA GENETICS ADNstandard S1 -- Jean Dupont

Overview OncoPortal Variants Patient's Disease (0) REPORTED 9/9 BRAF CDKN2A EGFR HRAS KRAS PIK3CA Solid Tumor Solution by Sophia somatic

TEST 42/42 GENES 360 VARIANTS 43 RETAINED 317 LOW CONFIDENCE VARIANT DEPTHS 30 DEPTH MIN 9385 DEPTH MAX

Patient Project Clinical Results Documents

**Interpretation Project** Delete Complete

Name Interpretation 2 Start Date 04/09/2018

Virtual Panel STS\_v1 (42 genes) End Date

Owner Jean Dupont Status draft

**Conclusion** Cancel Save Create Report Clinical Results

1. Genomic alterations associated to approved therapies in patient's disease:  
None Reported!

2. Other potentially actionable genomic alterations:  
 > KRAS p.(Gly13Asp) exon 2 - this mutation has been associated with Resistance or Non-Response to Chemotherapy, MEK Inhibitor in Non-small Cell Lung Carcinoma.  
 > BRAF p.(Val600Glu) exon 15 - this mutation has been associated with Sensitivity to BRAF Tyrosine Kinase Inhibitor, MEK Inhibitor in Non-small Cell Lung Carcinoma.  
 > EGFR p.(Thr790Met) exon 20 - this mutation has been associated with Sensitivity to 3rd generation EGFR Tyrosine Kinase inhibitor in Non-small Cell Lung Carcinoma.  
 > EGFR p.(Thr790Met) exon 20 - this mutation has been associated with Sensitivity to 3rd generation EGFR Tyrosine Kinase inhibitor in Non-small Cell Lung Carcinoma.  
 > EGFR p.(Leu858Arg) exon 21 - this mutation has been associated with Sensitivity to 2nd generation EGFR Tyrosine Kinase Inhibitor in Non-small Cell Lung Carcinoma.  
 > EGFR p.(Glu746\_Ala750del) exon 19 - this mutation has been associated with Sensitivity to 2nd generation EGFR Tyrosine Kinase Inhibitor in Non-small Cell Lung Carcinoma.  
 > KRAS p.(Gly12Asp) exon 2 - this mutation has been reported 5:

All Genes Retained Variant Low Confidence Va...

**Prediction**  
C: 334  
D: 3 B: 7 A: 16

**Pathogenicity Flags**  
1<sub>4c</sub> 4<sub>1x</sub> 5<sub>4c</sub>

**Actionability**  
40 DISEASES 63 DRUGS 0 TRIALS  
T4: 1 T2: 1 D: 1  
ACTIONABILITY

**Test Information**  
TEST ILL1XG154\_FFPE\_miniseq v5.3.20.1 GENHIGH1FSQ2

**Public Databases**

ExAC	r0.3.1	✓
G1000	v5.20130502	✓
ESP	5400	✓
COSMIC	v83	✓
ClinVar	v20171029	✓
CG69	837.20120813	✓
dbSNP	v150	✓
dbNSFP	v2.9	✓
GnomAD	r2.0.2	✓



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SCREENING GENES SNVs/INDELs CNVs FUSIONS WARNINGS Interpretation Scope STS\_v1

Variant List - sorted by: Prediction>Pathogenicity class>Gene

Retained Variants	P...	S...	T...	Gene	dbSNP	ID ClinVar	Depth	Community	Freq...	VF%	Processed Clin/Var rating	c.DNA	Coding consequence	ref	alt	Actionability
43	A 5	★	✓	SNP	BRAF rs113488022	rs113488022	7747	██████████	2973	9.8	Pathogenic/Likely_pathog...	c.1799T>A	missense	A	T	T1 T2 T3 T4
Highly Pathogenic	A 5	★	✓	INDEL	EGFR rs121913421	rs121913421	9010	██████████	765	1.5	drug_response	c.2235_2249de...	inframe_15	AGGAA...	A	T1 T2 T3 T4
Potentially Pathogenic	A 5	★	✓	SNP	KRAS rs121913529	rs121913529	5581	██████████	2329	6.7	Pathogenic/Likely_pathog...	c.35G>A	missense	C	T	T1 T2 T3 T4
Unknown Significance	A 4	★	✓	SNP	MAP2K1 rs730880503	rs730880503	6659	██████████	760	31.2	Pathogenic	c.355C>T	missense	C	T	T1 T2 T3 T4
Likely Benign	A	★	✓	SNP	KRAS rs112445441	rs112445441	5548	██████████	1839	13.7	Pathogenic/Likely_pathog...	c.38G>A	missense	C	T	T1 T2 T3 T4
Low Confidence Variants	A	★	✓	INDEL	CDKN2A		5842	██████████	592	8.8		c.68dupG	frameshift	ACCCC	ACCCCC	T1 T2 T3 T4
Flagged Variants	A		✓	SNP	CTNNB1 rs121913400	rs121913400	5771	██████████	668	31.9	Pathogenic/Likely_pathog...	c.98C>A	missense	C	A	T1 T2 T3 T4
	A		✓	INDEL	CTNNB1 rs587776850	rs587776850	5686	██████████	668	9.8	Pathogenic	c.133_135delTCT	inframe_3	CCTT	C	T1 T2 T3 T4
	A		✓	SNP	EGFR rs28929495	rs28929495	9248	██████████	805	23.8	Pathogenic	c.2155G>A	missense	G	A	T1 T2 T3 T4
	A		✓	SNP	KIT rs121913507	rs121913507	5173	██████████	549	8.4	Pathogenic/Likely_pathog...	c.2447A>T	missense	A	T	T1 T2 T3 T4
	A		✓	SNP	MAP2K1 rs1057519729	rs1057519729	7127	██████████	745	32.8	Pathogenic/Likely_pathog...	c.167A>C	missense	A	C	T1 T2 T3 T4
	A		✓	INDEL	MET		9385	██████████	474	6.5		c.713delT	frameshift	GTTTT	GTTT	T1 T2 T3 T4
	A		✓	SNP	HRAS rs121913254	rs121913254	4294	██████████	998	11.8	Pathogenic/Likely_pathog...	c.181C>A	missense	G	T	T1 T2 T3 T4
	A		✓	SNP	PIK3CA rs121913279	rs121913279	6482	██████████	1268	16.7	Pathogenic/Likely_pathog...	c.3140A>G	missense	A	G	T1 T2 T3 T4
	A	★	✓	SNP	PIK3CA rs104886003	rs104886003	5368	██████████	1134	7.9	Pathogenic/Likely_pathog...	c.1633G>A	missense	G	A	T1 T2 T3 T4
	B	★	✓	SNP	EGFR rs121434568	rs121434568	8999	██████████	928	2.5	drug_response	c.2573T>G	missense	T	G	T1 T2 T3 T4
	B		✓	SNP	ERBB2		6326	██████████	276	4.7		c.2411G>A	missense	G	A	T1 T2 T3 T4
	B		✓	SNP	RAF1		6176	██████████	138	5.1		c.1279A>C	missense	T	G	T1 T2 T3 T4
	B		✓	SNP	RET		6578	██████████	138	5.3		c.2720A>C	missense	A	C	T1 T2 T3 T4
	C 1		✓	SNP	ALK rs3795850	rs3795850	5902	██████████	5750	9.7	Benign	c.3375C>A	synonymous	G	T	T1 T2 T3 T4
	C 1		✓	SNP	FGFR3 rs7688609	rs7688609	6554	██████████	19036	99.9		c.1953G>A	synonymous	G	A	T1 T2 T3 T4
	C 1		✓	SNP	FOXL2 rs61750361	rs61750361	7221	██████████	659	6.6	Benign	c.501C>T	synonymous	G	A	T1 T2 T3 T4



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SCREENING | GENES | SNVs/INDELS | CNVs | FUSIONS | WARNINGS | Interpretation Scope STS\_v1

Virtual Panels: STS\_v1 [root] 42 D  
 cancer pulmon 1  
 mama 4  
 Test 1 7

select / unselect all Search:   Case sensitive  Exact match < >

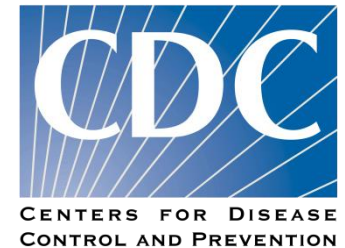
Filter	Chr	Name (IGV)	Molecular Variants										Structural Variants													
			Prediction		Pathogenicity			Types			Coding Consequences					CNV										
			A	B	C	5	4	3	2	1	SNV	INDEL	Total	FSFT	ALSE	INFR	SYN	SUTR	SUTR	INGE	INTR	Deletion	Amplification Duplication			
<input checked="" type="checkbox"/>	2	ALK									12	3	15											14		
<input checked="" type="checkbox"/>	10	RET									11	0	11				1								9	
<input checked="" type="checkbox"/>	7	EGFR									11	3	14				3	1	1						9	
<input checked="" type="checkbox"/>	1	NRAS									11	0	11				1						2		8	
<input checked="" type="checkbox"/>	5	TERT									8	4	12				1						3		8	
<input checked="" type="checkbox"/>	2	ERBB4									6	3	9												9	
<input checked="" type="checkbox"/>	19	GNA11									8	2	10												9	
<input checked="" type="checkbox"/>	17	ERBB2									4	1	5				2								3	
<input checked="" type="checkbox"/>	14	AKT1									5	1	6												6	
<input checked="" type="checkbox"/>	7	RAC1									7	0	7												7	
<input checked="" type="checkbox"/>	6	HIST1H3B									5	1	6										1		4	
<input checked="" type="checkbox"/>	11	HRAS									3	3	6												3	
<input checked="" type="checkbox"/>	6	ROS1									1	0	1												1	
<input checked="" type="checkbox"/>	2	SF3B1									2	1	3												3	
<input checked="" type="checkbox"/>	4	PDGFRA									14	4	18												16	
<input checked="" type="checkbox"/>	18	SMAD4									7	4	11												9	
<input checked="" type="checkbox"/>	15	MAP2K1									6	1	7				2								5	
<input checked="" type="checkbox"/>	17	H3F3B									3	1	4												1	
<input checked="" type="checkbox"/>	9	CDKN2A									5	2	7				1								3	
<input checked="" type="checkbox"/>	4	FBXW7									4	2	6												6	





# Our Collaborators

(Incomplete list )



# Genome Center at National Center for Disease Control and Public Health

## Projects at Genome Center

1. Completed project with G. Eliava Phage Institute : “Comparative Whole Genome Sequencing of Diagnostic *Brucella* Phages” Project was funded by DTRA, Manuscript is published
2. Completed project with Tbilisi Agrarian University : “Genetic characterization of mitochondrial DNA of Georgian wheat’s varieties” funded by AgrUni internal grant foundation, manuscript published
3. Completed project with Biotechnology Institute of National Academy of Sciences of the Kyrgyz Republic: “Microbiological Monitoring of Uranium-Contaminated Environments” funded by ISTC
4. Completed project with G. Eliava Phage Institute: “Characterization of mechanisms of adaptive phage-host co-evolution using next generation sequencing and phenotypic profiling “ funded by GRDF
5. Ongoing project with AIDS center “Genetic characteristic of hepatitis C virus in Georgia: implications for hepatitis C elimination program” Funded by CDC
6. Ongoing project G. Eliava Phage Institute: “Phage mediated antibiotic resistance gene transfer in marine, freshwater and extreme environments” funded by GRDF
7. Ongoing project with Batumi State University, Institute of Phytopathology “Whole Genome Sequencing of quarantine plant bacterial pathogen *Ralstonia Solanacearum* Isolated in Republic of Georgia” funded by GRDF and Rustaveli Foundation.



**Thank you for your attention !**

