Isolation of RNA and transcriptome analyses on acanthocephalans

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KICK-OFF MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 16th December2021

Project objectives:

To determine active cellular processes in acanthocephalans and fish intestine under different metal exposure regimes by profiling:

O4.1. metal distribution within proteins

O4.2. transcriptome and gene expression

Tasks:

RNA of appropriate concentration and quality isolated from acanthocephalans

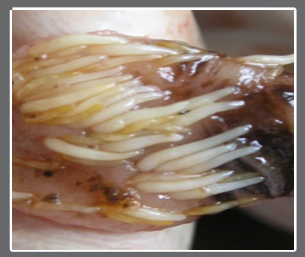
Our lab

De novo sequencing of transcriptome of acanthocephalans

Novogene
Advancing Genomics, Improving Life

Differences in gene expression in parasites from the reference and pollution impacted site

ENVIRONMENTAL SAMPLES



LN2/-80°C

6x specimens

6x samples

RNA Miniprep

Group 1

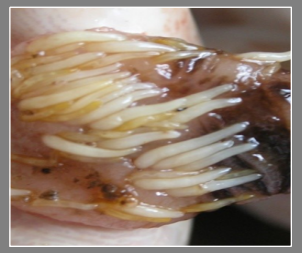
„reference" site (Krka spring)

Group 2

„polluted" site (Knin)

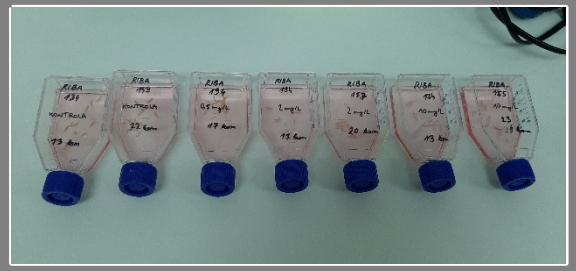
EXPERIMENTAL SAMPLES

Acantocephalans exposure to CdCl2, modified protocol *Brázová et al., 2012, Sensors*



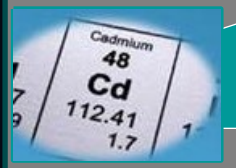
Live acanthocephala

PBS washing



Parasite culture, 50 mL flasks
RPMI medium + antibiotics:
penicillin, streptomycin
(100 U/mL)

Cd^{2+} exposure



Control

2 mg Cd^{2+}

10 mg Cd^{2+}

Incubation at 16 °C
3-5 days

EXPERIMENTAL SAMPLES

Daily analysis of motility (vitality) and integrity

6x specimens

3x samples

RNA Miniprep

Group 1

Control

Group 2

Treated (2mg Cd^{2+}, 10mgCd^{2+})

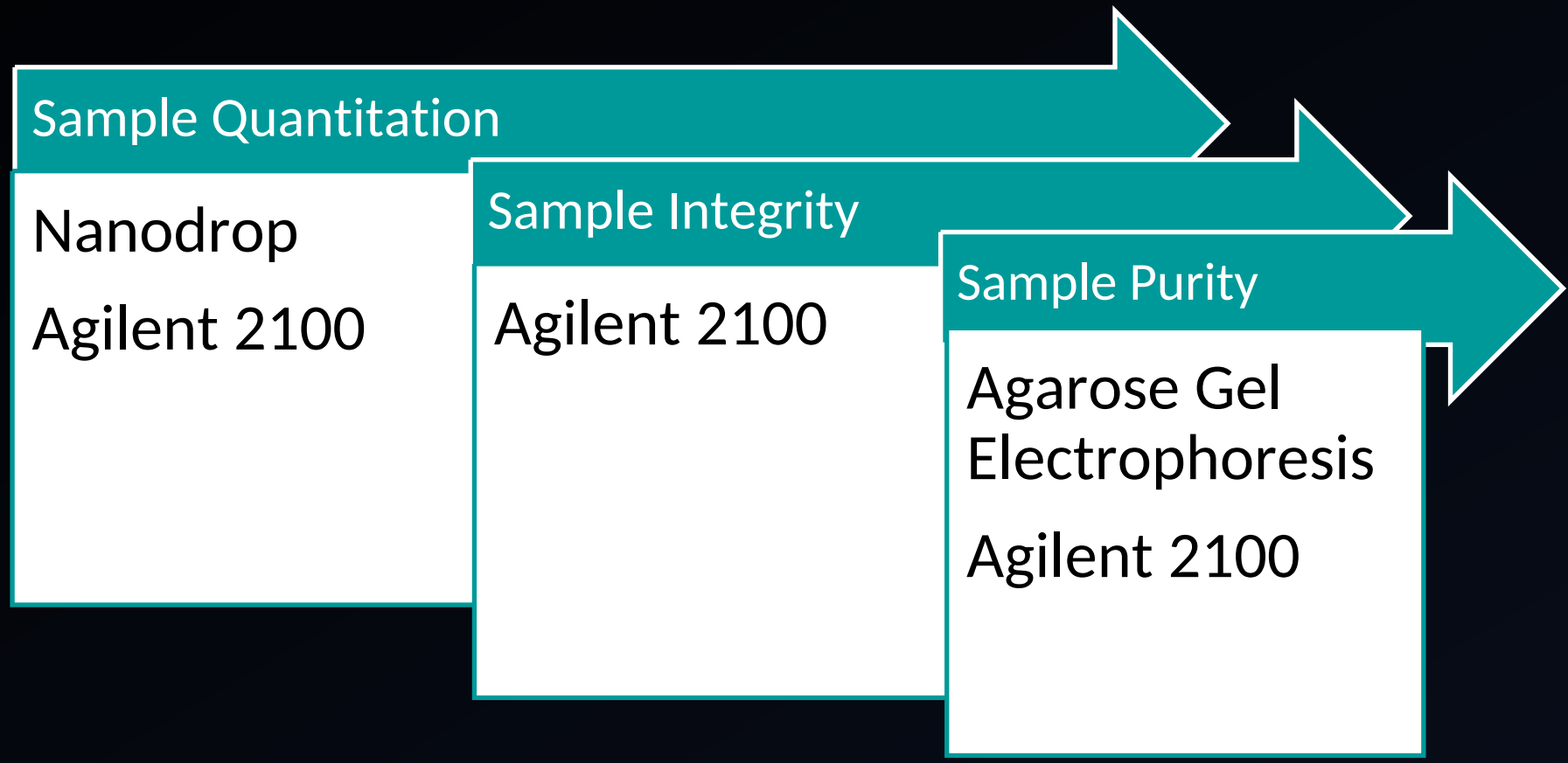
EXPERIMENTAL SAMPLES

Results of metal analysis

a) Digestion in HN03(2ml) and H2O2 (1ml) in the oven overnight (90°C)
b) ICP-MS analysis after samples dilution

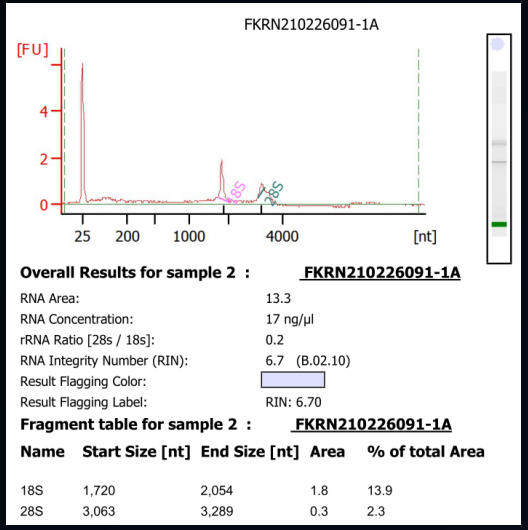
	Cd/ mg g^{-1}	Ag/ mg g^{-1}	Cu/ mg g^{-1}	Zn/ mg g^{-1}
Uncultured parasites from reference site	0,46	0,03	8,59	12,2
Cultured parasites from reference site untreated	0,32	0,046	9,91	11,3
Cultured parasites treated (3 days, 10 mg/mL)	188,6	0,038	15,4	12,3
Cultured parasites treated (5 days, 10 mg/mL)	267,0	0,017	3,42	10,8

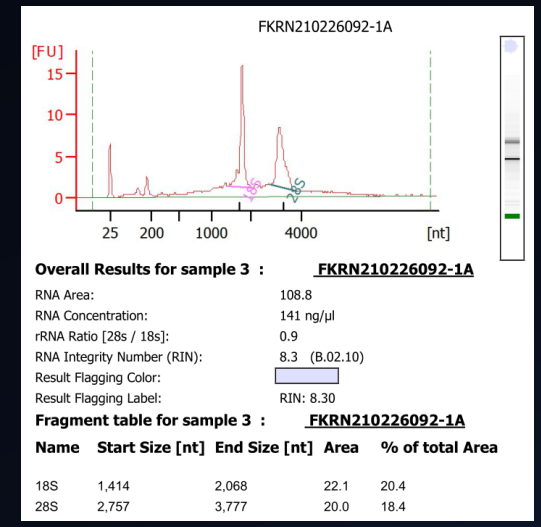
RNA isolation - QC Methods



Isolation of RNA of appropriate concentration and quality isolated from acanthocephalans

No.	Sample Name	Nucleic Acid ID	Concentration (ng/ul)	Volume (ul)	Total amount (ug)	Integrity value	Sample QC Results	Sample QC Memo	Sample volume for electrophoresis(ul)
1	TAC1P1	FKRN210226078-1A	34	35.00	1.19000	9.20	Hold	-,Severely impure	1.00
2	TAC2P1	FKRN210226079-1A	75	34.00	2.55000	7.60	Hold	-,Severely impure	1.00
3	RAC3P1	FKRN210226080-1A	7	82.00	0.57400	8.20	Pass	-	1.00
4	TAC4P1	FKRN210226081-1A	36	81.00	2.91600	8.90	Pass	-,Slightly impure	1.00
5	TAC6P1	FKRN210226082-1A	11	35.00	0.38500	6.30	Hold	Insufficient total amount,-	1.00
6	TAC7P1	FKRN210226083-1A	21	36.00	0.75600	7.50	Pass	-	1.00
7	TAC1P2	FKRN210226084-1A	21	83.00	1.74300	8.10	Pass	-	1.00
8	RAC2P2	FKRN210226085-1A	22	77.00	1.69400	8.90	Pass	-	1.00
9	TAC3P2	FKRN210226086-1A	18	82.00	1.47600	8.30	Pass	-	1.00
10	TAC4P2	FKRN210226087-1A	9	81.00	0.72900	9.00	Pass	-	2.00
11	TAC5P2	FKRN210226088-1A	81	34.00	2.75400	7.40	Hold	-,Severely impure	1.00
12	TAC6P2	FKRN210226089-1A	154	37.00	5.69800	8.40	Pass	-,Slightly impure	0.50
13	KONT143	FKRN210226090-1A	9	34.00	0.30600	1.00	Fail	Insufficient total amount,Degraded,-	2.00
14	KONN4	FKRN210226091-1A	17	81.00	1.37700	6.70	Pass	-	2.00
15	T155_10mgCd	FKRN210226092-1A	232	36.00	8.35200	8.30	Pass	-,Slightly impure	0.50
16	T157_2mgCd	FKRN210226093-1A	217	36.00	7.81200	7.30	Pass	-,Slightly impure	0.50





Results:

- First time transcriptome of *D. truttae*
- DEGs for „reference”/ „polluted” and „control” / „treated” groups
- Identification of genes included in metal pathways

Thanks:
Members of Laboratory for Biological Effects of Metals

Thank you for your attention!