Acanthocephalans and gene expression analyses

Irena Vardić Smrzlić, Ruđer Bošković Institute, Zagreb, Croatia

KICK-OFF MEETING

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

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Acanthocephalans

- Role of parasites in environmetal studies understimated
- Phylum Acanthocephala: endoparasites found in almost all marine, freshwater and terrestrial systems
- Complex life cycle including definitive and intermediate hosts
- Nightmare for taxonomy and systematics
- Research on Acanthocephala:

1) taxonomy and evolution
2) have a manipulative effect on intermediate hosts
3) have a potentially pathogenic effect on end hosts (fish)
4) as possible indicators of pollution in the aquatic environment

- Dentitruncus trutae: member of the Illiosentidae family with a worldwide distribution restricted to parts of southeast Europe.

a) b) c)

Figure 1. Female a) and male b) specimens of *D. trutae;* c) proboscis with spines

- In the Krka River it is found in brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*)
- D. truttae showed effective metal accumulation
- TRANSCRIPTOMICS – method for differentiate which genes are active
- Only one Acanthocephala species*, Pomphorhynchus laevis* – published genome and transcriptome

Project objectives:

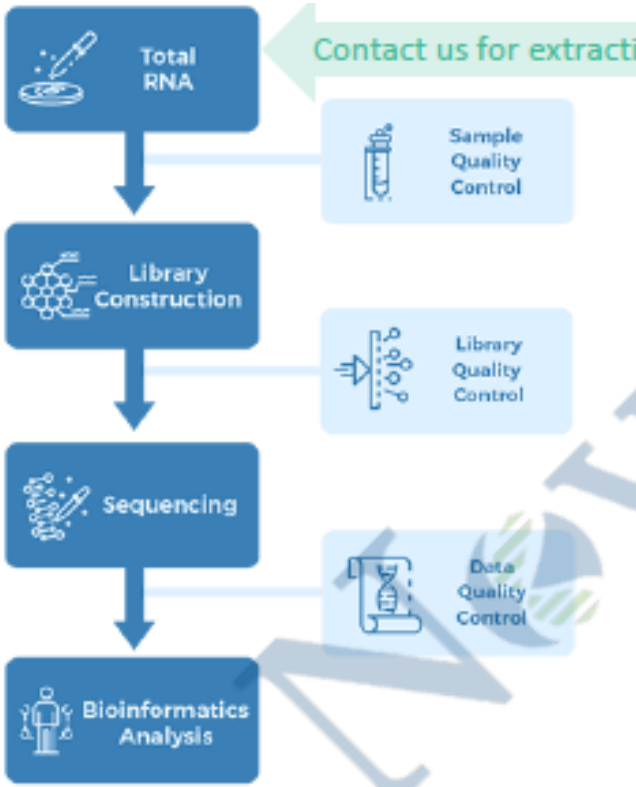
O4. To determine active cellular processes in acanthocephalans and fish intestine under different metal exposure regimes by profiling:

O4.1. metal distribution within cytosolic proteins;

O4.2. transcriptome and gene expression.

D1.7 RNA of appropriate concentration and quality isolated from acanthocephalans for transcriptome profiling, report prepared (*connected to **O4.2***)

D1.8 De novo sequencing of transcriptome of acanthocephalans and estimation of differences in gene expression in acanthocephalans from the reference and pollution impacted site conducted, report prepared (*connected to **O4.2***)

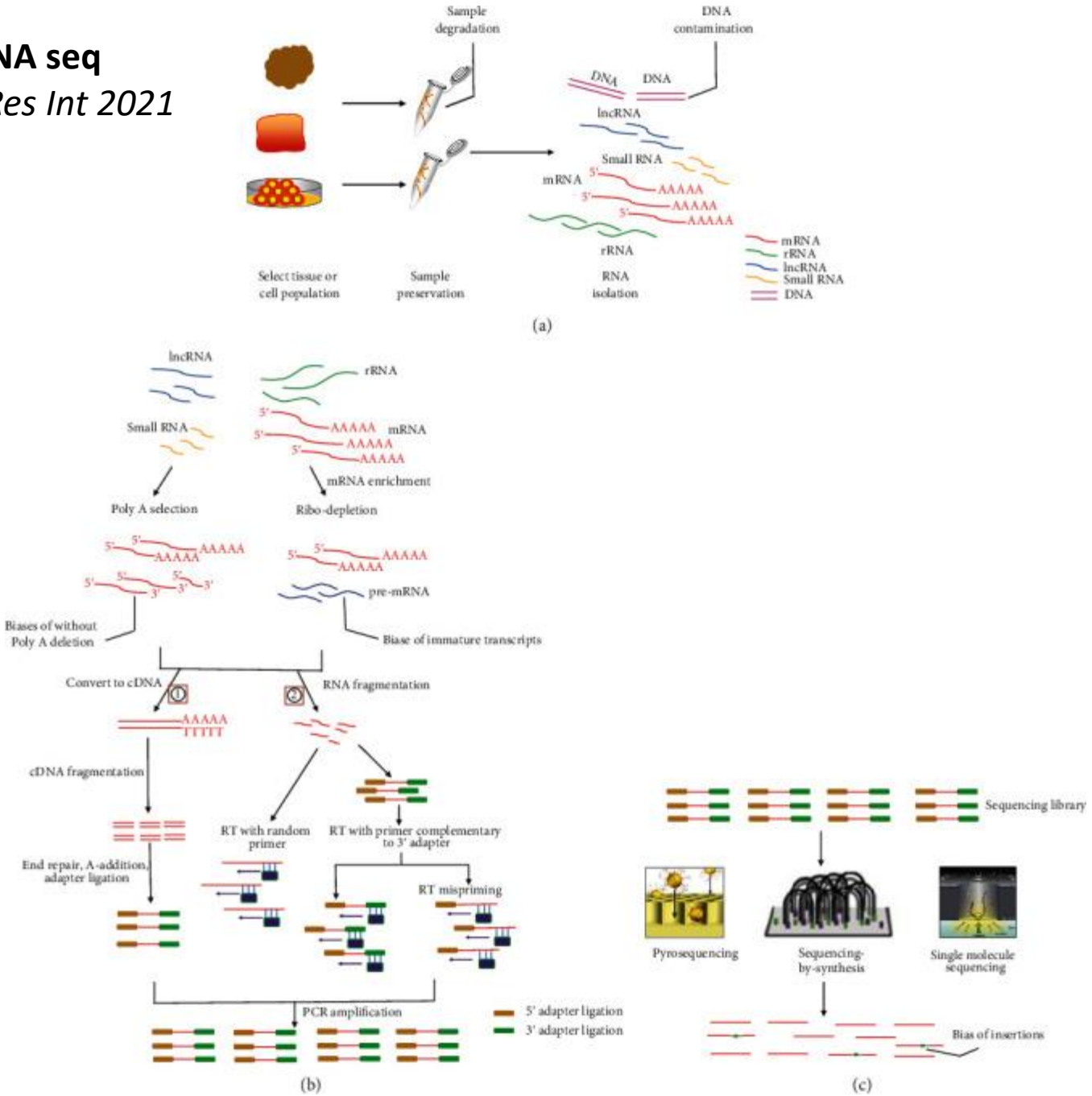


Recommended methods:

- TRIzol method
- RNA extraction kits
  (Commercially available kits)

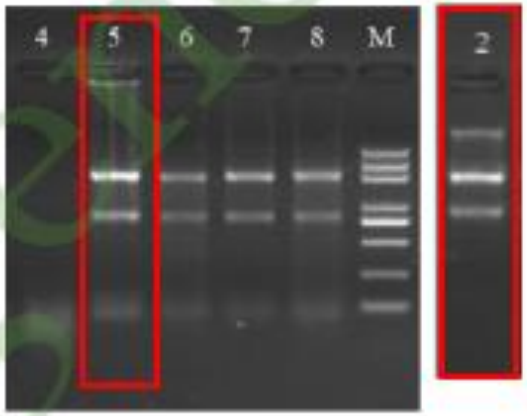
Bias in RNA seq

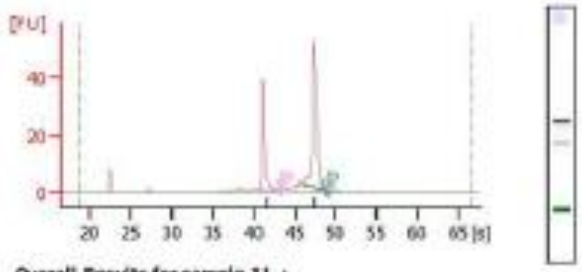
BioMed Res Int 2021



Sample
Quality
Control

Sample pre-QC	
1: 1% Agarose Gel Electrophoresis	
Contamination?	Degradation?
2: Nanodrop	
OD260/280≧1.8	OD260/230>2
3: Agilent 2100	
RIN≧6.8 (animal)	≧ 6.3 (Plant and Fungi)



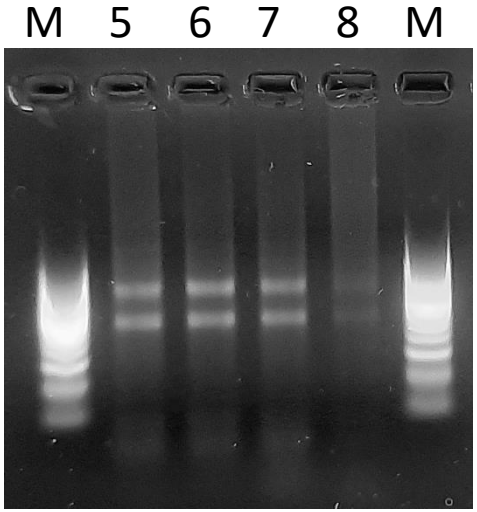


Overall Results for sample 11 :

RNA Area:	151.7
RNA Concentration:	110 ng/µl
rRNA Ratio [28s / 18s]:	2.0
RNA Integrity Number (RIN):	10 [8.02.08]
Result Flagging Color:	
Result Flagging Label:	RIN:10

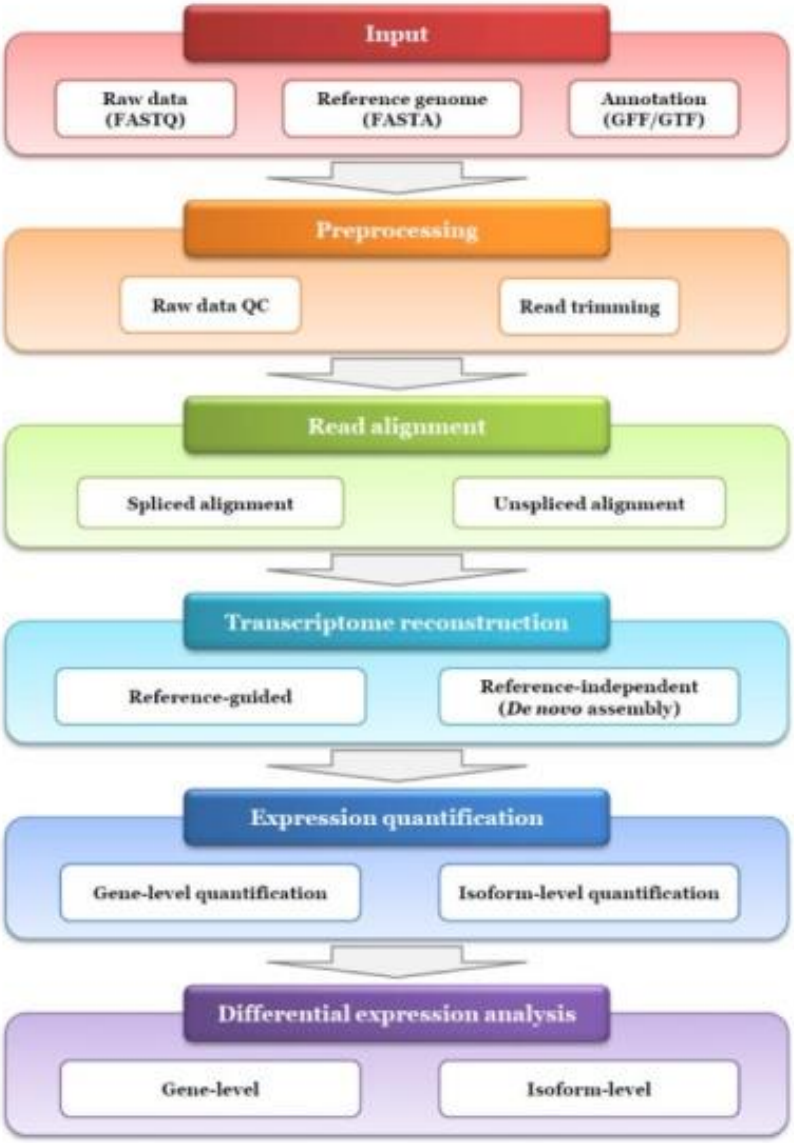
RNA were extracted by **Direct-zol RNA Miniprep (Zymo Research).**

SAMPLE	CONCENTRATION (ng/µl)	OD260/280	OD260/230	VOLUME (µL)
1	78.84	2.08	1.85	~46
2	102.29	2.14	2.17	~46
3	28.49	2.22	1.73	~80
4	67.82	2.08	2.30	~80
5	27.44	2.25	1.99	~80
6	31.16	2.01	2.28	~80
7	29.86	2.36	1.75	~80
8	20.76	2.27	1.76	~80
9	18.80	2.31	1.15	~80
10	10.93	2.10	0.90	~80
11	4.18	1.33	0.09	~80
12	11.52	2.03	0.71	~80



M – molecular marker, 1 kb

Data analyses



RNAseq blog.com

Summary:

- 1. „de novo” RNA sequencing from the reference site
- 2. differences between reference and „polluted” site
- 3. differences between exposed and control group of acanthocephalans

Thank you for your attention!