Protocol to pool RNA extraction material for sRNA (microRNA sequencing)

NMBU – 10012023

Pool of inputs

Context

RNA extracts were prepared from pools of stage-matched embryos (DevMap) or individual tissue samples (BodyMap) and sent to Novogene UK.

DevMap and BodyMap samples were provided as replicates. DevMap replicates (n=3) represent RNA collected from a pool of matched stage embryos. BodyMap replicates (n=3) represented RNA collected from individuals of the same gender and maturity state.

For sRNAseq, replicates were pooled in equimolar proportions by Novogene prior to library preparation according to the pooling strategy described below:

1) For DevMap, the pools represent different developmental stages, but disregard the replicate number
2) For BodyMap, the pools represent different tissues and maturity state, but disregard the replicate number or sex

NOTE 1: DevMap pooling was only performed for the "additional stages". Samples representing the "core stages" were processed as individual replicate samples.

NOTE 2: BodyMap gonad samples were not pooled. They were processed as individual samples preserving their unique gender, maturity state and replicate identity.

Table of the pooling:

Sample type	DevMap		
Replicates	1	2	3
Any stage (additional stages)	Pool		
Any stage (core stages)	No pool		

Sample type	BodyMap											
Maturity state	Immature Female			Immature Male			Mature Female			Mature Male		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
Brain	Pool						Pool					
Gill	Pool						Pool					
Head Kidney	Pool						Pool					
Liver	Pool						Pool					
Muscle	Pool						Pool					
Distal Intestine	Pool						Pool					
Gonad	No pool											