Method Comparison for RNA Extraction from Salamander Tissue.



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Plethodon metcalfi; Southern Gray-cheeked Salamander

Overall Scientific Purpose of the study .

- To explore in detail the metabolic physiology of salamanders during dormancy to gain insights into how salamanders will deal with warmer winters projected during future climate change.

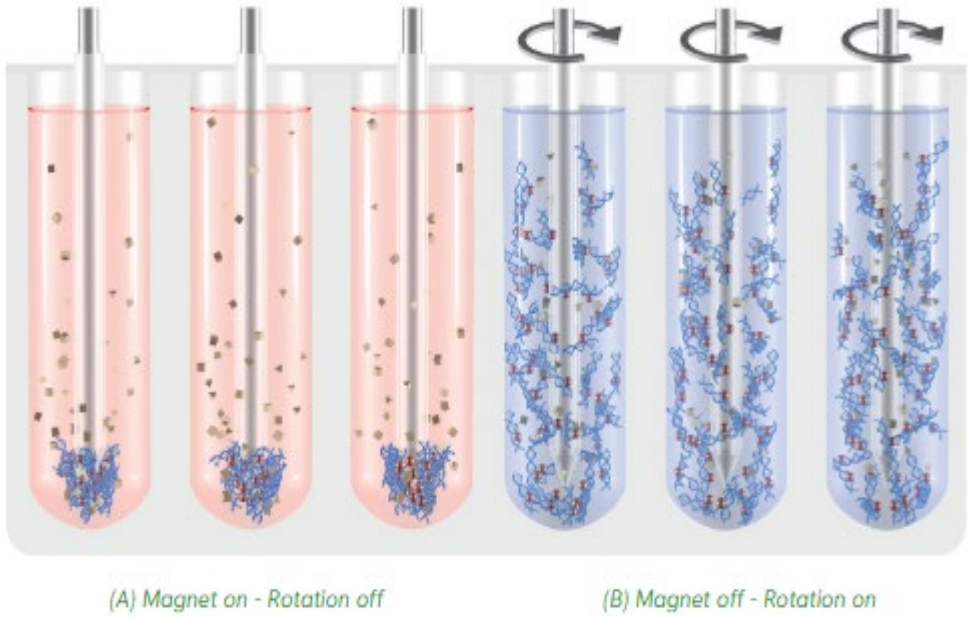
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RNA Extraction Methods

Method 1

Homogenization: Qiagen Tissuelyser in Revvity lysis buffer

RNA extraction: Revvity's chemagic 360

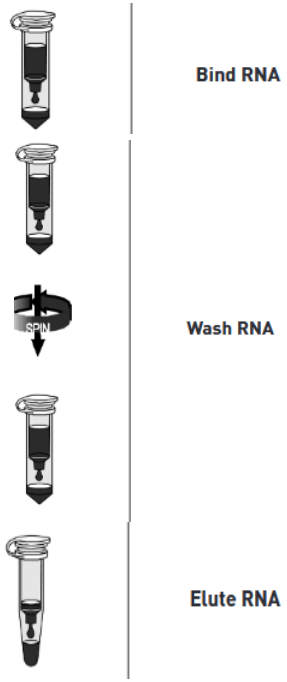


(A) Magnet on - Rotation off (B) Magnet off - Rotation on

Method 2

Homogenization: small battery-powered pestle in trizol

RNA extraction: PureLink RNA mini-kit





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Yield Results*

Sample Type	Median total Yield of non-degraded samples µg	% degraded samples	Method
Brain	2.35	0	Method 1
Brain	0.755	53	Method 2
Skin	7.03	0	Method 1
Skin	5.59	47	Method 2

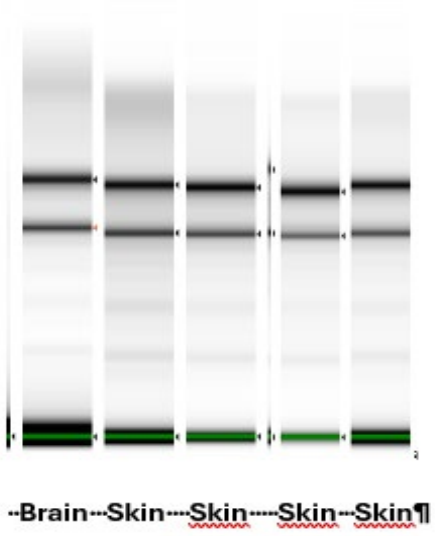
* Yields from GI tract and liver were equivalent with both methods



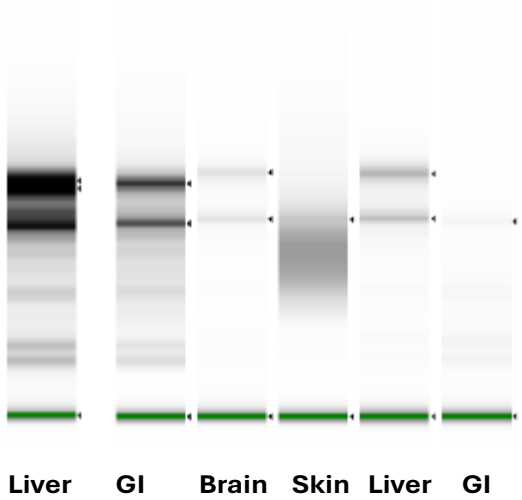
Quality Results

- 100% of RNA samples extracted with Method 1 had RINS >8.2.
- For the problematic brain and skin samples between 53% and 67% of the RNA was highly degraded when method 2 was used.
- The GI tract and liver samples which had equivalent yields with both extraction methods had lower degradation percentages 8% and 33%, respectively.

Method 1



Method 2





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Conclusions

Yields:

- Yields were much greater using method 1

Quality:

- RNA integrity using method 2 was inconsistent and a high percentage of the RNA extracted using this method was degraded.
- This is likely due to either the lysis method or the extraction method. Based on past experience, it is more likely due to the lysis method.
- Additional experiments were not conducted to determine if the lysis or the extraction protocol had the greatest effect.



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Authors:

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