

Supplemental Figures

A.

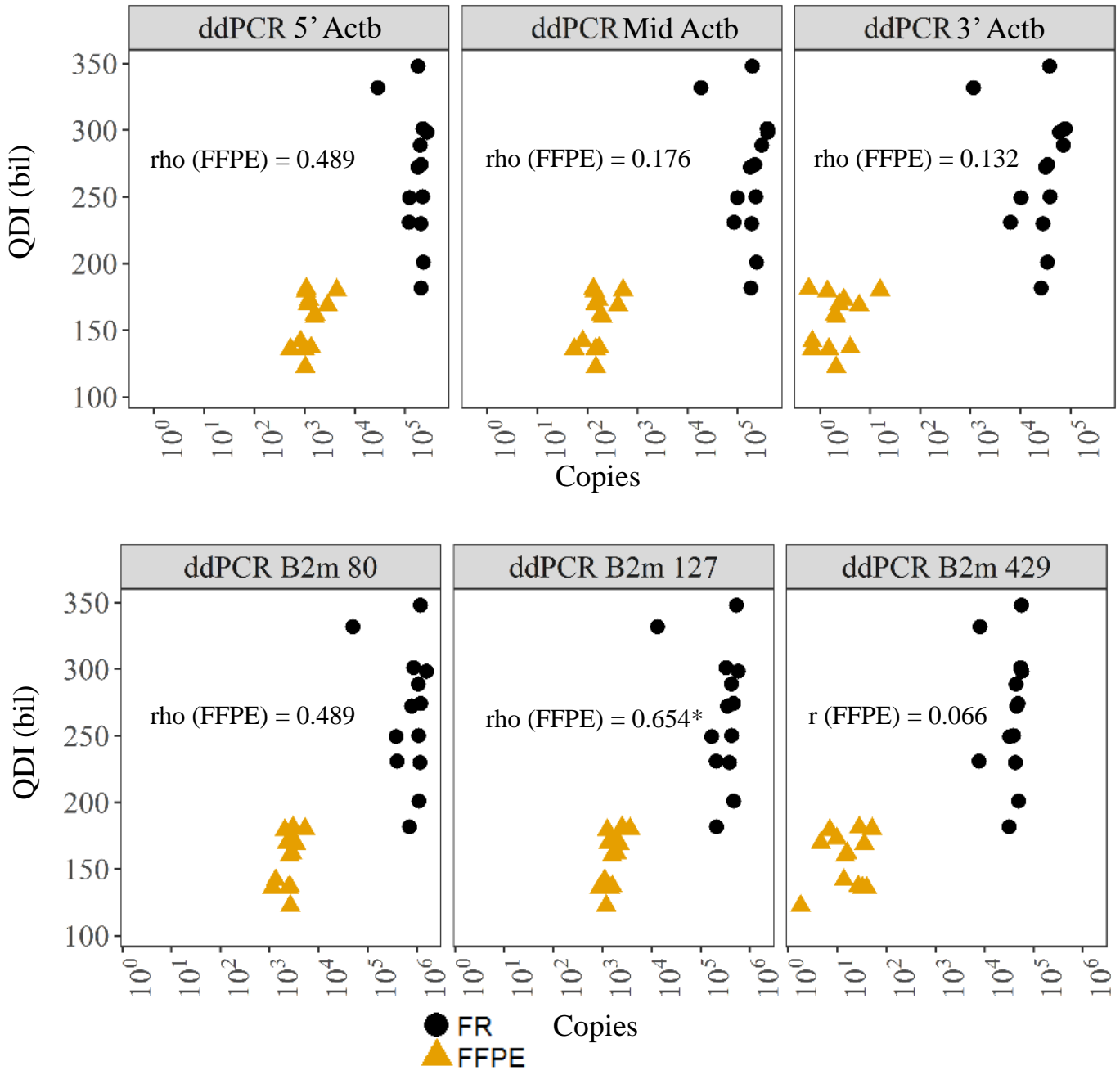
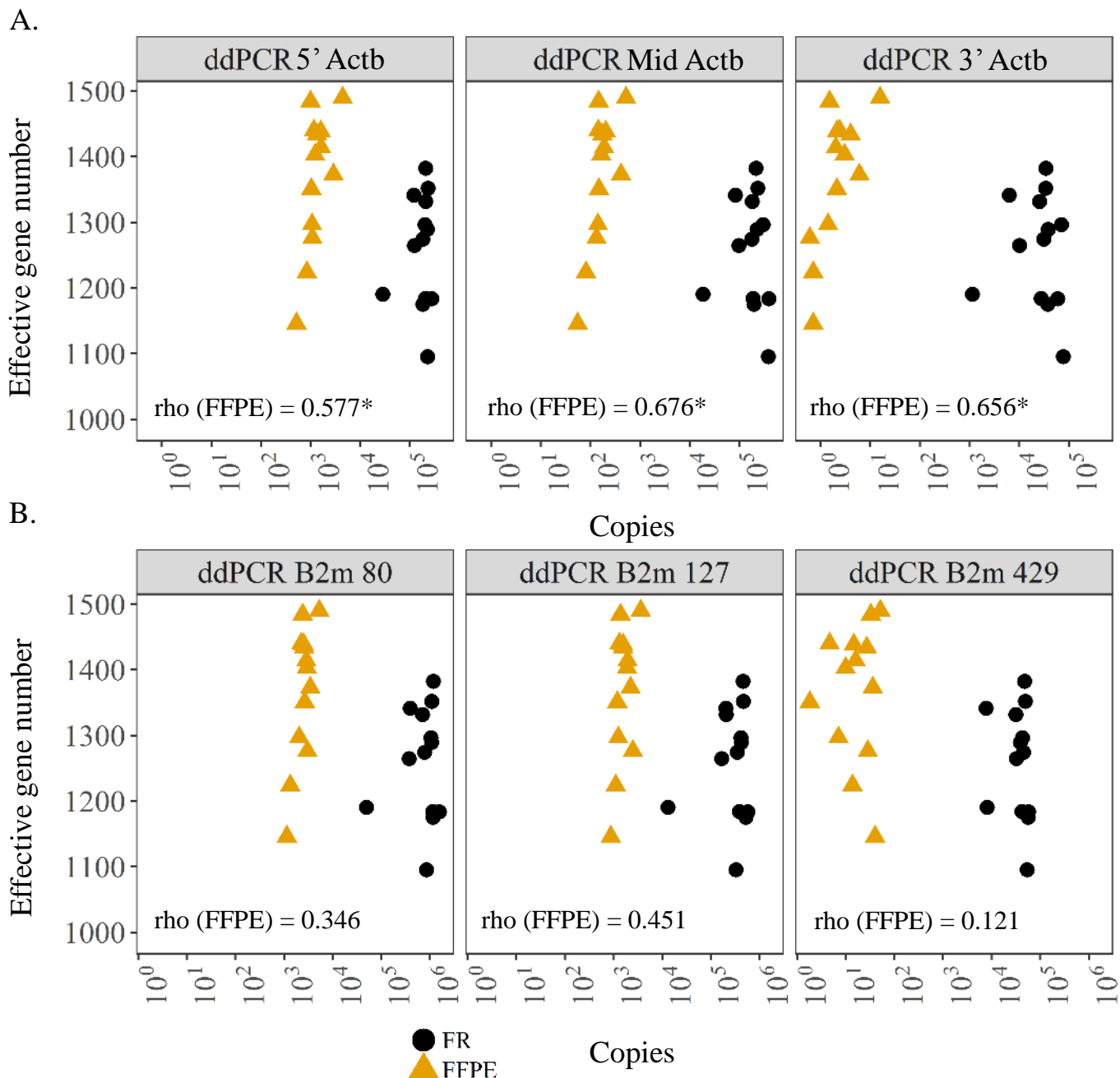


Figure S1. Study 1 on effects of 2 years in paraffin block (FFPE) relative to frozen (FR). Correlations between quantifiable RNA by digital drop PCR on two house keeping genes (*Actb* and *B2m*) and the combined RNA sequencing output of total genes counted multiplied by the amount of unique genes identified in the billions (QDI). (A) Copies of similarly sized *Actb* amplicons located at increasing distances from the 3' end of *Actb* mRNA (5'-*Actb*<Mid-*Actb*<3'-*Actb*). (B) Copies of *B2m* amplicons ranging in size (80, 127, and 429 base pairs) and location of amplification across *B2m* mRNA. rho indicates correlation coefficient for spearman test on only FFPE samples (* indicates p-value <0.05).



S2. Study 1 on effects of 2 years in paraffin block (FFPE) relative to frozen (FR). Correlations between quantifiable RNA by digital drop PCR on two house keeping genes (*Actb* and *B2m*) and the combined RNA sequencing output effective gene number. (A) Copies of similarly sized *Actb* amplicons located at increasing distances from the 3' end of *Actb* mRNA (5'-*Actb*<Mid-*Actb*<3'-*Actb*). (B) Copies of *B2m* amplicons ranging in size (80, 127, and 429 base pairs) and location of amplification across *B2m* mRNA. rho indicates correlation coefficient for spearman test on only FFPE samples (* indicates p-value <0.05).

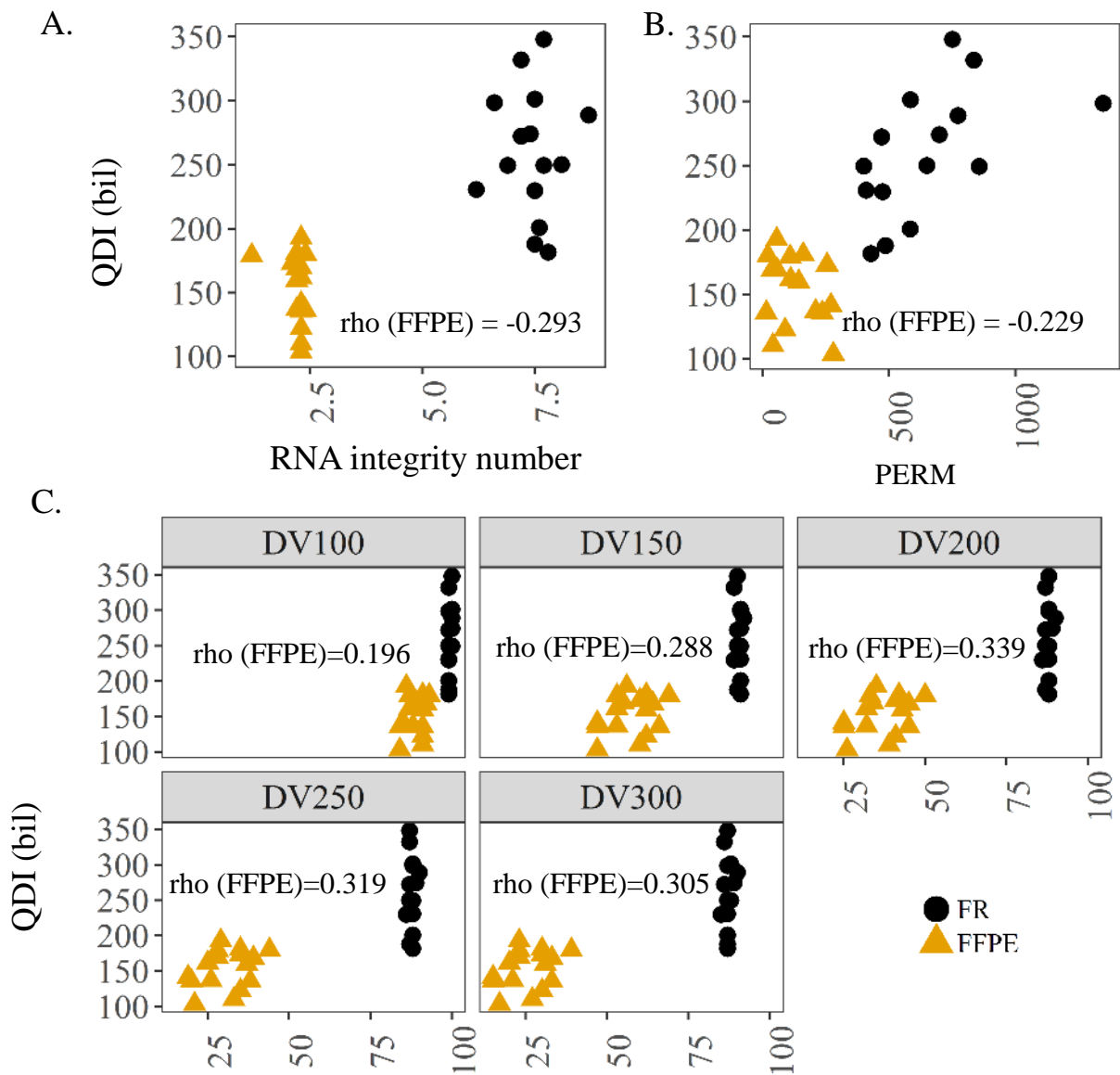


Figure S3. Study 1 on effects of 2 years in paraffin block (FFPE) relative to frozen (FR). Correlations between select pre sequencing quality metrics and the combined RNA sequencing output of total genes counted multiplied by the amount of unique genes identified in the billions or QDI. (A) RNA integrity number (RIN). (B) Paraffin embedded RNA metric (PERM). (C) Fragment analysis (DV) or percent of RNA fragments greater than 100, 150, 200, 250, or 300 nucleotides. rho indicates correlation coefficient for spearman test on only FFPE samples (* indicates p-value <0.05).

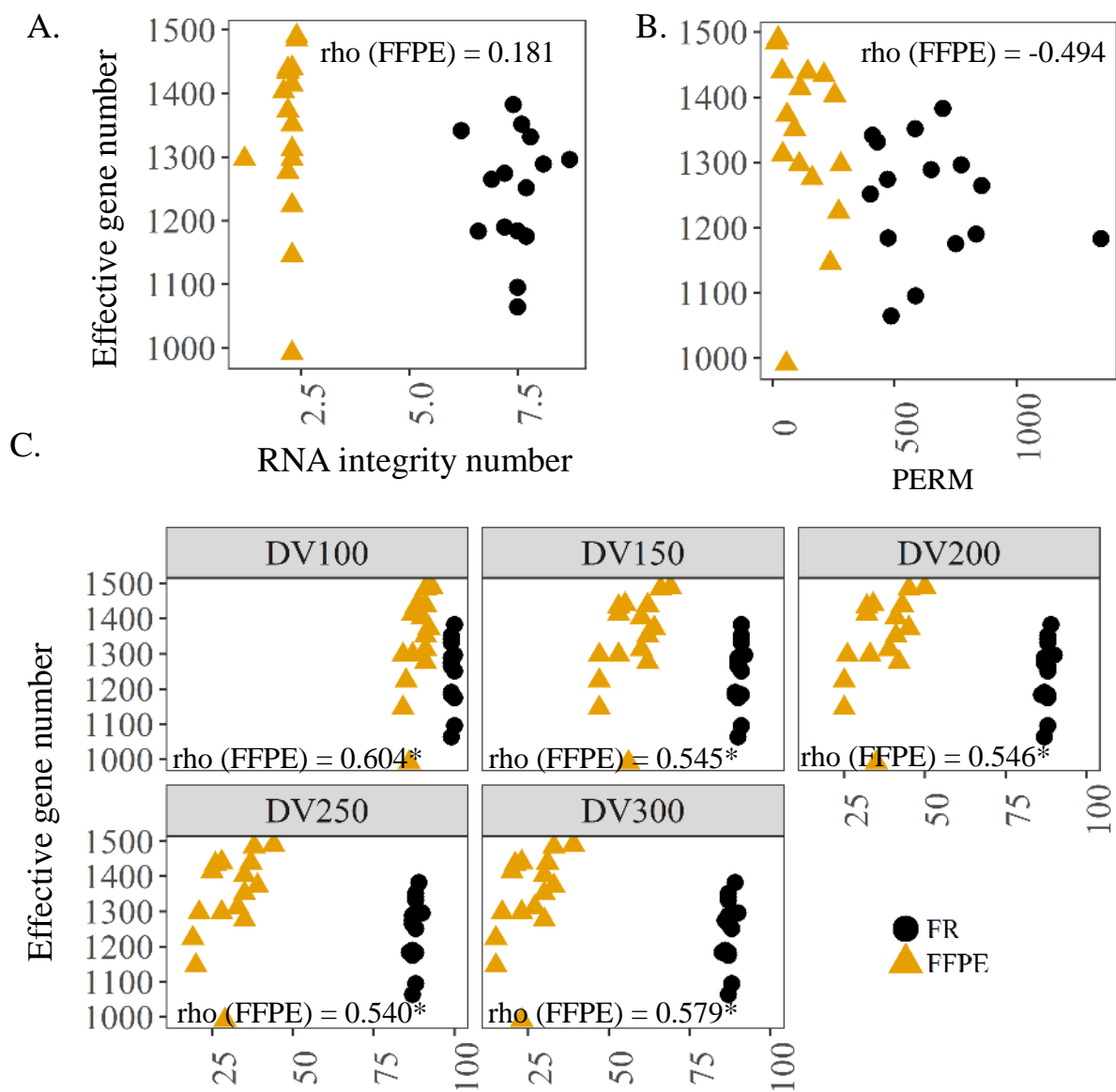


Figure S4. Study 1 on effects of 2 years in paraffin block (FFPE) relative to frozen (FR). Correlations between select pre sequencing quality metrics and effective gene number. (A) RNA integrity number (RIN). (B) Paraffin embedded RNA metric (PERM). (C) Fragment analysis (DV) or percent of RNA fragments greater than 100, 150, 200, 250, or 300 nucleotides. rho indicates correlation coefficient for spearman test on only FFPE samples (* indicates p-value < 0.05).

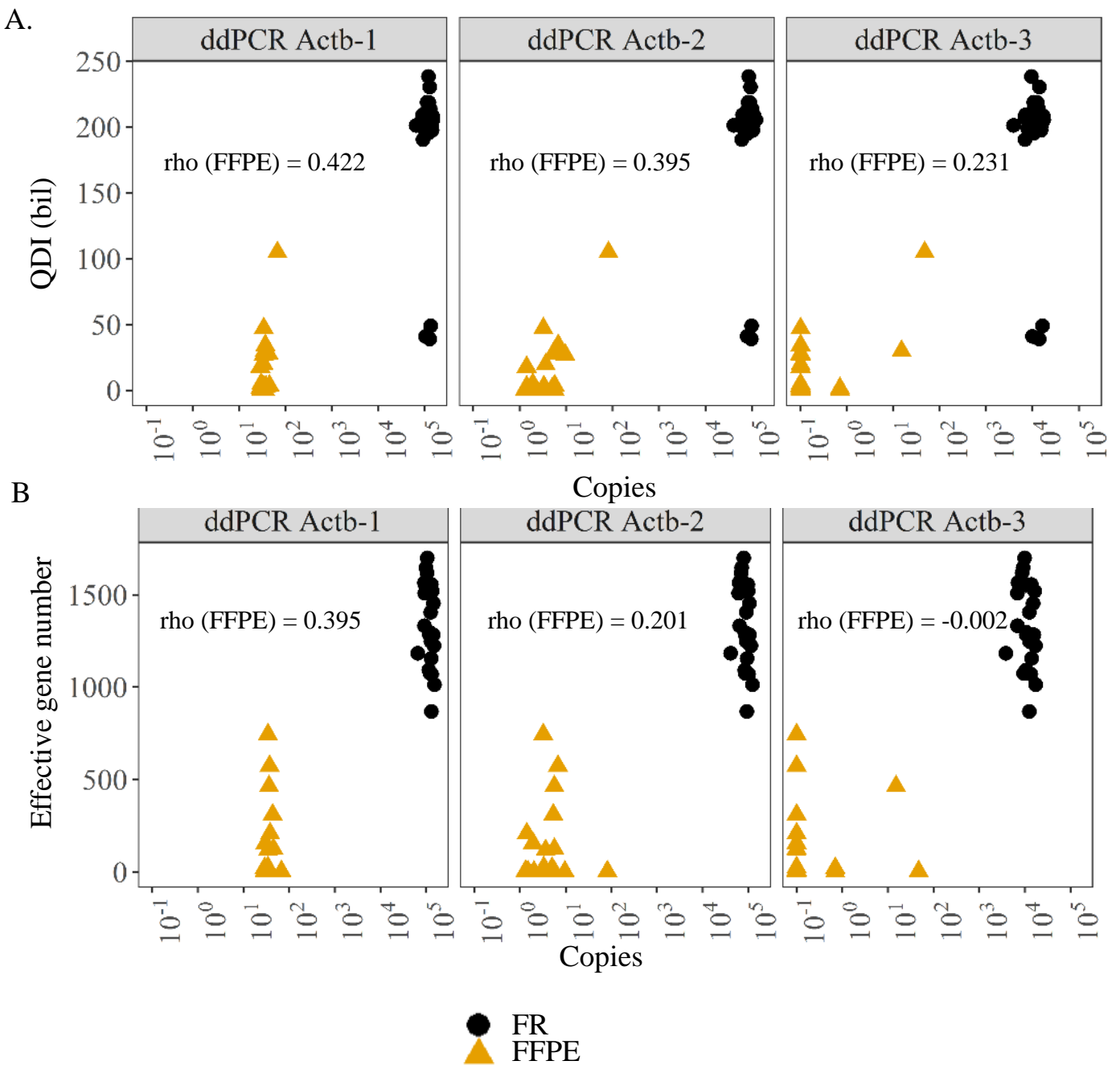


Figure S5. Study 2 on effects of over 21 years in paraffin block (FFPE) relative to frozen (FR). (A) Correlations of the RNA sequencing output QDI with copies of similarly sized *Actb* amplicons located at increasing distances from the 3' end of *Actb* mRNA (5'-*Actb*<Mid-*Actb*<3'-*Actb*). (B) Correlations of the RNA sequencing output effective gene number with copies of similarly sized *Actb* amplicons located at increasing distances from the 3' end of *Actb* mRNA (5'-*Actb*<Mid-*Actb*<3'-*Actb*). Offset of 0.01 applied to detections of zero copies so that samples with zero were still included in figure following the \log_{10} scaling. ρ indicates correlation coefficient for spearman test on only FFPE samples (* indicates p-value <0.05).

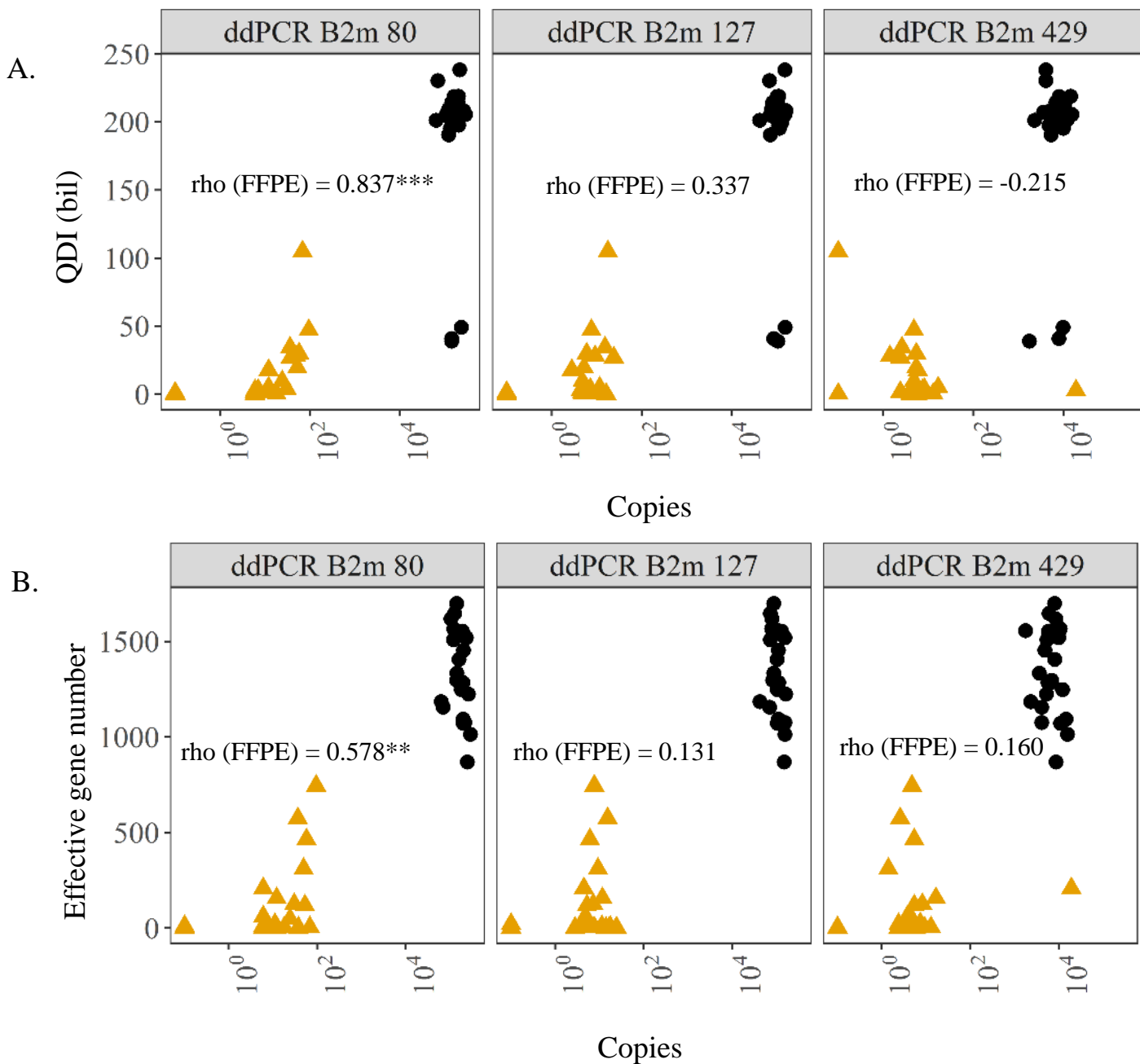


Figure S6. Study 2 on effects of over 21 years in paraffin block (FFPE) relative to frozen (FR). (A) Correlations of RNA sequencing output QDI with copies of *B2m* amplicons ranging in size (80, 127, and 429 base pairs) (B) Correlations of RNA sequencing output effective gene number and copies of *B2m* amplicons ranging in size (80, 127, and 429 base pairs). Offset of 0.01 applied to detections of zero copies so that samples with zero were still included in figure following the \log_{10} scaling. ρ indicates correlation coefficient for spearman test on only FFPE samples (** indicates p-value <0.01, *** p-value < 0.001).

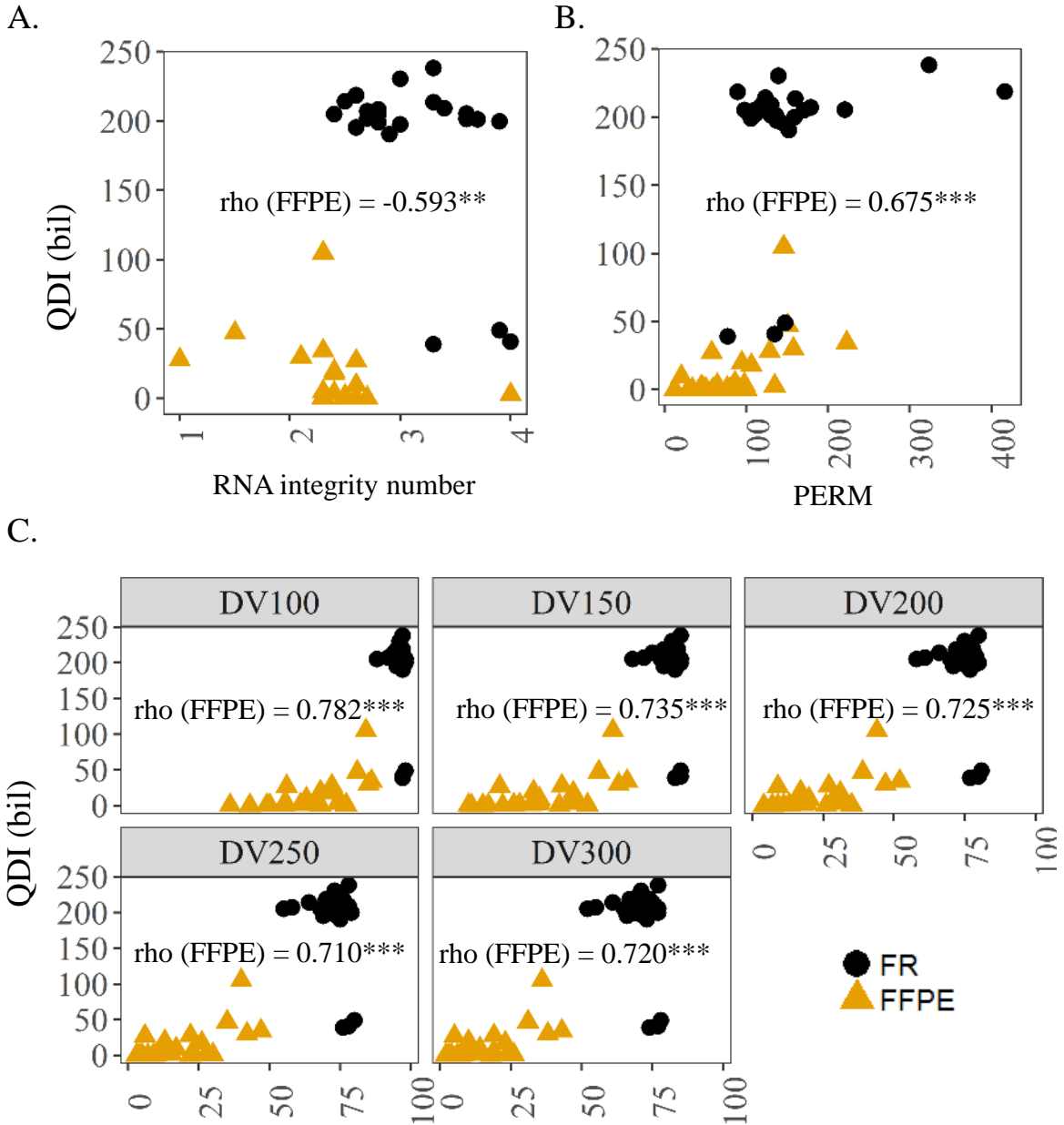


Figure S7. Study 2 on the effects of over 21 years in paraffin block (FFPE) relative to frozen (FR). Correlations between select pre sequencing quality metrics and the combined RNA sequencing output of total genes counted multiplied by the amount of unique genes identified in the billions. A) RNA integrity number (RIN). B) Paraffin embedded RNA metric (PERM). C) Fragment analysis (DV) or percent of RNA fragments greater than 100, 150, 200, 250, or 300 nucleotides. rho indicates correlation coefficient for spearman test on only FFPE samples (** indicates p-value <0.01, *** indicates p-value <0.001).

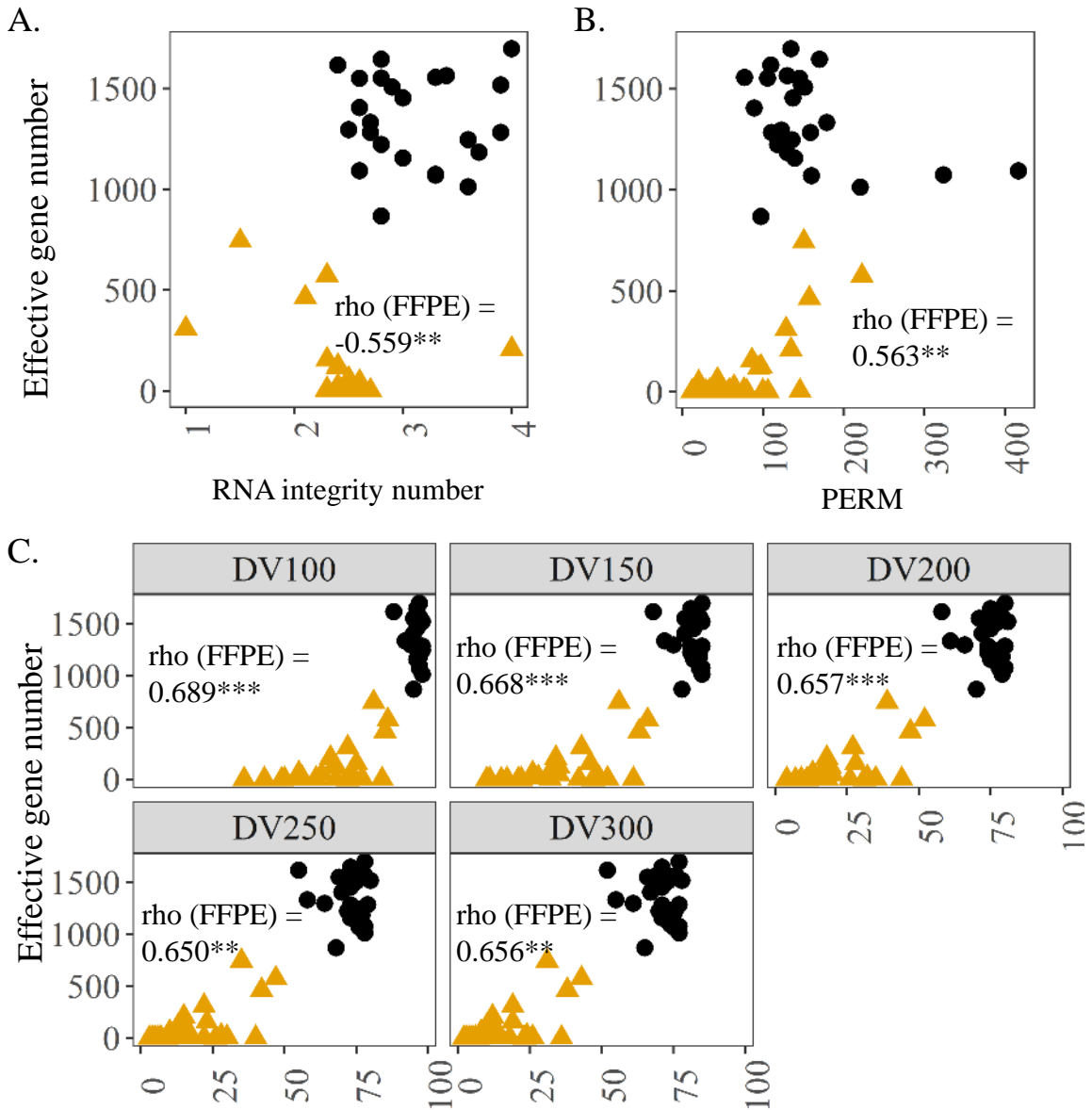
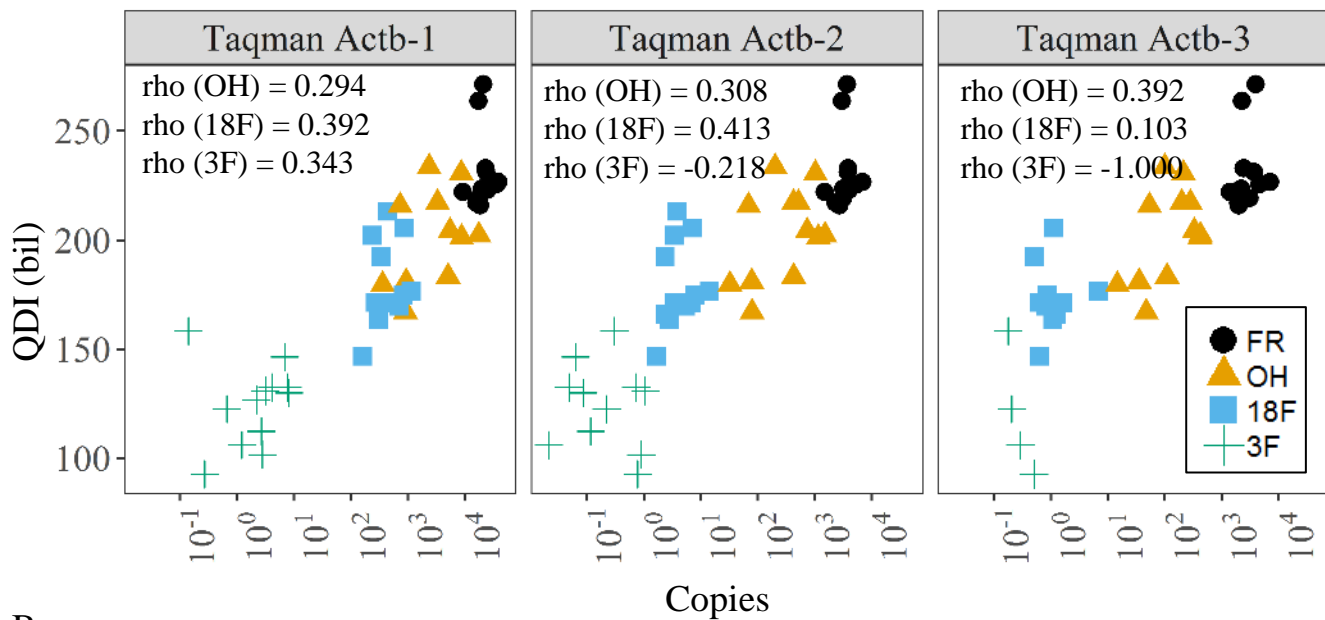


Figure S7. Study 2 on the effects of >21 years in paraffin block (FFPE) relative to frozen (FR) on correlations between select pre sequencing quality metrics and the RNA sequencing output effective gene number. (A) RNA integrity number (RIN). (B) Paraffin embedded RNA metric (PERM). (C) Fragment analysis (DV) or percent of RNA fragments greater than 100, 150, 200, 250, or 300 nucleotides. r indicates correlation coefficient for spearman test on only FFPE samples (** indicates p -value <0.01, *** indicates p -value <0.001).

A.



B.

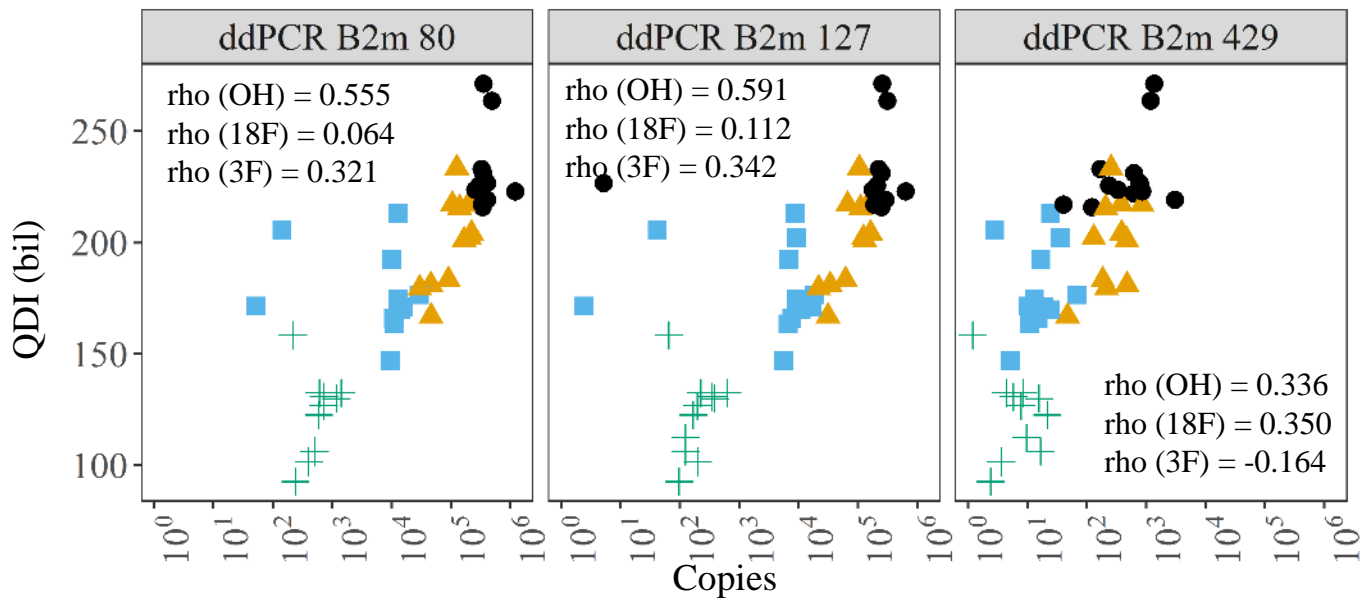
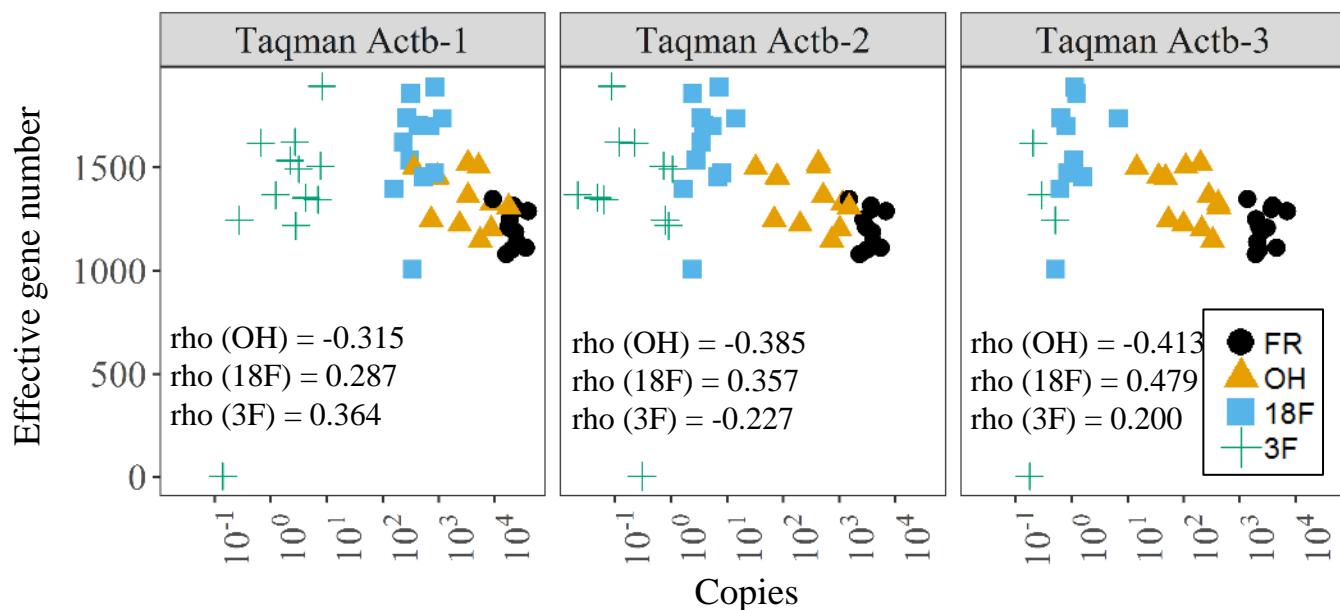


Figure S8. Study 3 on the effects of fixation and time in formalin relative to frozen (FR). Correlations between quantifiable RNA by digital drop PCR on two house keeping genes (*Actb* and *B2m*) and the combined RNA sequencing output of total genes counted multiplied by the amount of unique genes identified in the billions (QDI). Paired preservation groups as follows: 70% ethanol (OH) for 3 months, 18 hr. 10% formalin then 70% ethanol to 3 months (18F), or 3 months in 10% formalin (3F). (A) Copies of similarly sized *Actb* amplicons located at increasing distances from the 3' end of *Actb* mRNA (5'-*Actb*<*Mid-Actb*<3'-*Actb*). (B) Copies of *B2m* amplicons ranging in size (80, 127, and 429 base pairs) and location of amplification across *B2m* mRNA. rho indicates correlation coefficient for spearman test on each OH, 18F, and 3F samples separately (*p-value <0.05)

A.



B.

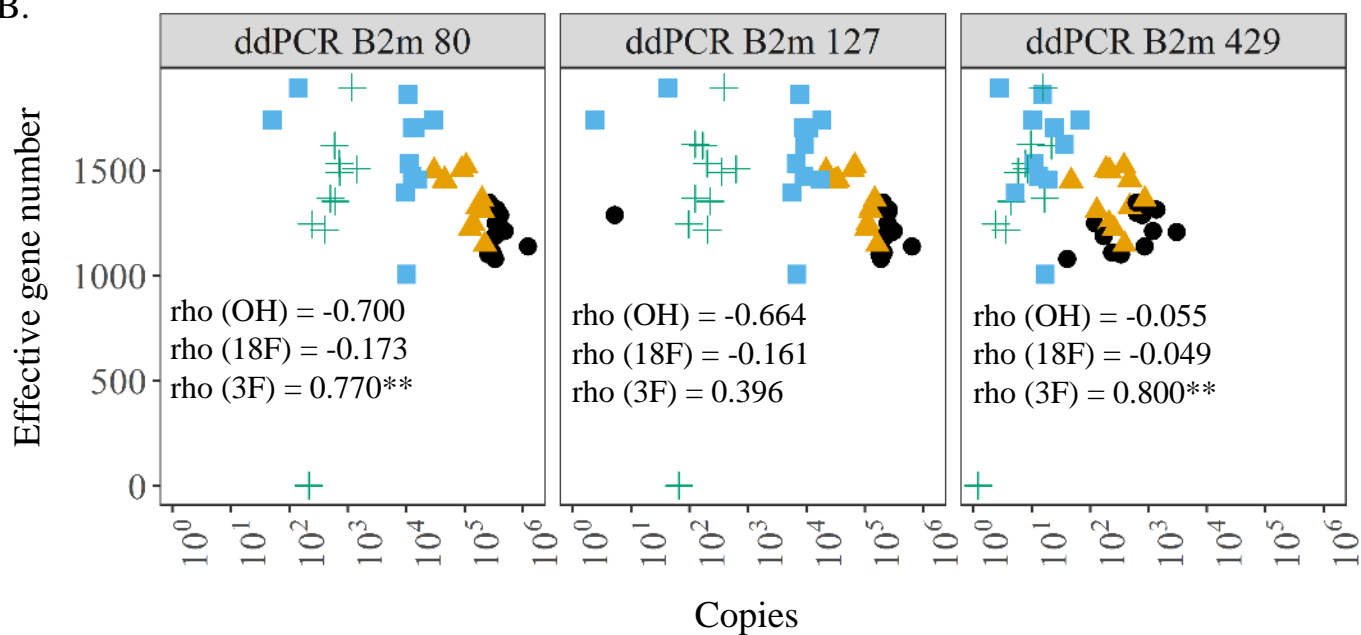
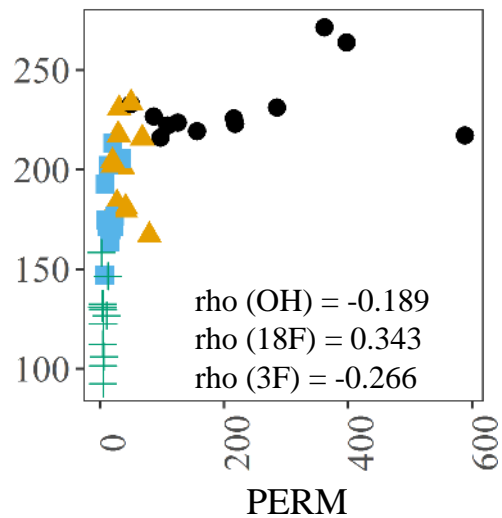
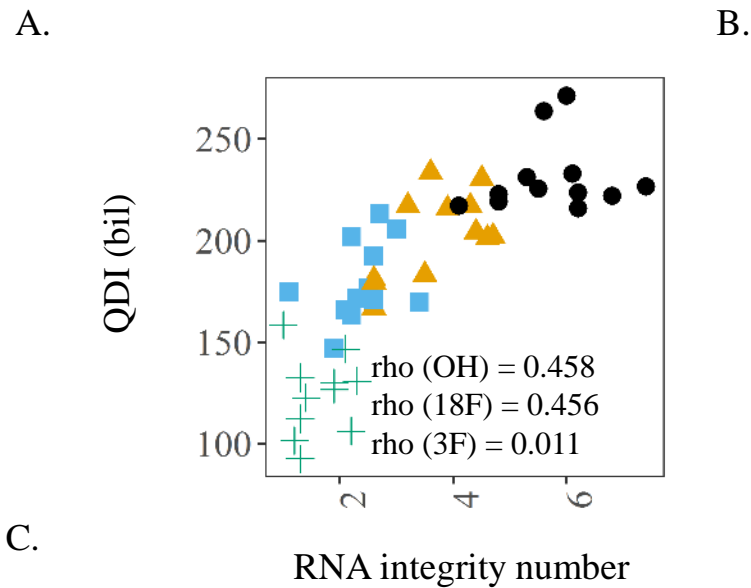


Figure S9. Study 3 on the effects of fixation and time in formalin relative to frozen (FR). Correlations between quantifiable RNA by digital drop PCR on two housekeeping genes (*Actb* and *B2m*) and the RNA sequencing output of effective gene number. Paired preservation groups as follows: 70% ethanol (OH) for 3 months, 18 hr. 10% formalin then 70% ethanol to 3 months (18F), or 3 months in 10% formalin (3F). (A) Copies of similarly sized *Actb* amplicons located at increasing distances from the 3' end of *Actb* mRNA (5'-*Actb*<Mid-*Actb*<3'-*Actb*). (B) Copies of *B2m* amplicons ranging in size (80, 127, and 429 base pairs) and location of amplification across *B2m* mRNA. rho indicates correlation coefficient for spearman test on each OH, 18F, and 3F samples separately (**p-value < 0.01)



C.

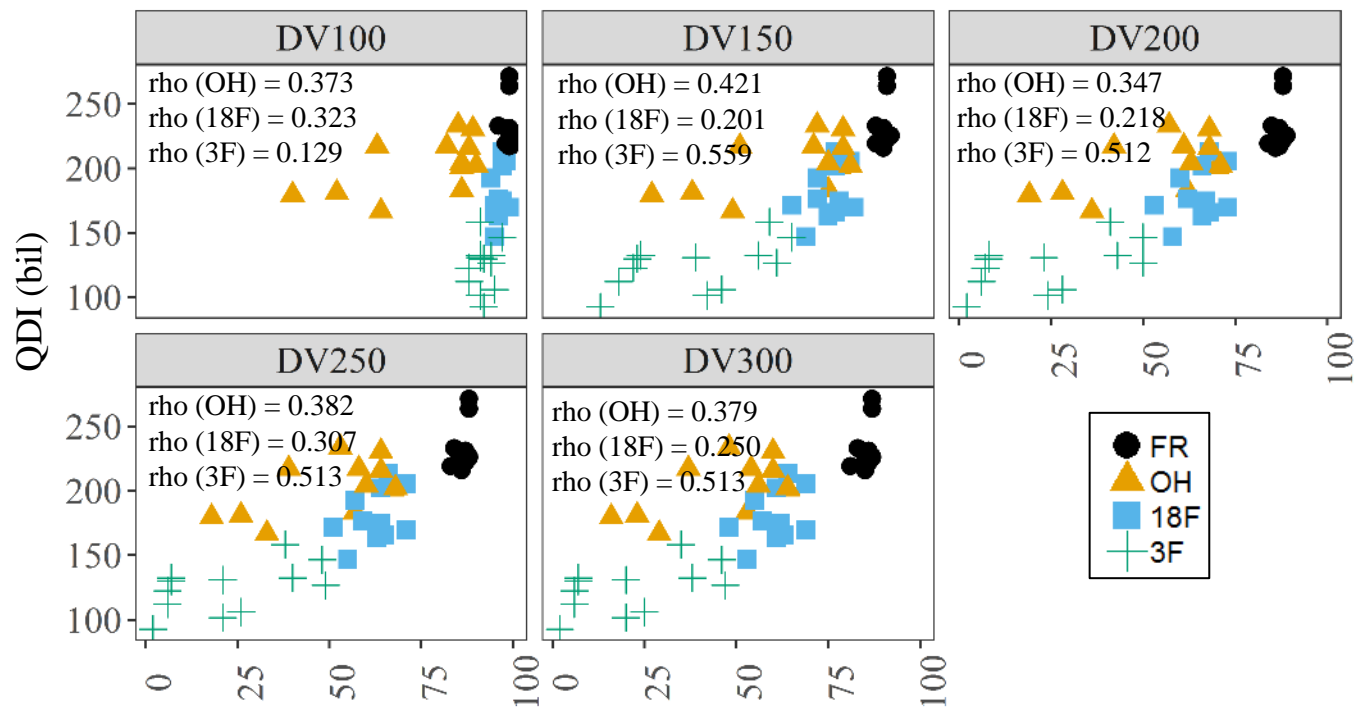
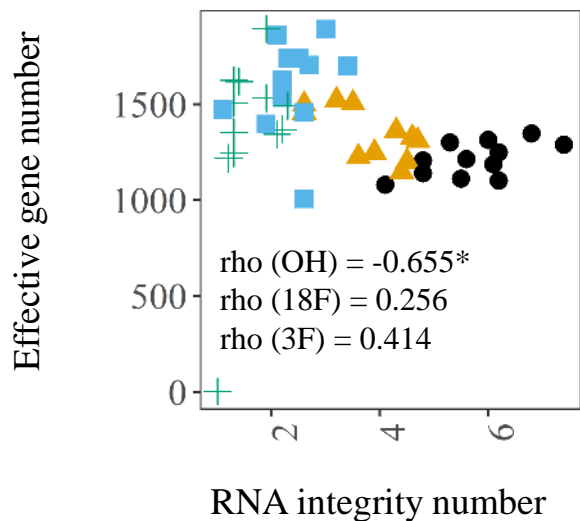
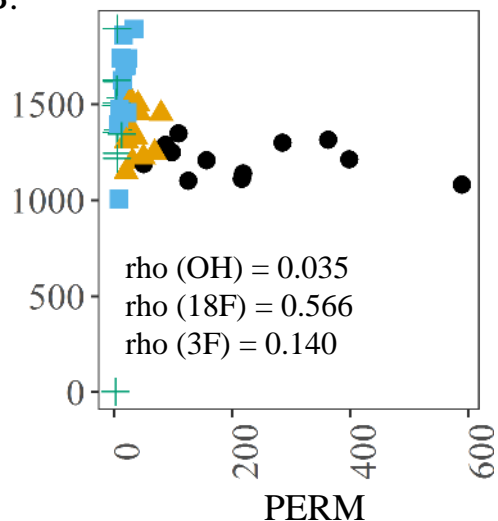


Figure S10. Study 3 on the effects of fixation and time in formalin relative to frozen (FR) controls. Correlations between select pre-sequencing quality metrics and the combined RNA sequencing output of total genes counted multiplied by the amount of unique genes identified in the billions. Paired preservation groups as follows: 70% ethanol (OH) for 3 months, 18 hr. 10% formalin then 70% ethanol to 3 months (18F), or 3 months in 10% formalin (3F). (A) RNA integrity number (RIN). (B) Paraffin embedded RNA metric (PERM). (C) Fragment analysis (DV) or percent of RNA fragments greater than 100, 150, 200, 250, or 300 nucleotides. ρ indicates correlation coefficient for spearman test on only FFPE samples (* indicates p-value <0.05).

A.



B.



C.

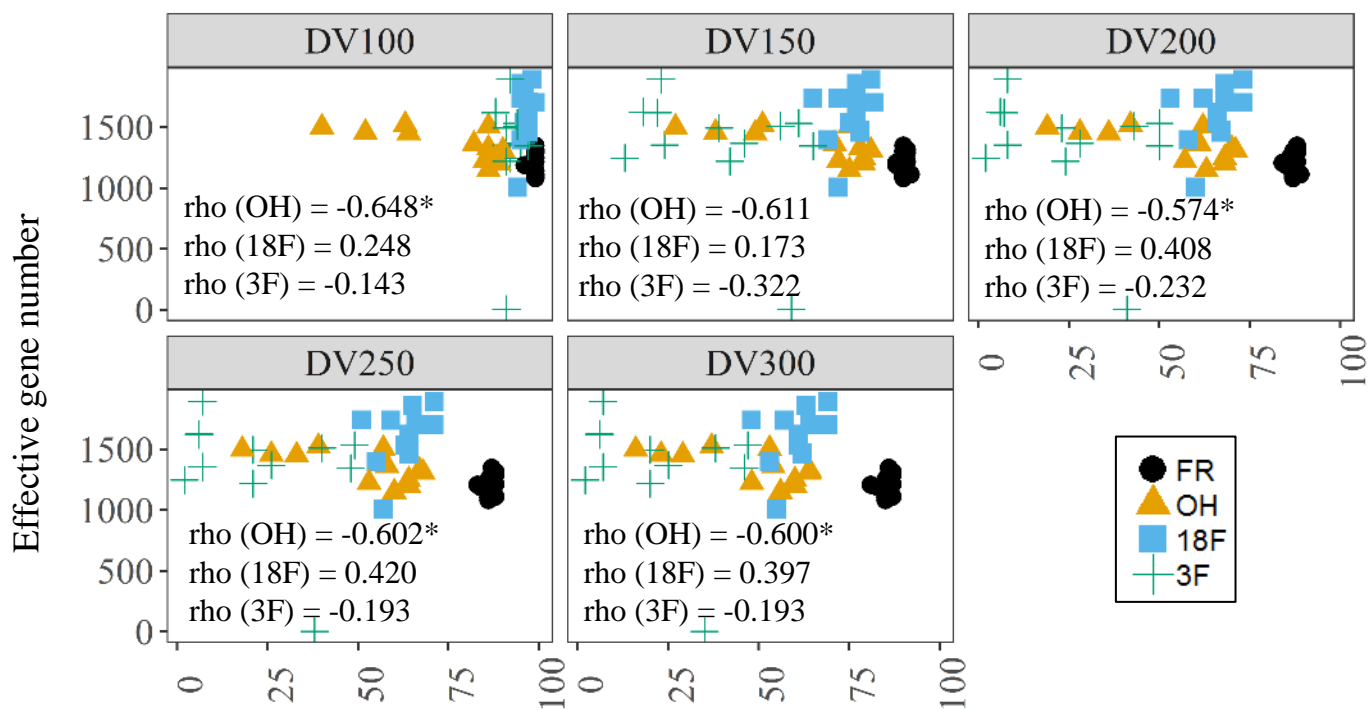
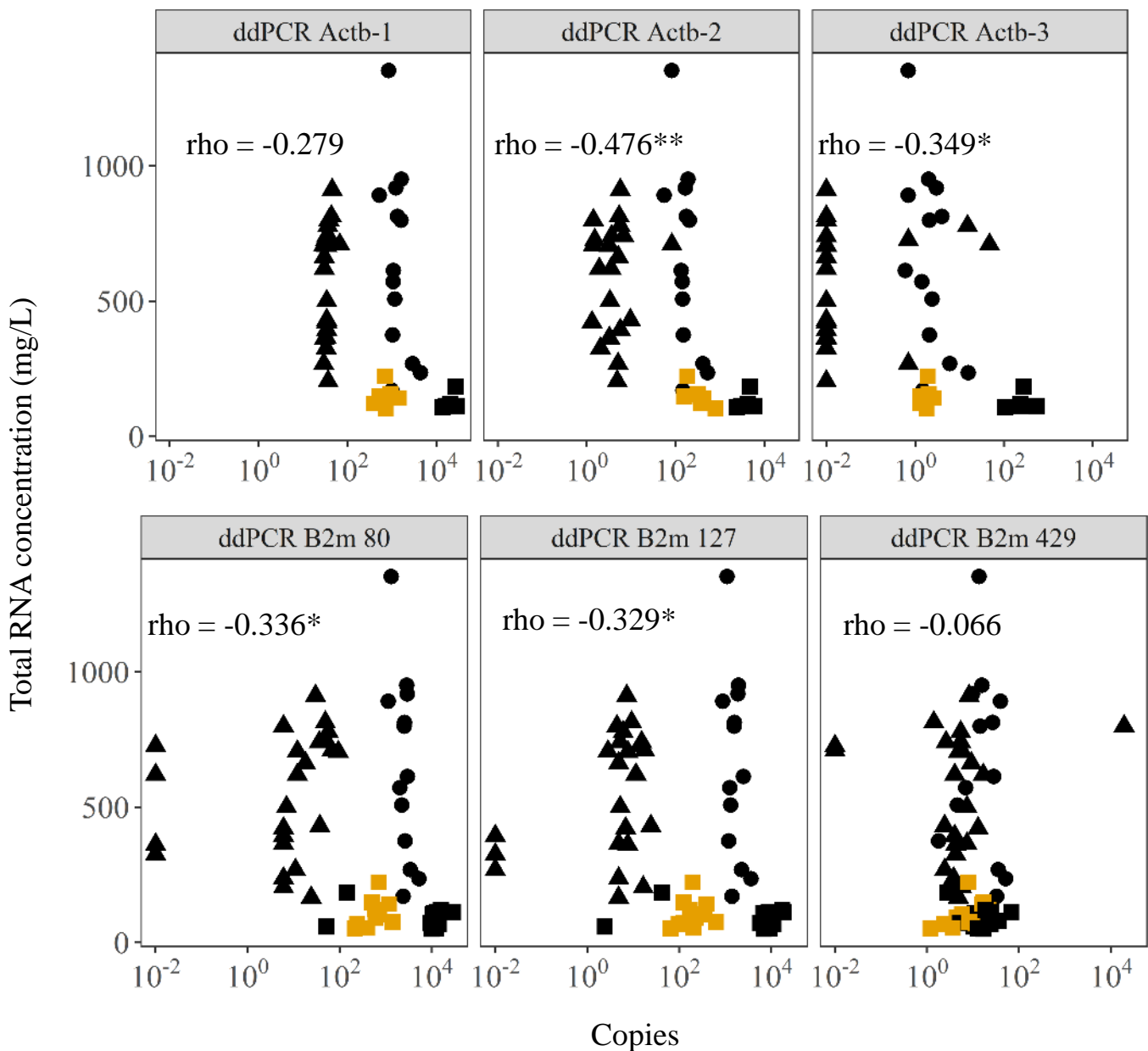


Figure S11. Study 3 on the effects of fixation and time in formalin relative to frozen (FR) controls. Correlations between select pre-sequencing quality metrics and the RNA sequencing output of effective gene number. Paired preservation groups as follows: 70% ethanol (OH) for 3 months, 18 hr. 10% formalin then 70% ethanol to 3 months (18F), or 3 months in 10% formalin (3F). (A) RNA integrity number (RIN). (B) Paraffin embedded RNA metric (PERM). (C) Fragment analysis (DV) or percent of RNA fragments greater than 100, 150, 200, 250, or 300 nucleotides. rho indicates correlation coefficient for spearman test on only FFPE samples (* indicates p-value <0.05).

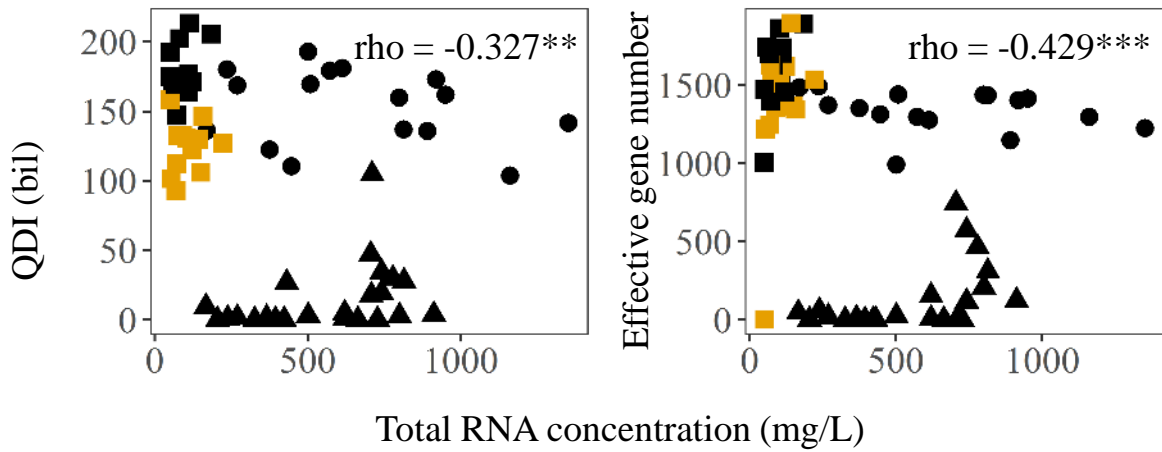
End of proposed manuscript figures. Two additional figure considerations...

Potential additional figures???



- Study 1 - 2 yr.
- ▲ Study 2 - 20 yr.
- Study 3 - Time in formalin

Black indicates 18 hr.
formalin fixation
Yellow indicates 3 mo.
formalin fixation



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