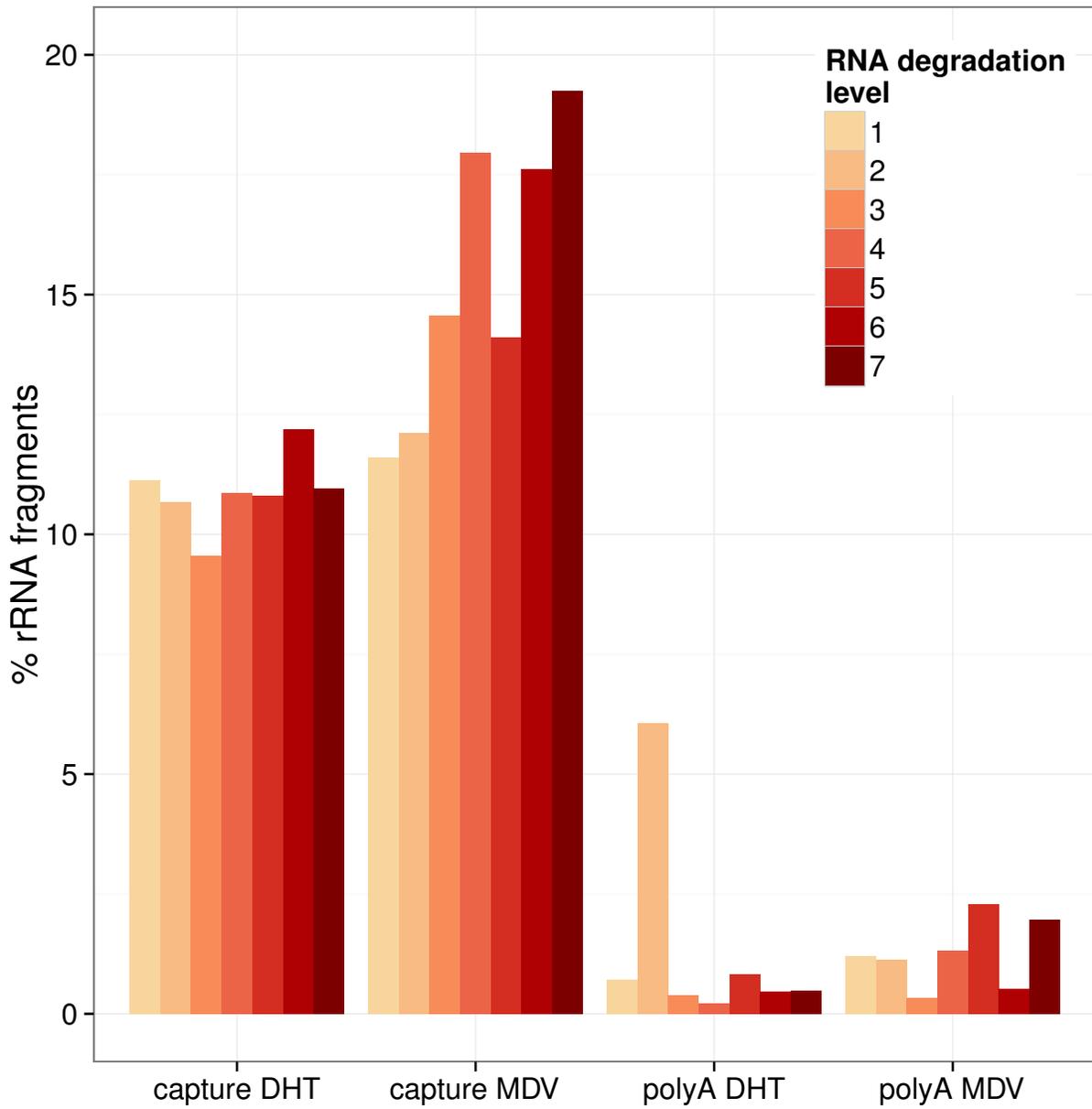
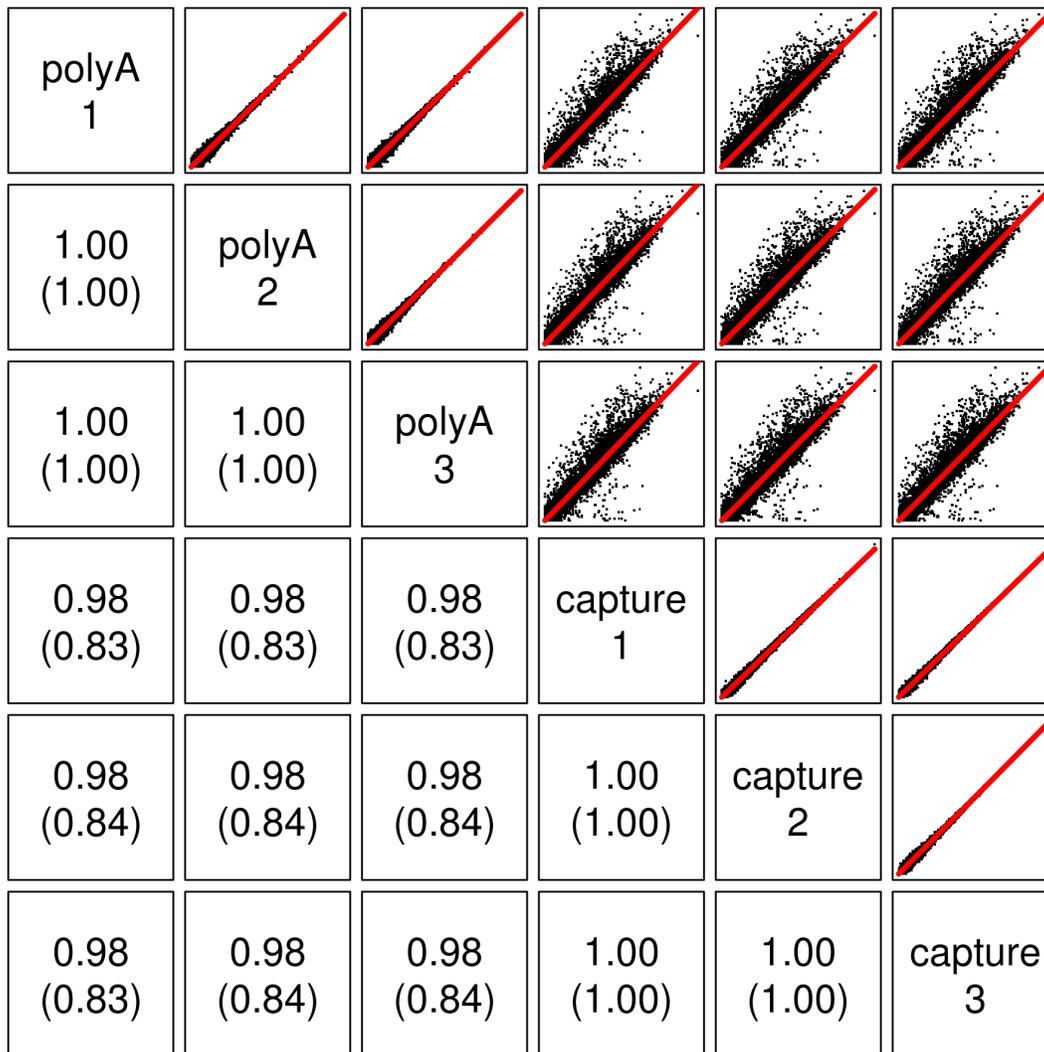


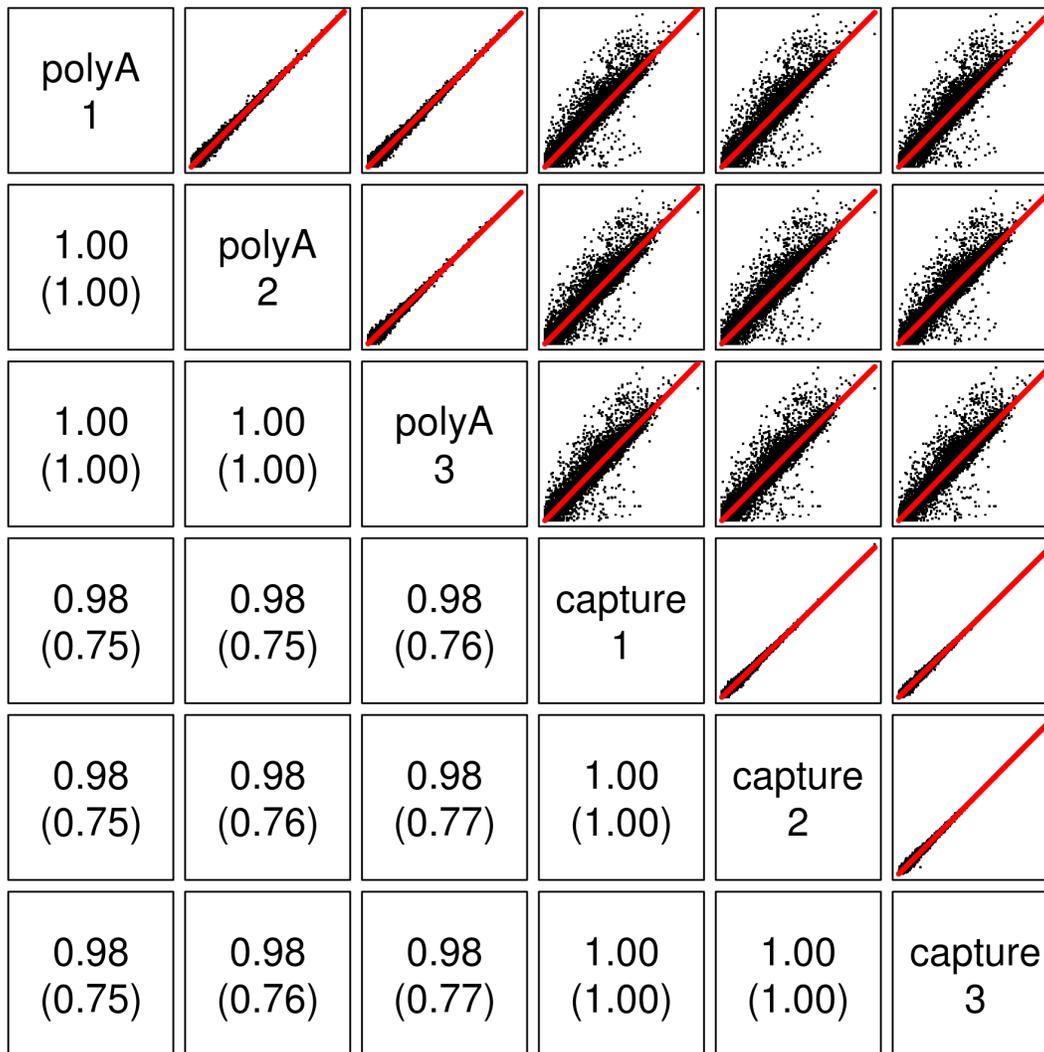
**Figure S1:** Correspondence of RNA degradation levels and RNA integrity numbers. Each point represents a library. Libraries with the same RNA degradation level were prepared from the same RNA isolate.



**Figure S2:** Role of RNA degradation levels on efficiency of rRNA depletion. Percentage of rRNA fragments was determined as function of RNA degradation level, library preparation protocol and treatment.

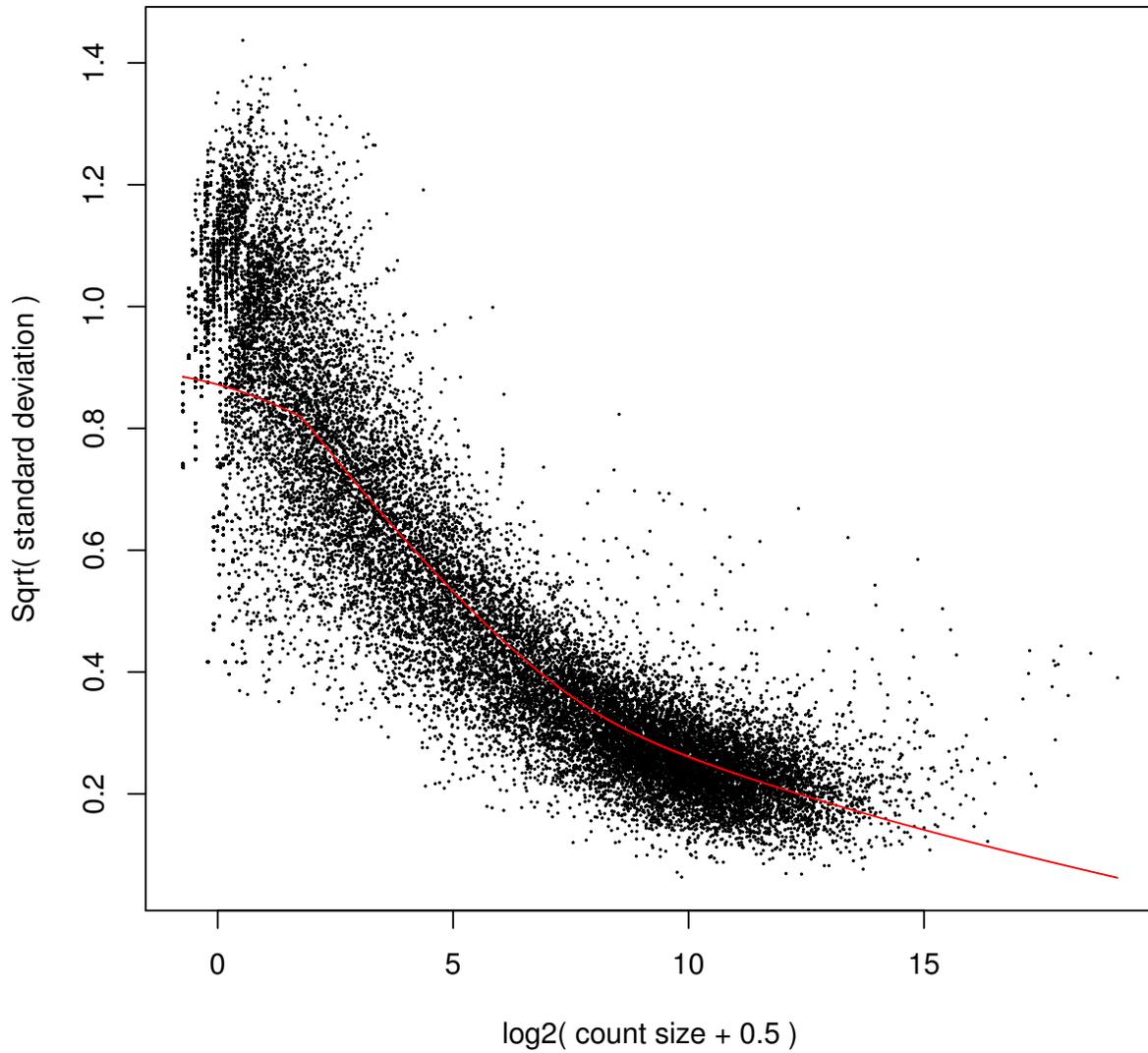


**Figure S3:** Technical reproducibility of DHT-treated libraries. Numbers in the lower panel are correlation coefficients of log<sub>2</sub>-transformed counts per million (CPM) that are plotted in the upper panel. Numbers in parentheses are correlation coefficients of un-transformed CPMs.



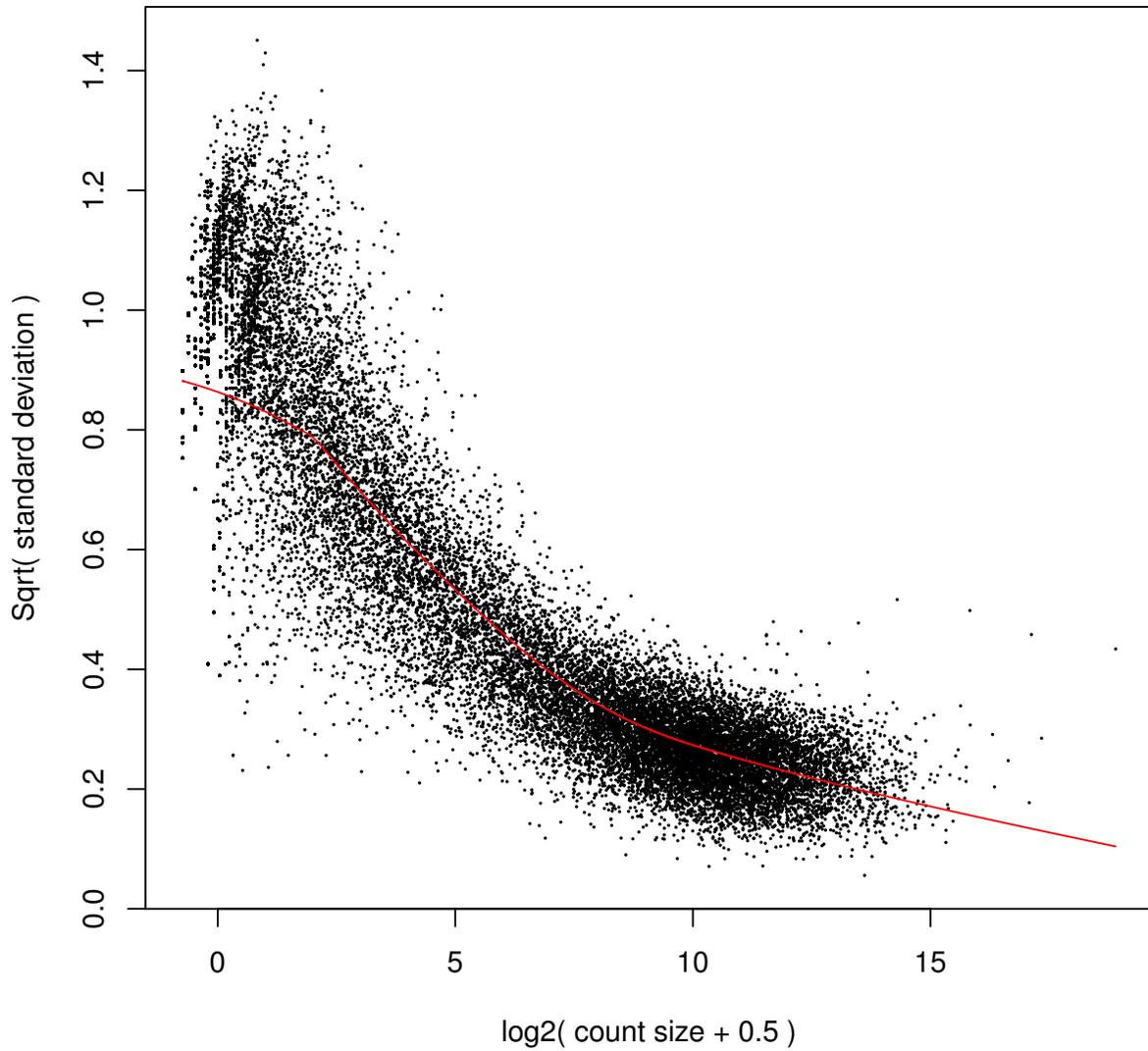
**Figure S4:** Technical reproducibility of MDV3100-treated libraries. Numbers in the lower panel are correlation coefficients of log<sub>2</sub>-transformed counts per million (CPM) that are plotted in the upper panel. Numbers in parentheses are correlation coefficients of un-transformed CPMs.

### voom: Mean-variance trend

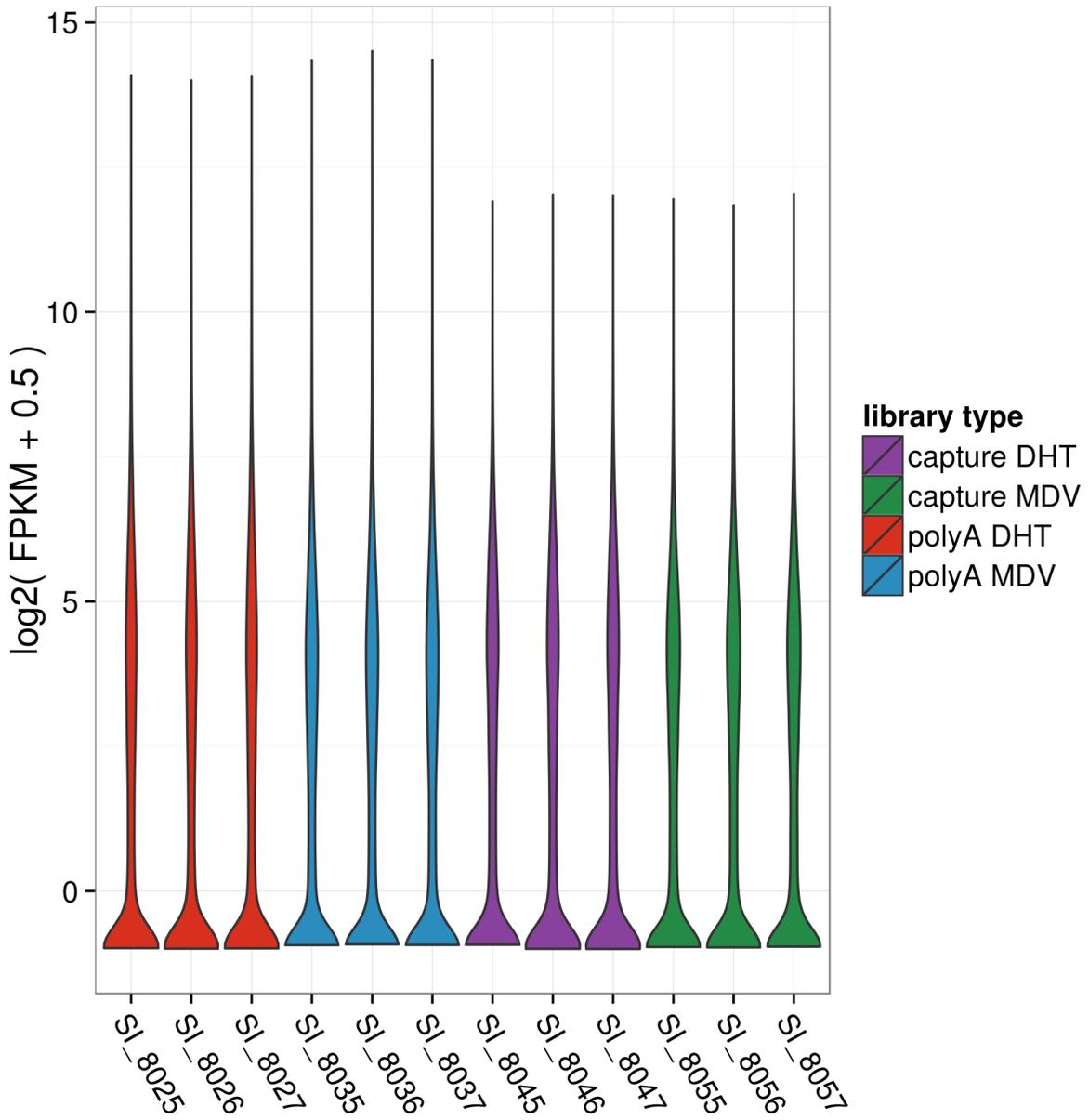


**Figure S5:** Mean-variance trend - poly(A) libraries. Shown is the trend between the average number of fragments assigned to a gene and the precision of measurement in technical replicates.

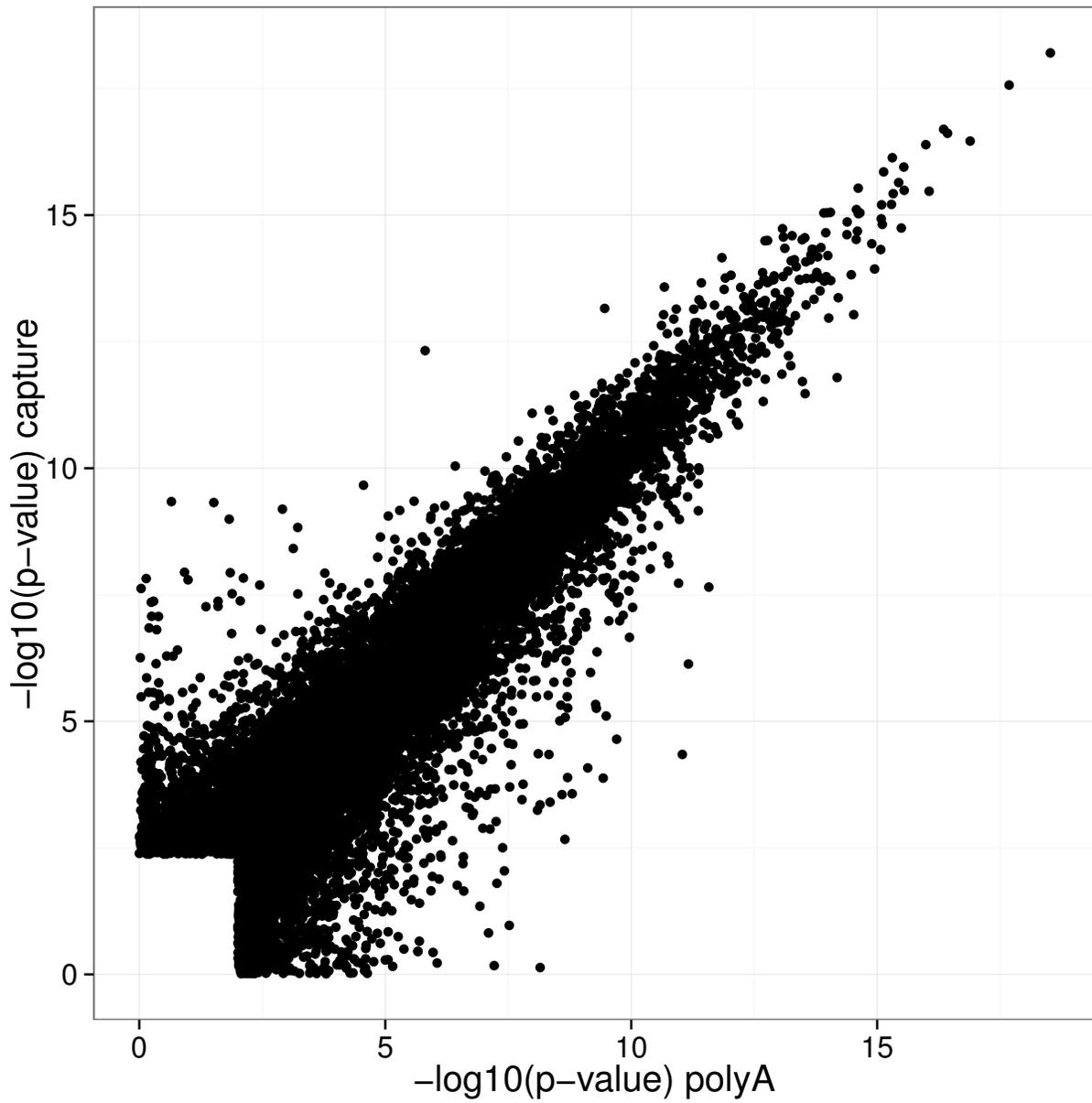
### voom: Mean-variance trend



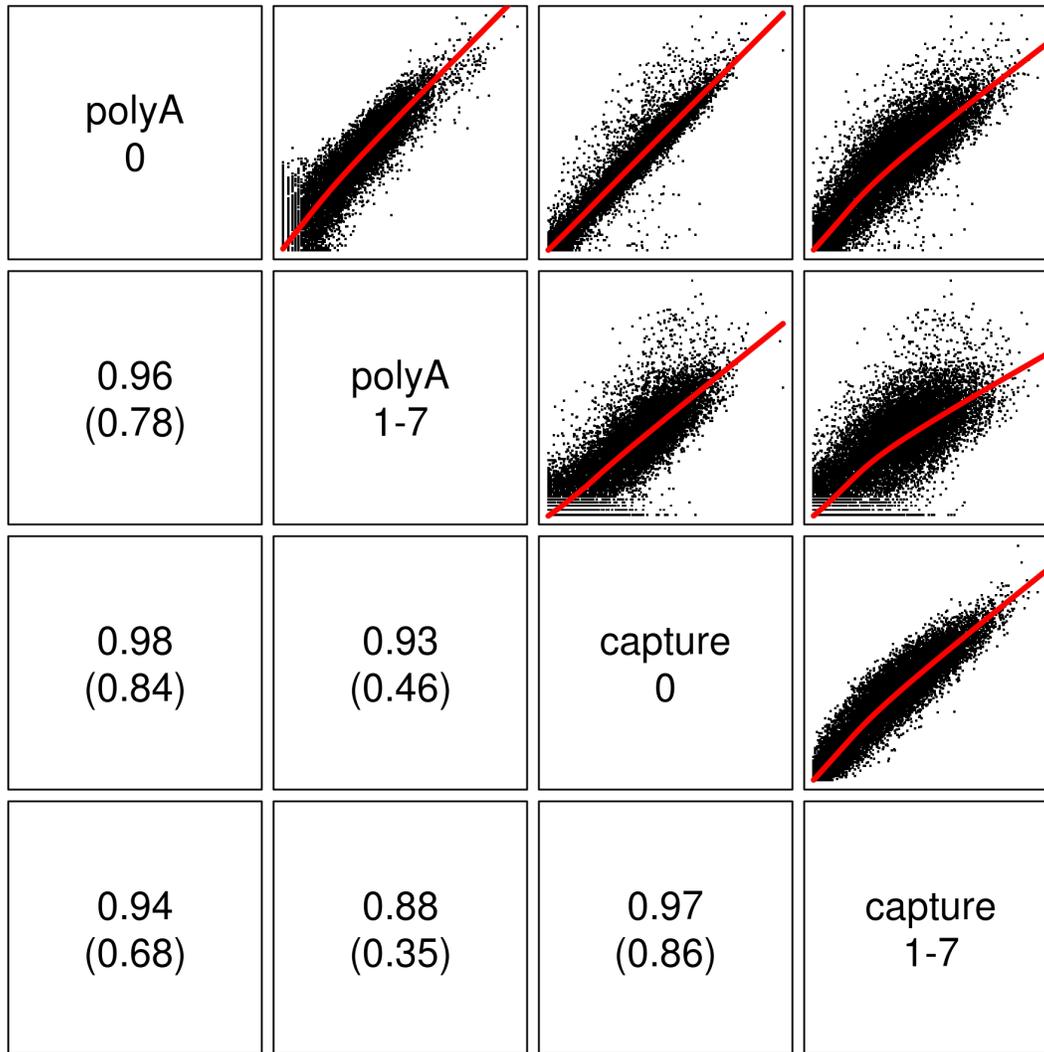
**Figure S6:** Mean-variance trend - capture libraries. Shown is the trend between the average number of fragments assigned to a gene and the precision of measurement in technical replicates.



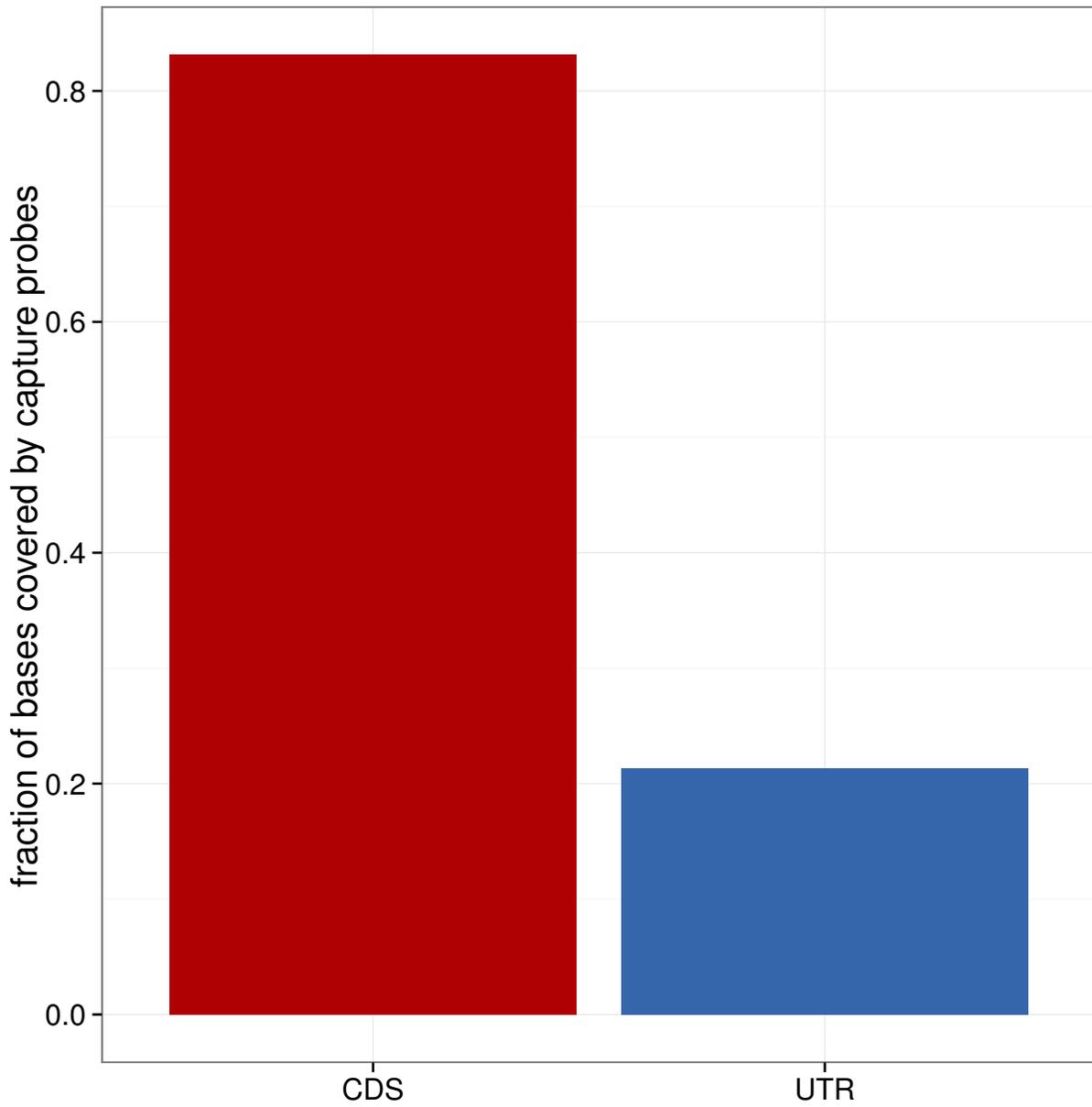
**Figure S7:** Dynamic range of gene expression levels. Violin plots of observed gene-level expression FPKMs by library type and treatment.



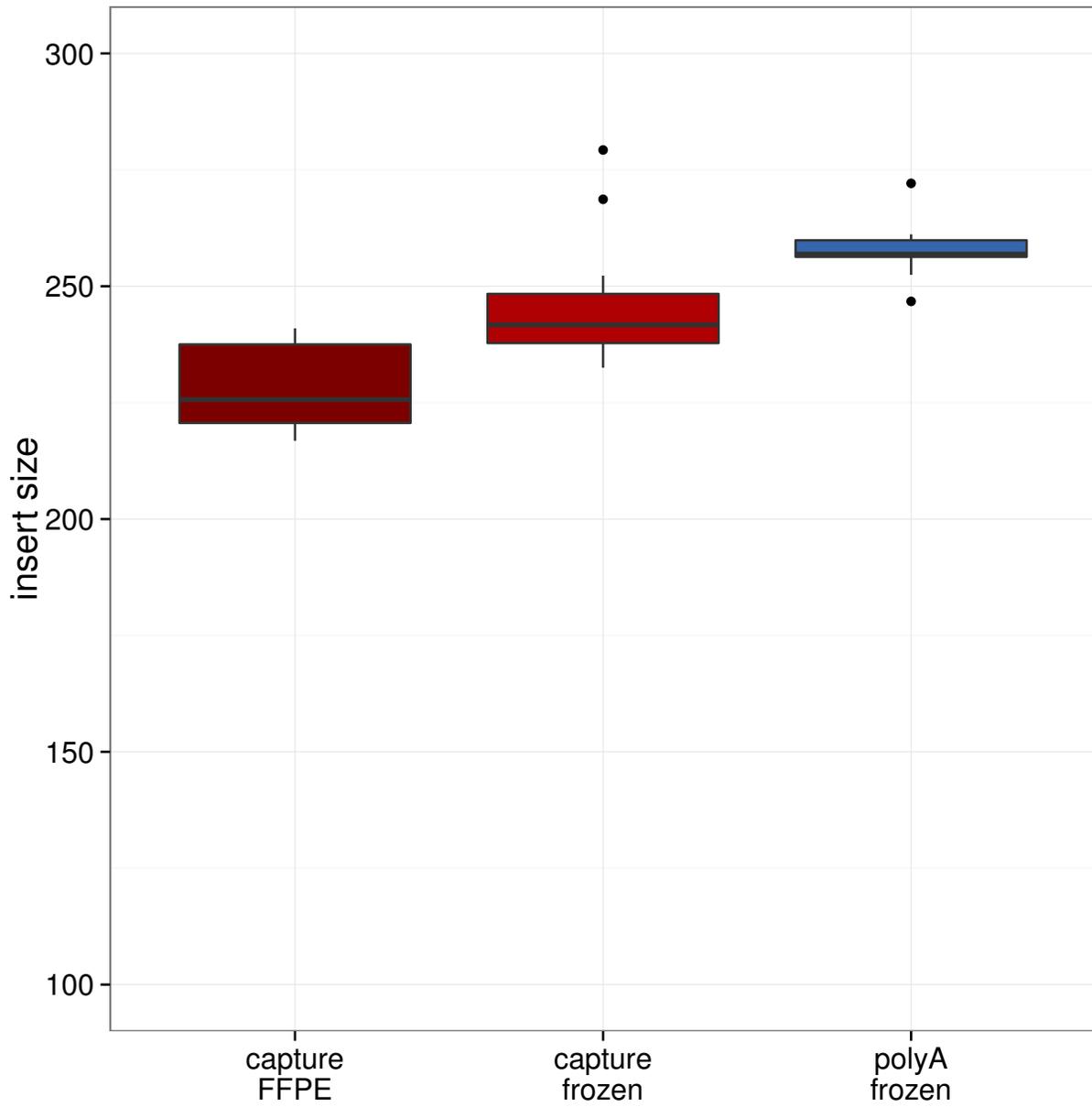
**Figure S8:** Correlation of significance,  $-\log_{10}(\text{p-value})$ , of differential expression DHT vs MDV (Enzalutamide) treatment.



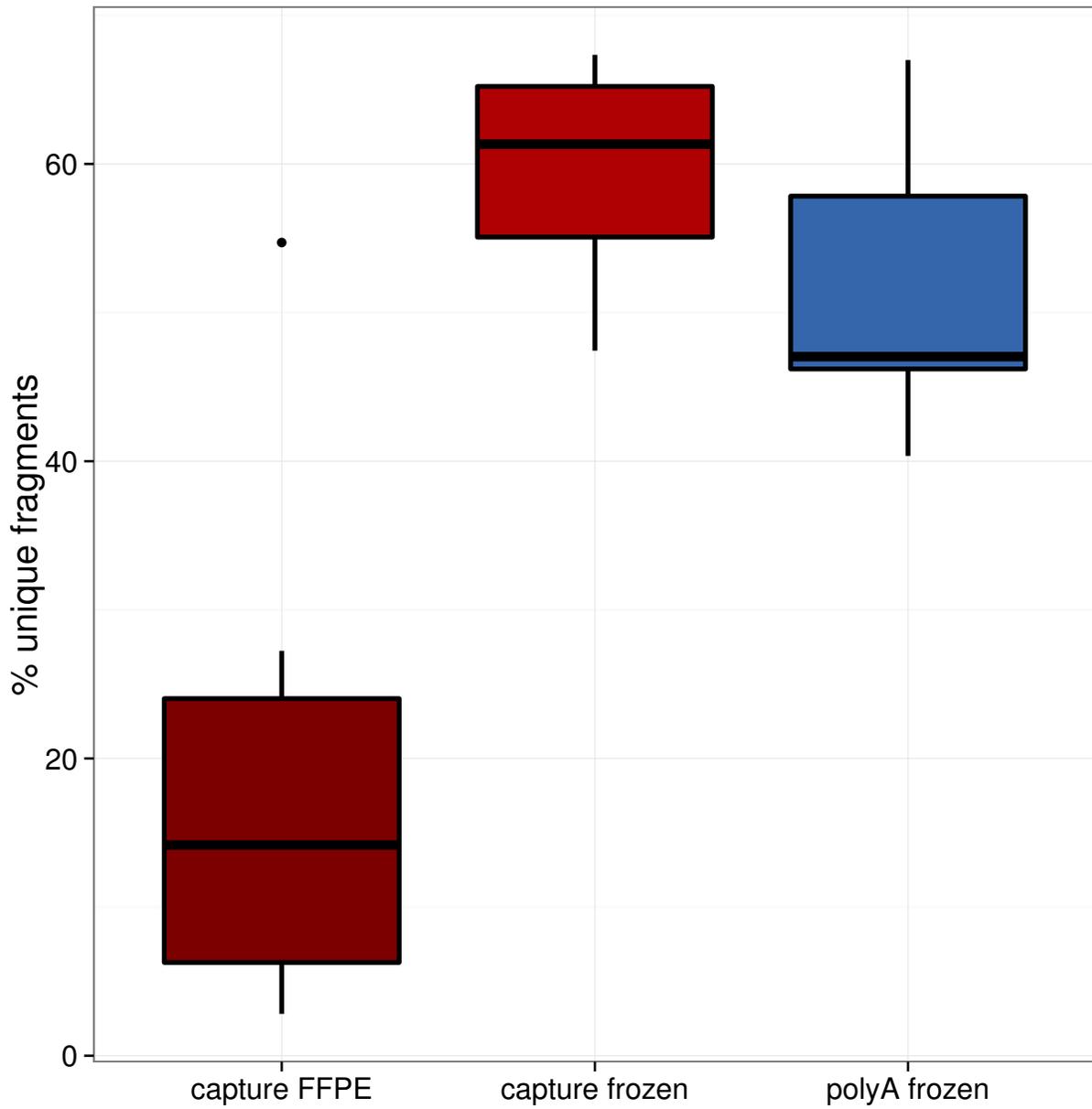
**Figure S9:** Effect of RNA degradation on the reproducibility libraries from DHT-treated VCaP cells. Numbers on the diagonal indicate RNA degradation levels.



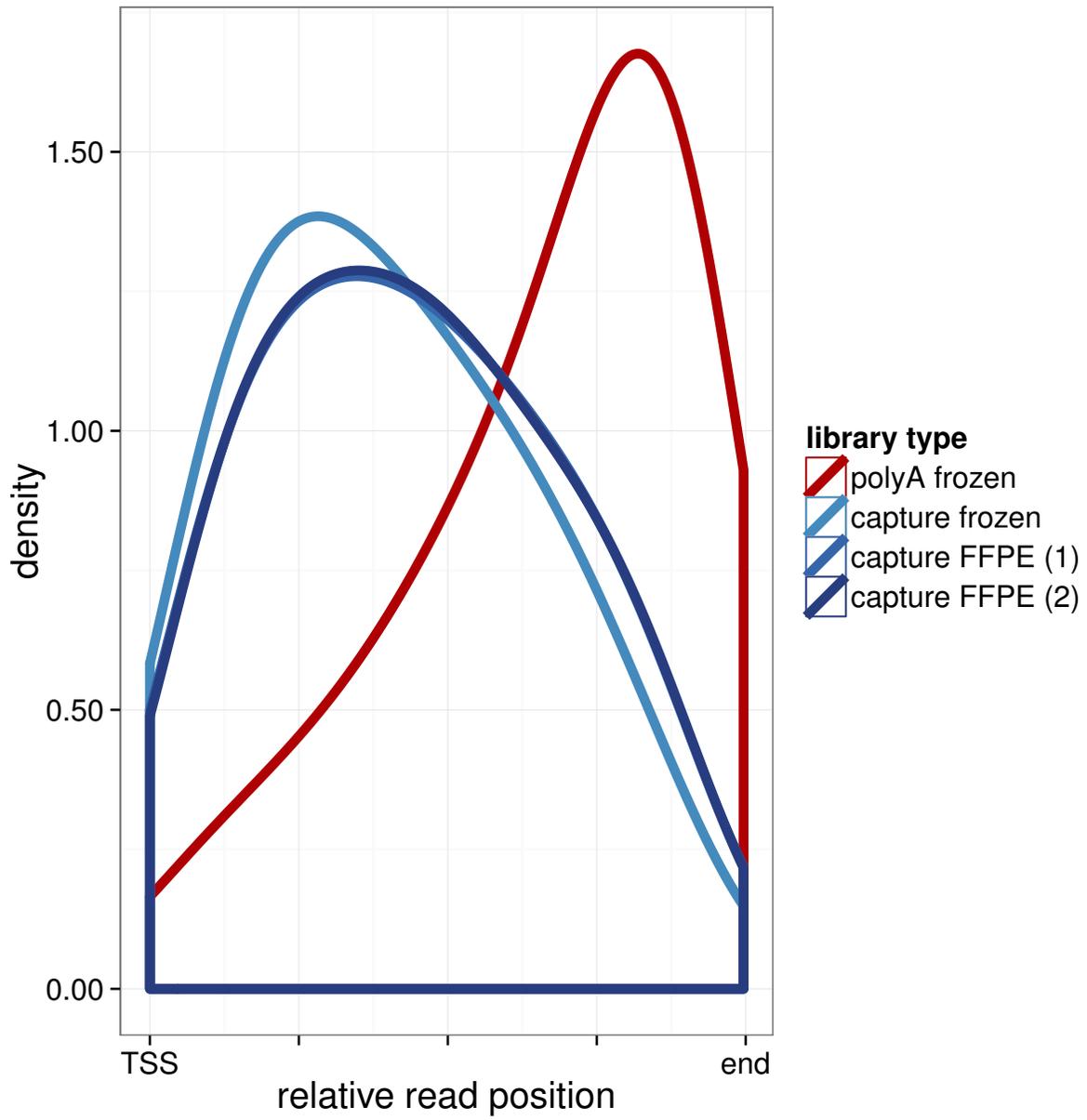
**Figure S10:** Fraction of covered bases of coding (CDS) and untranslated regions (UTR) by the capture probes.



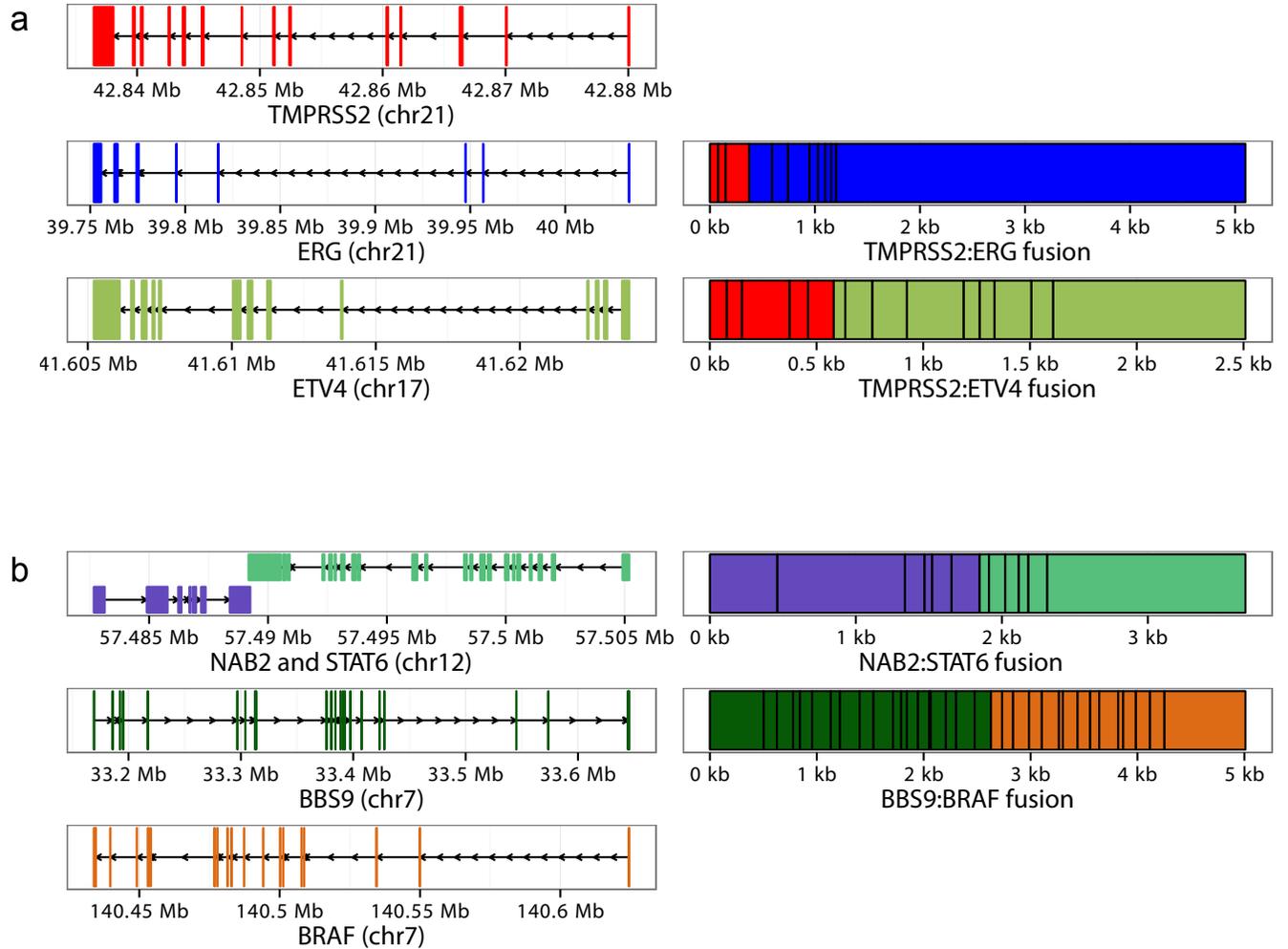
**Figure S11:** Distribution of fragment (insert) sizes by library type and source material (rapid autopsy cohort).



**Figure S12:** Library complexity as measured by the percentage of uniquely mapping fragments by library type and source material (rapid autopsy cohort).



**Figure S13:** Distribution of fragments along gene within multiple libraries from a single patient (rapid autopsy cohort).



**Figure S14:** (a) ETS-fusions in prostate cancer. The TMPRSS2-ERG fusion is the most common variant and involves the AR regulated TMPRSS2 and the ERG oncogenic transcription factor. A rare variant involving ETV4, a homologue of ERG, was found in one of the FFPE specimens. (b) Oncogenic fusions in a single aggressive case of solitary fibrous tumor (SFT). NAB2-STAT6 is the defining oncogenic fusion in SFT. The BBS9-BRAF fusion retains the kinase domain of BRAF and is likely translated and activating.

	gene_id	gene_name	gene_type	logFC	P.Value
1	ENSG00000198558.2	HIST1H4L	protein_coding	9.30	0.00
2	ENSG00000239002.2	SCARNA10	sense_intronic	9.26	0.00
3	ENSG00000124693.2	HIST1H3B	protein_coding	9.17	0.00
4	ENSG00000124529.3	HIST1H4B	protein_coding	8.98	0.00
5	ENSG00000270066.2	SCARNA2	lincRNA	8.80	0.00
6	ENSG00000252010.1	SCARNA5	snoRNA	8.57	0.00
7	ENSG00000182572.2	HIST1H3I	protein_coding	8.44	0.00
8	ENSG00000198518.5	HIST1H4E	protein_coding	8.31	0.00
9	ENSG00000184270.3	HIST2H2AB	protein_coding	8.21	0.00
10	ENSG00000251791.1	SCARNA6	snoRNA	7.63	0.00
11	ENSG00000196176.7	HIST1H4A	protein_coding	7.61	0.00
12	ENSG00000238741.1	SCARNA7	snoRNA	7.26	0.00
13	ENSG00000197061.3	HIST1H4C	protein_coding	7.25	0.00
14	ENSG00000198366.5	HIST1H3A	protein_coding	7.25	0.00
15	ENSG00000168298.4	HIST1H1E	protein_coding	7.19	0.00
16	ENSG00000256316.1	HIST1H3F	protein_coding	7.07	0.00
17	ENSG00000124575.5	HIST1H1D	protein_coding	7.04	0.00
18	ENSG00000197837.3	HIST4H4	protein_coding	6.90	0.00
19	ENSG00000188394.6	GPR21	protein_coding	6.35	0.00
20	ENSG00000184825.4	HIST1H2AH	protein_coding	6.21	0.00
21	ENSG00000196532.4	HIST1H3C	protein_coding	5.91	0.00
22	ENSG00000270141.2	TERC	lincRNA	5.90	0.00
23	ENSG00000137259.2	HIST1H2AB	protein_coding	5.69	0.00
24	ENSG00000252835.1	SCARNA21	snoRNA	5.66	0.00
25	ENSG00000196226.2	HIST1H2BB	protein_coding	5.29	0.00
26	ENSG00000239039.1	SNORD13	snoRNA	5.16	0.00
27	ENSG00000196787.2	HIST1H2AG	protein_coding	5.04	0.00
28	ENSG00000158406.2	HIST1H4H	protein_coding	4.99	0.00
29	ENSG00000254911.2	SCARNA9	antisense	4.92	0.00
30	ENSG00000200354.1	SNORA71D	snoRNA	4.80	0.00
31	ENSG00000120329.4	SLC25A2	protein_coding	4.68	0.00
32	ENSG00000228526.2	RP3-510D11.1	lincRNA	4.67	0.00
33	ENSG00000245532.4	NEAT1	lincRNA	4.66	0.00
34	ENSG00000221771.1	MIR1205	miRNA	4.66	0.00
35	ENSG00000213132.2	AC022498.1	protein_coding	4.64	0.00
36	ENSG00000197409.6	HIST1H3D	protein_coding	4.60	0.00
37	ENSG00000251992.1	SCARNA17	snoRNA	4.57	0.00
38	ENSG00000197714.4	ZNF460	protein_coding	4.50	0.00
39	ENSG00000207137.1	SNORD116-13	snoRNA	4.50	0.00
40	ENSG00000197846.3	HIST1H2BF	protein_coding	4.44	0.00
41	ENSG00000168274.3	HIST1H2AE	protein_coding	4.42	0.00
42	ENSG00000228768.1	AC003101.1	protein_coding	4.41	0.00
43	ENSG00000178404.5	DDC8	protein_coding	4.38	0.00
44	ENSG00000234188.1	AL121963.1	protein_coding	4.38	0.00
45	ENSG00000182611.3	HIST1H2AJ	protein_coding	4.36	0.00
46	ENSG00000184260.4	HIST2H2AC	protein_coding	4.33	0.00
47	ENSG00000200913.1	SNORD46	snoRNA	4.30	0.00
48	ENSG00000199032.1	MIR425	miRNA	4.30	0.00
49	ENSG00000209042.1	SNORD12C	snoRNA	4.26	0.00
50	ENSG00000196374.5	HIST1H2BM	protein_coding	4.20	0.00

**Table S1:** Genes with higher expression levels in capture vs poly(A) libraries.

	gene_id	gene_name	gene_type	logFC.polyA	logFC.capture
1	ENSG00000184012.7	TMPRSS2	protein_coding	4.00	3.39
2	ENSG00000163399.11	ATP1A1	protein_coding	2.87	2.51
3	ENSG00000135862.5	LAMC1	protein_coding	1.79	1.63
4	ENSG00000151503.8	NCAPD3	protein_coding	2.63	2.56
5	ENSG00000149428.14	HYOU1	protein_coding	2.23	2.08
6	ENSG00000155660.6	PDIA4	protein_coding	2.34	1.88
7	ENSG00000152377.8	SPOCK1	protein_coding	3.80	3.54
8	ENSG00000196091.8	MYBPC1	protein_coding	3.89	3.77
9	ENSG00000113716.8	HMGXB3	protein_coding	1.87	1.89
10	ENSG00000142515.10	KLK3	protein_coding	5.38	5.07
11	ENSG00000103942.8	HOMER2	protein_coding	2.21	1.73
12	ENSG00000112473.12	SLC39A7	protein_coding	1.94	1.68
13	ENSG00000134285.6	FKBP11	protein_coding	2.31	2.07
14	ENSG00000144481.12	TRPM8	protein_coding	2.87	2.50
15	ENSG00000149218.4	ENDOD1	protein_coding	2.98	2.85
16	ENSG00000150961.10	SEC24D	protein_coding	3.03	2.97
17	ENSG00000156113.16	KCNMA1	protein_coding	2.04	2.01
18	ENSG00000162174.8	ASRGL1	protein_coding	3.34	3.19
19	ENSG00000173482.12	PTPRM	protein_coding	2.65	2.63
20	ENSG00000114867.15	EIF4G1	protein_coding	0.88	0.76
21	ENSG00000187601.3	MAGEH1	protein_coding	4.11	3.53
22	ENSG00000157554.14	ERG	protein_coding	3.01	2.59
23	ENSG00000115758.8	ODC1	protein_coding	2.11	1.69
24	ENSG00000168280.12	KIF5C	protein_coding	1.66	1.56
25	ENSG00000138696.6	BMPR1B	protein_coding	2.36	2.17
26	ENSG0000019991.11	HGF	protein_coding	-4.70	-4.36
27	ENSG00000125266.6	EFNB2	protein_coding	-2.36	-2.05
28	ENSG00000135451.8	TROAP	protein_coding	-1.93	-1.86
29	ENSG00000138759.13	FRAS1	protein_coding	-2.35	-2.17
30	ENSG00000126562.12	WNK4	protein_coding	-4.43	-4.21
31	ENSG00000137642.8	SORL1	protein_coding	-1.93	-1.81
32	ENSG00000169083.11	AR	protein_coding	-2.15	-1.64
33	ENSG00000124406.12	ATP8A1	protein_coding	-1.90	-1.81
34	ENSG00000198901.9	PRC1	protein_coding	-1.70	-1.64
35	ENSG00000119866.16	BCL11A	protein_coding	-3.21	-3.15
36	ENSG00000137501.12	SYTL2	protein_coding	-4.99	-4.90
37	ENSG00000164104.7	HMGB2	protein_coding	-2.83	-2.68
38	ENSG00000163531.11	NFASC	protein_coding	-1.81	-1.87
39	ENSG00000139220.12	PPFIA2	protein_coding	-4.57	-4.43
40	ENSG00000139946.5	PELI2	protein_coding	-1.79	-1.97
41	ENSG00000146039.6	SLC17A4	protein_coding	-2.36	-2.29
42	ENSG00000155792.5	DEPTOR	protein_coding	-1.90	-2.03
43	ENSG0000010292.8	NCAPD2	protein_coding	-1.48	-1.43
44	ENSG00000136997.10	MYC	protein_coding	-1.47	-1.14
45	ENSG00000150764.9	DIXDC1	protein_coding	-2.36	-2.34
46	ENSG00000156218.8	ADAMTSL3	protein_coding	-4.39	-3.90
47	ENSG00000113369.4	ARRDC3	protein_coding	-2.15	-2.00
48	ENSG00000198363.11	ASPH	protein_coding	-2.05	-1.95
49	ENSG00000108846.11	ABCC3	protein_coding	-3.47	-3.13
50	ENSG00000112983.13	BRD8	protein_coding	-1.08	-0.92

**Table S2:** Genes with differential expression between DHT and MDV treatment.

	patient	frozen capture	frozen polyA	FFPE capture
1	WAF35	1	2	0
2	WAF57	1	1	0
3	GU08	0	1	1
4	WAF28	0	1	1
5	WAF18	1	1	0
6	WAF39	1	1	0
7	WAF20	1	1	0
8	WAF43	1	1	0
9	WAF56	1	1	2
10	WAF41	1	1	0
11	WAF4	1	0	1
12	WAF3	1	0	1
13	WAF11	1	0	1

**Table S3:** Number and type of libraries per rapid autopsy prostate sample.