

GGTACTCT
CCAUGAGAC
**Genomics &
Transcriptomics
Laboratory**

Sequencing Data Report

28.08.2017

1. Basic Information

BMFZ GTL contact information for NGS

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ProjectID	116-NGS-270617-0
Customer	PD Dr. Niegisch / Prof. Schulz
Company/Institute	Forschungslabor Urologie
Library Preparation Kit	TruSeq Stranded mRNA Library Prep Kit
Sequencing Chemistry	PE-410-1001 HiSeq® 3000/4000 PE Cluster Kit FC-410-1002 HiSeq® 3000/4000 SBS Kit (150 cycles)
Run Parameters	SR 1x150bp
Instrument Type	HiSeq3000/4000
Type of Analysis	Fastq only

Sample receipt:	27.06.2017	36 samples
Original Sample QC:	29.06.2017 – 30.06.2017	QC passed (as agreed upon with collaboration partner/customer)
Library Preparation:	24.07.2017 – 26.07.2017	QC passed
Sequencing run:	15.08.2017 – 18.08.2017	QC passed
Data transfer:	tba	

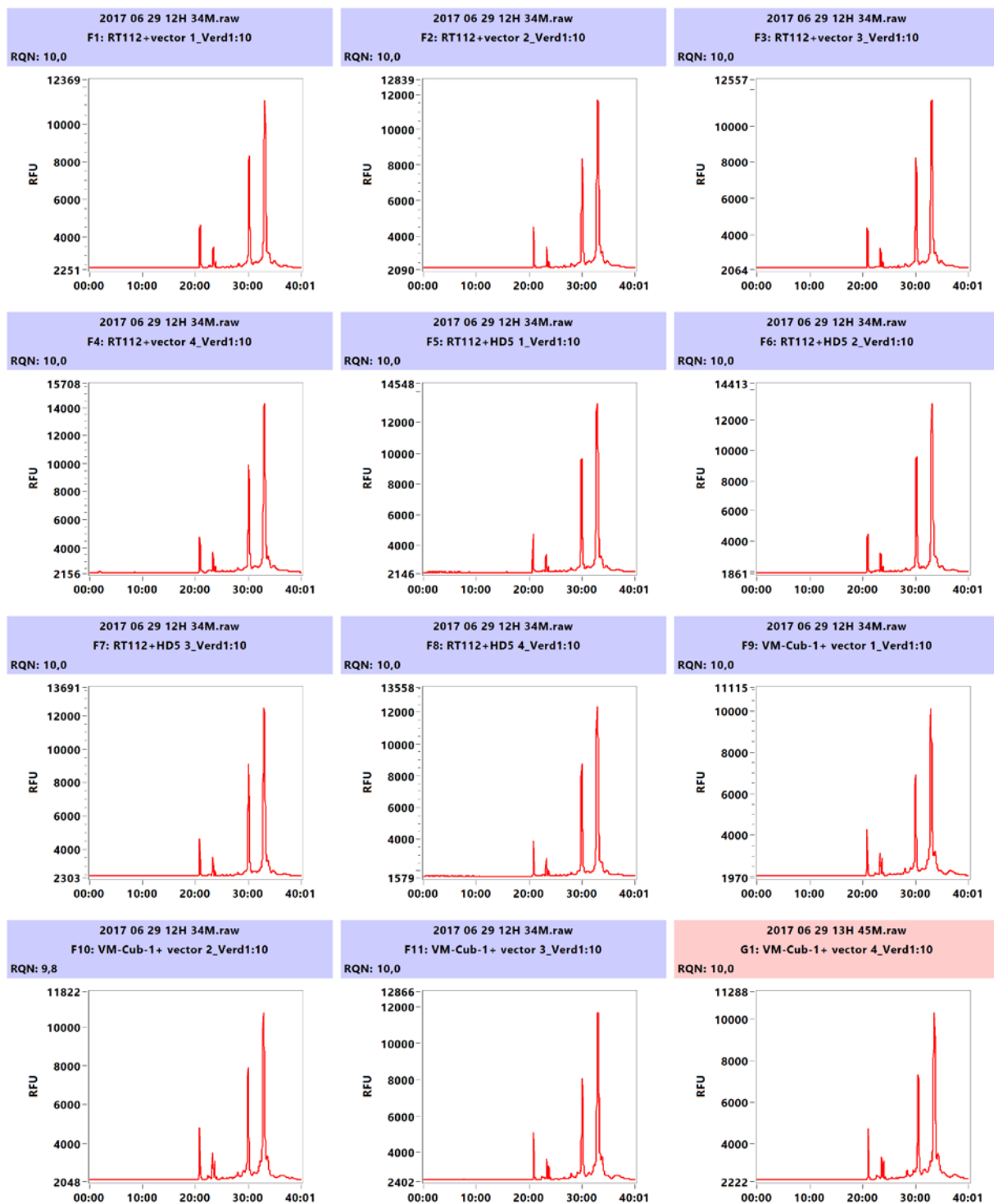
Comments:	- average reads per sample = 30.61 million - the sample with the lowest read count of 22.32 million reads is RT112_vector2 (see page 13)
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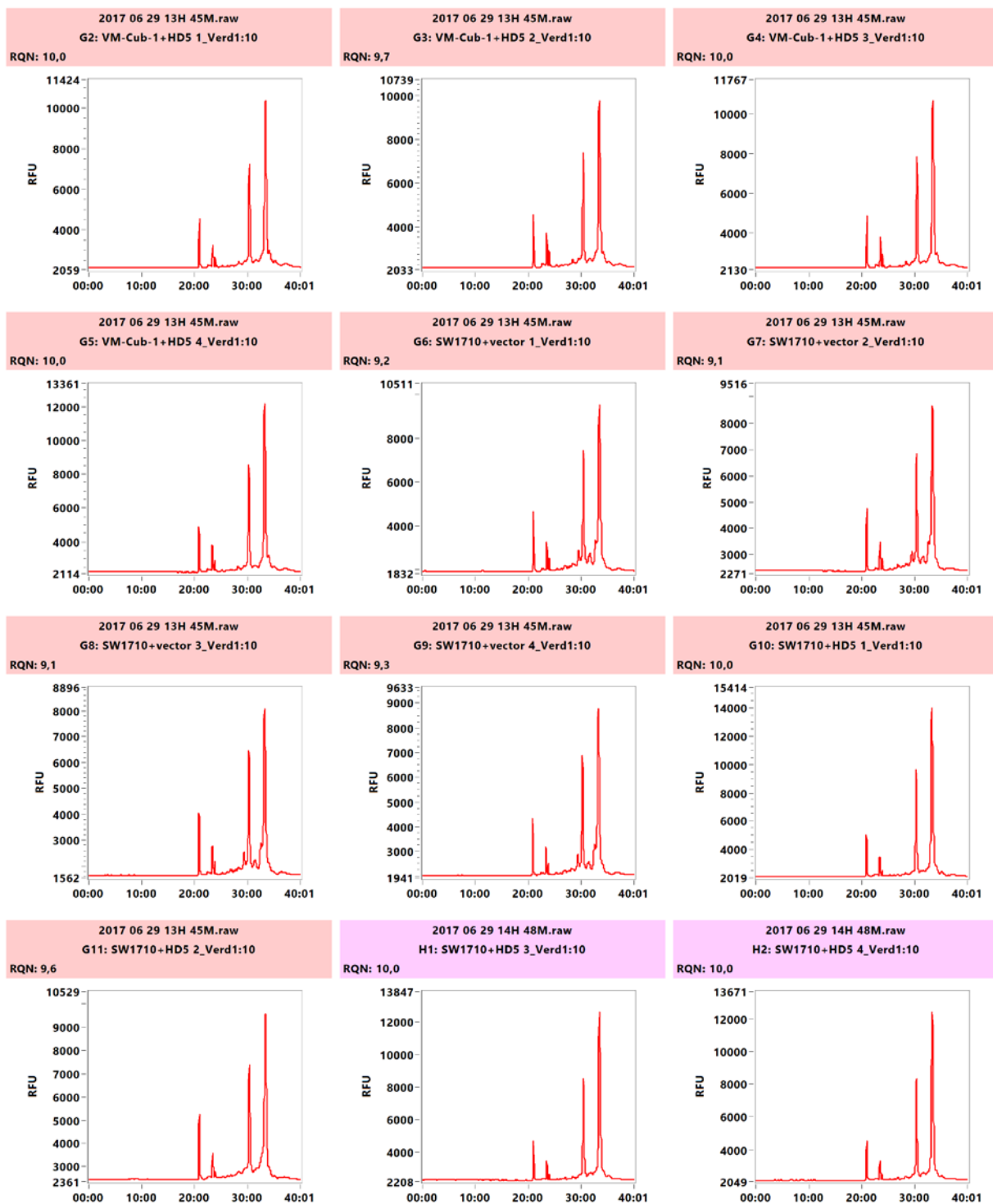
2. Original Sample QC

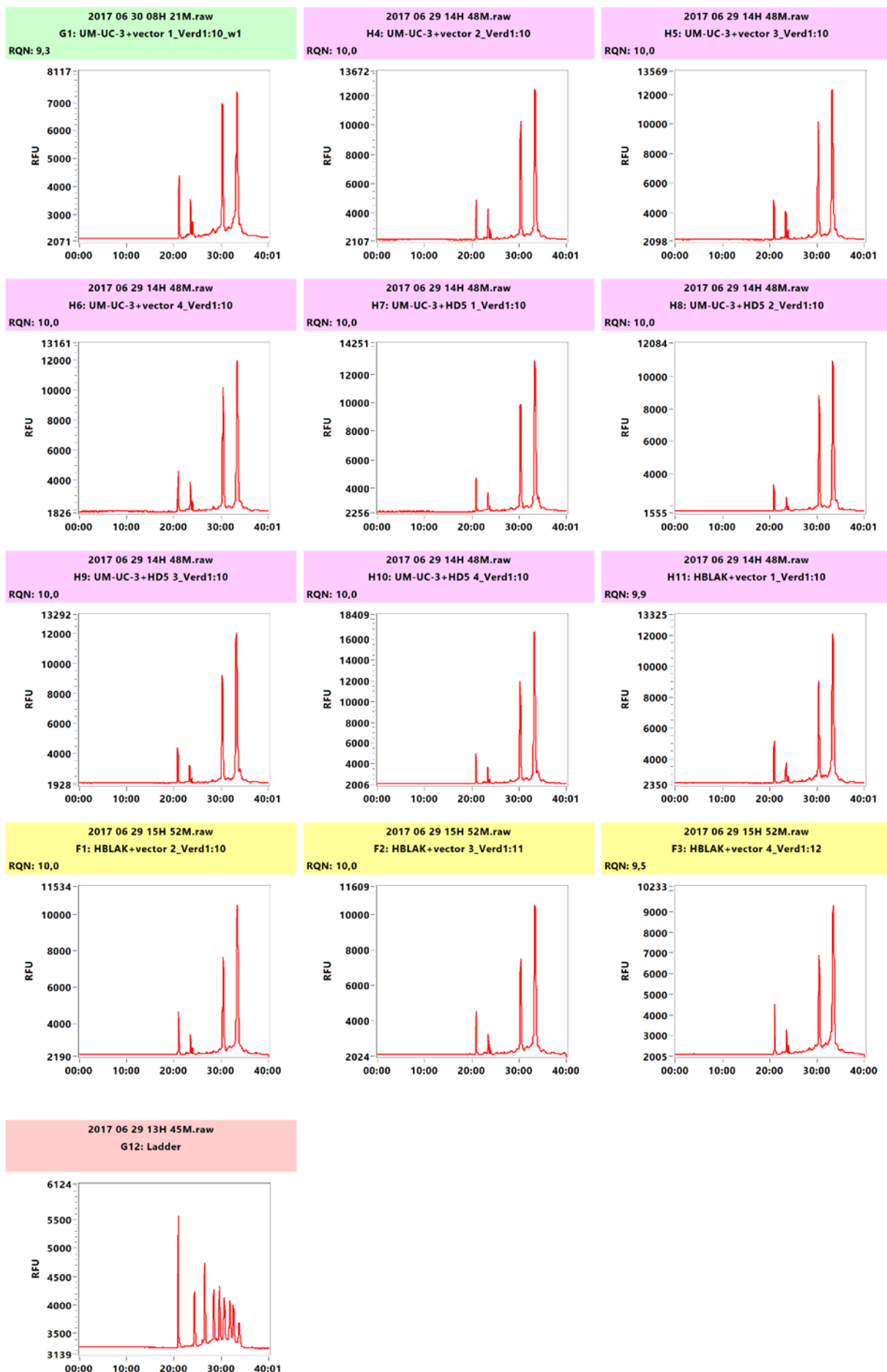
Remarks:

- The 260/280 and 260/230 ratios were determined by UV-Spectroscopy (NanoDrop 1000).
- Samples were diluted to ~5 ng/μL to fit into further downstream application. Concentration check with fluorometric assay. See below.
- The concentration of RNA was determined by Qubit RNA HS Assay, fluorescence-based quantification.
- Size distribution and quality of the RNA was checked by Fragment Analyzer using RNA Standard Sensitivity Assay. RNA Quality Number (RQN) indicates the Quality of RNA.
- The color code of the figures is due to the measurement setup and has therefore no further relevance (see below).

#	Sample	Concentration ND [ng/μL]	260/280	260/230	Concentration (dilution) Qubit [ng/μL]	RQN
1	RT112+vector 1	48.83	1.94	1.81	5.83	10.0
2	RT112+vector 2	52.44	1.82	2.29	6.44	10.0
3	RT112+vector 3	49.87	1.88	1.82	5.97	10.0
4	RT112+vector 4	50.28	1.89	1.85	5.83	10.0
5	RT112+HD5 1	47.76	1.96	2.26	5.64	10.0
6	RT112+HD5 2	48.54	1.90	2.26	5.92	10.0
7	RT112+HD5 3	49.31	1.87	2.25	5.64	10.0
8	RT112+HD5 4	48.39	1.99	1.62	5.98	10.0
9	VM-Cub-1+ vector 1	48.71	1.93	2.14	5.35	10.0
10	VM-Cub-1+ vector 2	46.12	1.92	2.13	5.06	9.8
11	VM-Cub-1+ vector 3	46.14	2.00	0.95	5.30	10.0
12	VM-Cub-1+ vector 4	47.29	1.91	2.29	5.67	10.0
13	VM-Cub-1+HD5 1	47.15	1.95	1.82	5.40	10.0
14	VM-Cub-1+HD5 2	45.76	1.92	2.15	5.16	9.7
15	VM-Cub-1+HD5 3	45.43	1.96	1.38	5.27	10.0
16	VM-Cub-1+HD5 4	46.84	1.93	2.08	5.49	10.0
17	SW1710+vector 1	48.06	1.92	2.24	5.51	9.2
18	SW1710+vector 2	46.28	1.92	2.05	5.44	9.1
19	SW1710+vector 3	46.64	1.89	2.20	5.41	9.1
20	SW1710+vector 4	44.24	1.95	1.58	5.34	9.3
21	SW1710+HD5 1	46.81	1.92	1.82	5.54	10.0
22	SW1710+HD5 2	48.57	1.92	2.26	5.69	9.6
23	SW1710+HD5 3	49.16	1.94	1.73	5.83	10.0
24	SW1710+HD5 4	51.73	1.88	2.03	6.02	10.0
25	UM-UC-3+vector 1	51.28	1.86	2.23	5.47	9.3
26	UM-UC-3+vector 2	49.47	1.85	2.06	5.80	10.0
27	UM-UC-3+vector 3	47.86	1.86	2.28	5.43	10.0
28	UM-UC-3+vector 4	46.35	1.88	2.21	5.90	10.0
29	UM-UC-3+HD5 1	51.48	1.84	2.33	5.89	10.0
30	UM-UC-3+HD5 2	50.46	1.82	2.25	5.68	10.0
31	UM-UC-3+HD5 3	52.15	1.89	1.86	6.05	10.0
32	UM-UC-3+HD5 4	53.28	1.87	1.97	6.83	10.0
33	HBLAK+vector 1	49.24	1.90	2.09	5.71	9.9
34	HBLAK+vector 2	49.32	1.91	2.16	6.28	10.0
35	HBLAK+vector 3	45.98	1.89	0.90	5.83	10.0
36	HBLAK+vector 4	47.28	1.97	2.13	5.48	9.5





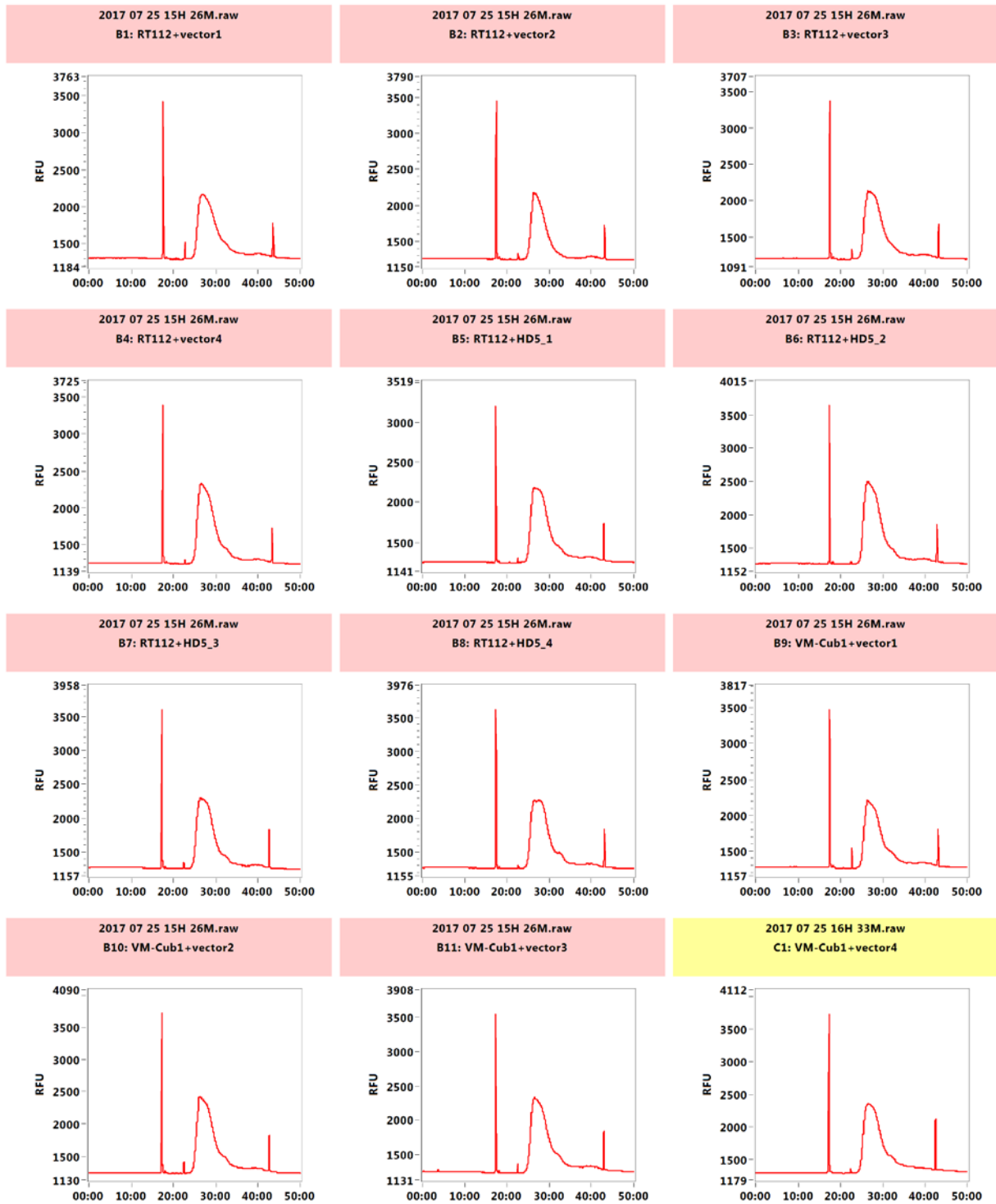


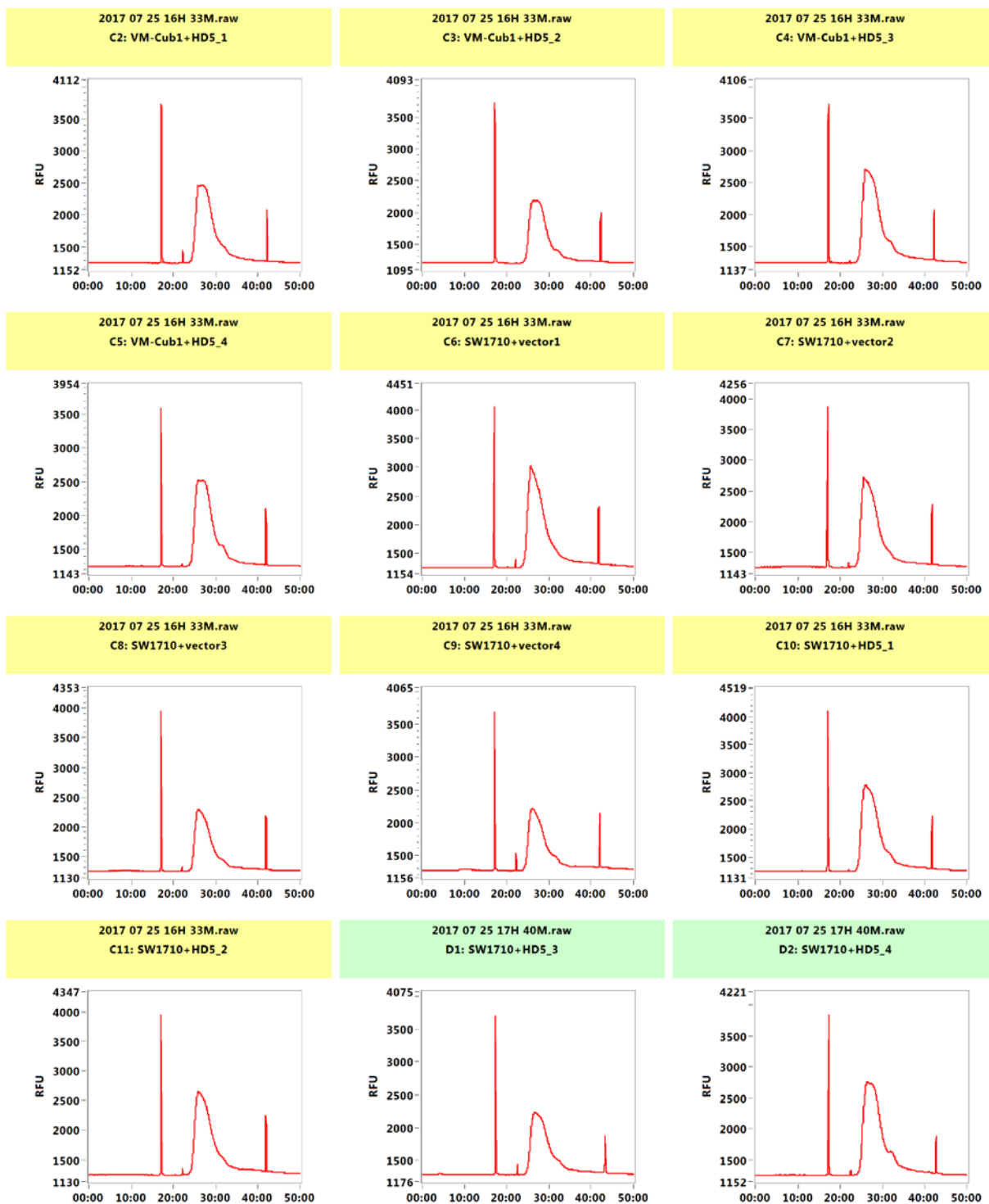
3. Library preparation

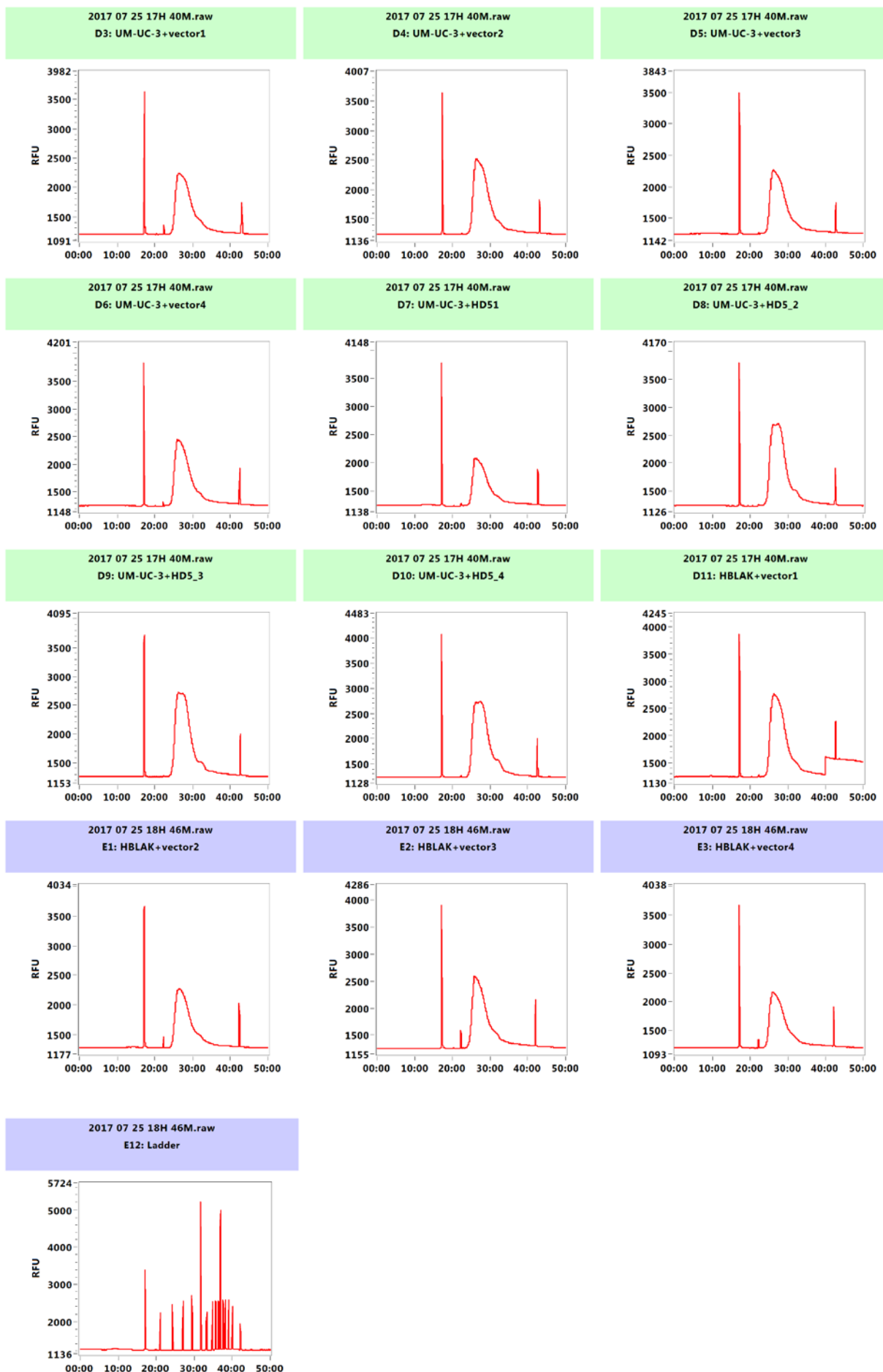
1. Normalization: Dilution to 250 ng in 50 μ L
2. Library Preparation: 50 μ L of total RNA sample input. The Library preparation was performed according to the TruSeq® Stranded mRNA Sample Preparation Guide (Part # 15031047 Rev. E).

4. Library QC

1. The library was first checked by NanoDrop.
2. 1:10 dilution in Resuspension Buffer (90 μ L RSB + 10 μ L library) to bring samples into Fragment Analyzer concentration range (<5 ng/ μ L).
3. Post library preparation QC was carried out using the Fragment Analyzer High Sensitivity NGS Fragment Analysis Kit (1 bp – 6,000 bp), DNF-474.
The color code of the figures is due to the measurement setup and has therefore no further relevance (see below).
4. Library normalization was performed using Qubit DNA HS Assay for concentration determination and Fragment Analyzer DNF-474 for average size information (dilution to 2 nM). Finally each time 12 of the 36 samples were pooled together into 3 pools in order to sequence them on 3 HiSeq3000 lanes.







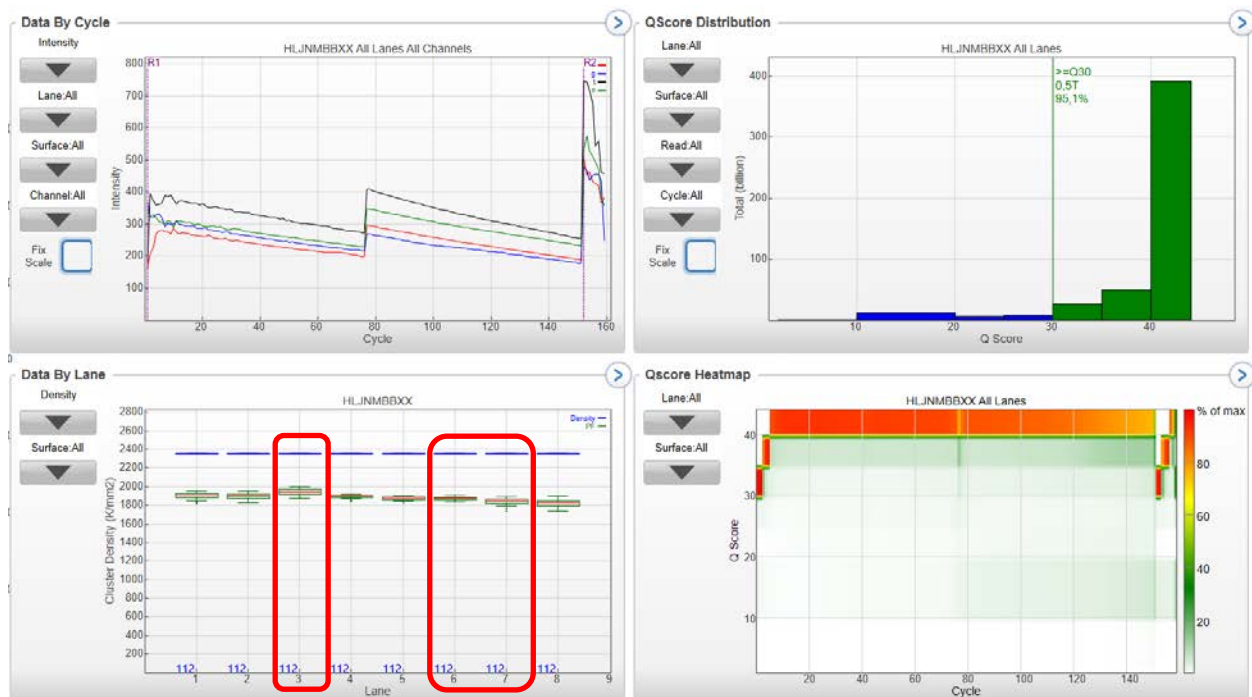
5. Sequencing QC

Sequencing run ID: 170816_J00150_0031_AHLJNMBBXX

Remarks:

- PhiX SpikeIn ~ 1%; represented by the aligned (%) value
- Error Rate has to be < 3%
- Phas/Prephas has to be < 0.3%
- Q30% has to be > 75%

Run Summary					
Lane	Level	Aligned (%)	Error Rate (%)	Phas/Prephas (%)	% >= Q30
3	Forward read	1.70 +/- 0.22	0.31 +/- 0.15	0.103 / 0.064	95.01
6	Forward read	1.97 +/- 0.04	0.27 +/- 0.14	0.104 / 0.067	94.60
7	Forward read	1.86 +/- 0.06	0.25 +/- 0.05	0.104 / 0.069	94.66
3	Reverse read	0.00 +/- 0.00	0.00 +/- 0.00	0.000 / 0.000	95.59
6	Reverse read	0.00 +/- 0.00	0.00 +/- 0.00	0.000 / 0.000	95.13
7	Reverse read	0.00 +/- 0.00	0.00 +/- 0.00	0.000 / 0.000	95.01



Lane 3

Lane 6 - 7

- All samples of this project were loaded/analyzed on lane 3, 6 and 7.

6. Reads mapped to index/ Index distribution

Lane	Sample	Barcode sequence	PF Clusters	% of the lane	% Perfect barcode	Yield (Mbases)	% >= Q30 bases	Mean Quality Score
3	HBLAK_vector1	CGTACG	29,499,846	7.39	100	4,454	95.43	39.33
3	HBLAK_vector2	GAGTGG	33,005,335	8.26	100	4,984	93.81	38.90
3	HBLAK_vector3	ACTGAT	40,816,980	10.22	100	6,163	93.45	38.81
3	HBLAK_vector4	ATTCCT	33,598,294	8.41	100	5,073	94.18	39.00
3	UM-UC-3_HD5_1	TAGCTT	31,454,551	7.87	100	4,750	95.27	39.29
3	UM-UC-3_HD5_2	GGCTAC	32,180,589	8.06	100	4,859	95.72	39.41
3	UM-UC-3_HD5_3	GTGGCC	35,056,571	8.78	100	5,294	95.98	39.48
3	UM-UC-3_HD5_4	GTTTCG	29,873,573	7.48	100	4,511	95.52	39.35
3	UM-UC-3_vector1	ATCACG	29,167,280	7.30	100	4,404	93.70	38.87
3	UM-UC-3_vector2	TTAGGC	26,066,076	6.53	100	3,936	96.02	39.49
3	UM-UC-3_vector3	ACTTGA	29,681,809	7.43	100	4,482	95.87	39.45
3	UM-UC-3_vector4	GATCAG	28,537,691	7.14	100	4,309	95.27	39.29
3	Undetermined	unknown	20,500,043	5.13	100	3,096	93.31	38.70
6	RT112_HD5_1	TAGCTT	33,360,147	8.68	100	5,037	94.97	39.20
6	RT112_HD5_2	GGCTAC	29,034,082	7.56	100	4,384	95.53	39.34
6	RT112_HD5_3	GTGGCC	32,093,808	8.36	100	4,846	95.37	39.30
6	RT112_HD5_4	GTTTCG	29,250,662	7.62	100	4,417	94.88	39.17
6	RT112_vector1	ATCACG	29,268,392	7.62	100	4,420	93.29	38.75
6	RT112_vector2	TTAGGC	22,321,159	5.81	100	3,370	94.91	39.19
6	RT112_vector3	ACTTGA	28,864,464	7.51	100	4,359	94.60	39.10
6	RT112_vector4	GATCAG	24,911,410	6.49	100	3,762	94.96	39.20
6	VM-Cub1_vector1	CGTACG	33,252,592	8.66	100	5,021	93.22	38.73
6	VM-Cub1_vector2	GAGTGG	35,575,795	9.26	100	5,372	94.07	38.95
6	VM-Cub1_vector3	ACTGAT	33,871,167	8.82	100	5,115	94.21	39.00
6	VM-Cub1_vector4	ATTCCT	31,998,304	8.33	100	4,832	94.73	39.13
6	Undetermined	unknown	20,314,102	5.29	100	3,067	93.46	38.76
6	SW1710_HD5_1	CGTACG	30,038,907	7.93	100	4,536	95.26	39.25
6	SW1710_HD5_2	GAGTGG	31,722,731	8.37	100	4,790	94.83	39.14
6	SW1710_HD5_3	ACTGAT	36,772,364	9.70	100	5,553	93.99	38.92
7	SW1710_HD5_4	ATTCCT	31,245,081	8.24	100	4,718	94.75	39.12
7	SW1710_vector1	TAGCTT	31,187,986	8.23	100	4,709	94.70	39.11
7	SW1710_vector2	GGCTAC	28,951,573	7.64	100	4,372	94.79	39.13
7	SW1710_vector3	GTGGCC	31,695,904	8.36	100	4,786	94.69	39.10
7	SW1710_vector4	GTTTCG	27,248,075	7.19	100	4,114	93.11	38.68
7	VM-Cub1_HD5_1	ATCACG	26,957,513	7.11	100	4,071	93.69	38.84
7	VM-Cub1_HD5_2	TTAGGC	28,914,931	7.63	100	4,366	95.45	39.31
7	VM-Cub1_HD5_3	ACTTGA	28,831,726	7.61	100	4,354	95.27	39.26
7	VM-Cub1_HD5_4	GATCAG	25,731,145	6.79	100	3,885	94.96	39.18
7	Undetermined	unknown	19,668,803	5.19	100	2,970	93.20	38.68

- Undetermined reads are all reads of a lane that do not match 100 % to the given barcode sequences.

7. Related files

QC step	File names
Sequencing Data Report: Summary	116-NGS-270617-0_Sequencing Data Report.pdf
Sample QC: Capillary gel electrophoresis of original sample /totalRNA (Fragment Analyzer using Total RNA Analysis Standard Sensitivity Assay)	116-NGS-270617-3 FA-ProbenQC.pdf
Library QC: Capillary gel electrophoresis of final library (Fragment Analyzer DNF-474-33 – NGS-HS)	116-NGS-270617-4_finaleLibrary.pdf
Sequencing Data: HiSeq3000 Data files	Fastq files for each sample and undetermined reads. Files can be found in the following location: \\116-NGS-270617-0\