Genomic Services Supporting Oncology: from Single Base to The Whole Genome

FRONTAGE

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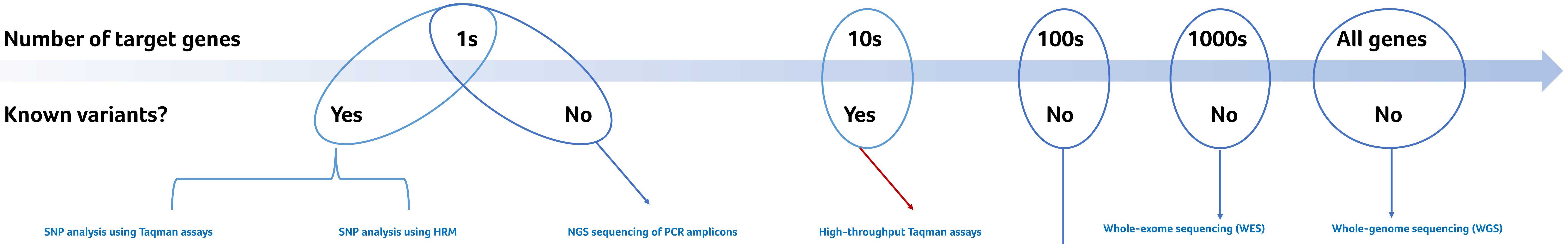
The rapid advances of genomic methods have revolutionized our understanding of the basis of cancer development and progression. In recent years, genomic methods for the analysis of sequence variants and the quantification of DNA/RNA have also become an indispensable part of drug discovery, development and clinical trials. Many areas now require genomic testing, including pharmacogenomics, pharmacokinetics, pharmacodynamics and drug safety.

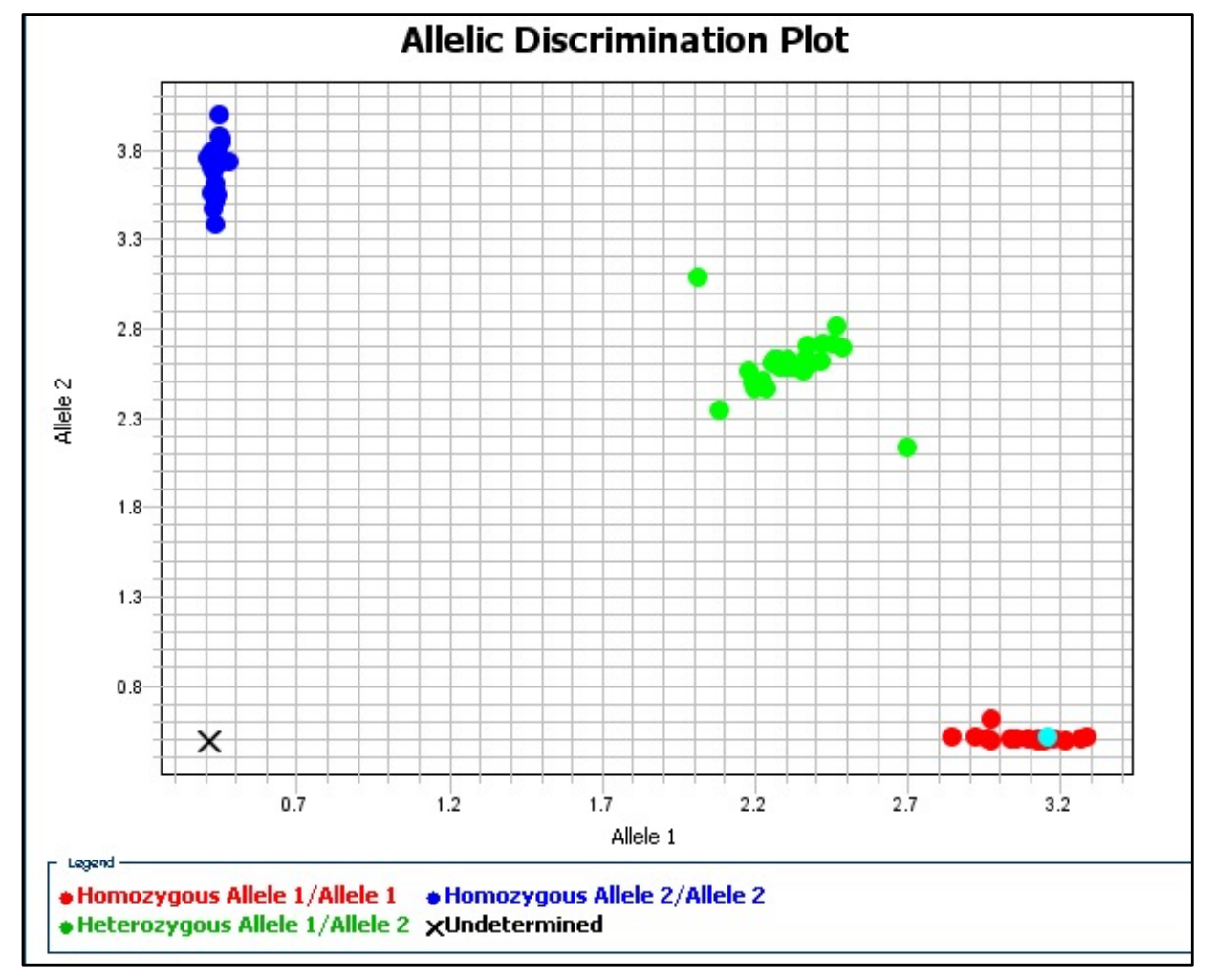
An enormous amount of diversity exists in the types of drugs and their targets, the mechanisms of action, the metabolic processes and the evaluation of drug efficacy, etc. In order to most effectively support cancer drug development, we have developed and optimized a comprehensive set of genomic assay platforms, method validation procedures, sample analysis pipelines and project management systems at Frontage Laboratories. These methods should allow us to provide high-quality and low-cost services tailored to specific projects.

PHARMACOGENOMIC ANALYSIS: FROM SINGLE SNPS TO GENOME-WIDE VARIANT ANALYSIS



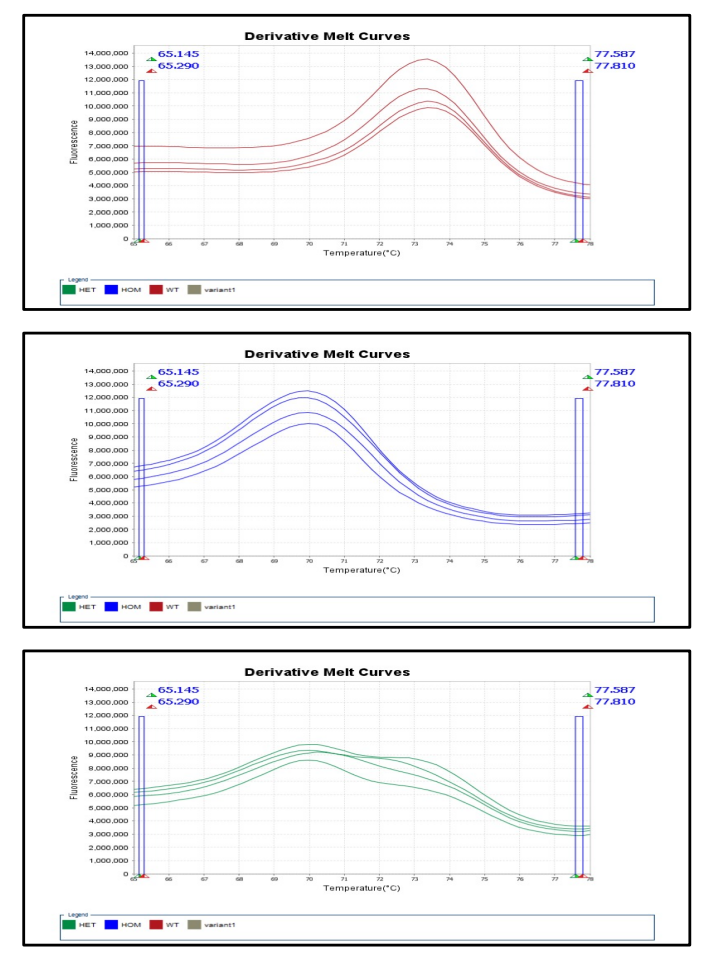
SNP analysis using Taqman assays

- Two Taqman probes with two different dyes are used in duplex qPCR assays
  - Probe 1 (Fam) matches allele 1 sequence
  - Probe 2 (Vic) matches allele 2 sequence
- 1/1: Fam only; 2/2: Vic only; 1/2: Fam + Vic



SNP analysis using HRM

- High resolution melting analysis
  - After PCR, temperature is raised to denature dsDNA
  - Fluorescent dye binds dsDNA
  - PCR products from different alleles denature at different temperatures
- Homozygous allele1 or allele 2: two distinct melting curves
- Heterozygous: a combination of the two melting curves



NGS sequencing of PCR amplicons

- Multiple SNPs and indels within the same gene
- Gene editing results and efficiency

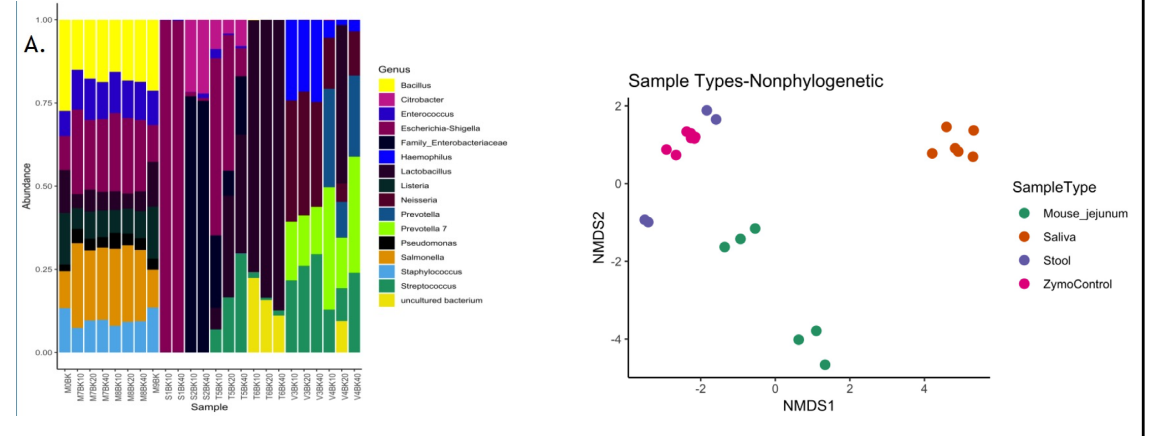
DNA extraction from edited cells → 1st PCR 2nd PCR → 3rd PCR (barcode PCR) → NGS

- Editing results: SNPs, indels, etc
- Editing efficiency: % of target cells edited

- Microbiome analysis by 16S rDNA sequencing

- The 16s rRNA gene contains hypervariable regions (HVR) flanked by highly conserved regions
- Conserved regions allow PCR amplification
- HVRs provide diversity information

Stool / Feces	Buccal swabs
Tissue biopsies	Oral biofilms
Cells	Soil
Saliva	Plants
Bronchial lavage	Food items
Sputum	



High-throughput Taqman assays

- Highly customizable and scalable
- Taqman array cards
- Taqman assay plates
- Rapid detection of dozens of SNPs

Whole-exome sequencing (WES)

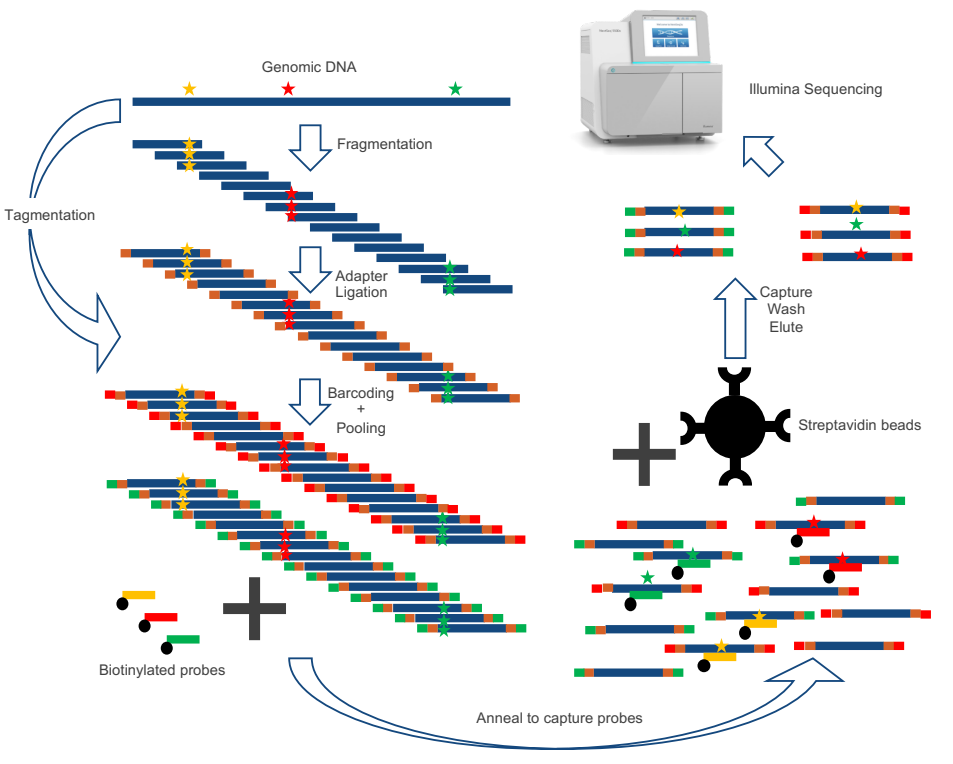
Whole-genome sequencing (WGS)

- Genome-wide data for SNPs and structural changes (indels, CNVs, fusions, translocations, etc)
- Comprehensive bioinformatic services
  - Data processing: processing of raw reads, sequencing alignment, variant calling, etc
  - Data analysis: variant filtering by quality and coverage, allele population frequency, probability of being pathogenic, pharmacogenomic databases and disease types to identify relevant and potentially pathogenic variants

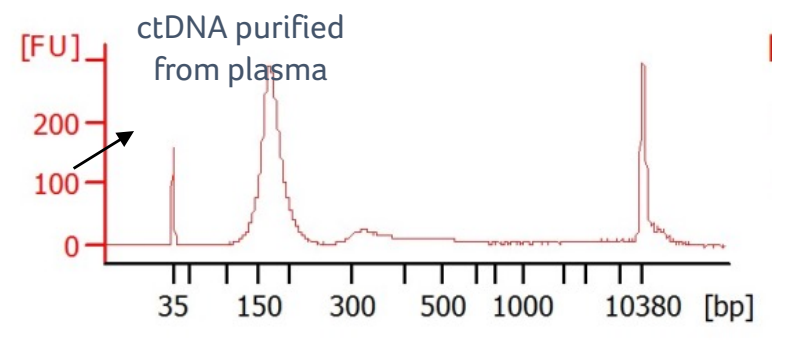
Target capture and NGS sequencing

- Selectively deep sequencing of a subset of genes or loci
- Flexible target selection
  - ➢ Customize panels to meet diverse needs
  - ➢ Commercially available panels
- Flexible sequencing depth depending on allele frequency

- DME profiling panels
- Oncogene panels
  - Tumor biopsy: tumor genomic DNA
  - Liquid biopsy: circulating tumor DNA (ctDNA) sequencing

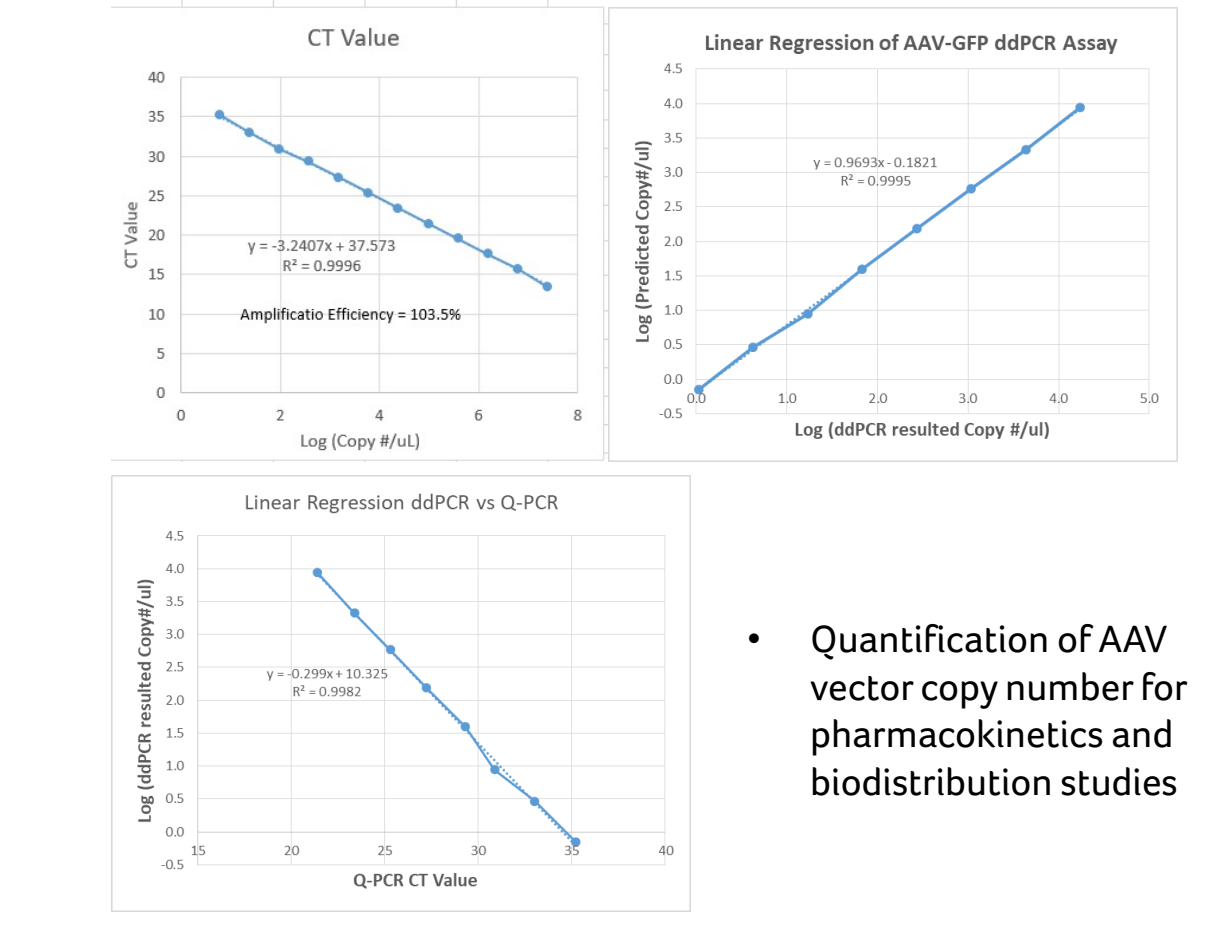


- **ctDNA sequencing**
  - ➢ Liquid biosy is non-invasive and low-cost compared to tumor biopsy and can be performed at any time
  - ➢ ctDNA can be extracted from blood or other biofluids for deep sequencing for the detection and quantification of mutant alleles



QUANTIFICATION OF DNA AND RNA: FROM SINGLE GENE TO WHOLE-TRANSCRIPTOMES

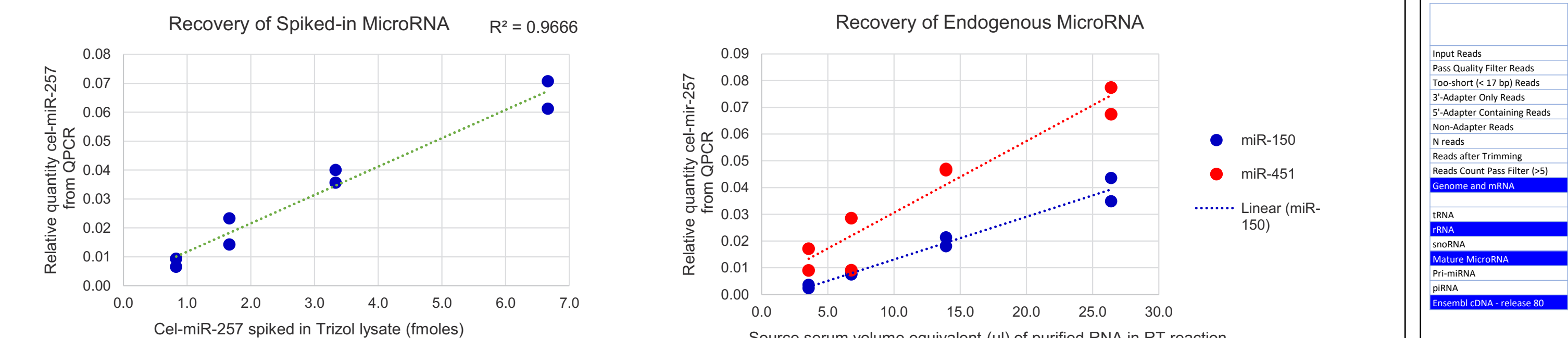
DNA copy number quantification by qPCR and ddPCR



- Quantification of AAV vector copy number for pharmacokinetics and biodistribution studies

Oligonucleotide quantification by multiple assay platforms

Quantification of synthetic and endogenous miRNAs by RT-qPCR

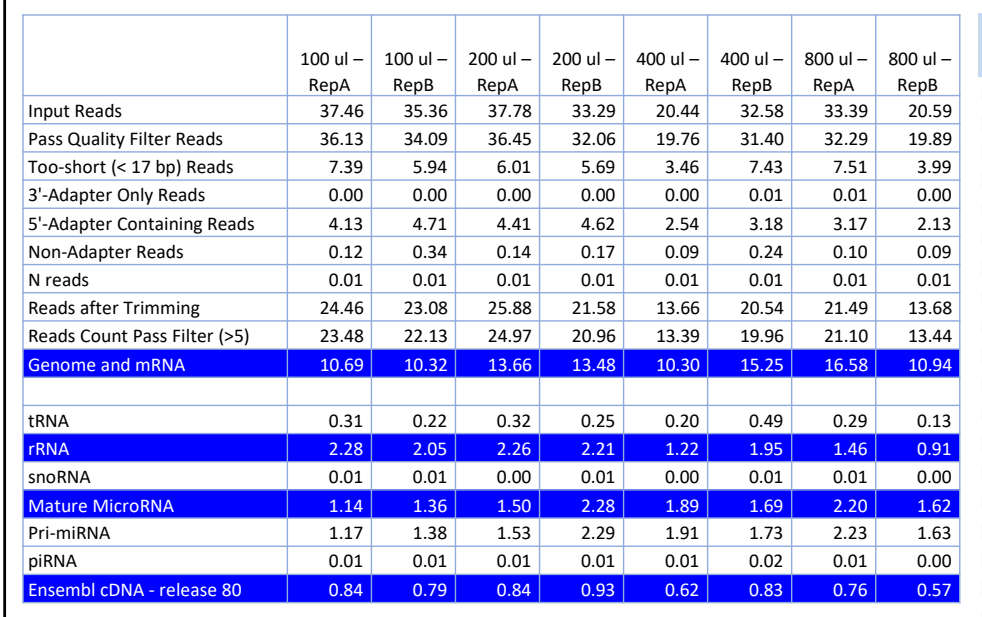


Relative quantity for panel A is based on 2^(Ct_{con} -Ct_{sample}), where Ct_{con} and Ct_{sample} represent the mean Ct value of a positive control RNA spiked into the RT reaction and for the spike in the extracted RNA sample, respectively.

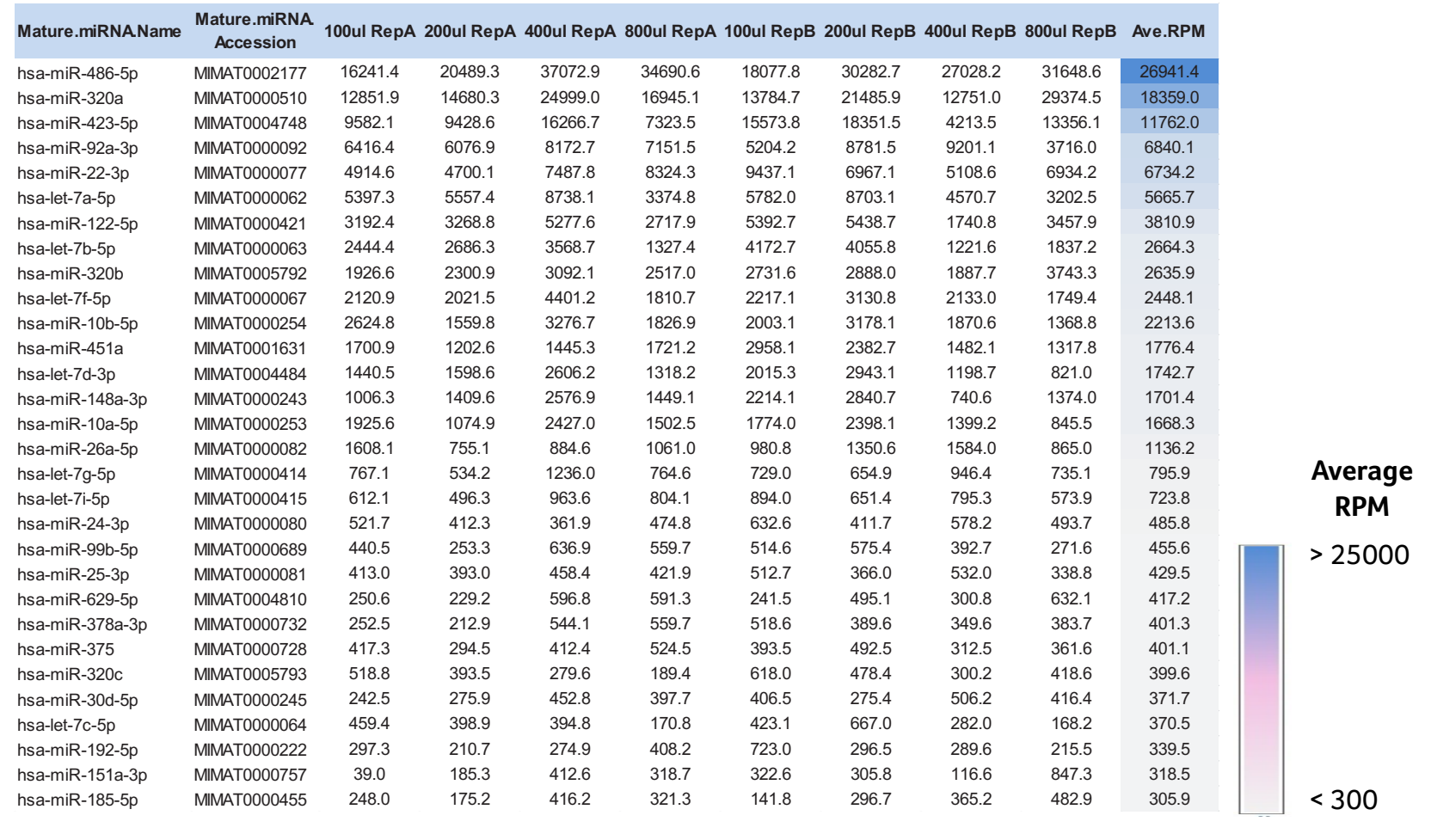
Relative quantity for panel B is based on 2^(Ct_{tissue} -Ct_{sample}), where Ct_{tissue} and Ct_{sample} represent the mean Ct value of a positive control tissue RNA pool and of the spike in the extracted RNA sample, respectively.

miRNA profiling and transcriptome analysis using NGS

miRNA profiling by miRNA-seq



	100 ul – RepA	100 ul – RepB	200 ul – RepA	200 ul – RepB	400 ul – RepA	400 ul – RepB	800 ul – RepA	800 ul – RepB
Input Reads	37.46	35.36	37.78	33.29	20.44	32.58	33.39	20.59
Pass Quality Filter Reads	36.13	34.09	36.45	32.06	19.76	31.40	32.29	19.89
Too-short (< 17 bp) Reads	7.39	5.94	6.01	5.69	3.46	7.43	7.51	3.99
3'-Adapter Only Reads	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
5'-Adapter Containing Reads	4.13	4.71	4.41	4.62	2.54	3.18	3.17	2.13
Non-Adapter Reads	0.12	0.34	0.14	0.17	0.09	0.24	0.10	0.09
N reads	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Reads after Trimming	24.46	23.08	25.88	21.58	13.66	20.54	21.49	13.68
Reads Count Pass Filter (>5)	23.48	22.13	24.97	20.96	13.39	19.96	21.10	13.44
Genome and mRNA	10.69	10.32	13.66	13.48	10.30	15.25	16.58	10.94
tRNA	0.31	0.22	0.32	0.25	0.20	0.49	0.29	0.13
rRNA	2.28	2.05	2.26	2.21	1.22	1.95	1.46	0.91
snoRNA	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.00
Mature MicroRNA	1.14	1.36	1.50	2.28	1.89	1.69	2.20	1.62
Pri-miRNA	1.17	1.38	1.53	2.29	1.91	1.73	2.23	1.63
piRNA	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00
Ensembl cDNA - release 80	0.84	0.79	0.84	0.93	0.62	0.83	0.76	0.57

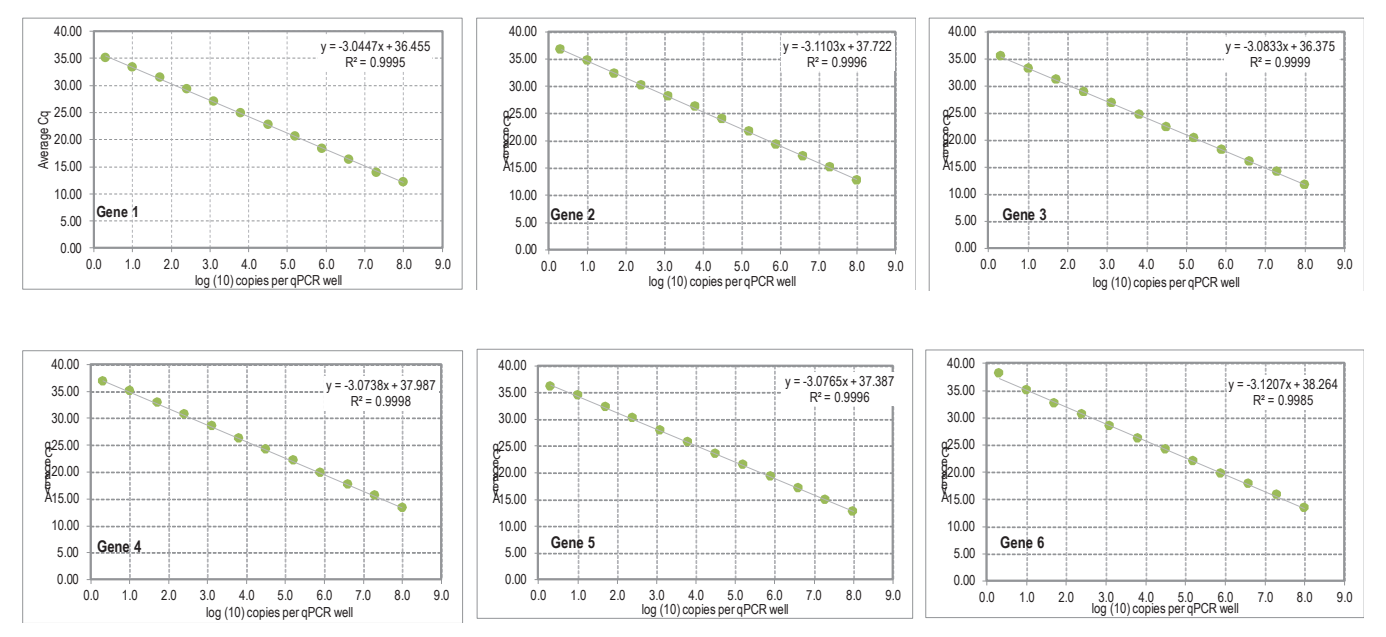


Mature.miRNA.Name	Mature.miRNA. Accession	100ul RepA	200ul RepA	400ul RepA	800ul RepA	100ul RepB	200ul RepB	400ul RepB	800ul RepB	Ave.RPM
hsa-miR-486-5p	MIMAT0002177	16241.4	20489.3	37072.9	34690.6	18077.8	30282.7	27028.2	31648.6	26941.4
hsa-miR-320a	MIMAT0000510	12851.9	14680.3	24999.0	16945.1	13784.7	21485.9	12751.0	29374.5	18359.0
hsa-miR-423-5p	MIMAT0004748	9582.1	9428.6	16266.7	7323.5	15573.8	18351.5	4213.5	13356.1	11762.0
hsa-miR-92a-3p	MIMAT0000092	6416.4	6076.9	8172.7	7151.5	5204.2	8781.5	9201.1	3716.0	6840.1
hsa-miR-22-3p	MIMAT0000077	4914.6	4700.1	7487.8	8324.3	9437.1	6967.1	5108.6	6934.2	6734.2
hsa-let-7a-5p	MIMAT0000062	5397.3	5557.4	8738.1	3374.8	5782.0	8703.1	4570.7	3202.5	5665.7
hsa-miR-122-5p	MIMAT0000421	3192.4	3268.8	5277.6	2717.9	5392.7	5438.7	1740.8	3457.9	3810.9
hsa-let-7b-5p	MIMAT0000063	2444.4	2686.3	3568.7	1327.4	4172.7	4055.8	1221.6	1837.2	2664.3
hsa-miR-320b	MIMAT0005792	1926.6	2300.9	3092.1	2517.0	2731.6	2888.0	1887.7	3743.3	2635.9
hsa-let-7f-5p	MIMAT0000067	2120.9	2021.5	4401.2	1810.7	2217.1	3130.8	2133.0	1749.4	2448.1
hsa-miR-10b-5p	MIMAT0000254	2624.8	1559.8	3276.7	1826.9	2003.1	3178.1	1870.6	1368.8	2213.6
hsa-miR-451a	MIMAT0001631	1700.9	1202.6	1445.3	1721.2	2958.1	2382.7	1482.1	1317.8	1776.4
hsa-let-7d-3p	MIMAT0004484	1440.5	1598.6	2606.2	1318.2	2015.3	2943.1	1198.7	821.0	1742.7
hsa-miR-148a-3p	MIMAT0000243	1006.3	1409.6	2576.9	1449.1	2214.1	2840.7	740.6	1374.0	1701.4
hsa-miR-10a-5p	MIMAT0000253	1925.6	1074.9	2427.0	1502.5	1774.0	2398.1	1399.2	845.5	1668.3
hsa-miR-26a-5p	MIMAT0000082	1608.1	755.1	884.6	1061.0	980.8	1350.6	1584.0	865.0	1136.2
hsa-let-7g-5p	MIMAT0000414	767.1	534.2	1236.0	764.6	729.0	654.9	946.4	735.1	795.9
hsa-let-7i-5p	MIMAT0000415	612.1	496.3	963.6	804.1	894.0	651.4	795.3	573.9	723.8
hsa-miR-24-3p	MIMAT0000080	521.7	412.3	361.9	474.8	632.6	411.7	578.2	493.7	485.8
hsa-miR-99b-5p	MIMAT0000689	440.5	253.3	636.9	559.7	514.6	575.4	392.7	271.6	455.6
hsa-miR-25-3p	MIMAT0000081	413.0	393.0	458.4	421.9	512.7	366.0	532.0	338.8	429.5
hsa-miR-629-5p	MIMAT0004810	250.6	229.2	596.8	591.3	241.5	495.1	300.8	632.1	417.2
hsa-miR-378a-3p	MIMAT0000732	252.5	212.9	544.1	559.7	518.6	389.6	349.6	383.7	401.3
hsa-miR-375	MIMAT0000728	417.3	294.5	412.4	524.5	393.5	492.5	312.5	361.6	401.1
hsa-miR-320c	MIMAT0005793	518.8	393.5	279.6	189.4	618.0	478.4	300.2	418.6	399.6
hsa-miR-30d-5p	MIMAT0000245	242.5	275.9	452.8	397.7	406.5	275.4	506.2	416.4	371.7
hsa-let-7c-5p	MIMAT0000064	459.4	398.9	394.8	170.8	423.1	667.0	282.0	168.2	370.5
hsa-miR-192-5p	MIMAT0000222	297.3	210.7	274.9	408.2	723.0	296.5	289.6	215.5	339.5
hsa-miR-151a-3p	MIMAT0000757	39.0	185.3	412.6	318.7	322.6	305.8	116.6	847.3	318.5
hsa-miR-185-5p	MIMAT0000455	248.0	175.2	416.2	321.3	141.8	296.7	365.2	482.9	305.9

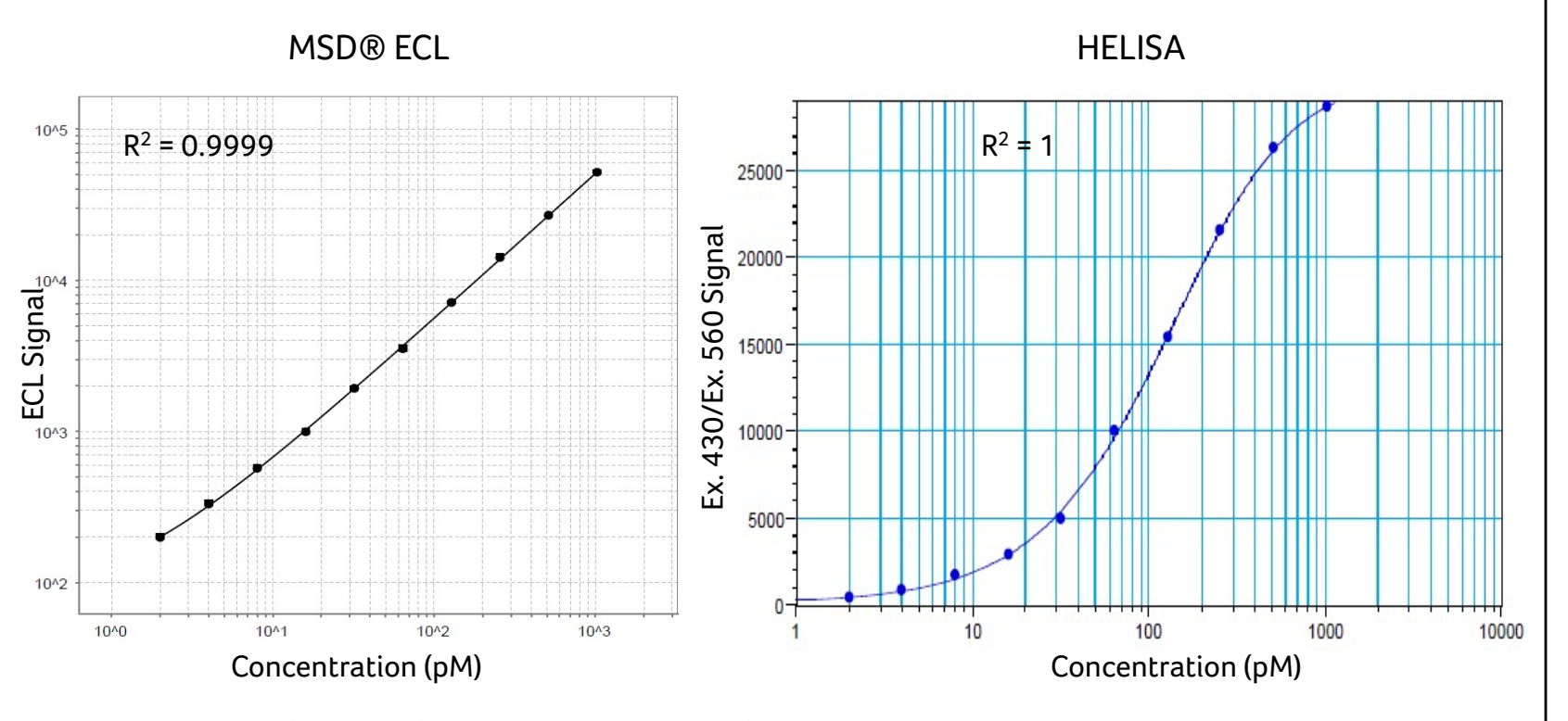
Isolation and sequencing of miRNAs from serum

Gene expression analysis by RT-qPCR and RT-ddPCR

- RT-qPCR assays for the expression of 6 human genes
  - Linear dynamic range of 10,000,000 copies.
  - Limit of detection: ~10 copies of cDNA per qPCR well.

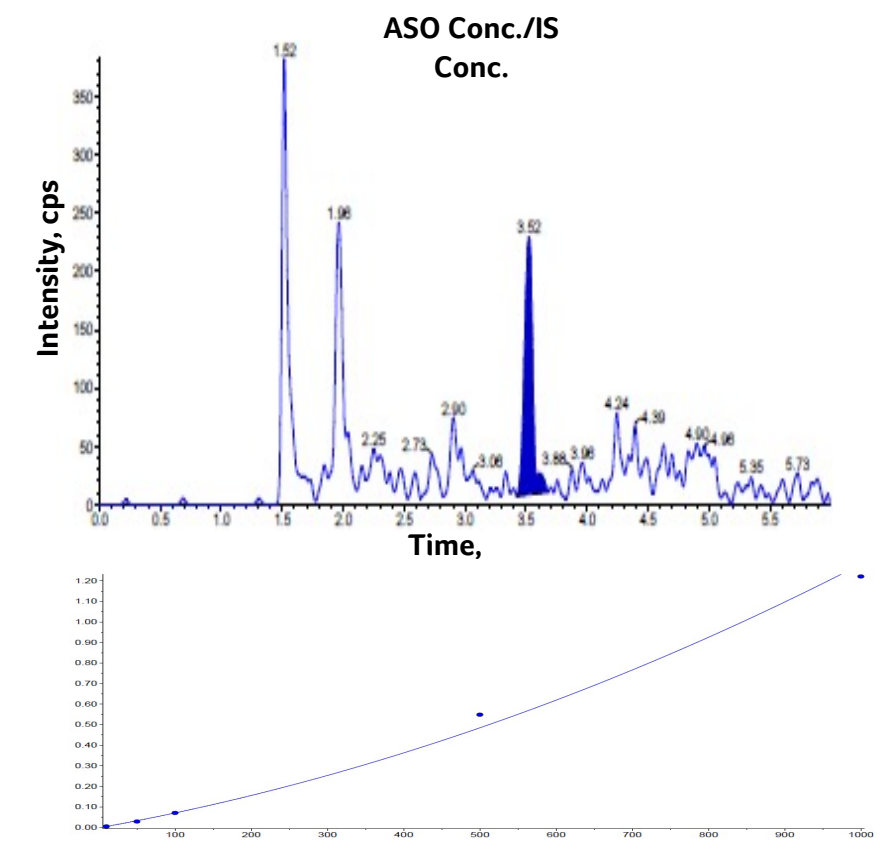


MSD® ECL and hybridization ELISA (HELISA)



- LLOD – 2 pM (12 pg/mL), LLOQ – 4 pM (24 pg/mL), ULOQ – 1,024 nM (~6.14 ng/mL)
- Ultra-high sensitivity for both MSD® ECL and HELISA methods
- Linear signal increase within the detection range

HPLC-MS, ion pairing Chromatography



HPLC-MS has sensitivity down to 10 nM only, but allows for tracking of both the therapeutic product and its metabolites

Transcriptome analysis by RNA-seq

- Optimized sample preparation protocols for limiting amounts of RNA, DNA, protein, and degraded starting materials (FFPE)
- Parsimonious consumption of samples and derivatives at each processing point

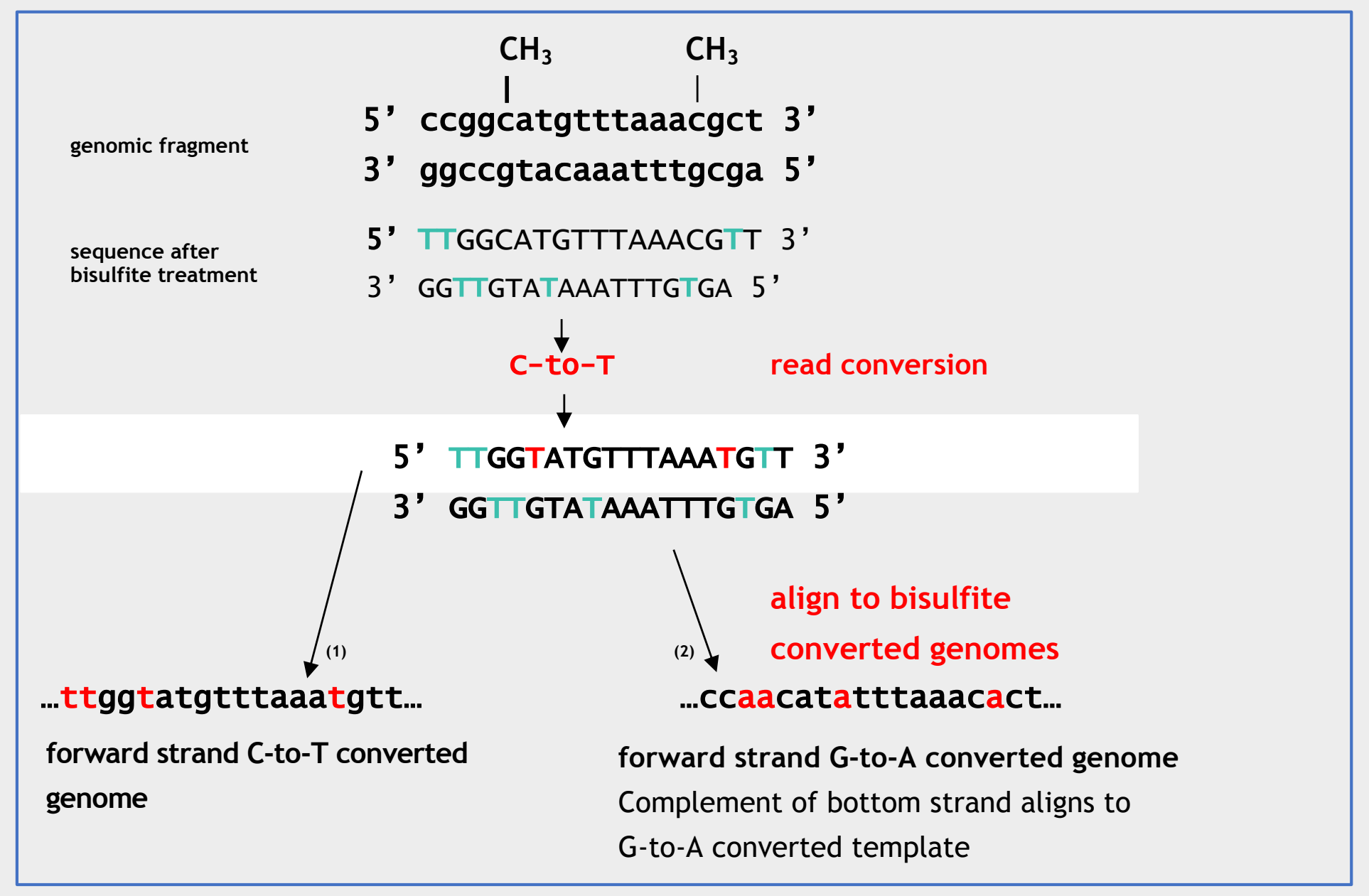
- Efficient workflows established for each service to ensure rapid turnarounds
- All GLP/ GCLP equipment undergoes continuous validation, calibration and maintenance schedule

- Customizable data analysis services, tailored to experimental objectives
- Post project support available with preparation of materials for regulatory submission

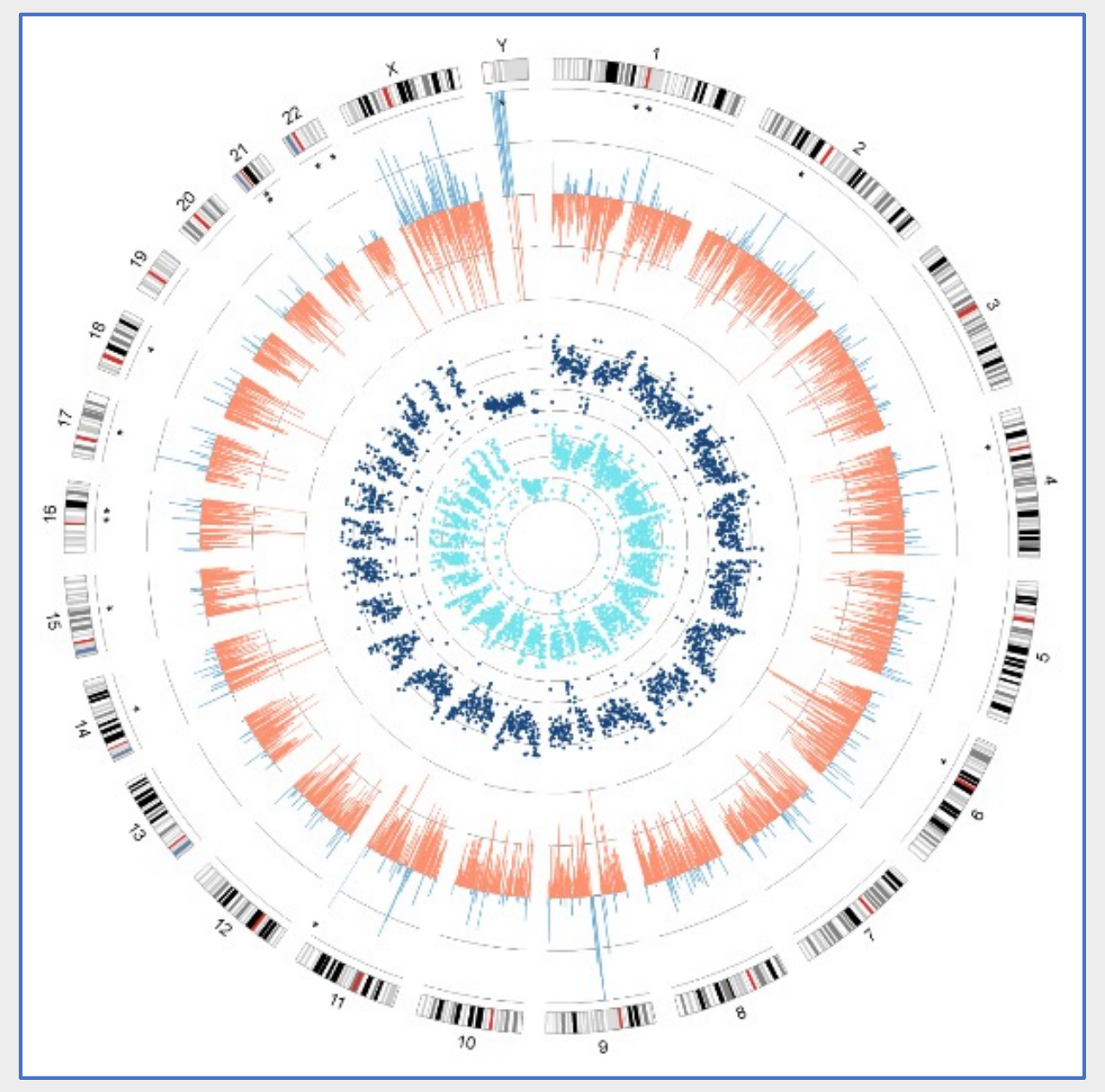
EPIGENOMIC ANALYSIS IN ONCOLOGY

DNA methylation analysis of ctDNA using bisulfite sequencing

Bisulfite library preparation



Sequencing and data analysis



ctDNA bisulfite sequencing results reveal genome-wide hypomethylation in PDAC compared to normal samples.

The outer Circos visualization shows the difference in % methylated CpGs between PDAC and Normal patients for each 1 Mbase tile in the genome. The blue and red bars indicate regions with higher and lower % methylation in PDAC vs. Normal, respectively. Regions with FDR < 0.1 are indicated by *. The gridlines indicate a methylation difference of 5%.

The inner scatter plots show the average number of methylated CpGs in each region across all samples for PDAC (dark blue; n=6) and Normal (light blue; n=6) groups. The gridlines indicate 50 methylated CpGs.