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MOLECULAR TARGETS AND CANCER THERAPEUTICS
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Whole exome (WES) and whole genome methylation sequencing (WGMS) of low-input cell-free DNA (cfDNA) to implement precision medicine in metastatic castration resistant prostate cancer (mCRPC)

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Presenting author disclosures

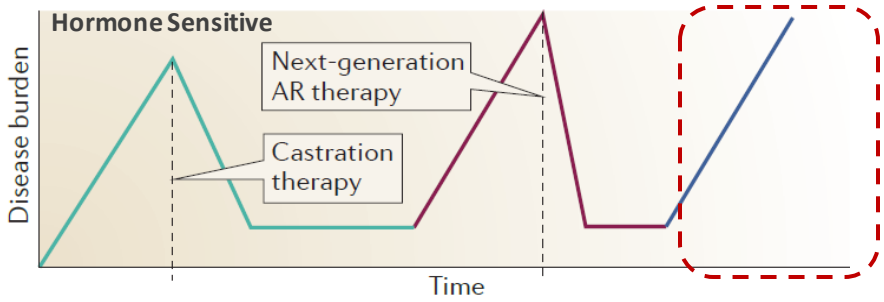
Raju Kandimalla

-I have no financial relationships to disclose.

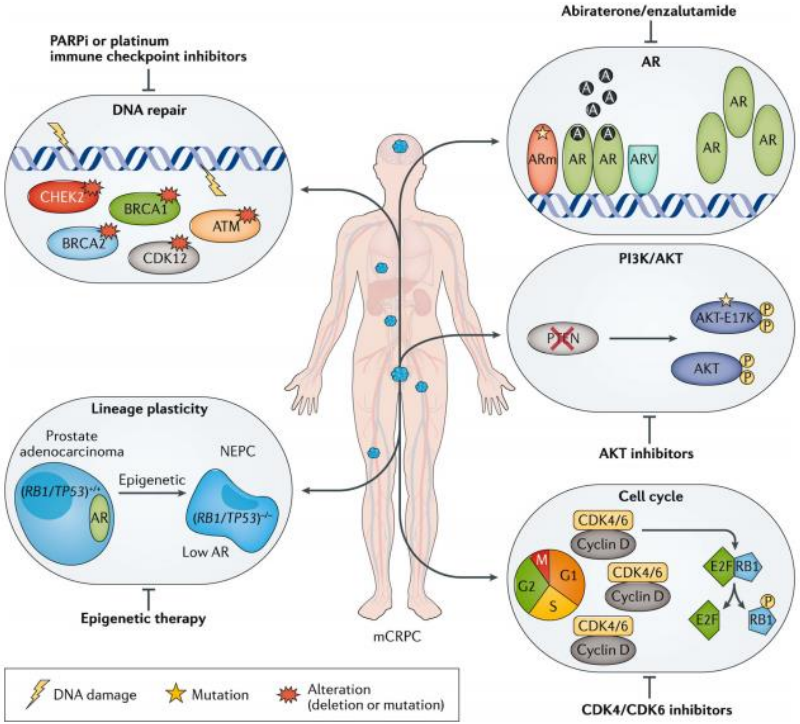
-and-I will not discuss off label use and/or investigational use in my presentation.

Impact of cfDNA in implementing precision medicine for patients with prostate cancer

Sequence of Prostate Cancer Treatment/Progression:



- Both genetic and epigenetic alterations (ex: DNA methylation/androgen receptor (AR) enhancer amplification) associated to resistance to second generation AR therapies
- Cell-free DNA (cfDNA) based targeted panels and CDx assays are already helping in implementing precision medicine
- cfDNA whole genome/epigenome profiling could add value in identifying novel resistance mechanisms and inform rational combinations
- *Current commonly used methodologies require relatively large amounts of cfDNA input material*



(Beltran et al. Nat Rev Urol 2019;(1)1041-1053)

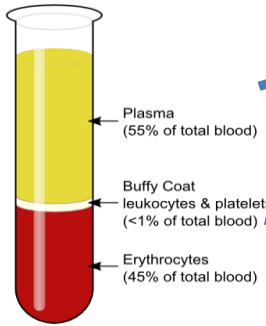
General workflow of plasma whole exome sequencing (WES) and targeted ATLAS sequencing

ID	Primary Diagnosis	Age	Active Treatment	Prior Treatment
P017685	CRPC	57	Trustuzumab	Mitoxantron, Cytoxan, Jevtana, Zytiga, Taxotere, Treslstar,
P017683	CRPC	68	Trelstar/Zytiga	Provenge
P017689	PC	72	Xtandi/Trelstar	Xtandi, Trelstar, Taxotere, Prolia
P018059	Healthy	69		
P017688	Healthy	57		
P017684	Healthy	63		

CRPC: Castration Resistant Prostate Cancer; PC: Prostate adenocarcinoma

Input amount:
30ng vs. 10ng

Panel:
WES vs. Atlas



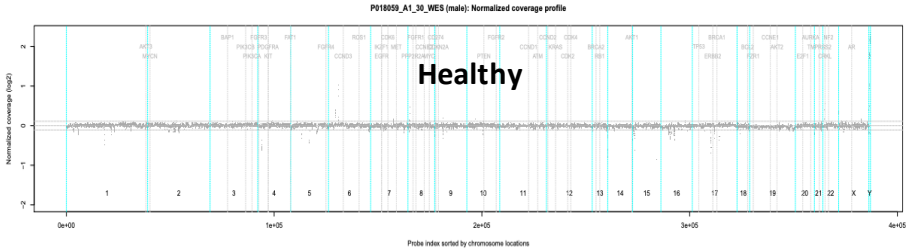
Plasma WES
(2500x depth)

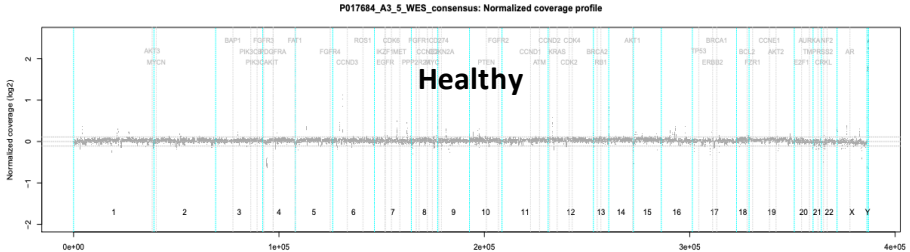
Plasma Atlas
(20000x depth)

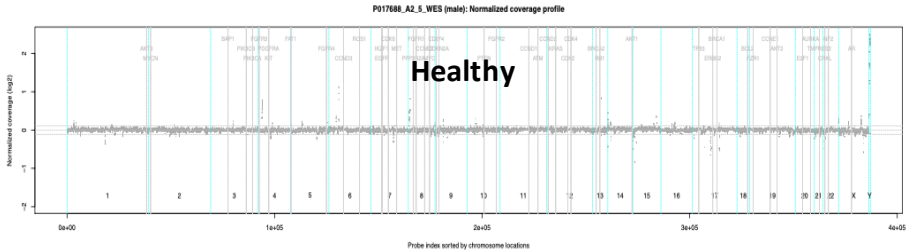
PBMC WES
(150x depth)

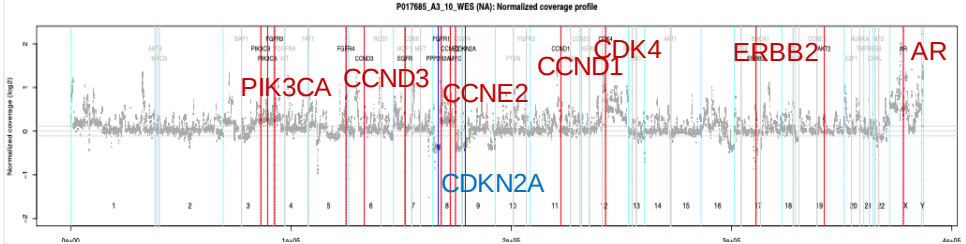
- Somatic mutations
- Pathogenic germline variants
- Copy number changes
- Fusions
- Tumor fraction
- TMB, MSI

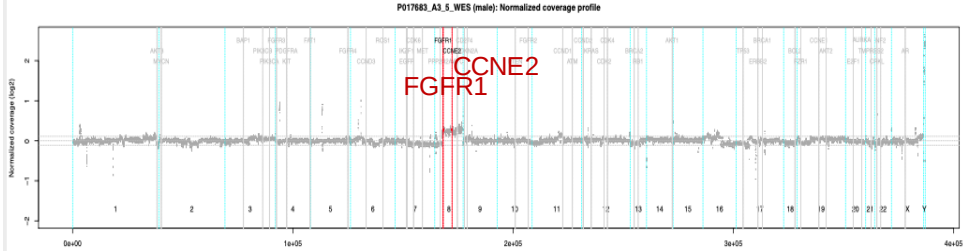
mCRPC patients showed distinct CNV profiles compared to healthy individuals

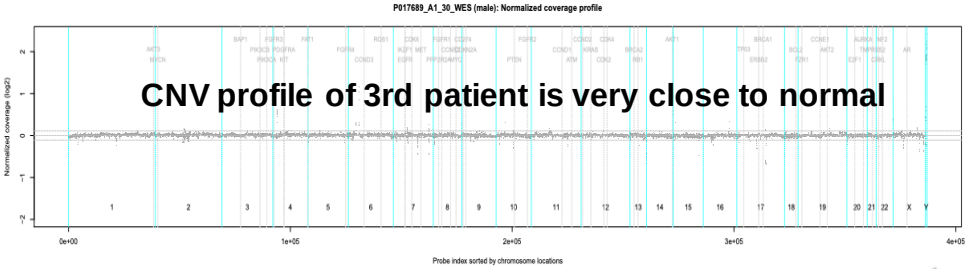












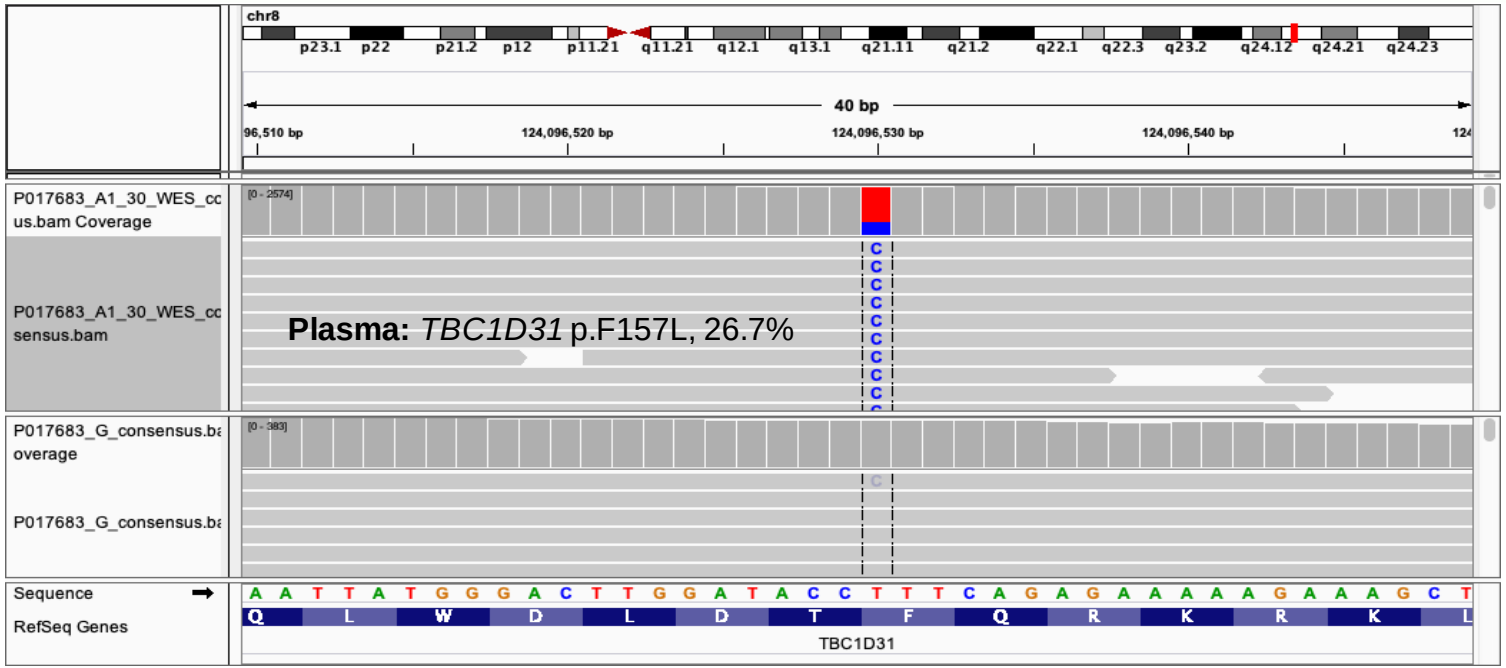
Plasma WES captures much more mutations than PredicineATLAS panel

	Plasma WES somatic mutations	Plasma WES synonymous mutations	Plasma WES mutations (AF > 1%)	ATLAS somatic mutations	ATLAS synonymous mutations	ATLAS mutations (AF < 1%)	ATLAS mutations (AF > 1%) by plasma WES	ATLAS mutations missed by plasma WES (MAF range)
P017685	***312***	65	274	***22***	3	10	100%	6 (0.27~0.59%)
P017683	***67***	9	57	***10***	1	4	100%	3 (0.23~0.43%)
P017689	***5***	1	5	***0***	0	0	NA	0
P017684	2	1	1	0	0	0	NA	0
P017688	2	1	1	0	0	0	NA	0
P018059	2	0	1	3	0	2	100%	2 (0.22~0.29%)

- Plasma WES captures much more mutations than PredicineATLAS panel
- All PredicineATLAS mutations ≥1% were also detected by plasma WES panel
- Two hot spots mutations in healthy donor P018059: TP53 p.R248Q (2.03%) and ATM p.R3008H (0.30%), which are likely CHIP mutations.
- Some low AF variants could be false positives due to the difficulties to differentiate somatic from germline/background at low AF for plasma WES.

AF: Allele Frequency

Tumor fraction estimation is more accurate in plasma WES



- Highest MAF (Mutant Allele Frequency) mutations detected by plasma WES panel: *TBC1D31* p.F157L, 26.7%
- Top mutations detected by PredicineATLAS panels: *STAG2* p.G66D 11.9%; *AR* p.T878A 9.27%

General workflow of plasma WGBS and PredicineECM

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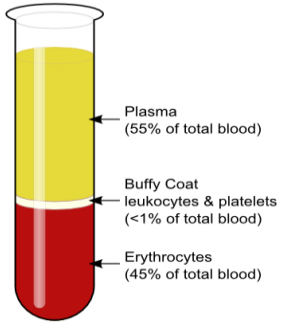
CRPC: Castration Resistant Prostate Cancer; PC: Prostate adenocarcinoma

Input amount:
2.5, 5, and 10ng

Panel:
PredicineECM vs. WGBS



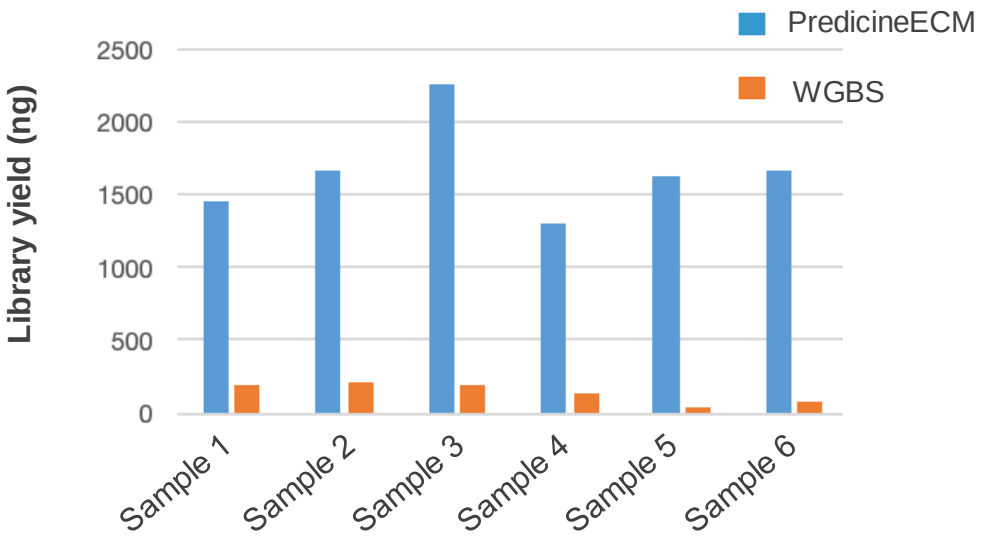
Plasma PredicineECM
(50x depth)
Converts methylated C to T

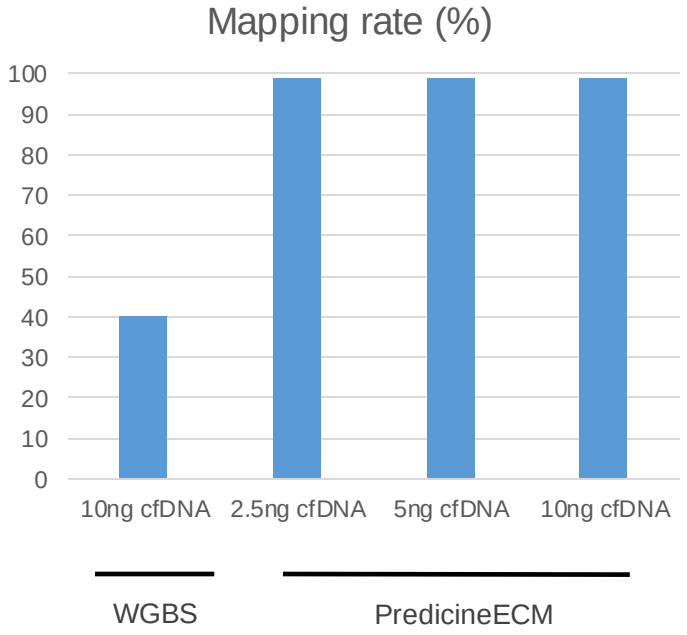
Plasma WGBS
(50x depth)
Converts Unmethylated C to T

DMR analysis

PredicineECM provides 10-fold higher library yield and 2-fold high mapping rate

Library yield with the same amount of DNA and same number of PCR cycles





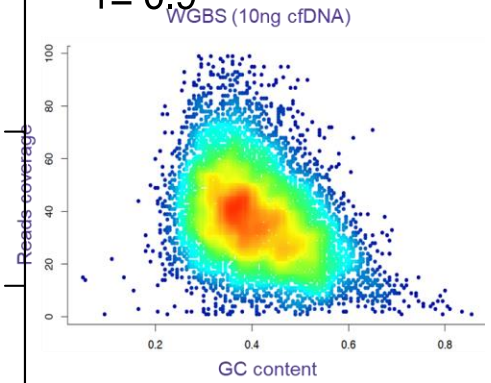
PredicineECM assay showed good coverage across different GC% regions and high correlation to WGBS

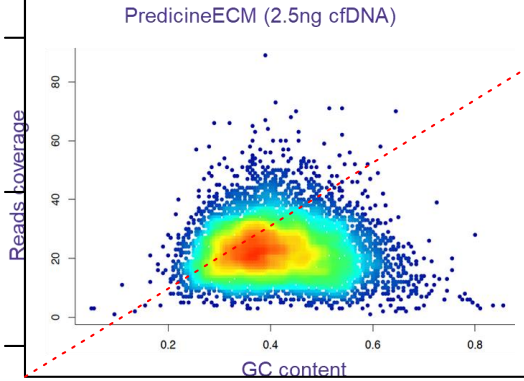
P001451_201006_10ng_TET3h , CpG number= 39741

Taps coversion rate

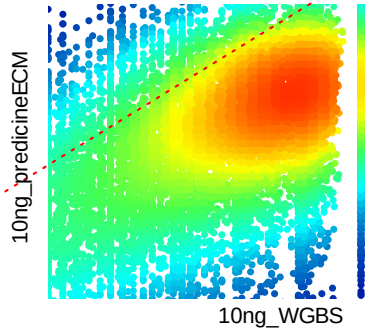
Coverage Distribution vs. GC content

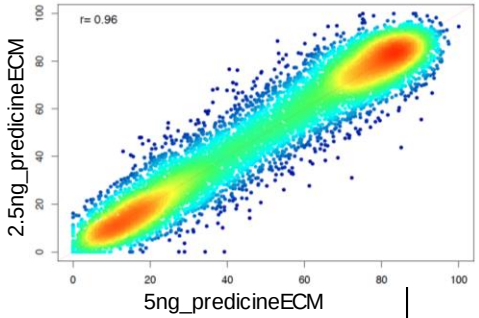
r= 0.9



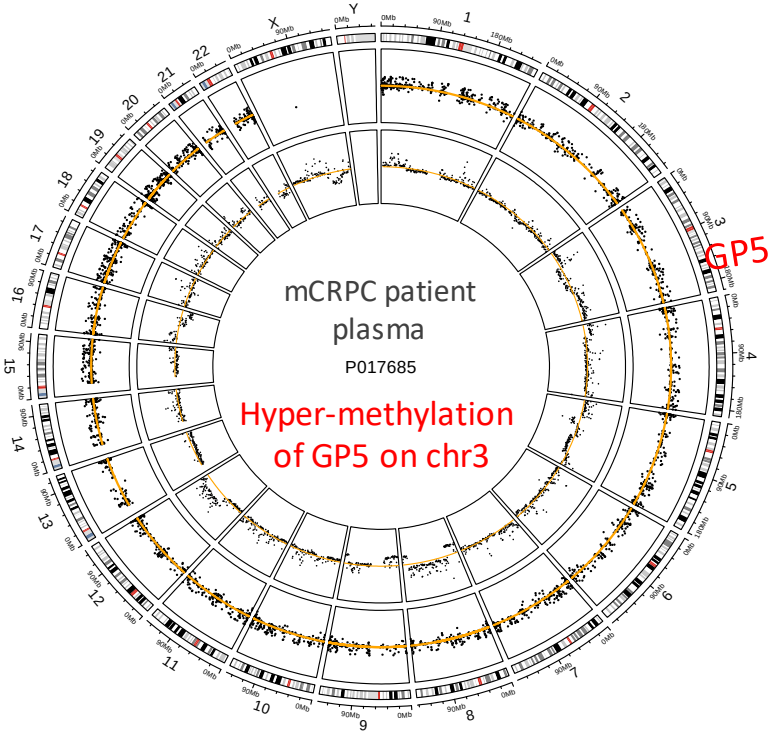


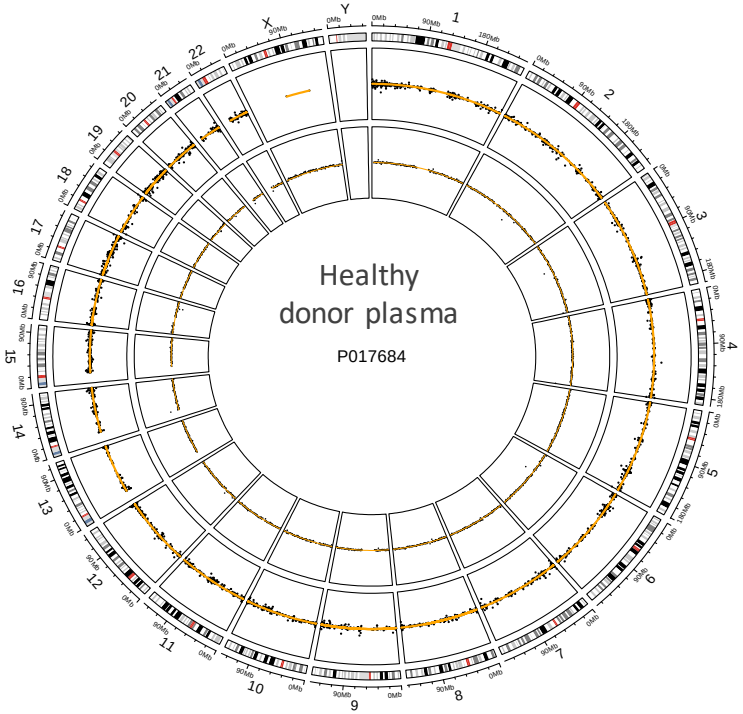
Methylation correlation between PredicineECM vs. WGBS





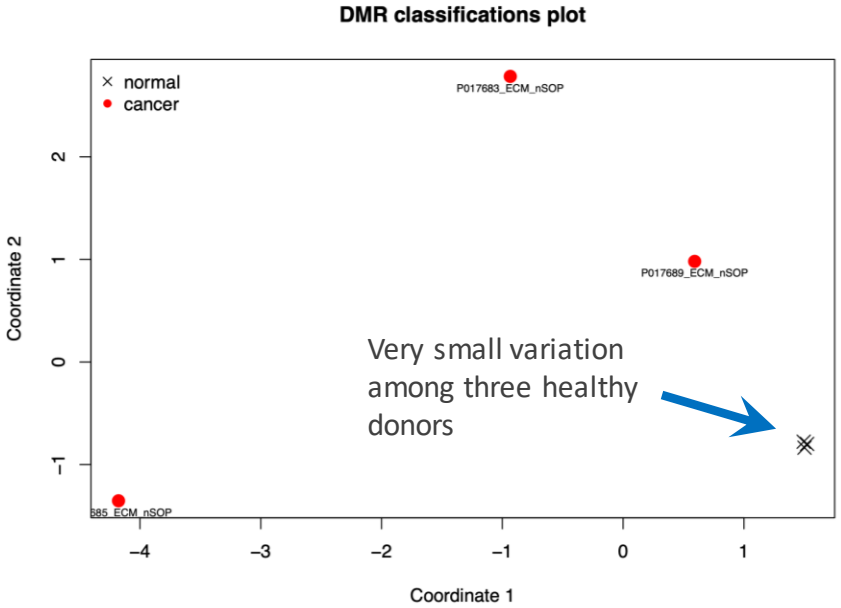
Circos plots showing the genome-wide DNA methylation and CNV profiles in mCRPC vs. Healthy

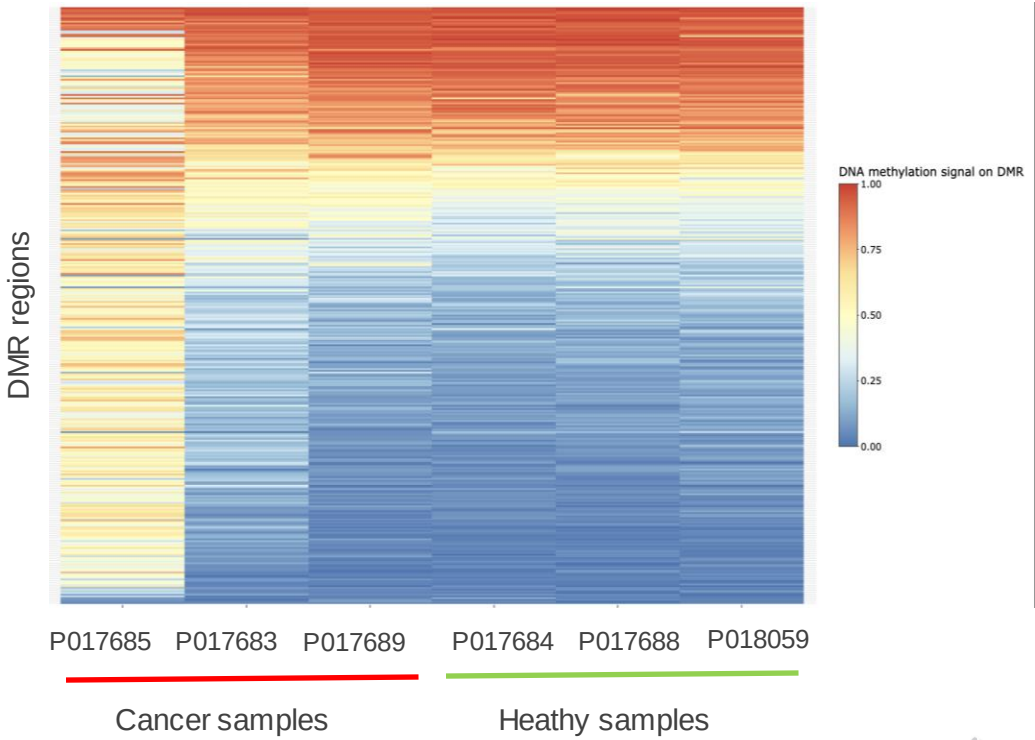




DMR analysis shows many hyper-methylated regions (red) in mCRPC sample, including one marker region GP5.

DNA methylation profiles are distinct between mCRPC and healthy plasma samples





Conclusions

Low-input Plasma cfDNA Whole Exome Sequencing

- Plasma low pass WES can provide more comprehensive mutation, CNV profile, and better tumor fraction estimation than ATLAS targeted panel
- Low pass plasma WES detects all ATLAS mutations > 1% LOD (Limit Of Detection)
- *Predicine WES+ (2500x WES with 1% LOD & 20000x for ATLAS panel with LOD 0.25%)*

Low-input Plasma Whole Genome Methylation Sequencing

- Using low cfDNA input (2.5, 5 and 10 ng cfDNA), PredicineECM assay demonstrated superior mapping quality and less GC bias than whole genome bisulfite sequencing (WGBS)
- PredicineECM methylation results clearly separated cancer vs. healthy plasma samples

Acknowledgements

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