In-depth Gene Expression Profiling of Seminomatous Testicular Germ Cell Tumors

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ABSTRACT

In depth Whole Transcriptome Analysis by RNA-Seq was used to compare the expression profile of testicular germ cell tumors of seminoma histology and normal testis **(**Poster #2, Abstract #3561). From this analysis we identified a large number of genes that showed differential expression with statistically high significance, (p<0.01, >2X FC). The list included genes related to stem cell pluripotency (*NANOG, POU5F1*), proliferation (*KRAS, CCND2*), a number of non-coding RNAs (snoRNAs, miRNA precursors, *XIST*), and testicular cancer related genes (*LDHB, AKAP4*). From this list we identified a subset of genes for validation and further screening. We used the QuantStudio™ 12K Flex Real-Time PCR OpenArray® platform to quantitatively screen up to 112 coding and non-coding genes (including controls) using samples from different malignant testicular germ cell tumors (GCC): seminoma (SE) and non-seminoma (NS) and normal tissue (N).

We used a complete work flow solution from sample prep to next-generation sequencing (NGS) to qPCR to compare the expression profile of normal testis and seminoma type germ cell tumors.

MATERIALS AND METHODS

A. Profiling on NGS Ion Proton™ Platform

B. Validation/Screening on OpenArray® Platform

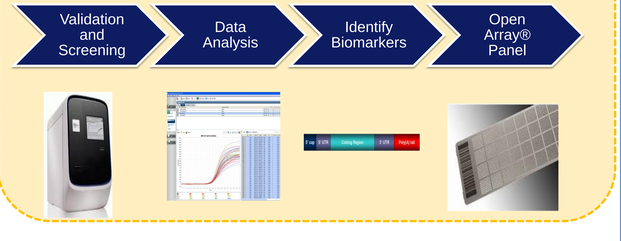


Figure 1. Complete Workflow. The complete workflow from profiling with sequencing on the Ion Torrent™ Proton™ System (A) to screening and qPCR validation on the QuantStudio™ OpenArray® Platform (B).

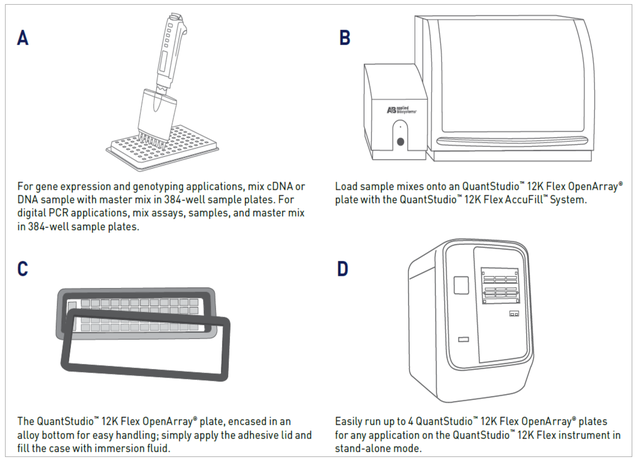
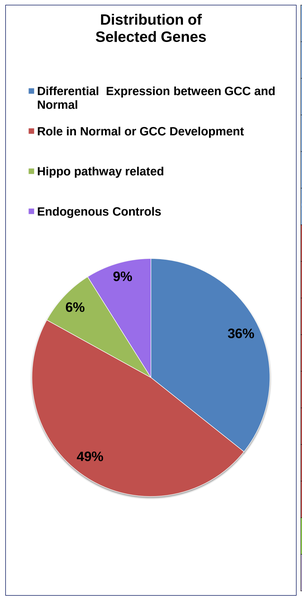


Figure 2. The QuantStudio™ 12K Flex Real-time PCR OpenArray® System. Custom 112 format OpenArray® plates are were used to screen 112 genes for each sample. 24 Samples(14 SE, 7 NS, and 3 N) were screened in triplicate on four custom OpenArray® plates. 200ng of total RNA (converted to cDNA) was used to allow ~1ng per through-hole qPCR reaction.



CCPG1	CD53	CDX1	CYP17A1	DPPA5	DSP	ELOVL6
EPHB4	ESRP1	FAM162B	GSG1	IGF2	L1TD1	LDHC
MOB1A	MOB1B	MSH2	MYCL	MYCN	NGEF	NLRP9
OSBPL3	PDPN	PIK3AP1	PIK3C2B	PIM2	PLCZ1	PQLC3
PTPN12	SERPINA5	SLA	SNX10	SOX13	SOX30	SOX9
SYNJ2BP	TET1	TFCP2L1	YWHAZ	ZNF107	AKAP4	ALPL
ALPPL2	ARHGDIB	BCAR4	CARD11	CASP8	CCND2	CD44
CPEB1	CTSC	CXCL9	DICER1	DNAAF1	DSCC1	EFEMP1
CECR1	ETV1	ETV4	ETV5	FMN2	HDAC9	HELLS
IGF2BP1	JUP	KIT	KRAS	LASP1	LDHB	LIN28A
MDM2	MSH6	NANOG	NANOG3	NFE2L3	NLRP7	NRCAM
PODXL	POU5F1	PRDM1	PRDM14	PROM1	PTCHD1	RAB15
RPRM	SET	SIRT1	SOX2	SOX15	SOX17	SPRY4
SUSD2	TFAP2C	XIST	ZFP42	TEAD4	WNT3	DLG3
FZD10	PPP1CB	FRMD6	FZD5	GAPDH	HPRT1	USP32
BCR	CASC3	ATP5B	ARF1	ALAS1	IPO8	GUSB

Figure 3. Distribution of 112 genes spotted on QuantStudio™ OpenArray® Panel. Genes selected had consistent differential expression between normal and GCC samples, and are suggested to play a role in in normal and aberrant gonadal development.

RESULTS

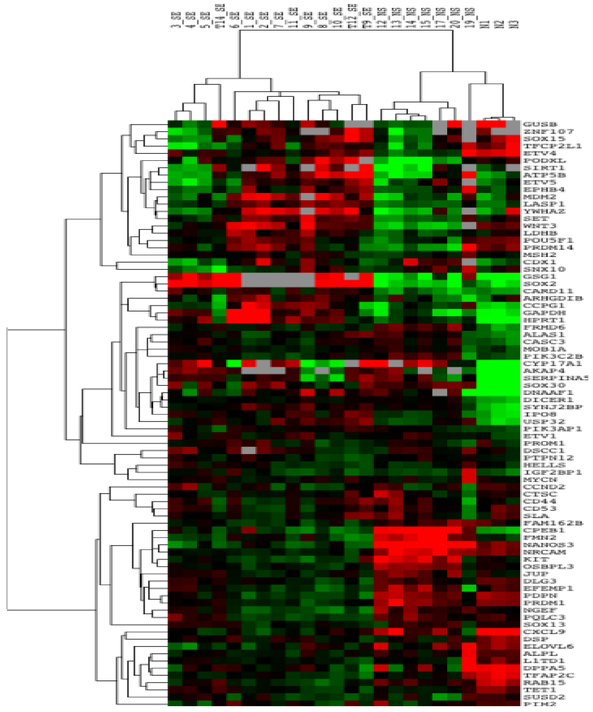


Figure 4. Cluster Analysis of OpenArray® Panel Screening Data. Expression levels were normalized to USP32 (most stable in samples according to transcriptome sequencing and qPCR data), and hierarchically clustered using an unsupervised centroid algorithm. Green means ΔΔCt is greater than the median of that gene in all samples; red means ΔΔCt is lower than median; grey is undetectable. SE samples are separated from NS and N samples using these selected genes.

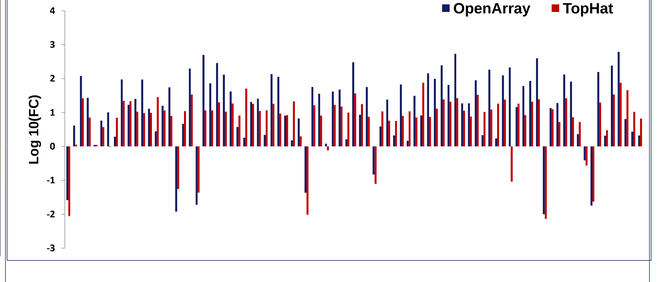


Figure 5. Comparison of Results using Open Array and NGS. Fold change comparison between normal (3 samples) and SE (3 samples) that were run on Open Array and NGS for 112 genes. qPCR expression levels were normalized with USP32. 94% of the assays showed good directional concordance. Blue – OpenArray® panel FC (fold change) for qPCR; Red,-TopHat, FC for NGS.

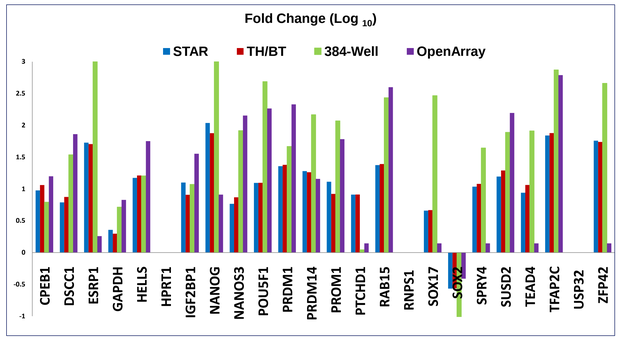


Figure 6. Comparison of FC Across Three Different Platforms. A subset of 20 TaqMan® Gene Expression Assays comparison between 3 platforms: NGS (STAR and TopHat2/BowTie2 aligners), TaqMan® Assays on 384-well plate and OpenArray® panel. qPCR expression levels were normalized to USP32, HPRT, and RNPS1.

CONCLUSIONS

1. We successfully used the Ion Proton™ System with Ambion® RNA library preparation kits for Whole Transcriptome sequencing on normal and seminoma cancer samples (Poster # 2, Abstract 3561) following by gene expression validation and screening on QuantStudio™ OpenArray® platform (Figure 1 and 2).
2. From NGS data we identified 112 genes of interest that had p<0.05 and >2 fold differential for screening on the QuantStudio™ 12K Flex Real-Time qPCR Systems OpenArray® Platform (Figure 3).
3. Data from 24 samples show that the non-seminoma, seminoma, and normal samples cluster separately and can be differentiated (Figure 4).
4. Fold change across three different platforms, NGS and OpenArray® and TaqMan® Assays on plates showed good directional concordance (Figure 5 and 6).

ACKNOWLEDGEMENTS

We would like to thank Dr. Stephen Jackson for his great support with data analysis and generating the heat map.

TRADEMARKS/LICENSING

The QuantStudio™ 12K Flex Real-Time PCR System is For Research Use Only. Not for use in diagnostic procedures.