Supplemental Material

Analytical Validation of the RT-PCR Assay: Assay performance was tested for intra-assay coefficient of variation (CV) using 6 replicates in 3 independent experiments, and inter-assay CV across 6-log dilutions, each in triplicate, by 3 different technologists. A CV below 15% is considered an acceptable level. The dynamic linearity of the assay was tested over a log range of dilutions from 0.1 pg to 1,000 ng RNA of human prostate LNCaP cell line. The linear correlation coefficient was calculated, and the concordance between a standard PCR platform (Eppendorf Realplex) and the multiplex PCR assay was tested with 5 serial dilutions.

Each assay was tested in single runs and as a multiplex run to exclude interference in reverse transcription and inter-primer interference. The analytical sensitivity of each assay was evaluated using LNCaP prostate cancer cells spiked at 10, 100, 500, and 1,000 cells per 2.5 ml of volunteer blood.

The RT-PCR analysis of each gene transcript detection from 10 LNCaP cells spiked in 2.5ml of healthy volunteer blood demonstrated CV<6% .

Figure S1. Analytical validation of the RT-PCR assay. The dynamic linearity range and the limit of detection were established based on LNCaP dilutions from 1 pg to 100 ng.

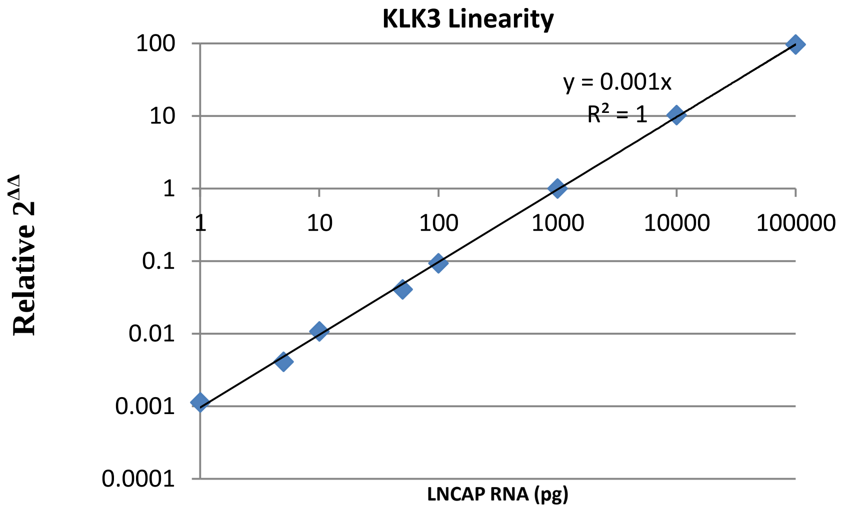

Table S1. Performance of the multiplex detection of *KLK3*, *KLK2*, *HOXB13*, *GRHL2*, and *FOXA1* transcripts: Assay performance was tested for intra- and inter-assay coefficient of variation (CV) and dynamic linear correlation and a reportable range was established for the multiplex detection using LNCaP RNA. Percentage of volunteer controls and CRCP patients with detectable transcripts, per gene. *, n=6

	Assay CV		Linear Correlation	Reportable Range	p-value	C_t Threshold	Detectable signal (%)	
	Intra- (n=18)	Inter- (n=42)					Controls (n=51)	Patients (n=97)
KLK3	1.05%	2.13%	1	1 pg - 100 ng	< *0.001*	26.9	0%	55%
KLK2	0.27%*	2.98%	0.999	5 pg - 100 ng	< *0.001*	40	0%	39%
HOXB13	1.02%	1.29%	1	5 pg - 100 ng	< *0.001*	34.4	6%	53%
GRHL2	0.98%	1.08%	1	10 pg - 100 ng	< *0.001*	40	0%	29%
FOXA1	0.88%	1.26%	0.999	50 pg - 100 ng	< *0.001*	40	0%	42%

Table S2. Distribution of the original 130 genes. Filtering steps.

S2.A Gene expression enriched ≥4-fold in prostate tissue compared to PBMC in TiGER (Tissue-specific Gene Expression and Regulation) database(12) resulted in 130 genes.

Symbol	Enrichment factor (TiGER)
UPK3A	25.5235
SEMG1	24.1104
PRAC	23.9283
SEMG2	23.6152

MSMB	23.2264
EDC3	23.2264
KLK2	**22.7748**
TGM4	21.6653
KLK4	20.2061
OR51E2	20.1094
ACPP	19.693
TARP	18.4554
KLK3	**18.3136**
NKX3-1	18.0329
PPIL5	17.8664
HOXB13	**16.8346**
POTEG	16.0703
RLN1	15.3141
PCDHB8	14.5848
PCDHB13	14.5848
SLC45A3	14.4948
XCL2	13.9219
FOXA1	**13.629**
TRPM8	13.6125
TMPRSS2	12.8894
SRD5A2	12.7617
PATE1	12.7617
GLTSCR1	12.6767
LOC150519	12.6488
ATP5SL	12.4288
AR	12.2513
C5orf37	12.1727
NWD1	12.0111
ANO7	11.9641
SLC39A2	11.7801
TEPP	11.6016
DOCK9	11.2079
MPHOSPH10	10.9386
CYP24A1	10.9386
TDRD6	10.7984
ARHGAP6	10.7846
FCER1A	10.7467
ZNF554	10.6348
SPRR4	10.5097
FLJ20184	10.4414
C21orf84	10.2094
CPNE4	10.176
ALOX15B	10.0092
ZNF35	9.96039
KCNJ5	9.9258
C6orf141	9.81673
PLA2G4F	9.40339
ACTG2	9.37597
SULT2B1	9.32589
LMAN1L	9.21681

C10orf125	9.00829
CSTA	8.84814
SATB1	8.70801
BMPR1B	8.36114
PAGE4	8.24605
AMACR	8.18935
PTGFR	8.12111
EPC1	8.02543
NEFH	8.01319
LPAR3	7.97609
KCNC2	7.81331
OR51E1	7.76802
TTC8	7.7482
SCGB3A1	7.65705
CYP4F8	7.65705
LOC643837	7.63523
ZNF552	7.53152
DNAJC19	7.52943
PIP	7.50691
NPY	7.38838
WNT5B	7.37345
CD177	7.29242
RPL22L1	7.25897
TMEM220	7.14658
MYH11	7.13156
SLC2A12	7.04096
TMEFF2	7.01195
TTLL3	7.00997
FOLH1	6.99838
DNAH5	6.96095
DNAH8	6.96095
SCUBE2	6.93231
RDH11	6.92832
SLC14A1	6.83023
ERGIC1	6.78767
ZNF761	6.6583
PSCA	6.63611
TSC22D2	6.55028
IGF1	6.52926
EMILIN2	6.52926
EPHX2	6.51663
PAEP	6.49689
NME4	6.4031
S1PR5	6.38087
OPLAH	6.38087
ITGA8	6.25065
ZNF613	6.16084
PART1	6.04504
TNFRSF10D	5.9251
PDE9A	5.91849
SLC30A4	5.74278

C1orf116	5.73784
HOXA13	5.67189
ABCC4	5.60563
HIST1H4H	5.60272
DLK2	5.58326
TRAPPC6B	5.5257
STEAP1	5.51859
AQP2	5.51859
PCP4	5.46932
MST1R	5.43943
GRHL2	**5.38569**
ZG16B	5.37337
FAM83A	5.34213
MARVELD2	5.23559
CTSG	5.22071
AZGP1	5.19122
PTGS2	5.18446
LCAT	5.16931
RPL39	5.1047
HOXD9	5.1047
PSEN2	5.1047
ING2	5.1047
SLC26A7	5.1047
SCGB1A1	5.02101

S2.B Top 30 genes expressed in prostate cancer metastatic tissue (Prostate Cancer Genomic Project(13)).

Symbol	Enrichment factor (TiGER)	Mets (average)
OR51E2	20.1094	12.98
KLK2	**22.7748**	**12.08**
KLK3	**18.3136**	**12.06**
LOC643837	7.63523	11.71
IGF1	6.52926	11.3
SLC30A4	5.74278	11.06
SLC45A3	14.4948	10.96
TMEM220	7.14658	10.77
AMACR	8.18935	10.74
MARVELD2	5.23559	10.71
ERGIC1	6.78767	10.63
NKX3-1	18.0329	10.51
FOXA1	**13.629**	**10.41**
AR	12.2513	10.38
FOLH1	6.99838	10.34
RPL39	5.1047	10.29
ZNF552	7.53152	10.17
RDH11	6.92832	10
ACPP	19.693	9.98
HOXB13	**16.8346**	**9.94**
NME4	6.4031	9.94
ABCC4	5.60563	9.81
ZNF554	10.6348	9.69
KLK4	20.2061	9.65
NPY	7.38838	9.65
HIST1H4H	5.60272	9.6
KCNJ5	9.9258	9.55
ZG16B	5.37337	9.52
GRHL2	**5.38569**	**9.51**
TARP	18.4554	9.46

S2.C Top 10 genes expressed in prostate tissue but not in PBMC or in >2 tissues other than prostate tissue. (Novartis Gene Expression Database(14)).

Symbol	**Enrichment factor (TiGER)**	**Mets (average)**	**GNF tissues**	**RT-qPCR signal in PBMC**	**Final selection**
KLK2	22.7748	12.08	prostate only	NO	YES
KLK3	18.3136	12.06	prostate only	NO	YES
SLC45A3	14.4948	10.96	prostate only	YES	NO
NKX3-1	18.0329	10.51	prostate, trachea	YES	NO
FOXA1	13.629	10.41	prostate, lung, liver	NO	YES
FOLH1	6.99838	10.34	prostate only	YES	NO
ACPP	19.693	9.98	prostate only	YES	NO
HOXB13	16.8346	9.94	prostate only	NO	YES
NPY	7.38838	9.65	prostate only	YES	NO
GRHL2	5.38569	9.51	prostate, placenta	NO	YES