Supplementary Figure 1. Study design

From our earlier Genome-wide methylation study, 21 CGIs which were most methylated in bladder cancer compared to control urines were selected for the urine assay development.

Marker Selection

Technical performance of these 21 CGIs were tested in bladder cancer cell lines using BS-SNaPshot assay.

Results: 8 CGIs were selected that performed best regarding PCR efficiency and probe signal.

Testing

Tested the methylation of these 8 CGI's in an independent set of 48 FFPE tumors, 70 urines from healthy individuals, and 101 preTUR urines from recurrent tumors derived from primary Ta G1/G2 tumors (Test set).

Results: Logistic regression enabled us to find a best combination of 3 CGIs which showed a sensitivity of 68% at 90% specificity in detecting recurrent tumors.

Validation

The 3 gene methylation panel was validated in an independent set of 95 preTUR urines from recurrent tumors derived from primary Ta G1/G2 tumors (Validation set).

Results: Methylation assay consisting of a combination of 3 markers showed a sensitivity of 74% at 77% specificity in the validation set.

Results: Combination of the methylation assay with the FGFR3 assay resulted in a sensitivity of 79% in the detection of recurrent bladder tumors in voided urine (AUC=0.89).

Testing methylation assay in other urines

Results: The 3-plex methylation assay was tested in 39 primary tumor urines (Sensitivity 80%) and 40 urines from recurrence free patients (22% false positives). This assay was also tested in a subset of urines from other urological symptoms: 1) 25 High leucocyte count urines 2) 18 Cystitis urines 3) 30 lower urinary tract symptoms 4) 14 Prostate cancer urines and 5) 3 Renal cancer urines.