



(43) International Publication Date
12 September 2014 (12.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/138396 A1

- (51) **International Patent Classification:**
C12Q 1/68 (2006.01) *G01N 33/574* (2006.01)
- (21) **International Application Number:**
PCT/US2014/021199
- (22) **International Filing Date:**
6 March 2014 (06.03.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/773,724 6 March 2013 (06.03.2013) US
- (71) **Applicant:** CEPHEID [US/US]; 904 Caribbean Drive, Sunnyvale, California 94089 (US).
- (72) **Inventors:** MCCANN, Leena; 904 Caribbean Drive, Sunnyvale, California 94089 (US). WALLACE, Stacey Ellen; 904 Caribbean Drive, Sunnyvale, California 94089 (US). LAI, Edwin Wei-Lung; 904 Caribbean Drive, Sunnyvale, California 94089 (US). HIGUCHI, Russell; 904 Caribbean Drive, Sunnyvale, California 94089 (US). LYKKE, Chris; 904 Caribbean Drive, Sunnyvale, California 94089 (US).
- (74) **Agent:** SCARR, Rebecca B.; 2275 Deming Way, Suite 310, Middleton, Wisconsin 53562 (US).

(81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) **Title:** METHODS OF DETECTING BLADDER CANCER

(57) **Abstract:** Compositions and methods for detecting bladder cancer are provided. In some embodiments, methods of detecting low grade bladder cancer are provided. In some embodiments, methods of monitoring recurrence of bladder cancer are provided. The methods comprise detecting androgen receptor (AR) and/or uroplakin 1B (UPK1B). Methods for monitoring therapy with androgen receptor antagonists by determining the expression of AR and/or UPK1B are also provided. In some embodiments the expression is determined in urine samples.



WO 2014/138396 A1

METHODS OF DETECTING BLADDER CANCER

1. FIELD OF THE INVENTION

[001] Compositions and methods for detecting bladder cancer are provided. In particular, bladder cancer markers and panels of markers useful in the detection of bladder cancer are provided.

2. BACKGROUND

[002] 386,000 cases of bladder cancer are diagnosed globally each year, including 70,500 cases per year in the United States. The incidence of bladder cancer is three times higher in men than in women. The highest incidence and prevalence are found in the European Union, North America, North Africa, and the Middle East

[003] Smoking is the greatest risk factor for bladder cancer. Additional risk factors include chemical exposure, chemotherapy (such as Cytoxan), radiation treatment, and chronic bladder infection.

[004] Bladder tumors include papillary tumors, which are urothelial carcinomas that grow narrow, finger-like projections; and nonpapillary (sessile) tumors, such as carcinoma-in-situ, which are less common but have a high risk of becoming invasive.

[005] Symptoms of bladder cancer can include abdominal pain, blood in the urine, bone pain or tenderness, fatigue, painful urination, frequent urination, urinary urgency, incontinence, and weight loss. Diagnosis is generally based on imaging, urinalysis, and/or biopsy.

[006] The prognosis for bladder cancer depends on the stage of cancer at diagnosis. The prognosis for early tumors is favorable, while the prognosis for advanced tumors is poor. Long-term follow up is recommended to detect cancer recurrence, which occurs in up to 70% of bladder cancers. For the first two years, cystoscopy and urine cytology are recommended every 3 to 4 months, and then at longer intervals in subsequent years, often for the patient's lifetime. These methods are invasive and costly, making bladder cancer one of the most expensive cancers to treat from diagnosis until death.

[007] Existing non-invasive diagnostic tests include ImmunoCyt™ (Scimedx, Denville, NJ) and UroVysion® (Abbott Molecular, Abbott Park, IL). ImmunoCyt™ is a cytology assay that uses a cocktail of three monoclonal antibodies labeled with fluorescent markers to detect certain cellular markers of bladder cancer in exfoliated cells isolated from urine samples. ImmunoCyt™ is used in conjunction with standard urine cytology to improve cytology's sensitivity at detecting tumor cells. UroVysion® is also a cytology-based assay, which detects aneuploidy in certain chromosomes via fluorescent in situ

hybridization (FISH). Determination of the results is conducted by enumerating signals through microscopic examination of the nucleus of cells in urine.

[008] Improved methods for early detection of bladder cancer are needed. In particular, an accurate urine-based diagnostic test that does not rely on cytology could reduce the need for costly and invasive cystoscopy and labor-intensive and potentially subjective cytology assays.

3. SUMMARY

[009] Compositions and methods for detecting bladder cancer are provided. In particular, bladder cancer markers and panels of markers useful in the detection of bladder cancer are provided. In some embodiments, the levels of androgen receptor (AR) and/or uroplakin 1B (UPK1B) mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA, are measured, for example, by quantitative RT-PCR, and the results can be used to determine whether or not a subject has bladder cancer. In some embodiments, the levels of AR and/or UPK1B mRNA, and optionally at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, are normalized to an endogenous control. In some embodiments, the endogenous control is ABL mRNA. In some embodiments, an endogenous control is selected that is expected to be expressed at similar levels in bladder urothelial cells from subjects with and without bladder cancer. In some embodiments, the sample is a urine sample. In some embodiments, the present methods are used to monitor subjects with a history of bladder cancer for tumor recurrence. In some embodiments, the subject has been treated with Bacillus Calmette-Guerin (BCG) within the past three months. In some embodiments, the present methods are used to detect bladder cancer in subjects with no history of bladder cancer. In some such embodiments, the subjects have symptoms of bladder cancer. Nonlimiting exemplary symptoms of bladder cancer include abdominal pain, blood in the urine, bone pain or tenderness, fatigue, painful urination, frequent urination, urinary urgency, incontinence, and weight loss. In some embodiments, the bladder cancer is low grade bladder cancer.

[0010] In some embodiments, methods for detecting the presence of bladder cancer in a subject are provided. In some embodiments, a method comprises detecting the levels of each marker of a set of bladder cancer markers in a sample from the subject, wherein the set of bladder cancer markers comprises androgen receptor (AR) or uroplakin 1B (UPK1B), or

both AR and UPK1B. In some embodiments, detection of an elevated level of AR and/or UPK1B indicates the presence of bladder cancer in the subject.

[0011] In some embodiments, methods of monitoring anti-androgen therapy in a subject with bladder cancer are provided. In some embodiments, the methods comprise detecting the levels of each marker of a set of bladder cancer markers in a sample from the subject at a first time point, wherein the set of bladder cancer markers comprises androgen receptor (AR), or uroplakin 1B (UPK1B), or both AR and UPK1B. In some embodiments, a decrease in the level of the at least one marker at the first time point relative to a second time point indicates that the subject is responding to anti-androgen therapy. In some embodiments, the methods comprise comparing the level of the at least one marker at the first time point to the level of the at least one marker at the second time point. In some embodiments, the subject is undergoing anti-androgen therapy at the first time point. In some embodiments, the second time point is prior to the subject beginning anti-androgen therapy. In some embodiments, the subject is undergoing anti-androgen therapy at the second time point.

[0012] In some embodiments, the set of bladder cancer markers further comprises at least one marker selected from corticotrophin releasing hormone (CRH), insulin-like growth factor 2 (IGF2), keratin 20 (KRT20) and annexin 10 (ANXA10). In some embodiments, the set of bladder cancer markers comprises: a) AR, CRH, IGF2, KRT20, and ANXA10; b) AR, CRH, IGF2, and KRT20; c) UPK1B, CRH, IGF2, KRT20, and ANXA10; d) UPK1B, CRH, IGF2, and KRT20; e) AR, UPK1B, CRH, IGF2, KRT20, and ANXA10; f) AR, UPK1B, CRH, IGF2, and KRT20; g) UPK1B, CRH, IGF2, and ANXA10; or h) UPK1B, CRH, KRT20, and ANXA10.

[0013] In some embodiments, a method further comprises detecting an endogenous control. In some embodiments, the endogenous control is selected from ABL, GUSB, GAPDH, TUBB, and UPK1a. In some embodiments, the endogenous control is ABL. In some embodiments, a method comprises detecting an exogenous control. In some embodiments, the exogenous control is an RNA. In some such embodiments, the exogenous control is an Armored RNA[®]. In some embodiments, the method comprises detecting in a single multiplex reaction: a) AR, CRH, IGF2, KRT20, ANXA10, an endogenous control, and an exogenous control; b) AR, CRH, IGF2, KRT20, an endogenous control, and an exogenous control; c) UPK1B, CRH, IGF2, KRT20, ANXA10, an endogenous control, and an exogenous control; d) UPK1B, CRH, IGF2, KRT20, an endogenous control, and an exogenous control; e) AR, UPK1B, CRH, IGF2, KRT20, and

ANXA10; f) AR, UPK1B, CRH, IGF2, KRT20, an endogenous control, and an exogenous control; g) UPK1B, CRH, IGF2, ANXA10, an endogenous control, and an exogenous control; or h) UPK1B, CRH, KRT20, ANXA10, an endogenous control, and an exogenous control. In some embodiments, the method comprises detecting in a single multiplex reaction: a) AR, CRH, IGF2, KRT20, ANXA10, ABL, and an exogenous control; b) AR, CRH, IGF2, KRT20, ABL, and an exogenous control; c) UPK1B, CRH, IGF2, KRT20, ANXA10, ABL, and an exogenous control; d) UPK1B, CRH, IGF2, KRT20, ABL, and an exogenous control; e) AR, UPK1B, CRH, IGF2, KRT20, and ANXA10; f) AR, UPK1B, CRH, IGF2, KRT20, ABL, and an exogenous control; g) UPK1B, CRH, IGF2, ANXA10, ABL, and an exogenous control; or h) UPK1B, CRH, KRT20, ANXA10, ABL, and an exogenous control.

[0014] In some embodiments, detecting comprises RT-PCR. In some embodiments, detecting comprises quantitative RT-PCR. In some embodiments, a method comprises comparing a Ct value or a Δ Ct value to a threshold Ct value or Δ Ct value. In some embodiments, Δ Ct is the Ct value for the endogenous control minus the Ct value for the marker. In some embodiments, the RT-PCR reaction takes less than three hours or less than 2 hours from an initial denaturation step through a final extension step.

[0015] In some embodiments, a method comprises contacting RNA from the sample with a set of bladder cancer marker primer pairs, wherein the set of bladder cancer marker primer pairs comprises a first primer pair for detecting AR, or a first primer pair for detecting UPK1B, or a first primer pair for detecting AR and a second primer pair for detecting UPK1B. In some embodiments, the set of bladder cancer marker primer pairs comprises a third primer pair for detecting a marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A. In some embodiments, the set of bladder cancer marker primer pairs comprises a fourth primer pair for detecting a marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, wherein the third primer pair and the fourth primer pair detect different markers. In some embodiments, the set of bladder cancer marker primer pairs comprises a fifth primer pair for detecting a marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, wherein the third primer pair, the fourth primer pair, and the fifth primer pair detect different markers. In some embodiments, the set of bladder cancer marker primer pairs comprises a separate primer pair for detecting each marker in a set of markers selected from a) AR, CRH, IGF2, KRT20, and ANXA10; b) AR, CRH, IGF2, and KRT20; c) UPK1B, CRH, IGF2, KRT20, and ANXA10; d) UPK1B, CRH, IGF2, and KRT20; e) AR, UPK1B, CRH, IGF2, KRT20,

and ANXA10; f) AR, UPK1B, CRH, IGF2, and KRT20; g) UPK1B, CRH, IGF2, and ANXA10; or h) UPK1B, CRH, KRT20, and ANXA10. In some embodiments, the set of bladder cancer marker primer pairs comprises a separate primer pair for detecting each marker and control in a set selected from a) AR, CRH, IGF2, KRT20, ANXA10, an endogenous control, and an exogenous control; b) AR, CRH, IGF2, KRT20, an endogenous control, and an exogenous control; c) UPK1B, CRH, IGF2, KRT20, ANXA10, an endogenous control, and an exogenous control; d) UPK1B, CRH, IGF2, KRT20, an endogenous control, and an exogenous control; e) AR, UPK1B, CRH, IGF2, KRT20, and ANXA10; f) AR, UPK1B, CRH, IGF2, KRT20, an endogenous control, and an exogenous control; g) UPK1B, CRH, IGF2, ANXA10, an endogenous control, and an exogenous control; or h) UPK1B, CRH, KRT20, ANXA10, an endogenous control, and an exogenous control. In some embodiments, the set of bladder cancer marker primer pairs comprises a separate primer pair for detecting each marker and control in a set selected from: a) AR, CRH, IGF2, KRT20, ANXA10, ABL, and an exogenous control; b) AR, CRH, IGF2, KRT20, ABL, and an exogenous control; c) UPK1B, CRH, IGF2, KRT20, ANXA10, ABL, and an exogenous control; d) UPK1B, CRH, IGF2, KRT20, ABL, and an exogenous control; e) AR, UPK1B, CRH, IGF2, KRT20, and ANXA10; f) AR, UPK1B, CRH, IGF2, KRT20, ABL, and an exogenous control; g) UPK1B, CRH, IGF2, ANXA10, ABL, and an exogenous control; or h) UPK1B, CRH, KRT20, ANXA10, ABL, and an exogenous control.

[0016] In some embodiments, wherein each bladder cancer marker primer pair produces an amplicon that is 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, or 50 to 150 nucleotides long. In some embodiments, at least one, at least two, or at least three bladder cancer marker primer pair spans an intron in the genomic sequence. In some embodiments, each bladder cancer marker primer pair spans an intron in the genomic sequence.

[0017] In some embodiments, the primer pair for detecting AR comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 54 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 55, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the primer pair for detecting UPK1B comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13,

at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 51 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 52, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

[0018] In some embodiments, a method further comprises contacting RNA from the sample with an endogenous control primer pair. In some such embodiments, the endogenous control primer pair is for detecting an endogenous control is selected from ABL, GUSB, GAPDH, TUBB, and UPK1a. In some embodiments, the endogenous control primer pair is for detecting ABL. In some embodiments, the endogenous control primer pair comprises a first primer comprising SEQ ID NO: 8 and a second primer comprising SEQ ID NO: 9, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control primer pair comprises a first primer comprising SEQ ID NO: 41 and a second primer comprising SEQ ID NO: 42, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control primer pair comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 8 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 9, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control primer pair comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 41 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 42, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

[0019] In some embodiments, a method comprises contacting RNA from the sample with an exogenous control primer pair. In some such embodiments, the exogenous control primer pair is for detecting an exogenous RNA. In some embodiments, the exogenous control primer pair comprises a first primer comprising SEQ ID NO: 23 and a second primer comprising SEQ ID NO: 24, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the exogenous control

primer pair comprises a first primer comprising SEQ ID NO: 44 and a second primer comprising SEQ ID NO: 45, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the exogenous control primer pair comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 23 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 24, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the exogenous control primer pair comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 44 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 45, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

[0020] In some embodiments, the method comprises forming a set of bladder cancer marker amplicons, wherein the set of bladder cancer marker amplicons comprises an AR amplicon, or a UPK1B amplicon, or both an AR amplicon and a UPK1B amplicon, and contacting the bladder cancer marker amplicons with a set of bladder cancer marker probes, wherein the set of bladder cancer marker probes comprises a first probe for detecting the AR amplicon, or a first probe for detecting the UPK1B amplicon, or a first probe for detecting the AR amplicon and a second probe for detecting the UPK1B amplicon. In some embodiments, the set of bladder cancer marker amplicons comprises a third amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, a ANXA10 amplicon, a UPK2 amplicon, a MGEA5 amplicon, and a PIK3A amplicon, and wherein the set of bladder cancer marker probes comprises a third probe for detecting the third amplicon. In some embodiments, the set of bladder cancer marker amplicons comprises a fourth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, a ANXA10 amplicon, a UPK2 amplicon, a MGEA5 amplicon, and a PIK3A amplicon, wherein the set of bladder cancer marker probes comprises a fourth probe for detecting the fourth amplicon, and wherein the third amplicon and the fourth amplicon are different. In some embodiments, the set of bladder cancer marker amplicons comprises a fifth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, a ANXA10 amplicon, a UPK2 amplicon, a MGEA5 amplicon, and a PIK3A amplicon, wherein the set

of bladder cancer marker probes comprises a fifth probe for detecting the fifth amplicon, and wherein the third amplicon, the fourth amplicon, and the fifth amplicon are different.

[0021] In some embodiments, the probe for detecting AR comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 56, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the probe for detecting UPK1B comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 53, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, each bladder cancer marker probe comprises a dye, and wherein each dye is detectably different from the other three labels. In some embodiments, each bladder cancer marker probe comprises a fluorescent dye and a quencher molecule.

[0022] In some embodiments, a method comprises forming an endogenous control amplicon, and contacting the endogenous control amplicon with an endogenous control probe. In some embodiments, the endogenous control probe comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 10, 11, 12, or 43, wherein the endogenous control probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control probe comprises a dye that is detectably different from the dyes of the bladder cancer marker probes.

[0023] In some embodiments, a method comprises forming an exogenous control amplicon, and contacting the exogenous control amplicon with an exogenous control probe. In some embodiments, the exogenous control probe comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 25 or 46, wherein the exogenous control probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the exogenous control probe comprises a dye that is detectably different from the dyes of the bladder cancer marker probes and the endogenous control probe.

[0024] In some embodiments, the set of bladder cancer markers are detected in a single multiplex reaction. In some embodiments, the method comprises using linear discriminant analysis (LDA) to combine two or more of the bladder cancer marker levels into a combined score. In some embodiments, LDA is used to combine all of the bladder cancer marker levels into a combined score.

[0025] In some embodiments, the sample comprises urothelial cells. In some embodiments, the sample is selected from a urine sample and a bladder washing sample. In some embodiments, the subject has a history of bladder cancer. In some embodiments, the subject is being monitored for recurrence of bladder cancer.

[0026] In some embodiments, compositions are provided. In some embodiments, a composition comprises a set of bladder cancer marker primer pairs, wherein the set of bladder cancer marker primer pairs comprises a first primer pair for detecting AR, or a first primer pair for detecting UPK1B, or a first primer pair for detecting AR and a second primer pair for detecting UPK1B. In some embodiments, the set of bladder cancer marker primer pairs comprises a third primer pair for detecting a marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A. In some embodiments, the set of bladder cancer marker primer pairs comprises a fourth primer pair for detecting a marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, wherein the third primer pair and the fourth primer pair detect different markers. In some embodiments, the set of bladder cancer marker primer pairs comprises a fifth primer pair for detecting a marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, wherein the third primer pair, the fourth primer pair, and the fifth primer pair detect different markers.

[0027] In some embodiments, each bladder cancer marker primer pair produces an amplicon that is 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, or 50 to 150 nucleotides long. In some embodiments, at least one, at least two, or at least three bladder cancer marker primer pair spans an intron in the genomic sequence. In some embodiments, each bladder cancer marker primer pair spans an intron in the genomic sequence.

[0028] In some embodiments, the primer pair for detecting AR comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 54 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 55, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the primer pair for detecting UPK1B comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 51 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ

ID NO: 52, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

[0029] In some embodiments, a composition further comprises a set of bladder cancer marker probes, wherein the set of bladder cancer marker probes comprises a first probe for detecting an AR amplicon, or a first probe for detecting a UPK1B amplicon, or a first probe for detecting an AR amplicon and a second probe for detecting a UPK1B amplicon. In some embodiments, the set of bladder cancer marker probes comprises a third probe for detecting a third amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, a ANXA10 amplicon, a UPK2 amplicon, a MGEA5 amplicon, and a PIK3A amplicon. In some embodiments, the set of bladder cancer marker probes comprises a fourth probe for detecting a fourth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, a ANXA10 amplicon, a UPK2 amplicon, a MGEA5 amplicon, and a PIK3A amplicon, wherein the third amplicon and the fourth amplicon are different. In some embodiments, the set of bladder cancer marker probes comprises fourth probe for detecting a fifth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, a ANXA10 amplicon, a UPK2 amplicon, a MGEA5 amplicon, and a PIK3A amplicon, wherein the third amplicon, the fourth amplicon, and the fifth amplicon are different.

[0030] In some embodiments, the probe for detecting AR comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 56, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the probe for detecting UPK1B comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 53, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, each bladder cancer marker probe comprises a dye, and wherein each dye is detectably different from the other three labels. In some embodiments, each bladder cancer marker probe comprises a fluorescent dye and a quencher molecule.

[0031] In some embodiments, a composition further comprises an endogenous control primer pair for detecting an endogenous control. In some embodiments, the endogenous control is selected from ABL, GUSB, GAPDH, TUBB, and UPK1a. In some embodiments, the endogenous control is ABL. In some embodiments, the endogenous control primer pair comprises a first primer comprising SEQ ID NO: 8 and a second primer

comprising SEQ ID NO: 9, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control primer pair comprises a first primer comprising SEQ ID NO: 41 and a second primer comprising SEQ ID NO: 42, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control primer pair comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 8 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 9, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control primer pair comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 41 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 42, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

[0032] In some embodiments, a composition further comprises an endogenous control probe for detecting an endogenous control amplicon. In some embodiments, the endogenous control is selected from ABL, GUSB, GAPDH, TUBB, and UPK1a. In some embodiments, the endogenous control is ABL. In some embodiments, the endogenous control probe comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 10, 11, 12, or 43, wherein the endogenous control probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long. In some embodiments, the endogenous control probe comprises a dye that is detectably different from the dyes of the bladder cancer marker probes.

[0033] In some embodiments, a composition is a lyophilized composition. In some embodiments, the composition is a solution. In some embodiments, the composition further comprises urothelial cells. In some embodiments, the urothelial cells are from a urine sample.

[0034] Further embodiments and details of the inventions are described below.

4. DETAILED DESCRIPTION

4.1. Definitions

[0035] To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

[0036] As used herein, the terms “detect”, “detecting” or “detection” may describe either the general act of discovering or discerning or the specific observation of a detectably labeled composition.

[0037] As used herein, the term “detectably different” refers to a set of labels (such as dyes) that can be detected and distinguished simultaneously.

[0038] As used herein, the terms “patient” and “subject” are used interchangeably to refer to a human. In some embodiments, the methods described herein may be used on samples from non-human animals.

[0039] As used herein, “bladder cancer” is a tumor, such as a transitional cell carcinoma, arising from the lining of the bladder, and includes low grade and high grade bladder cancers, as well as metastatic bladder cancer. “Low grade bladder cancer” refers to superficial tumors that project into the interior of the bladder cavity. Low grade bladder cancers have a high rate of recurrence. “High grade bladder cancer” refers to a fast-growing and/or invasive tumor that invades the bladder wall. High grade bladder cancers have the potential to spread (i.e., metastasize) to other areas of the body. “Metastatic bladder cancer” refers to invasive bladder cancer that has spread to one or more locations in the body beyond the bladder.

[0040] As used herein, the terms “oligonucleotide,” “polynucleotide,” “nucleic acid molecule,” and the like, refer to nucleic acid-containing molecules, including but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-

2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

[0041] As used herein, the term “oligonucleotide,” refers to a single-stranded polynucleotide having fewer than 500 nucleotides. In some embodiments, an oligonucleotide is 8 to 200, 8 to 100, 12 to 200, 12 to 100, 12 to 75, or 12 to 50 nucleotides long. Oligonucleotides may be referred to by their length, for example, a 24 residue oligonucleotide may be referred to as a “24-mer.”

[0042] As used herein, the term “complementary” to a target RNA (or target region thereof), and the percentage of “complementarity” of the probe sequence to that of the target RNA sequence is the percentage “identity” to the sequence of target RNA or to the reverse complement of the sequence of the target RNA. In determining the degree of “complementarity” between probes used in the compositions described herein (or regions thereof) and a target RNA, such as those disclosed herein, the degree of “complementarity” is expressed as the percentage identity between the sequence of the probe (or region thereof) and sequence of the target RNA or the reverse complement of the sequence of the target RNA that best aligns therewith. The percentage is calculated by counting the number of aligned bases that are identical as between the 2 sequences, dividing by the total number of contiguous nucleotides in the probe, and multiplying by 100. When the term “complementary” is used, the subject oligonucleotide is at least 90% complementary to the target molecule, unless indicated otherwise. In some embodiments, the subject oligonucleotide is at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to the target molecule.

[0043] A “primer” or “probe” as used herein, refers to an oligonucleotide that comprises a region that is complementary to a sequence of at least 8 contiguous nucleotides of a target nucleic acid molecule, such as an mRNA or a DNA reverse-transcribed from an mRNA. In some embodiments, a primer or probe comprises a region that is complementary to a sequence of at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of a target molecule. When a primer or probe comprises a region that is “complementary to at least x contiguous nucleotides of a target molecule,” the primer or probe is at least 95% complementary to at least x contiguous nucleotides of the target

molecule. In some embodiments, the primer or probe is at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to the target molecule.

[0044] A “sample,” as used herein, includes urine samples (including samples derived from urine samples), and other types of human samples. As used herein, urine samples include, but are not limited to, whole urine, a sample comprising cells from a urine sample, a sample comprising the cell pellet isolated by centrifugation of a urine sample, a sample comprising cells isolated by filtration of a urine sample, and the like. In some embodiments, a urine sample comprises a preservative, such as a preservative that causes damage, such as lysis, of red and/or white blood cells. In some embodiments, a sample is a human sample other than a urine sample, such as a tissue sample (including a bladder tissue and/or bladder tumor sample), a blood sample (including whole blood, serum, plasma, etc.), etc. In some embodiments, a sample is a bladder washing sample.

[0045] As used herein, “corticotrophin releasing hormone” or “CRH” refers to an mRNA that encodes CRH, as well as the CRH protein. In some embodiments, CRH is human CRH. Nonlimiting exemplary human CRH mRNA sequences are found at GenBank Accession No. NM_000756, and at SEQ ID NO: 1.

[0046] As used herein, “insulin-like growth factor 2” or “IGF2” refers to an mRNA that encodes IGF2, as well as the IGF2 protein. In some embodiments, IGF2 is human IGF2. Nonlimiting exemplary human IGF2 mRNA sequences are shown in SEQ ID NOs: 2 to 4.

[0047] As used herein, “keratin 20” or “KRT20” refers to an mRNA that encodes KRT20, as well as the KRT20 protein. In some embodiments, KRT20 is human KRT20. Nonlimiting exemplary human KRT20 mRNA sequences are found at GenBank Accession No. NM_019010, and at SEQ ID NO: 5.

[0048] As used herein, “annexin A10” or “ANXA10” refers to an mRNA that encodes ANXA10, as well as the ANXA10 protein. In some embodiments, ANXA10 is human ANXA10. Nonlimiting exemplary human ANXA10 mRNA sequences are found at GenBank Accession No. NM_007193, and at SEQ ID NO: 6.

[0049] As used herein, “androgen receptor” or “AR” refers to an mRNA that encodes AR, as well as the AR protein. In some embodiments, AR is human AR. Nonlimiting exemplary human AR mRNA sequences are found at GenBank Accession Nos. NM_000044 and NM_001011645, and at SEQ ID NOs: 49 and 57.

[0050] As used herein, “uroplakin 1B” or “UPK1B” refers to an mRNA that encodes UPK1B, as well as the UPK1B protein. In some embodiments, UPK1B is human UPK1B.

Nonlimiting exemplary human UPK1B mRNA sequences are found at GenBank Accession No. NM_006952 and at SEQ ID NO: 50.

[0051] As used herein, “uroplakin 2” or “UPK2” refers to an mRNA that encodes UPK2, as well as the UPK2 protein. In some embodiments, UPK2 is human UPK2. Nonlimiting exemplary human UPK2 mRNA sequences are found at GenBank Accession No. NM_006760.3, and at SEQ ID NO: 58.

[0052] As used herein, “meningioma expressed antigen 5” or “MGEA5” refers to an mRNA that encodes MGEA5, as well as the MGEA5 protein. In some embodiments, MGEA5 is human MGEA5. Nonlimiting exemplary human MGEA5 mRNA sequences are found at GenBank Accession Nos. NM_001142434.1 and NM_012215.3, and at SEQ ID NOs: 59 and 60.

[0053] As used herein, “phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha” or “PIK3CA” refers to an mRNA that encodes PIK3CA, as well as the PIK3CA protein. In some embodiments, PIK3CA is human PIK3CA. Nonlimiting exemplary human PIK3CA mRNA sequences are found at GenBank Accession No. NM_006218.2, and at SEQ ID NO: 61.

[0054] An “endogenous control,” as used herein refers to a moiety that is naturally present in the sample to be used for detection, and which can be used to normalize the levels of the bladder cancer markers described herein (including, but not limited to, AR, UPK1B, CRH, IGF2, KRT20, and ANXA10). Thus, an endogenous control is typically a moiety that is present at similar levels from cell to cell, and at similar levels in cells from subjects with bladder cancer and cells from subjects without bladder cancer. In some embodiments, an endogenous control is an RNA (such as an mRNA, tRNA, ribosomal RNA, etc.). Nonlimiting exemplary endogenous controls include ABL mRNA, GUSB mRNA, GAPDH mRNA, TUBB mRNA, and UPK1a mRNA. Nonlimiting exemplary human ABL mRNA sequences are found at GenBank Accession No. NM_007313, and at SEQ ID NO: 7. In some embodiments, an endogenous control is selected that can be detected in the same manner as the bladder cancer markers are detected and, in some embodiments, simultaneously with the bladder cancer markers. In some embodiments, in addition to being an endogenous control indicative of sample sufficiency (i.e., indicative of a sufficient number of cells in a sample for detecting levels of the bladder cancer markers), an endogenous control may also indicate elevated levels of urothelial cells in a sample, which may itself be an indicator of bladder cancer.

[0055] An “exogenous control,” as used herein, refers to a moiety that is added to a sample to be used for detection. An exogenous control is typically selected that is not expected to be present in the sample to be used for detection, or is present at very low levels in the sample such that the amount of the moiety naturally present in the sample is either undetectable or is detectable at a much lower level than the amount added to the sample as an exogenous control. In some embodiments, an exogenous control comprises a nucleotide sequence that is not expected to be present in the sample type used for detection of the bladder cancer markers. In some embodiments, an exogenous control comprises a nucleotide sequence that is not known to be present in the species from whom the sample is taken. In some embodiments, an exogenous control comprises a nucleotide sequence from a different species than the subject from whom the sample was taken. In some embodiments, an exogenous control comprises a nucleotide sequence that is not known to be present in any species. In some embodiments, an exogenous control is selected that can be detected in the same manner as the bladder cancer markers are detected and, in some embodiments, simultaneously with the bladder cancer markers. In some embodiments, an exogenous control is an RNA. In some embodiments, an RNA is an Armored RNA[®], which comprises RNA packaged in a bacteriophage protective coat. *See, e.g., WalkerPeach et al., Clin. Chem.* 45:12: 2079-2085 (1999).

[0056] In the sequences herein, “U” and “T” are used interchangeably, such that both letters indicate a uracil or thymine at that position. One skilled in the art will understand from the context and/or intended use whether a uracil or thymine is intended and/or should be used at that position in the sequence. For example, one skilled in the art would understand that native RNA molecules typically include uracil, while native DNA molecules typically include thymine. Thus, where an RNA sequence includes “T”, one skilled in the art would understand that that position in the native RNA is likely a uracil.

[0057] In the present disclosure, “a sequence selected from” encompasses both “one sequence selected from” and “one or more sequences selected from.” Thus, when “a sequence selected from” is used, it is to be understood that one, or more than one, of the listed sequences may be chosen.

[0058] In the present disclosure, a method that comprises detecting a “a set of bladder cancer markers consisting of...” involves detection of only the bladder cancer markers of the set, and not any further bladder cancer markers. The method may comprise additional components or steps, however, such as detecting endogenous and/or exogenous controls. Similarly, a method or composition that comprises “a set of bladder cancer marker

primer pairs” and/or “a set of bladder cancer marker probes” can include primer pairs and/or probes for only the bladder cancer markers of the set, and not for any other bladder cancer markers. The method or composition may comprise additional components, however, such as one or more endogenous control primer pairs and/or one or more exogenous control primer pairs.

4.2. Detecting bladder cancer

[0059] The present inventors have developed an assay for detecting bladder cancer that involves detecting androgen receptor (AR) and/or uroplakin 1B (UPK1B), and optionally, at least one marker selected from CRH, IGF2, KRT20 ANXA10, UPK2, MGEA5, and PIK3A. The presently described assays have several advantages over existing and previously described diagnostics for bladder cancer. For example, the present assays do not rely on cytology, which can be costly, and requires trained cytologists for accurate interpretation of results. Instead, the present assays rely on the polymerase chain reaction (PCR), and can be carried out in a substantially automated manner, for example, using the GeneXpert[®] system (Cepheid, Sunnyvale, CA). The present assays can be completed in under 3 hours, and in some embodiments, under 2 hours, using an automated system, for example, the GeneXpert[®] system. Existing tests can require several days for a laboratory to complete and send results. In addition, the present assay can be carried out on much smaller volumes of urine (in some embodiments, 5 ml or less). Thus, the present assays, which rely on PCR rather than cytology, allows for a fast, one-pot reaction for diagnosis of bladder cancer, which in many instances can be carried out at the point of care using an automated system such as GeneXpert[®].

4.2.1. General methods

[0060] Compositions and methods for detecting bladder cancer are provided. In some embodiments, compositions and methods for detecting low grade bladder cancer are provided. In some embodiments, compositions and methods of detecting high grade bladder cancer are provided. In some embodiments, compositions and methods for monitoring the recurrence of bladder cancer are provided.

[0061] In some embodiments, a method of detecting bladder cancer comprises detecting the level of AR in a sample. In some embodiments, a method of detecting bladder cancer comprises detecting the level of UPK1B in a sample. In some embodiments, a method of detecting bladder cancer comprises detecting the level of AR and UPK1B in a sample. In some embodiments, a method of detecting bladder cancer comprises detecting

the level of at least one additional marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A. In some embodiments, a method of detecting bladder cancer comprises detecting the levels of a set of bladder cancer markers comprising, or consisting of, AR, CRH, IGF2, KRT20, and ANXA10; or UPK1B, CRH, IGF2, KRT20, and ANXA10; or AR, CRH, IGF2, and KRT20; or UPK1B, CRH, IGF2, and KRT20; or UPK1B, CRH, IGF2, and ANXA10.

[0062] In some embodiments, a method of detecting bladder cancer further comprises detecting the level of at least one endogenous control. In some embodiments, a method of detecting bladder cancer further comprises detecting the level of at least one exogenous control. In some embodiments, a method of detecting bladder cancer further comprises detecting the levels of at least one endogenous control and at least one exogenous control.

[0063] In some embodiments, a method of detecting bladder cancer comprises detecting the level of AR mRNA in a sample. In some embodiments, a method of detecting bladder cancer comprises detecting the level of UPK1B mRNA in a sample. In some embodiments, a method of detecting bladder cancer comprises detecting the level of AR mRNA and UPK1B mRNA in a sample. In some embodiments, a method of detecting bladder cancer comprises detecting the level of at least one additional marker selected from CRH, IGF2, KRT20, and ANXA10 mRNA. In some embodiments, a method of detecting bladder cancer comprises detecting the levels of a set of bladder cancer markers comprising, or consisting of, AR, CRH, IGF2, KRT20, and ANXA10 mRNA; or UPK1B, CRH, IGF2, KRT20, and ANXA10 mRNA; or AR, CRH, IGF2, and KRT20 mRNA; or UPK1B, CRH, IGF2, and KRT20 mRNA; or UPK1B, CRH, IGF2, and ANXA10 mRNA.

[0064] In some embodiments, a method of detecting bladder cancer further comprises detecting the level of at least one endogenous control RNA. In some embodiments, a method of detecting bladder cancer further comprises detecting the level of at least one exogenous control RNA. In some embodiments, a method of detecting bladder cancer further comprises detecting the levels of at least one endogenous control RNA and at least one exogenous control RNA.

[0065] In the present disclosure, the term “target RNA” is used for convenience to refer to AR and UPK1B, and also to other target RNAs, such as CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNAs and exogenous and/or endogenous control RNAs. Thus, it is to be understood that when a discussion is presented in terms of a target

RNA, that discussion is specifically intended to encompass AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNAs, and/or other target RNAs.

[0066] In some embodiments, the level of one or more target RNAs is detected in a urine sample. In some embodiments, the level of one or more target RNAs is determined in a urine sample that has been preserved in a manner that causes damage, such as lysis, to red blood cells and/or white blood cells. In some embodiments, the level of one or more target RNAs is detected in urothelial cells isolated from a urine sample, either with or without preservative treatment. In some embodiments, the urothelial cells are isolated by filtration.

[0067] In some embodiments, detection of an elevated level of AR mRNA in a sample from a subject indicates the presence of bladder cancer in the subject. In some embodiments, detection of an elevated level of UPK1B in a sample from a subject indicates the presence of bladder cancer in the subject. In some embodiments, detection of an elevated level of one or more target RNAs selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A in a sample from a subject indicates the presence of bladder cancer in the subject. In some embodiments, the detecting is done quantitatively. In other embodiments, the detecting is done qualitatively. In some embodiments, detecting a target RNA comprises forming a complex comprising a polynucleotide and a nucleic acid selected from a target RNA, a DNA amplicon of a target RNA, and a complement of a target RNA. In some embodiments, detecting a target RNA comprises RT-PCR. In some embodiments, detecting a target RNA comprises quantitative RT-PCT. In some embodiments, the level of the target RNA is compared to a normal or control level of the target RNA.

[0068] In some embodiments, the levels of target RNAs, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA, can be measured in samples collected at one or more times from a patient to monitor the status or progression of bladder cancer in the patient. In some embodiments, a patient with a history of bladder cancer, such as a history of low grade bladder cancer or a history of high grade bladder cancer, is monitored by detecting the levels of AR and/or UPK1B mRNA, and optionally at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, at regular or semi-regular intervals. In some such embodiments, the patient is monitored by detecting the levels of the target RNAs at least once per month, at least once every two months, at least once every three months, at least once every four months, at least once every five months, at least once every six months, at least once every nine months, at least once per year, or at least once every two years.

[0069] In some embodiments, the levels of target RNAs, such as UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA, can be measured in samples collected at one or more times from a patient to monitor response to anti-androgen therapy. Nonlimiting exemplary anti-androgen agents include flutamide (Eulexin®), bicalutamide (Casodex®), and nilutamide (Nilandron®). In some embodiments, a patient undergoing anti-androgen therapy for bladder cancer is monitored by detecting the levels of AR and/or UPK1B mRNA, and optionally at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, at regular or semi-regular intervals. In some such embodiments, the patient is monitored by detecting the levels of the target RNAs at least once per month, at least once every two months, at least once every three months, at least once every four months, at least once every five months, at least once every six months, at least once every nine months, at least once per year, or at least once every two years. In some embodiments, a reduction in the level of AR and/or UPK1B during treatment indicates that the bladder cancer is responding to the anti-androgen therapy.

[0070] In some embodiments, a sample to be tested is a urine sample (such as a voided urine sample), or is derived from a urine sample. In some embodiments, a preservative is added to the urine sample, for example, to damage (e.g., lyse) red and/or white blood cells present in the urine sample. By damaging or lysing red and/or white blood cells prior to isolation of urothelial cells, contamination by the red and/or white blood cells can be reduced. In some embodiments, the urine sample is centrifuged to concentrate the urothelial cells. In some embodiments, the urine sample is filtered to isolate the urothelial cells from other urine and preservative materials. In some such embodiments, the filter is part of a GeneXpert cartridge (Cepheid, Sunnyvale, CA).

[0071] In some embodiments, less than 5 ml, less than 4 ml, less than 3 ml, or less than 2 ml of urine are used in the present methods. In some embodiments, the urine sample is analyzed without a centrifugation step. Thus, in some embodiments, the present methods are carried out in the absence of centrifugation. In some embodiments, a larger volume of urine may be used, and in some such embodiments, a centrifugation step may be used to concentrate the urothelial cells prior to analysis.

[0072] In some embodiments, the sample to be tested is another bodily fluid, such as blood, sputum, mucus, saliva, semen, etc. In some embodiments, a sample to be tested is a blood sample. In some embodiments, the blood sample is whole blood. In some embodiments, the blood sample is a sample of blood cells. In some embodiments, the blood sample is plasma. In some embodiments, the blood sample is serum.

[0073] The clinical sample to be tested is, in some embodiments, fresh (i.e., never frozen). In other embodiments, the sample is a frozen specimen. In some embodiments, the sample is a tissue sample, such as a formalin-fixed paraffin embedded sample. In some embodiments, the sample is a liquid cytology sample.

[0074] In some embodiments, the methods described herein are used for early detection of bladder cancer in a sample of urothelial cells, such as those obtained from voided urine. In some embodiments, the methods described herein are used for monitoring for recurrence of bladder cancer using a sample of urothelial cells, such as those obtained from voided urine.

[0075] In some embodiments, the sample to be tested is obtained from an individual who has one or more of the following risk factors: history of smoking, hematuria, history of bladder or other cancers, and exposure to known carcinogens such as benzene. In some embodiments, the sample is obtained from an individual who has diagnostic signs or clinical symptoms that may be associated with bladder cancer, such as blood in the urine, frequent urination, urinary urgency, incontinence, difficulty urinating, abdominal pain, unexplained weight loss and/or loss of appetite. In some embodiments, the sample to be tested is obtained from an individual who has previously been diagnosed with low grade or high grade bladder cancer. In some such embodiments, the individual is monitored for recurrence of bladder cancer.

[0076] Bladder cancer can be divided into stages, which indicate the growth pattern of the primary tumor. Table A shows the stages of bladder cancer according to the American Joint Committee on Cancer (AJCC). The stages shown cover only the “T” portion of the “TNM” system. The “T” portion refers to the primary tumor, while “N” refers to spread of the cancer to the lymph nodes, and “M” refers to whether the cancer has metastasized to distant sites.

Table A: Bladder cancer stages

Stage	description
T0	No evidence of primary tumor
Ta	Non-invasive papillary carcinoma
Tis / CIS	Non-invasive flat carcinoma (carcinoma <i>in situ</i>)
T1	Tumor has grown from the lining of the bladder into the connective tissue, but has not grown into the muscle layer of the bladder
T2	Tumor has grown into the muscle layer
T2a	Tumor has grown into the inner half of the muscle layer
T2b	Tumor has grown into the outer half of the muscle layer
T3	Tumor has grown through the muscle layer of the bladder and into the fatty

	tissue that surrounds it
T3a	Tumor's spread to fatty tissue can only be seen under a microscope
T3b	Tumor's spread to fatty tissue can be seen on imaging tests or can be seen or felt by surgeon
T4	Tumor has spread beyond the fatty tissue to nearby organs or structures
T4a	Tumor has spread to the stroma of the prostate, or to the uterus and/or vagina
T4b	Tumor has spread to the pelvic wall or abdominal wall

[0077] In some embodiments, methods described herein can be used for routine screening of healthy individuals with no risk factors. In some embodiments, methods described herein are used to screen asymptomatic individuals having one or more of the above-described risk factors.

[0078] In some embodiments, the methods described herein can be used to detect low grade bladder cancer. In some embodiments, the methods described herein can be used to detect high grade bladder cancer. In some embodiments, the methods described herein detect at least 15%, at least 17%, at least 20%, at least 22%, at least 25%, at least 27%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, or at least 35% of low grade bladder cancers. In some embodiments, the methods described herein detect at least 85%, at least 87%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of high grade bladder cancers.

[0079] In some embodiments, the methods described herein can be used to assess the effectiveness of a treatment for bladder cancer in a patient. In some embodiments, target RNA levels, such as the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, are determined at various times during the treatment, and are compared to target RNA levels from an archival sample taken from the patient before the beginning of treatment. In some embodiments, target RNA levels are compared to target RNA levels from an archival normal sample taken from the patient. Ideally, target RNA levels in the normal sample evidence no aberrant changes in target RNA levels.

[0080] In some embodiments, use of the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, for monitoring recurrence of bladder cancer is provided. In some embodiments, an elevated level of one or more, two or more, three or more, or four or more, five or more, or all six mRNAs indicates that bladder cancer has recurred in the patient.

[0081] In any of the embodiments described herein, RNA levels may be detected concurrently or simultaneously in the same or separate assay reactions. In some embodiments, RNA levels are detected at different times, *e.g.*, in serial assay reactions.

[0082] In some embodiments, a method comprises detecting the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A in a sample from a subject, wherein detection of a level of any one of the mRNAs that is greater than a normal level of the RNA indicates the presence of bladder cancer in the subject. In some embodiments, detection of elevated levels of two, three, four, five, or six bladder cancer marker mRNAs indicates the presence of bladder cancer in the subject. In some embodiments, detection of elevated levels of at least two, at least three, at least four, or at least five of the bladder cancer marker mRNAs indicates a greater risk of high grade bladder cancer.

[0083] In some embodiments, a method of facilitating diagnosis of bladder cancer in a subject is provided. Such methods comprise detecting the level of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A in a sample from the subject. In some embodiments, information concerning the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A in the sample from the subject is communicated to a medical practitioner. A “medical practitioner,” as used herein, refers to an individual or entity that diagnoses and/or treats patients, such as a hospital, a clinic, a physician’s office, a physician, a nurse, or an agent of any of the aforementioned entities and individuals. In some embodiments, detecting the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A is carried out at a laboratory that has received the subject’s sample from the medical practitioner or agent of the medical practitioner. The laboratory carries out the detection by any method, including those described herein, and then communicates the results to the medical practitioner. A result is “communicated,” as used herein, when it is provided by any means to the medical practitioner. In some embodiments, such communication may be oral or written, may be by telephone, in person, by e-mail, by mail or other courier, or may be made by directly depositing the information into, *e.g.*, a database accessible by the medical practitioner, including databases not controlled by the medical practitioner. In some embodiments, the information is maintained in electronic form. In some embodiments, the information can be stored in a memory or other computer readable medium, such as RAM, ROM, EEPROM,

flash memory, computer chips, digital video discs (DVD), compact discs (CDs), hard disk drives (HDD), magnetic tape, etc.

[0084] In some embodiments, methods of detecting the presence bladder cancer are provided. In some embodiments, methods of diagnosing bladder cancer are provided. In some embodiments, the method comprises obtaining a sample from a subject and providing the sample to a laboratory for detection of levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20 and ANXA10 in the sample. In some embodiments, the method further comprises receiving a communication from the laboratory that indicates the level of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A in the sample. In some embodiments, bladder cancer is present if the level of any one of the four mRNAs is greater than a normal or control level of the mRNA. A “laboratory,” as used herein, is any facility that detects the levels of target RNA in a sample by any method, including the methods described herein, and communicates the level to a medical practitioner. In some embodiments, a laboratory is under the control of a medical practitioner. In some embodiments, a laboratory is not under the control of the medical practitioner.

[0085] When a laboratory communicates the level of a target RNA to a medical practitioner, in some embodiments, the laboratory communicates a numerical value representing the level of the RNA in the sample, with or without providing a numerical value for a normal level. In some embodiments, the laboratory communicates the level of the RNA by providing a qualitative value, such as “high,” “low,” “elevated,” “decreased,” “positive” (such as “AR positive” or “AR and CRH positive”), etc. In some embodiments, the laboratory communicates a suggested diagnosis, such as “bladder cancer positive” or “positive for cancer,” and the like; or simply “cancer positive” or “cancer negative.”

[0086] As used herein, when a method relates to detecting bladder cancer, determining the presence of bladder cancer, monitoring for bladder cancer, and/or diagnosing bladder cancer, the method includes activities in which the steps of the method are carried out, but the result is negative for the presence of bladder cancer. That is, detecting, determining, monitoring, and diagnosing bladder cancer include instances of carrying out the methods that result in either positive or negative results.

[0087] In some embodiments, more than one RNA is detected simultaneously in a single reaction. In some embodiments, AR mRNA and at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A are detected simultaneously in

a single reaction. In some embodiments, UPK1B mRNA and at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A are detected simultaneously in a single reaction. In some embodiments, AR and UPK1B mRNA and at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A are detected simultaneously in a single reaction. In some embodiments, at least one endogenous control and/or at least one exogenous control are detected simultaneously with the bladder cancer markers in a single reaction.

4.2.2. Exemplary controls

[0088] In some embodiments, a normal level (a “control”) of a target RNA, such as AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, can be determined as an average level or range that is characteristic of normal urothelial cells or other reference material, against which the level measured in the sample can be compared. The determined average or range of a target RNA in normal subjects can be used as a benchmark for detecting above-normal levels of the target RNA that are indicative of bladder cancer. In some embodiments, normal levels of a target RNA can be determined using individual or pooled RNA-containing samples from one or more individuals, such as from normal urothelial cells isolated from urine of healthy individuals.

[0089] In some embodiments, determining a normal level of a target RNA comprises detecting a complex comprising a polynucleotide for detection hybridized to a nucleic acid selected from a target RNA, a DNA amplicon of the target RNA, and a complement of the target RNA. That is, in some embodiments, a normal level can be determined by detecting a DNA amplicon of the target RNA, or a complement of the target RNA rather than the target RNA itself. In some embodiments, a normal level of such a complex is determined and used as a control. The normal level of the complex, in some embodiments, correlates to the normal level of the target RNA. Thus, when a normal level of a target is discussed herein, that level can, in some embodiments, be determined by detecting such a complex.

[0090] In some embodiments, a normal level of a target RNA is, or has been, determined by the same method as the level of the target RNA from a patient sample. In some such embodiments, the method is RT-PCR (such as real-time RT-PCR, quantitative RT-PCR, etc.).

[0091] In some embodiments, a control comprises RNA from cells of a single individual, *e.g.*, from normal urothelial cells isolated from urine of a healthy individual. In some embodiments, a control comprises RNA from blood, such as whole blood or serum, of a single individual. In some embodiments, a control comprises RNA from a pool of cells from multiple individuals. In some embodiments, a control comprises RNA from a pool of urine from multiple individuals. In some embodiments, a control comprises commercially-available human RNA (see, for example, Ambion). In some embodiments, a normal level or normal range has already been predetermined prior to testing a sample for an elevated level.

[0092] In some embodiments, the normal level of a target RNA can be determined from one or more continuous cell lines, typically cell lines previously shown to have levels of RNAs that approximate the levels in normal urothelial cells.

[0093] In some embodiments, quantitation of target RNA levels requires assumptions to be made about the total RNA per cell and the extent of sample loss during sample preparation. In order to correct for differences between different samples or between samples that are prepared under different conditions, the quantities of target RNAs in some embodiments are normalized to the levels of at least one endogenous control and/or at least one exogenous control.

[0094] In some embodiments, a control RNA is an endogenous control RNA. An endogenous control RNA may be any RNA suitable for the purpose, for example, RNAs that are present at approximately constant levels from cell to cell and in urothelial cells from both bladder cancer and non-bladder cancer patients. Nonlimiting exemplary endogenous control RNAs include ABL, GUSB, GAPDH, TUBB, and UPK1a. In some embodiments, one endogenous control is used for normalization. In some embodiments, more than one endogenous control is used for normalization. In some embodiments, an endogenous control may also indicate elevated levels of urothelial cells in a sample, which may be an indicator of bladder cancer. Thus, in some embodiments, an endogenous control may not only indicate sample sufficiency, but may also indicate elevated numbers of cells in a urine sample, which is itself an indicator of bladder cancer.

[0095] In some embodiments, a control RNA is an exogenous control RNA. In some such embodiments, the exogenous control RNA is an Armored RNA[®], which is protected by a bacteriophage coat. An exogenous control RNA may, in some embodiments, be used to determine if the detection assay reaction has failed, and therefore the results are not meaningful. For example, if an exogenous control RNA is not amplified in the assay

reaction, then a negative result for the target RNAs is likely not meaningful because the levels reflect the reaction failing rather than the target RNA levels being low. Reaction failure can occur for any number of reasons, including, but not limited to, the presence of a reaction inhibitor in the sample (an “inhibitory sample”), compromised reagents, the presence of an RNase, etc. An exogenous RNA control may be added at any stage of the sample collection and analysis. For example, in some embodiments, the exogenous control RNA is added to the sample at the time preservative is added, is added to the sample when it is received by the diagnostic laboratory, is added to the sample immediately prior to analysis, or is added to the sample during analysis (as a nonlimiting example, during or after lysis of the urothelial cells but before addition of the amplification reagents).

[0096] In some embodiments, the level of a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, is compared to a reference level, e.g., from a confirmed bladder cancer. In some such embodiments, a similar level of a target RNA relative to the reference sample indicates bladder cancer.

[0097] In some embodiments, a level of a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, that is at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater than a normal level of the respective target RNA indicates the presence of bladder cancer. In some embodiments, a level of a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, that is at least about two-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold greater than a normal level of the respective target RNA indicates the presence of bladder cancer.

[0098] In some embodiments, a control level of a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, , UPK2, MGEA5, or PIK3A mRNA, is determined contemporaneously, such as in the same assay or batch of assays, as the level of the target RNA in a sample. In some embodiments, a control level of a target RNA is not determined contemporaneously as the level of the target RNA in a sample. In some such embodiments, the control level has been determined previously.

[0099] In some embodiments, the level of an endogenous control and/or an exogenous control is determined contemporaneously, such as in the same assay or batch of assays, as the level of the target RNA in a sample. In some embodiments, an assay comprises reagents for determining the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and

PIK3A, and an endogenous control simultaneously in the same assay reaction. In some embodiments, an assay comprises reagents for determining the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, and an exogenous control simultaneously in the same assay reaction. In some embodiments, an assay comprises reagents for determining the levels of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A, an endogenous control, and an exogenous control simultaneously in the same assay reaction. In some such embodiments, for example, an assay reaction comprises primer sets for amplifying AR and/or UPK1B, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, , UPK2, MGEA5, and PIK3A a primer set for amplifying an endogenous control and/or a primer set for amplifying an exogenous control, and detectably different labeled probes for detecting the amplification products (such as, for example, TaqMan[®] probes with detectably different dyes for each different amplicon to be detected).

[00100] In some embodiments, the level of a target RNA is not compared to a control level, for example, when it is known that the target RNA is present at very low levels, or not at all, in normal cells. In such embodiments, detection of a high level of the target RNA in a sample is indicative of bladder cancer.

4.2.3. Exemplary sample preparation

4.2.3.1. Exemplary urine preservatives

[00101] In some embodiments, a preservative is added to the urine sample. In some embodiments, the preservative is added within one hour, two hours, three hours, or six hours of the time the urine sample was collected (e.g., voided). In some embodiments, a preservative is added to the urine sample within one hour, two hours, three hours, or six hours before the sample is analyzed by the methods described herein.

[00102] In some embodiments, a preservative causes damage, such as lysis, of red blood cells and/or white blood cells, but does not damage urothelial cells. Red blood cells and/or white blood cells may be present in the urine as a result of a tumor and/or infection. In some such embodiments, adding the preservative allows for improved enrichment of the urothelial cells, for example by filtration. In some embodiments, a preservative lowers the pH of the urine sample and improves solubility of urine salts. In some such embodiments, the preservative facilitates passage of the salts through a filter in a filtration step. A desirable pH of preserved urine to be passed through a filter is between

about 2.5 and 4. In some embodiments, a desirable pH of preserved urine is between about 2.7 and 3.7. In some embodiments, a desirable pH of preserved urine is between about 3 and 3.5. In some embodiments, a desirable pH of preserved urine is about 3.2.

[00103] In some embodiments a preservative is added such that the urine/preservative sample comprises 0.875M to 2.625M guanidine hydrochloride, 0.25% to 0.75% N-acetyl-L-cysteine, 6.25 to 18.75 mM sodium citrate, and 0.625% to 1.875% Tween-20, and has a pH of 3 to 3.5. In some embodiments a preservative is added such that the urine/preservative sample comprises about 1.75 M guanidine hydrochloride, about 0.5% N-acetyl-L-cysteine, about 12.5 mM sodium citrate, and about 1.25% Tween-20, and has a pH of about 3.2.

[00104] A nonlimiting exemplary commercial preservative is PreservCyt (Hologic, Bedford, MA).

4.2.3.2. Exemplary cell enrichment

[00105] In some embodiments, urothelial cells are enriched by centrifugation. In some such embodiments, the cell pellet is resuspended in the supernatant and/or a preservative. Resuspension of the cell pellet can be used to adjust the concentration of cells in solution. The resuspended cell pellet may be used (for example, with lysis) in the methods described herein, or may be subject to an additional enrichment step, such as filtration.

[00106] In some embodiments, urothelial cells are enriched by filtration. Nonlimiting exemplary filter pore sizes that may be suitable for capturing urothelial cells include 0.8 μm , 2 μm , 8 μm , and 10 μm . In some embodiments, a filter pore size is selected that allows pass-through of red blood cells and/or white blood cells, while retaining most urothelial cells. In some embodiments, a filter is located within a GeneXpert cartridge designed for carrying out a bladder cancer diagnostic assay described herein.

4.2.3.3. Exemplary mRNA preparation

[00107] Target RNA can be prepared by any appropriate method. Total RNA can be isolated by any method, including, but not limited to, the protocols set forth in Wilkinson, M. (1988) Nucl. Acids Res. 16(22):10,933; and Wilkinson, M. (1988) Nucl. Acids Res. 16(22): 10934, or by using commercially-available kits or reagents, such as the TRIzol[®] reagent (Invitrogen), Total RNA Extraction Kit (iNtRON Biotechnology), Total RNA Purification Kit (Norgen Biotek Corp.), RNAqueous[™] (Ambion), MagMAX[™] (Ambion), RecoverAll[™] (Ambion), RNeasy (Qiagen), etc.

[00108] In some embodiments, RNA levels are measured in a sample in which RNA has not first been purified from the cells. In some such embodiments, the cells are subject to a lysis step to release the RNA. Nonlimiting exemplary lysis methods include sonication (for example, for 2-15 seconds, 8-18 μ m at 36 kHz); chemical lysis, for example, using a detergent; and various commercially available lysis reagents (such as Rneasy lysis buffer, Qiagen). In some embodiments, RNA levels are measured in a sample in which RNA has been isolated.

[00109] In some embodiments, RNA is modified before a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, is detected. In some embodiments, all of the RNA in the sample is modified. In some embodiments, just the particular target RNAs to be analyzed are modified, e.g., in a sequence-specific manner. In some embodiments, RNA is reverse transcribed. In some such embodiments, RNA is reverse transcribed using MMLV reverse transcriptase. Nonlimiting exemplary conditions for reverse transcribing RNA using MMLV reverse transcriptase include incubation from 5 to 20 minutes at 40°C to 50°C.

[00110] When a target RNA is reverse transcribed, a DNA complement of the target RNA is formed. In some embodiments, the complement of a target RNA is detected rather than a target RNA itself (or a DNA copy of the RNA itself). Thus, when the methods discussed herein indicate that a target RNA is detected, or the level of a target RNA is determined, such detection or determination may be carried out on a complement of a target RNA instead of, or in addition to, the target RNA itself. In some embodiments, when the complement of a target RNA is detected rather than the target RNA, a polynucleotide for detection is used that is complementary to the complement of the target RNA. In some such embodiments, a polynucleotide for detection comprises at least a portion that is identical in sequence to the target RNA, although it may contain thymidine in place of uridine, and/or comprise other modified nucleotides.

4.2.4. Exemplary analytical methods

[00111] As described above, methods are presented for detecting bladder cancer. The methods comprise detecting a panel of bladder cancer markers comprises AR and/or UPK1B, and optionally, at least one marker selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A. In some embodiments, the method further comprises detecting at least one endogenous control and/or at least one exogenous control. In some embodiments, detection of an elevated level of at least one, at least two, at least

three, at least four, at least five, or six bladder cancer markers indicates the presence of bladder cancer. In some embodiments, the bladder cancer is low grade bladder cancer. In some embodiments, the bladder cancer is high grade bladder cancer. In some embodiments, the bladder cancer is a recurrence of bladder cancer in a patient with a history of bladder cancer.

[00112] Any analytical procedure capable of permitting specific and quantifiable (or semi-quantifiable) detection of a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNAs, may be used in the methods herein presented. Such analytical procedures include, but are not limited to, RT-PCR methods, and other methods known to those skilled in the art.

[00113] In some embodiments, the method of detecting a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, comprises amplifying cDNA complementary to the target RNA. Such amplification can be accomplished by any method. Exemplary methods include, but are not limited to, real time PCR, endpoint PCR, and amplification using T7 polymerase from a T7 promoter annealed to a cDNA, such as provided by the SenseAmp Plus™ Kit available at Implen, Germany.

[00114] When a target RNA or a cDNA complementary to a target RNA is amplified, in some embodiments, a DNA amplicon of the target RNA is formed. A DNA amplicon may be single stranded or double-stranded. In some embodiments, when a DNA amplicon is single-stranded, the sequence of the DNA amplicon is related to the target RNA in either the sense or antisense orientation. In some embodiments, a DNA amplicon of a target RNA is detected rather than the target RNA itself. Thus, when the methods discussed herein indicate that a target RNA is detected, or the level of a target RNA is determined, such detection or determination may be carried out on a DNA amplicon of the target RNA instead of, or in addition to, the target RNA itself. In some embodiments, when the DNA amplicon of the target RNA is detected rather than the target RNA, a polynucleotide for detection is used that is complementary to the complement of the target RNA. In some embodiments, when the DNA amplicon of the target RNA is detected rather than the target RNA, a polynucleotide for detection is used that is complementary to the target RNA. Further, in some embodiments, multiple polynucleotides for detection may be used, and some polynucleotides may be complementary to the target RNA and some polynucleotides may be complementary to the complement of the target RNA.

[00115] In some embodiments, the method of detecting one or more target RNAs, , such as AR and/or UPK1B mRNA, and optionally, at least one mRNA selected

from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A, comprises RT-PCR, as described below. In some embodiments, detecting one or more target RNAs comprises real-time monitoring of an RT-PCR reaction, which can be accomplished by any method. Such methods include, but are not limited to, the use of TaqMan[®], Molecular beacon, or Scorpion probes (i.e., energy transfer (ET) probes, such as FRET probes) and the use of intercalating dyes, such as SYBR green, EvaGreen, thiazole orange, YO-PRO, TO-PRO, etc.

[00116] Nonlimiting exemplary conditions for amplifying cDNA that has been reverse transcribed from the target RNAs are as follows. An exemplary cycle comprises an initial denaturation at 90°C to 100°C for 2 to 5 minutes, followed by cycling that comprises denaturation at 90°C to 100°C for 1 to 10 seconds, annealing at 60°C to 70°C for 10 to 30 seconds, and extension at 60°C to 75°C for 10 to 40 seconds. In some embodiments, for the first cycle following the initial denaturation step, the cycle denaturation step is omitted. In some embodiments, Taq polymerase is used for amplification. In some embodiments, the cycle is carried out at least 10 times, at least 15 times, at least 20 times, at least 25 times, at least 30 times, at least 35 times, or at least 45 times. In some such embodiments, Taq is used with a hot start function. In some embodiments, the amplification reaction occurs in a GeneXpert cartridge, and amplification of the four bladder cancer marker target RNAs occurs in the same reaction. In some embodiments, detection of AR and/or UPK1B mRNA, and optionally, at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A occurs in less than 3 hours, less than 2.5 hours, or less than 2 hours, from initial denaturation through the last extension.

[00117] In some embodiments, detection of a target RNA comprises forming a complex comprising a polynucleotide that is complementary to a target RNA or to a complement thereof, and a nucleic acid selected from the target RNA, a DNA amplicon of the target RNA, and a complement of the target RNA. Thus, in some embodiments, the polynucleotide forms a complex with a target RNA. In some embodiments, the polynucleotide forms a complex with a complement of the target RNA, such as a cDNA that has been reverse transcribed from the target RNA. In some embodiments, the polynucleotide forms a complex with a DNA amplicon of the target RNA. When a double-stranded DNA amplicon is part of a complex, as used herein, the complex may comprise one or both strands of the DNA amplicon. Thus, in some embodiments, a complex comprises only one strand of the DNA amplicon. In some embodiments, a complex is a triplex and comprises the polynucleotide and both strands of the DNA amplicon. In some

embodiments, the complex is formed by hybridization between the polynucleotide and the target RNA, complement of the target RNA, or DNA amplicon of the target RNA. The polynucleotide, in some embodiments, is a primer or probe.

[00118] In some embodiments, a method comprises detecting the complex. In some embodiments, the complex does not have to be associated at the time of detection. That is, in some embodiments, a complex is formed, the complex is then dissociated or destroyed in some manner, and components from the complex are detected. An example of such a system is a TaqMan[®] assay. In some embodiments, when the polynucleotide is a primer, detection of the complex may comprise amplification of the target RNA, a complement of the target RNA, or a DNA amplicon of a target RNA.

[00119] In some embodiments the analytical method used for detecting at least one target RNA in the methods set forth herein includes real-time quantitative RT-PCR. In some embodiments, the analytical method used for detecting at least one target RNA includes the use of a TaqMan[®] probe. The assay uses energy transfer (“ET”), such as fluorescence resonance energy transfer (“FRET”), to detect and quantitate the synthesized PCR product. Typically, the TaqMan[®] probe comprises a fluorescent dye molecule coupled to the 5'-end and a quencher molecule coupled to the 3'-end, such that the dye and the quencher are in close proximity, allowing the quencher to suppress the fluorescence signal of the dye via FRET. When the polymerase replicates the chimeric amplicon template to which the TaqMan[®] probe is bound, the 5'-nuclease of the polymerase cleaves the probe, decoupling the dye and the quencher so that the dye signal (such as fluorescence) is detected. Signal (such as fluorescence) increases with each RT-PCR cycle proportionally to the amount of probe that is cleaved.

[00120] In some embodiments, quantitation of the results of real-time RT-PCR assays is done by constructing a standard curve from a nucleic acid of known concentration and then extrapolating quantitative information for target RNAs of unknown concentration. In some embodiments, the nucleic acid used for generating a standard curve is an RNA (for example, an endogenous control, or an exogenous control). In some embodiments, the nucleic acid used for generating a standard curve is a purified double-stranded plasmid DNA or a single-stranded DNA generated in vitro.

[00121] In some embodiments, where the amplification efficiencies of the target nucleic acids and the endogenous reference are approximately equal, quantitation is accomplished by the comparative Ct (cycle threshold, e.g., the number of PCR cycles required for the fluorescence signal to rise above background) method. Ct values are

inversely proportional to the amount of nucleic acid target in a sample. In some embodiments, Ct values of a target RNA can be compared with a control or calibrator, such as an exogenous control RNA. In some embodiments, the Ct values of the exogenous control and the target RNA are normalized to an appropriate endogenous control. Nonlimiting exemplary endogenous controls are discussed herein.

[00122] In some embodiments, a threshold Ct (or a “cutoff Ct”) value for a target RNA, below which bladder cancer is indicated, has previously been determined. In such embodiments, a control sample may not be assayed concurrently with the test sample. In some embodiments, as discussed herein, a Δ Ct threshold value is determined, above which bladder cancer is indicated, has previously been determined.

[00123] In addition to the TaqMan[®] assays, other real-time RT-PCR chemistries useful for detecting and quantitating PCR products in the methods presented herein include, but are not limited to, Molecular Beacons, Scorpion probes and intercalating dyes, such as SYBR Green, EvaGreen, thiazole orange, YO-PRO, TO-PRO, etc., which are discussed below.

[00124] In various embodiments, real-time RT-PCR detection is utilized to detect, in a single multiplex reaction, four bladder cancer markers of the panel described herein, and optionally, at least one endogenous control and/or at least one exogenous control. In various embodiments, real-time RT-PCR detection is utilized to detect, in a single multiplex reaction, five bladder cancer markers of the panel described herein, and optionally, at least one endogenous control and/or at least one exogenous control. In some multiplex embodiments, a plurality of probes, such as TaqMan[®] probes, each specific for a different RNA target, is used. In some embodiments, each target RNA-specific probe is spectrally distinguishable from the other probes used in the same multiplex reaction. A nonlimiting exemplary seven-color multiplex system is described, *e.g.*, in Lee et al., *BioTechniques*, 27: 342-349.

[00125] In some embodiments, quantitation of real-time RT PCR products is accomplished using a dye that binds to double-stranded DNA products, such as SYBR Green, EvaGreen, thiazole orange, YO-PRO, TO-PRO, etc. In some embodiments, the assay is the QuantiTect SYBR Green PCR assay from Qiagen. In this assay, total RNA is first isolated from a sample. Total RNA is subsequently poly-adenylated at the 3'-end and reverse transcribed using a universal primer with poly-dT at the 5'-end. In some embodiments, a single reverse transcription reaction is sufficient to assay multiple target RNAs. Real-time RT-PCR is then accomplished using target RNA-specific primers and an

miScript Universal Primer, which comprises a poly-dT sequence at the 5'-end. SYBR Green dye binds non-specifically to double-stranded DNA and upon excitation, emits light. In some embodiments, buffer conditions that promote highly-specific annealing of primers to the PCR template (e.g., available in the QuantiTect SYBR Green PCR Kit from Qiagen) can be used to avoid the formation of non-specific DNA duplexes and primer dimers that will bind SYBR Green and negatively affect quantitation. Thus, as PCR product accumulates, the signal from SYBR Green increases, allowing quantitation of specific products.

[00126] Real-time RT-PCR is performed using any RT-PCR instrumentation available in the art. Typically, instrumentation used in real-time RT-PCR data collection and analysis comprises a thermal cycler, optics for fluorescence excitation and emission collection, and optionally a computer and data acquisition and analysis software.

[00127] In some embodiments, the analytical method used in the methods described herein is a DASL[®] (cDNA-mediated Annealing, Selection, Extension, and Ligation) Assay. In some embodiments, total RNA is isolated from a sample to be analyzed by any method. Total RNA may then be polyadenylated (> 18 A residues are added to the 3'-ends of the RNAs in the reaction mixture). The RNA is reverse transcribed using a biotin-labeled DNA primer that comprises from the 5' to the 3' end, a sequence that includes a PCR primer site and a poly-dT region that binds to the poly-dA tail of the sample RNA. The resulting biotinylated cDNA transcripts are then hybridized to a solid support via a biotin-streptavidin interaction and contacted with one or more target RNA-specific polynucleotides. The target RNA-specific polynucleotides comprise, from the 5'-end to the 3'-end, a region comprising a PCR primer site, region comprising an address sequence, and a target RNA-specific sequence.

[00128] In some DASL[®] embodiments, the target RNA-specific sequence comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 contiguous nucleotides having a sequence that is the same as, or complementary to, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 contiguous nucleotides of a bladder cancer marker target RNA, an endogenous control RNA, or an exogenous control RNA.

[00129] After hybridization, the target RNA-specific polynucleotide is extended, and the extended products are then eluted from the immobilized cDNA array. A second PCR reaction using a fluorescently-labeled universal primer generates a

fluorescently-labeled DNA comprising the target RNA-specific sequence. The labeled PCR products are then hybridized to a microbead array for detection and quantitation.

[00130] In some embodiments, the analytical method used for detecting and quantifying the levels of the at least one target RNA in the methods described herein is a bead-based flow cytometric assay. *See* Lu J. et al. (2005) *Nature* 435:834-838, which is incorporated herein by reference in its entirety. An example of a bead-based flow cytometric assay is the xMAP[®] technology of Luminex, Inc. (See <http://www.luminexcorp.com/technology/index.html>). In some embodiments, total RNA is isolated from a sample and is then labeled with biotin. The labeled RNA is then hybridized to target RNA-specific capture probes (e.g., FlexmiR[™] products sold by Luminex, Inc. at www.luminexcorp.com/products/assays/index.html) that are covalently bound to microbeads, each of which is labeled with 2 dyes having different fluorescence intensities. A streptavidin-bound reporter molecule (e.g., streptavidin-phycoerythrin, also known as “SAPE”) is attached to the captured target RNA and the unique signal of each bead is read using flow cytometry. In some embodiments, the RNA sample is first polyadenylated, and is subsequently labeled with a biotinylated 3DNA[™] dendrimer (i.e., a multiple-arm DNA with numerous biotin molecules bound thereto), using a bridging polynucleotide that is complementary to the 3'-end of the poly-dA tail of the sample RNA and to the 5'-end of the polynucleotide attached to the biotinylated dendrimer. The streptavidin-bound reporter molecule is then attached to the biotinylated dendrimer before analysis by flow cytometry. In some embodiments, biotin-labeled RNA is first exposed to SAPE, and the RNA/SAPE complex is subsequently exposed to an anti-phycoerythrin antibody attached to a DNA dendrimer, which can be bound to as many as 900 biotin molecules. This allows multiple SAPE molecules to bind to the biotinylated dendrimer through the biotin-streptavidin interaction, thus increasing the signal from the assay.

[00131] In some embodiments, the analytical method used for detecting and quantifying the levels of the at least one target RNA in the methods described herein is by gel electrophoresis and detection with labeled probes (e.g., probes labeled with a radioactive or chemiluminescent label), such as by Northern blotting. In some embodiments, total RNA is isolated from the sample, and then is size-separated by SDS polyacrylamide gel electrophoresis. The separated RNA is then blotted onto a membrane and hybridized to radiolabeled complementary probes. In some embodiments, exemplary probes contain one or more affinity-enhancing nucleotide analogs as discussed below, such as locked nucleic acid (“LNA”) analogs, which contain a bicyclic sugar moiety instead of deoxyribose or

ribose sugars. *See, e.g.,* Várallyay, E. et al. (2008) Nature Protocols 3(2):190-196, which is incorporated herein by reference in its entirety.

[00132] In some embodiments, detection and quantification of one or more target RNAs is accomplished using microfluidic devices and single-molecule detection. In some embodiments, target RNAs in a sample of isolated total RNA are hybridized to two probes, one which is complementary to nucleic acids at the 5'-end of the target RNA and the second which is complementary to the 3'-end of the target RNA. Each probe comprises, in some embodiments, one or more affinity-enhancing nucleotide analogs, such as LNA nucleotide analogs and each is labeled with a different fluorescent dye having different fluorescence emission spectra (i.e., detectably different dyes). The sample is then flowed through a microfluidic capillary in which multiple lasers excite the fluorescent probes, such that a unique coincident burst of photons identifies a particular target RNA, and the number of particular unique coincident bursts of photons can be counted to quantify the amount of the target RNA in the sample. In some alternative embodiments, a target RNA-specific probe can be labeled with 3 or more distinct labels selected from, e.g., fluorophores, electron spin labels, etc., and then hybridized to an RNA sample.

[00133] Optionally, the sample RNA is modified before hybridization. The target RNA/probe duplex is then passed through channels in a microfluidic device and that comprise detectors that record the unique signal of the 3 labels. In this way, individual molecules are detected by their unique signal and counted. *See* U.S. Patent Nos. 7,402,422 and 7,351,538 to Fuchs et al., U.S. Genomics, Inc., each of which is incorporated herein by reference in its entirety.

4.2.5. Exemplary Automation and Systems

[00134] In some embodiments, gene expression is detected using an automated sample handling and/or analysis platform. In some embodiments, commercially available automated analysis platforms are utilized. For example, in some embodiments, the GeneXpert[®] system (Cepheid, Sunnyvale, CA) is utilized.

[00135] The present invention is illustrated for use with the GeneXpert system. Exemplary sample preparation and analysis methods are described below. However, the present invention is not limited to a particular detection method or analysis platform. One of skill in the art recognizes that any number of platforms and methods may be utilized.

[00136] The GeneXpert[®] utilizes a self-contained, single use cartridge. Sample extraction, amplification, and detection may all carried out within this self-contained “laboratory in a cartridge.” (See e.g., US Patents 5,958,349, 6,403,037, 6,440,725, 6,783,736, 6,818,185; each of which is herein incorporated by reference in its entirety.)

[00137] Components of the cartridge include, but are not limited to, processing chambers containing reagents, filters, and capture technologies useful to extract, purify, and amplify target nucleic acids. A valve enables fluid transfer from chamber to chamber and contain nucleic acids lysis and filtration components. An optical window enables real-time optical detection. A reaction tube enables very rapid thermal cycling.

[00138] In some embodiments, the GenXpert[®] system includes a plurality of modules for scalability. Each module includes a plurality of cartridges, along with sample handling and analysis components.

[00139] In some embodiments, the GeneXpert[®] sample preparation method utilizes filtration in order to capture and concentrate cells from urine. In some embodiments, a filter pore size of 0.8 μm is utilized. This size facilitates capture of all cells in urine. In other embodiments, pore sizes of 0.5 to 10 μm , 0.5 to 5 μm , 0.8 to 10 μm , 0.8 to 5 μm , 0.8 to 2 μm , 2 to 5 μm , 2 to 10 μm , 2 to 8 μm , 5 to 8 μm , or 5 to 10 μm are utilized. Certain filters (such as 5 μm , 8 μm , and 10 μm) allow the removal of most red and white blood cells from the sample while capturing the larger urothelial cells, which are the assay target cells. In some embodiments, this sample preparation method improves assay specificity by removing white blood cells that may be present due to infection or inflammation. In some instances, sample preparation methods such as centrifugation of whole urine followed by RNA isolation from the urine pellet do not allow for removal of white blood cells. In some embodiments, the efficiency of cell capture by filtration is higher compared to centrifugation, and may provide more consistent results.

[00140] After the cells from the urine are captured on the filter, in some embodiments, they are washed and then lysed using sonication (2-15 seconds, 8-16 μm at 36 kHz). The cell lysate is then collected and used to reconstitute the RT-PCR reagents, which are present in the cartridge as lyophilized particles.

[00141] In some embodiments, RT-PCR is used to amplify and analyze the presence or expression levels of the bladder cancer markers. In some embodiments, the reverse transcription uses MMLV RT enzyme and an incubation of 5 to 20 minutes at 40°C to 50°C. In some embodiments, the PCR uses Taq polymerase with hot start function, such as AptaTaq (Roche). In some embodiments, the initial denaturation is at 90°C to 100°C for

2 to 5 minutes; the cycling denaturation temperature is 90°C to 100°C for 1 to 10 seconds; the cycling anneal temperature is 60°C to 70°C for 10 to 30 seconds; and the cycling extend temperature is 60°C to 75°C for 10 to 40 seconds; and up to 50 cycles are performed.

[00142] The present invention is not limited to particular primer and/or probe sequences. Exemplary amplification primers and detection probes are described in the Examples.

[00143] In some embodiments, an off-line centrifugation is used to improve assay results with samples with low cellular content. The sample, with or without the preservative added, is centrifuged and the supernatant removed. The pellet is then resuspended in a smaller volume of either supernatant or the preservative. The resuspended pellet is then added to a GeneXpert[®] cartridge as previously described.

4.2.6. Exemplary Data Analysis

[00144] In some embodiments, the level of a target RNA, such as AR and/or UPK1B mRNA, and optionally, at least one of CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA, is normalized to an endogenous control RNA. Normalization may comprise, for example, determination of the difference of the level of the target RNA to the level of the endogenous control RNA. In some such embodiments, the level of the RNAs are represented by a Ct value obtained from quantitative PCR. In some such embodiments, the difference is expressed as ΔCt . ΔCt may be calculated as $Ct[\text{target RNA}] - Ct[\text{endogenous control}]$ or $Ct[\text{endogenous control}] - Ct[\text{target RNA}]$. In certain embodiments, $\Delta Ct = Ct[\text{endogenous control}] - Ct[\text{marker}]$. In some embodiments, a threshold ΔCt value is set, above or below which bladder cancer is indicated. In some such embodiments, the ΔCt threshold is set as the ΔCt value below which 95% of normal samples are correctly characterized. In some such embodiments, a ΔCt value that is higher than the threshold ΔCt value is indicative of bladder cancer.

[00145] In some embodiments, linear discriminant analysis (LDA) is used, for example, to combine two or more of the markers into a single combined score. In some such embodiments, a single threshold value is used for the markers included in the LDA. In some embodiments, the Ct or ΔCt of each marker is weighted in the LDA equation. In some embodiments, at least two markers are given different weighting factors in the equation. In some embodiments, each marker is given a different weighting factor in the equation. In some embodiments, the weighting factors are determined such that the LDA equation results in a selected sensitivity and/or specificity, e.g., along a ROC curve, for a

sample dataset. In some embodiments, a standardized LDA equation is used. In some such embodiments, by using linear discriminate analysis, misclassifications (such as false negatives and false positives) are reduced and/or minimized. A nonlimiting exemplary standardized LDA equation may have the following form: $LDA = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5$, wherein $x_1 = (\text{endogenous control Ct} - p)/P$, $x_2 = (\text{marker 1 } \Delta\text{Ct} - q)/Q$, $x_3 = (\text{marker 2 } \Delta\text{Ct} - r)/R$, $x_4 = (\text{marker 3 } \Delta\text{Ct} - s)/S$, $x_5 = (\text{marker 4 } \Delta\text{Ct} - t)/T$; wherein $\Delta\text{Ct} = \text{endogenous control Ct} - \text{marker Ct}$; and wherein $\beta_1, \beta_2, \beta_3, \beta_4$, and β_5 are weighting factors; β_0 is the intercept; p, q, r, s , and t are the mean Ct or ΔCt values for the endogenous control, marker 1, marker 2, marker 3, and marker 4, respectively, from a sample data set; and P, Q, R, S , and T are the standard deviation of the Ct or ΔCt values for the endogenous control, marker 1, marker 2, marker 3, and marker 4, respectively, from the sample data set. In some embodiments, β_2 conditionally accounts for β_1 , etc., through regression. In some embodiments, an LDA cutoff is set such that a sample is considered positive if the LDA is less than or equal to the cutoff. In some embodiments, an LDA cutoff is set such that a sample is considered positive if the LDA is less than the cutoff. In some embodiments, an LDA cutoff is set such that a sample is considered positive if the LDA is greater than or equal to the cutoff. In some embodiments, an LDA cutoff is set such that a sample is considered positive if the LDA is greater than the cutoff.

[00146] A nonlimiting exemplary LDA equation for a four marker combination of KRT20, ANXA10, CRH, and IGF2, is as follows:

$$LDA = -2.94 + 1.60 * x_1 - 1.05 * x_2 - 0.38 * x_3 - 0.71 * x_4 - 1.74 * x_5$$

wherein $x_1 = (\text{ABL Ct} - 31.7)/2.6$, $x_2 = (\text{ANXA10 } \Delta\text{Ct} - (-7.8))/5.2$, $x_3 = (\text{KRT20 } \Delta\text{Ct} - (-3.2))/4.6$, $x_4 = (\text{CRH } \Delta\text{Ct} - (-9.4))/5.3$, and $x_5 = (\text{IGF2 } \Delta\text{Ct} - (-6.2))/6.3$. For the above equation, in some embodiments, a sample is classified as positive if $LDA \leq -2$. With this cutoff, in some embodiments, corresponding specificities of 90-95% yield sensitivities of 74%-85%.

[00147] A nonlimiting exemplary LDA equation for a four marker combination of UPK1B, ANXA10, CRH, and IGF2, is as follows:

$$LDA = -2.39 + 6.79 * x_1 - 3.31 * x_2 - 1.87 * x_3 - 3.90 * x_4 - 4.65 * x_5$$

wherein $x_1 = (\text{ABL Ct} - 31.7)/2.6$, $x_2 = (\text{ANXA10 } \Delta\text{Ct} - (-9.14))/4.9$, $x_3 = (\text{UPK1B } \Delta\text{Ct} - (-10.1))/4.6$, $x_4 = (\text{CRH } \Delta\text{Ct} - (-10.1))/4.7$, and $x_5 = (\text{IGF2 } \Delta\text{Ct} - (-8.2))/6.0$. For the above equation, in some embodiments, a sample is classified as positive if $LDA \leq +2$. With this cutoff, in some embodiments, corresponding specificities of 90-95% yield sensitivities of as high as 89%.

[00148] In some embodiments, a computer-based analysis program is used to translate the raw data generated by the detection assay (*e.g.*, the expression level of the bladder cancer markers described herein) into data of predictive value for a clinician. The clinician can access the predictive data using any suitable means. Thus, in some embodiments, the present invention provides the further benefit that the clinician, who is not likely to be trained in genetics or molecular biology, need not understand the raw data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject.

[00149] The present invention contemplates any method capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information provides, medical personal, and subjects. For example, in some embodiments of the present invention, a sample (*e.g.*, a biopsy or a serum or urine sample) is obtained from a subject and submitted to a profiling service (*e.g.*, clinical lab at a medical facility, genomic profiling business, etc.), located in any part of the world (*e.g.*, in a country different than the country where the subject resides or where the information is ultimately used) to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves (*e.g.*, a urine sample) and directly send it to a profiling center. Where the sample comprises previously determined biological information, the information may be directly sent to the profiling service by the subject (*e.g.*, an information card containing the information may be scanned by a computer and the data transmitted to a computer of the profiling center using an electronic communication systems). Once received by the profiling service, the sample is processed and a profile is produced (*i.e.*, expression data), specific for the diagnostic or prognostic information desired for the subject.

[00150] The profile data is then prepared in a format suitable for interpretation by a treating clinician. For example, rather than providing raw expression data, the prepared format may represent a diagnosis or risk assessment (*e.g.*, expression level of the bladder cancer markers described herein or diagnosis of bladder cancer) for the subject, along with recommendations for particular treatment options. The data may be displayed to the clinician by any suitable method. For example, in some embodiments, the profiling service generates a report that can be printed for the clinician (*e.g.*, at the point of care) or displayed to the clinician on a computer monitor.

[00151] In some embodiments, the information is first analyzed at the point of care or at a regional facility. The raw data is then sent to a central processing facility for further analysis and/or to convert the raw data to information useful for a clinician or patient. The central processing facility provides the advantage of privacy (all data is stored in a central facility with uniform security protocols), speed, and uniformity of data analysis. The central processing facility can then control the fate of the data following treatment of the subject. For example, using an electronic communication system, the central facility can provide data to the clinician, the subject, or researchers.

[00152] In some embodiments, the subject is able to directly access the data using the electronic communication system. The subject may chose further intervention or counseling based on the results. In some embodiments, the data is used for research use. For example, the data may be used to further optimize the inclusion or elimination of markers as useful indicators of a particular condition or stage of disease or as a companion diagnostic to determine a treatment course of action.

4.2.7. Exemplary polynucleotides

[00153] In some embodiments, polynucleotides are provided. In some embodiments, synthetic polynucleotides are provided. Synthetic polynucleotides, as used herein, refer to polynucleotides that have been synthesized in vitro either chemically or enzymatically. Chemical synthesis of polynucleotides includes, but is not limited to, synthesis using polynucleotide synthesizers, such as OligoPilot (GE Healthcare), ABI 3900 DNA Synthesizer (Applied Biosystems), and the like. Enzymatic synthesis includes, but is not limited, to producing polynucleotides by enzymatic amplification, e.g., PCR. A polynucleotide may comprise one or more nucleotide analogs (i.e., modified nucleotides) discussed herein.

[00154] In some embodiments, a polynucleotide is provided that comprises a region that is identical to, or complementary to, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at elast 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of a sequence selected from AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA. In some embodiments, a polynucleotide is provided that comprises a region that is identical to, or complementary to, a span of 6 to 100, 8 to 100, 8 to 75, 8 to 50, 8 to 40, or 8 to 30 contiguous nucleotides of a sequence selected from AR, UPK1B, CRH, IGF2, KRT20,

ANXA10 mRNA, UPK2, MGEA5, and PIK3A. Nonlimiting exemplary polynucleotides are shown in Tables 1 and 6.

[00155] In various embodiments, a polynucleotide comprises fewer than 500, fewer than 300, fewer than 200, fewer than 150, fewer than 100, fewer than 75, fewer than 50, fewer than 40, or fewer than 30 nucleotides. In various embodiments, a polynucleotide is between 6 and 200, between 8 and 200, between 8 and 150, between 8 and 100, between 8 and 75, between 8 and 50, between 8 and 40, or between 8 and 30 nucleotides long.

[00156] In some embodiments, the polynucleotide is a primer. In some embodiments, the primer is labeled with a detectable moiety. In some embodiments, a primer is not labeled. A primer, as used herein, is a polynucleotide that is capable of specifically hybridizing to a target RNA or to a cDNA reverse transcribed from the target RNA or to an amplicon that has been amplified from a target RNA or a cDNA (collectively referred to as “template”), and, in the presence of the template, a polymerase and suitable buffers and reagents, can be extended to form a primer extension product.

[00157] In some embodiments, the polynucleotide is a probe. In some embodiments, the probe is labeled with a detectable moiety. A detectable moiety, as used herein, includes both directly detectable moieties, such as fluorescent dyes, and indirectly detectable moieties, such as members of binding pairs. When the detectable moiety is a member of a binding pair, in some embodiments, the probe can be detectable by incubating the probe with a detectable label bound to the second member of the binding pair. In some embodiments, a probe is not labeled, such as when a probe is a capture probe, e.g., on a microarray or bead. In some embodiments, a probe is not extendable, e.g., by a polymerase. In other embodiments, a probe is extendable.

[00158] In some embodiments, the polynucleotide is a FRET probe that in some embodiments is labeled at the 5'-end with a fluorescent dye (donor) and at the 3'-end with a quencher (acceptor), a chemical group that absorbs (i.e., suppresses) fluorescence emission from the dye when the groups are in close proximity (i.e., attached to the same probe). In other embodiments, the dye and quencher are not at the ends of the FRET probe. Thus, in some embodiments, the emission spectrum of the dye should overlap considerably with the absorption spectrum of the quencher.

4.2.7.1. Exemplary polynucleotide modifications

[00159] In some embodiments, the methods of detecting at least one target RNA described herein employ one or more polynucleotides that have been modified, such

as polynucleotides comprising one or more affinity-enhancing nucleotide analogs. Modified polynucleotides useful in the methods described herein include primers for reverse transcription, PCR amplification primers, and probes. In some embodiments, the incorporation of affinity-enhancing nucleotides increases the binding affinity and specificity of a polynucleotide for its target nucleic acid as compared to polynucleotides that contain only deoxyribonucleotides, and allows for the use of shorter polynucleotides or for shorter regions of complementarity between the polynucleotide and the target nucleic acid.

[00160] In some embodiments, affinity-enhancing nucleotide analogs include nucleotides comprising one or more base modifications, sugar modifications and/or backbone modifications.

[00161] In some embodiments, modified bases for use in affinity-enhancing nucleotide analogs include 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, 2-chloro-6-aminopurine, xanthine and hypoxanthine.

[00162] In some embodiments, affinity-enhancing nucleotide analogs include nucleotides having modified sugars such as 2'-substituted sugars, such as 2'-O-alkyl-ribose sugars, 2'-amino-deoxyribose sugars, 2'-fluoro-deoxyribose sugars, 2'-fluoro-arabinose sugars, and 2'-O-methoxyethyl-ribose (2'MOE) sugars. In some embodiments, modified sugars are arabinose sugars, or d-arabino-hexitol sugars.

[00163] In some embodiments, affinity-enhancing nucleotide analogs include backbone modifications such as the use of peptide nucleic acids (PNA; *e.g.*, an oligomer including nucleobases linked together by an amino acid backbone). Other backbone modifications include phosphorothioate linkages, phosphodiester modified nucleic acids, combinations of phosphodiester and phosphorothioate nucleic acid, methylphosphonate, alkylphosphonates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, carboxymethyl esters, methylphosphorothioate, phosphorodithioate, p-ethoxy, and combinations thereof.

[00164] In some embodiments, a polynucleotide includes at least one affinity-enhancing nucleotide analog that has a modified base, at least nucleotide (which may be the same nucleotide) that has a modified sugar, and/or at least one internucleotide linkage that is non-naturally occurring.

[00165] In some embodiments, an affinity-enhancing nucleotide analog contains a locked nucleic acid ("LNA") sugar, which is a bicyclic sugar. In some embodiments, a polynucleotide for use in the methods described herein comprises one or

more nucleotides having an LNA sugar. In some embodiments, a polynucleotide contains one or more regions consisting of nucleotides with LNA sugars. In other embodiments, a polynucleotide contains nucleotides with LNA sugars interspersed with deoxyribonucleotides. *See, e.g.,* Frieden, M. *et al.* (2008) *Curr. Pharm. Des.* 14(11):1138-1142.

4.2.7.2. Exemplary primers

[00166] In some embodiments, a primer is provided. In some embodiments, a primer is identical to, or complementary to, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of a sequence selected from AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA. In some embodiments, a primer is provided that comprises a region that is identical to, or complementary to, a span of 6 to 100, 8 to 100, 8 to 75, 8 to 50, 8 to 40, or 8 to 30 contiguous nucleotides of a sequence selected from AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA. Nonlimiting exemplary primers are shown in Tables 1 and 6. In some embodiments, a primer may also comprise portions or regions that are not identical or complementary to the target RNA. In some embodiments, a region of a primer that is identical or complementary to a target RNA is contiguous, such that any region of a primer that is not identical or complementary to the target RNA does not disrupt the identical or complementary region.

[00167] In some embodiments, a primer comprises a portion that is identically present in a target RNA. In some such embodiments, a primer that comprises a region that is identically present in the target RNA is capable of selectively hybridizing to a cDNA that has been reverse transcribed from the RNA, or to an amplicon that has been produced by amplification of the target RNA or cDNA. In some embodiments, the primer is complementary to a sufficient portion of the cDNA or amplicon such that it selectively hybridizes to the cDNA or amplicon under the conditions of the particular assay being used.

[00168] As used herein, “selectively hybridize” means that a polynucleotide, such as a primer or probe, will hybridize to a particular nucleic acid in a sample with at least 5-fold greater affinity than it will hybridize to another nucleic acid present in the same sample that has a different nucleotide sequence in the hybridizing region. Exemplary hybridization conditions are discussed herein, for example, in the context of a reverse

transcription reaction or a PCR amplification reaction. In some embodiments, a polynucleotide will hybridize to a particular nucleic acid in a sample with at least 10-fold greater affinity than it will hybridize to another nucleic acid present in the same sample that has a different nucleotide sequence in the hybridizing region.

[00169] In some embodiments, a primer is used to reverse transcribe a target RNA, for example, as discussed herein. In some embodiments, a primer is used to amplify a target RNA or a cDNA reverse transcribed therefrom. Such amplification, in some embodiments, is quantitative PCR, for example, as discussed herein. In some embodiments, a primer comprises a detectable moiety.

[00170] In some embodiments, primer pairs are provided. Such primer pairs are designed to amplify a portion of a target mRNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, or an endogenous control RNA, or an exogenous control RNA. In some embodiments, a primer pair is designed to produce an amplicon that is 50 to 1500 nucleotides long, 50 to 1000 nucleotides long, 50 to 750 nucleotides long, 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, 50 to 150 nucleotides long, or 50 to 100 nucleotides long. Nonlimiting exemplary primer pairs are shown in Tables 1 and 6. In some embodiments, a primer pair is designed that spans an intron in the genomic sequence so that the mRNA, without the intron, is more preferably amplified than the genomic sequence. By “spans an intron” is meant that one primer of the primer pair is complementary to a sequence in the mRNA or a cDNA reverse transcribed from the mRNA that is at least partially located 5' to an intron in the genomic sequence and one primer of the primer pair is complementary to a sequence in the mRNA or a cDNA reverse transcribed from the mRNA that is at least partially located 3' to the same intron in the genomic sequence. In some embodiments, one primer of the primer pair is complementary to a sequence in the mRNA or a cDNA reverse transcribed from the mRNA that is located 5' to an intron in the genomic sequence and one primer of the primer pair is complementary to a sequence in the mRNA or a cDNA reverse transcribed from the mRNA that is located 3' to the same intron in the genomic sequence. In some embodiments, one of the primers in the primer pair may be complementary to a sequence in the mRNA or a cDNA reverse transcribed from the mRNA that is spliced together when the intron is removed such that the contiguous complementary sequence is not found in the genomic sequence. A primer pair comprising such a primer is still considered to span an intron.

4.2.7.3. Exemplary probes

[00171] In various embodiments, methods of detecting the presence of bladder cancer comprise hybridizing nucleic acids of a sample with a probe. In some embodiments, the probe comprises a portion that is complementary to a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. In some embodiments, the probe comprises a portion that is identically present in the target RNA. In some such embodiments, a probe that is complementary to a target RNA is complementary to a sufficient portion of the target RNA such that it selectively hybridizes to the target RNA under the conditions of the particular assay being used. In some embodiments, a probe that is complementary to a target RNA comprises a region that is complementary to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of the target RNA, such as CRH, IGF2, KRT20, ANXA10, , UPK2, MGEA5, or PIK3A mRNA. Nonlimiting exemplary probes are shown in Tables 1 and 6. A probe that is complementary to a target RNA may also comprise portions or regions that are not complementary to the target RNA. In some embodiments, a region of a probe that is complementary to a target RNA is contiguous, such that any region of a probe that is not complementary to the target RNA does not disrupt the complementary region.

[00172] In some embodiments, the probe comprises a portion that is identically present in the target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. In some such embodiments, a probe that comprises a region that is identically present in the target RNA is capable of selectively hybridizing to a cDNA that has been reverse transcribed from the RNA, or to an amplicon that has been produced by amplification of the target RNA or cDNA. In some embodiments, the probe is complementary to a sufficient portion of the cDNA or amplicon such that it selectively hybridizes to the cDNA or amplicon under the conditions of the particular assay being used. In some embodiments, a probe that is complementary to a cDNA or amplicon comprises a region that is complementary to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of the cDNA or amplicon. A probe that is complementary to a cDNA or amplicon may also comprise portions or regions that are not complementary to the cDNA or amplicon. In some embodiments, a region of a probe that is

complementary to a cDNA or amplicon is contiguous, such that any region of a probe that is not complementary to the cDNA or amplicon does not disrupt the complementary region.

[00173] In some embodiments, the method of detectably quantifying one or more target RNAs comprises: (a) reverse transcribing a target RNA to produce a cDNA that is complementary to the target RNA; (b) amplifying the cDNA from (a); and (c) detecting the amount of a target RNA using real time RT-PCR and a detection probe (which may be simultaneous with the amplification step (b)).

[00174] As described above, in some embodiments, real time RT-PCR detection may be performed using a FRET probe, which includes, but is not limited to, a TaqMan[®] probe, a Molecular beacon probe and a Scorpion probe. In some embodiments, the real time RT-PCR detection and quantification is performed with a TaqMan[®] probe, *i.e.*, a linear probe that typically has a fluorescent dye covalently bound at one end of the DNA and a quencher molecule covalently bound elsewhere, such as at the other end of, the DNA. The FRET probe comprises a sequence that is complementary to a region of the cDNA such that, when the FRET probe is hybridized to the cDNA, the dye fluorescence is quenched, and when the probe is digested during amplification of the cDNA, the dye is released from the probe and produces a fluorescence signal. In such embodiments, the amount of target RNA in the sample is proportional to the amount of fluorescence measured during cDNA amplification.

[00175] The TaqMan[®] probe typically comprises a region of contiguous nucleotides having a sequence that is complementary to a region of a target RNA or its complementary cDNA that is reverse transcribed from the target RNA template (*i.e.*, the sequence of the probe region is complementary to or identically present in the target RNA to be detected) such that the probe is specifically hybridizable to the resulting PCR amplicon. In some embodiments, the probe comprises a region of at least 6 contiguous nucleotides having a sequence that is fully complementary to or identically present in a region of a cDNA that has been reverse transcribed from a target RNA template, such as comprising a region of at least 8 contiguous nucleotides, at least 10 contiguous nucleotides, at least 12 contiguous nucleotides, at least 14 contiguous nucleotides, or at least 16 contiguous nucleotides having a sequence that is complementary to or identically present in a region of a cDNA reverse transcribed from a target RNA to be detected.

[00176] In some embodiments, the region of the cDNA that has a sequence that is complementary to the TaqMan[®] probe sequence is at or near the center of the cDNA molecule. In some embodiments, there are independently at least 2 nucleotides, such as at

least 3 nucleotides, such as at least 4 nucleotides, such as at least 5 nucleotides of the cDNA at the 5'-end and at the 3'-end of the region of complementarity.

[00177] In some embodiments, Molecular Beacons can be used to detect and quantitate PCR products. Like TaqMan[®] probes, Molecular Beacons use FRET to detect and quantitate a PCR product via a probe having a fluorescent dye and a quencher attached at the ends of the probe. Unlike TaqMan[®] probes, Molecular Beacons remain intact during the PCR cycles. Molecular Beacon probes form a stem-loop structure when free in solution, thereby allowing the dye and quencher to be in close enough proximity to cause fluorescence quenching. When the Molecular Beacon hybridizes to a target, the stem-loop structure is abolished so that the dye and the quencher become separated in space and the dye fluoresces. Molecular Beacons are available, e.g., from Gene Link[™] (see <http://www.genelink.com/newsite/products/mbintro.asp>).

[00178] In some embodiments, Scorpion probes can be used as both sequence-specific primers and for PCR product detection and quantitation. Like Molecular Beacons, Scorpion probes form a stem-loop structure when not hybridized to a target nucleic acid. However, unlike Molecular Beacons, a Scorpion probe achieves both sequence-specific priming and PCR product detection. A fluorescent dye molecule is attached to the 5'-end of the Scorpion probe, and a quencher is attached elsewhere, such as to the 3'-end. The 3' portion of the probe is complementary to the extension product of the PCR primer, and this complementary portion is linked to the 5'-end of the probe by a non-amplifiable moiety. After the Scorpion primer is extended, the target-specific sequence of the probe binds to its complement within the extended amplicon, thus opening up the stem-loop structure and allowing the dye on the 5'-end to fluoresce and generate a signal. Scorpion probes are available from, e.g., Premier Biosoft International (see http://www.premierbiosoft.com/tech_notes/Scorpion.html).

[00179] In some embodiments, labels that can be used on the FRET probes include colorimetric and fluorescent dyes such as Alexa Fluor dyes, BODIPY dyes, such as BODIPY FL; Cascade Blue; Cascade Yellow; coumarin and its derivatives, such as 7-amino-4-methylcoumarin, aminocoumarin and hydroxycoumarin; cyanine dyes, such as Cy3 and Cy5; eosins and erythrosins; fluorescein and its derivatives, such as fluorescein isothiocyanate; macrocyclic chelates of lanthanide ions, such as Quantum Dye[™]; Marina Blue; Oregon Green; rhodamine dyes, such as rhodamine red, tetramethylrhodamine and rhodamine 6G; Texas Red; fluorescent energy transfer dyes, such as thiazole orange-ethidium heterodimer; and, TOTAB.

[00180] Specific examples of dyes include, but are not limited to, those identified above and the following: Alexa Fluor 350, Alexa Fluor 405, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 500, Alexa Fluor 514, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 610, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, and, Alexa Fluor 750; amine-reactive BODIPY dyes, such as BODIPY 493/503, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/655, BODIPY FL, BODIPY R6G, BODIPY TMR, and, BODIPY-TR; Cy3, Cy5, 6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, SYPRO, TAMRA, 2', 4', 5', 7'-Tetrabromosulfonefluorescein, and TET.

[00181] Examples of dye/quencher pairs (i.e., donor/acceptor pairs) include, but are not limited to, fluorescein/tetramethylrhodamine; IAEDANS/fluorescein; EDANS/dabcyl; fluorescein/fluorescein; BODIPY FL/BODIPY FL; fluorescein/QSY 7 or QSY 9 dyes. When the donor and acceptor are the same, FRET may be detected, in some embodiments, by fluorescence depolarization. Certain specific examples of dye/quencher pairs (i.e., donor/acceptor pairs) include, but are not limited to, Alexa Fluor 350/Alexa Fluor 488; Alexa Fluor 488/Alexa Fluor 546; Alexa Fluor 488/Alexa Fluor 555; Alexa Fluor 488/Alexa Fluor 568; Alexa Fluor 488/Alexa Fluor 594; Alexa Fluor 488/Alexa Fluor 647; Alexa Fluor 546/Alexa Fluor 568; Alexa Fluor 546/Alexa Fluor 594; Alexa Fluor 546/Alexa Fluor 647; Alexa Fluor 555/Alexa Fluor 594; Alexa Fluor 555/Alexa Fluor 647; Alexa Fluor 568/Alexa Fluor 647; Alexa Fluor 594/Alexa Fluor 647; Alexa Fluor 350/QSY 35; Alexa Fluor 350/dabcyl; Alexa Fluor 488/QSY 35; Alexa Fluor 488/dabcyl; Alexa Fluor 488/QSY 7 or QSY 9; Alexa Fluor 555/QSY 7 or QSY 9; Alexa Fluor 568/QSY 7 or QSY 9; Alexa Fluor 568/QSY 21; Alexa Fluor 594/QSY 21; and Alexa Fluor 647/QSY 21. In some instances, the same quencher may be used for multiple dyes, for example, a broad spectrum quencher, such as an Iowa Black[®] quencher (Integrated DNA Technologies, Coralville, IA) or a Black Hole Quencher[™] (BHQ[™]; Sigma-Aldrich, St. Louis, MO).

[00182] In some embodiments, for example, in a multiplex reaction in which two or more moieties (such as amplicons) are detected simultaneously, each probe comprises a detectably different dye such that the dyes may be distinguished when detected simultaneously in the same reaction. One skilled in the art can select a set of detectably different dyes for use in a multiplex reaction.

[00183] Specific examples of fluorescently labeled ribonucleotides useful in the preparation of RT-PCR probes for use in some embodiments of the methods described herein are available from Molecular Probes (Invitrogen), and these include, Alexa Fluor 488-5-UTP, Fluorescein-12-UTP, BODIPY FL-14-UTP, BODIPY TMR-14-UTP, Tetramethylrhodamine-6-UTP, Alexa Fluor 546-14-UTP, Texas Red-5-UTP, and BODIPY TR-14-UTP. Other fluorescent ribonucleotides are available from Amersham Biosciences (GE Healthcare), such as Cy3-UTP and Cy5-UTP.

[00184] Examples of fluorescently labeled deoxyribonucleotides useful in the preparation of RT-PCR probes for use in the methods described herein include Dinitrophenyl (DNP)-1'-dUTP, Cascade Blue-7-dUTP, Alexa Fluor 488-5-dUTP, Fluorescein-12-dUTP, Oregon Green 488-5-dUTP, BODIPY FL-14-dUTP, Rhodamine Green-5-dUTP, Alexa Fluor 532-5-dUTP, BODIPY TMR-14-dUTP, Tetramethylrhodamine-6-dUTP, Alexa Fluor 546-14-dUTP, Alexa Fluor 568-5-dUTP, Texas Red-12-dUTP, Texas Red-5-dUTP, BODIPY TR-14-dUTP, Alexa Fluor 594-5-dUTP, BODIPY 630/650-14-dUTP, BODIPY 650/665-14-dUTP; Alexa Fluor 488-7-OBEA-dCTP, Alexa Fluor 546-16-OBEA-dCTP, Alexa Fluor 594-7-OBEA-dCTP, Alexa Fluor 647-12-OBEA-dCTP. Fluorescently labeled nucleotides are commercially available and can be purchased from, e.g., Invitrogen.

[00185] In some embodiments, dyes and other moieties, such as quenchers, are introduced into polynucleotide used in the methods described herein, such as FRET probes, via modified nucleotides. A "modified nucleotide" refers to a nucleotide that has been chemically modified, but still functions as a nucleotide. In some embodiments, the modified nucleotide has a chemical moiety, such as a dye or quencher, covalently attached, and can be introduced into a polynucleotide, for example, by way of solid phase synthesis of the polynucleotide. In other embodiments, the modified nucleotide includes one or more reactive groups that can react with a dye or quencher before, during, or after incorporation of the modified nucleotide into the nucleic acid. In specific embodiments, the modified nucleotide is an amine-modified nucleotide, *i.e.*, a nucleotide that has been modified to have a reactive amine group. In some embodiments, the modified nucleotide comprises a modified base moiety, such as uridine, adenosine, guanosine, and/or cytosine. In specific embodiments, the amine-modified nucleotide is selected from 5-(3-aminoallyl)-UTP; 8-[(4-amino)butyl]-amino-ATP and 8-[(6-amino)butyl]-amino-ATP; N6-(4-amino)butyl-ATP, N6-(6-amino)butyl-ATP, N4-[2,2-oxy-bis-(ethylamine)]-CTP; N6-(6-Amino)hexyl-ATP; 8-[(6-Amino)hexyl]-amino-ATP; 5-propargylamino-CTP, 5-propargylamino-UTP. In some

embodiments, nucleotides with different nucleobase moieties are similarly modified, for example, 5-(3-aminoallyl)-GTP instead of 5-(3-aminoallyl)-UTP. Many amine modified nucleotides are commercially available from, e.g., Applied Biosystems, Sigma, Jena Bioscience and TriLink.

[00186] Exemplary detectable moieties also include, but are not limited to, members of binding pairs. In some such embodiments, a first member of a binding pair is linked to a polynucleotide. The second member of the binding pair is linked to a detectable label, such as a fluorescent label. When the polynucleotide linked to the first member of the binding pair is incubated with the second member of the binding pair linked to the detectable label, the first and second members of the binding pair associate and the polynucleotide can be detected. Exemplary binding pairs include, but are not limited to, biotin and streptavidin, antibodies and antigens, etc.

[00187] In some embodiments, multiple target RNAs are detected in a single multiplex reaction. In some such embodiments, each probe that is targeted to a unique cDNA is spectrally distinguishable when released from the probe. Thus, each target RNA is detected by a unique fluorescence signal.

[00188] One skilled in the art can select a suitable detection method for a selected assay, e.g., a real-time RT-PCR assay. The selected detection method need not be a method described above, and may be any method.

4.3. Exemplary compositions and kits

[00189] In another aspect, compositions are provided. In some embodiments, compositions are provided for use in the methods described herein.

[00190] In some embodiments, compositions are provided that comprise at least one target RNA-specific primer. The term “target RNA-specific primer” encompasses primers that have a region of contiguous nucleotides having a sequence that is (i) identically present in a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, or (ii) complementary to the sequence of a region of contiguous nucleotides found in a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. In some embodiments, a composition is provided that comprises at least one pair of target RNA-specific primers. The term “pair of target RNA-specific primers” encompasses pairs of primers that are suitable for amplifying a defined region of a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. A pair of target RNA-specific primers typically comprises a

first primer that comprises a sequence that is identical to the sequence of a region of a target RNA (although the primer will typically comprise DNA or modified nucleosides rather than RNA) and a second primer that comprises a sequence that is complementary to a region of a target RNA. A pair of primers is typically suitable for amplifying a region of a target mRNA that is 50 to 1500 nucleotides long, 50 to 1000 nucleotides long, 50 to 750 nucleotides long, 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, 50 to 150 nucleotides long, or 50 to 100 nucleotides long. Nonlimiting exemplary primers, and pairs of primers, are shown in Tables 1 and 6.

[00191] In some embodiments, a composition comprises at least one, at least two, at least three, at least four, at least five, or six pairs of target RNA-specific primers, one pair for amplifying each of AR and/or UPK1B mRNA, and at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A. In some embodiments, a composition additionally comprises a pair of target RNA-specific primers for amplifying an endogenous control RNA and/or one pair of target RNA-specific primers for amplifying an exogenous control RNA.

[00192] In some embodiments, a composition comprises at least one target RNA-specific probe. The term “target RNA-specific probe” encompasses probes that have a region of contiguous nucleotides having a sequence that is (i) identically present in a target RNA, such as such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA, or (ii) complementary to the sequence of a region of contiguous nucleotides found in a target RNA, such as such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. Nonlimiting exemplary target-specific probes are shown in Tables 1 and 6.

[00193] In some embodiments, a composition (including a composition described above that comprises one or more pairs of target RNA-specific primers) comprises at least one, at least two, at least three, at least four, at least five, or six probes, one probe for detecting each of AR and/or UPK1B, , and at least one mRNA selected from CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A. In some embodiments, a composition additionally comprises a probe for detecting an endogenous control RNA and/or a probe for detecting an exogenous control RNA.

[00194] In some embodiments, a composition is an aqueous composition. In some embodiments, the aqueous composition comprises a buffering component, such as phosphate, tris, HEPES, etc., and/or additional components, as discussed below. In some

embodiments, a composition is dry, for example, lyophilized, and suitable for reconstitution by addition of fluid. A dry composition may include one or more buffering components and/or additional components.

[00195] In some embodiments, a composition further comprises one or more additional components. Additional components include, but are not limited to, salts, such as NaCl, KCl, and MgCl₂; polymerases, including thermostable polymerases such as Taq; dNTPs; reverse transcriptases, such as MMLV reverse transcriptase; RNase inhibitors; bovine serum albumin (BSA) and the like; reducing agents, such as β -mercaptoethanol; EDTA and the like; etc. One skilled in the art can select suitable composition components depending on the intended use of the composition.

[00196] In some embodiments, compositions are provided that comprise at least one polynucleotide for detecting at least one target RNA. In some embodiments, the polynucleotide is used as a primer for a reverse transcriptase reaction. In some embodiments, the polynucleotide is used as a primer for amplification. In some embodiments, the polynucleotide is used as a primer for RT-PCR. In some embodiments, the polynucleotide is used as a probe for detecting at least one target RNA. In some embodiments, the polynucleotide is detectably labeled. In some embodiments, the polynucleotide is a FRET probe. In some embodiments, the polynucleotide is a TaqMan[®] probe, a Molecular Beacon, or a Scorpion probe.

[00197] In some embodiments, a composition comprises at least one FRET probe having a sequence that is identically present in, or complementary to a region of, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. In some embodiments, a FRET probe is labeled with a donor/acceptor pair such that when the probe is digested during the PCR reaction, it produces a unique fluorescence emission that is associated with a specific target RNA. In some embodiments, when a composition comprises multiple FRET probes, each probe is labeled with a different donor/acceptor pair such that when the probe is digested during the PCR reaction, each one produces a unique fluorescence emission that is associated with a specific probe sequence and/or target RNA. In some embodiments, the sequence of the FRET probe is complementary to a target region of a target RNA. In other embodiments, the FRET probe has a sequence that comprises one or more base mismatches when compared to the sequence of the best-aligned target region of a target RNA.

[00198] In some embodiments, a composition comprises a FRET probe consisting of at least 8, at least 9, at least 10, at least 11, at least 13, at least 14, at least 15, at

least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 nucleotides, wherein at least a portion of the sequence is identically present in, or complementary to a region of, AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. In some embodiments, at least 8, at least 9, at least 10, at least 11, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 nucleotides of the FRET probe are identically present in, or complementary to a region of, AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. In some embodiments, the FRET probe has a sequence with one, two or three base mismatches when compared to the sequence or complement of AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA.

[00199] In some embodiments, a kit comprises a polynucleotide discussed above. In some embodiments, a kit comprises at least one primer and/or probe discussed above. In some embodiments, a kit comprises at least one polymerase, such as a thermostable polymerase. In some embodiments, a kit comprises dNTPs. In some embodiments, kits for use in the real time RT-PCR methods described herein comprise one or more target RNA-specific FRET probes and/or one or more primers for reverse transcription of target RNAs and/or one or more primers for amplification of target RNAs or cDNAs reverse transcribed therefrom.

[00200] In some embodiments, one or more of the primers and/or probes is “linear”. A “linear” primer refers to a polynucleotide that is a single stranded molecule, and typically does not comprise a short region of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to another region within the same polynucleotide such that the primer forms an internal duplex. In some embodiments, the primers for use in reverse transcription comprise a region of at least 4, such as at least 5, such as at least 6, such as at least 7 or more contiguous nucleotides at the 3'-end that has a sequence that is complementary to region of at least 4, such as at least 5, such as at least 6, such as at least 7 or more contiguous nucleotides at the 5'-end of a target RNA.

[00201] In some embodiments, a kit comprises one or more pairs of linear primers (a “forward primer” and a “reverse primer”) for amplification of a cDNA reverse transcribed from a target RNA, such as AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, or PIK3A mRNA. Accordingly, in some embodiments, a first primer comprises a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at

least 22, at least 23, at least 24, or at least 25 contiguous nucleotides having a sequence that is identical to the sequence of a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides at a first location in the mRNA. Furthermore, in some embodiments, a second primer comprises a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides having a sequence that is complementary to the sequence of a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides at a second location in the mRNA, such that a PCR reaction using the two primers results in an amplicon extending from the first location of the mRNA to the second location of the mRNA.

[00202] In some embodiments, the kit comprises at least two, at least three, or at least four sets of primers, each of which is for amplification of a cDNA that is reverse transcribed from a different target RNA, including AR, UPK1B, CRH, IGF2, KRT20, ANXA10, UPK2, MGEA5, and PIK3A mRNA. In some embodiments, the kit further comprises at least one set of primers for amplifying a control RNA, such as an endogenous control and/or an exogenous control.

[00203] In some embodiments, probes and/or primers for use in the compositions described herein comprise deoxyribonucleotides. In some embodiments, probes and/or primers for use in the compositions described herein comprise deoxyribonucleotides and one or more nucleotide analogs, such as LNA analogs or other duplex-stabilizing nucleotide analogs described above. In some embodiments, probes and/or primers for use in the compositions described herein comprise all nucleotide analogs. In some embodiments, the probes and/or primers comprise one or more duplex-stabilizing nucleotide analogs, such as LNA analogs, in the region of complementarity.

[00204] In some embodiments, the kits for use in real time RT-PCR methods described herein further comprise reagents for use in the reverse transcription and amplification reactions. In some embodiments, the kits comprise enzymes such as reverse transcriptase, and a heat stable DNA polymerase, such as Taq polymerase. In some embodiments, the kits further comprise deoxyribonucleotide triphosphates (dNTP) for use in

reverse transcription and amplification. In further embodiments, the kits comprise buffers optimized for specific hybridization of the probes and primers.

[00205] The following examples are for illustration purposes only, and are not meant to be limiting in any way.

5. EXAMPLES

5.1. Example 1: Detection of high grade and low grade bladder cancer

[00206] More than 30 mRNA markers and 20 microRNA markers were evaluated in both bladder tissue and urine samples to determine the most accurate panel for detection of bladder cancer. Based on results from over 200 urine samples using eight markers and the GeneXpert system (Cepheid, Sunnyvale, CA), a panel consisting of CRH, IGF2, KRT20, and ANXA10 mRNA markers (with or without at least one endogenous and/or at least one exogenous control) was selected.

[00207] Various reaction compositions were designed for use in the GeneXpert[®] system, for detecting various combinations of CRH, IGF2, KRT20, and ANXA10 mRNA. Table 1 shows the sequences of the primers and probes used to detect each of the target RNAs by quantitative RT-PCR in the various reaction compositions.

Table 1: Primer and probe sequences

oligo name	target	sequence	SEQ ID NO	Reagent Formul. ("TSR")
ABLa3a4 PrmrFwd4	ABL	GATCAACACTGCTTCTGATGGCAA	8	CL3, CL4, CL1
ABLa3a4 PrmrRev1	ABL	CCACCGTTGAATGATGATGAACCAA	9	CL3, CL4, CL1
ABLa3a4 Probe1	ABL	F4-CCTCCGAGAGCCGCTTCAAC-Q4	10	CL3
ABL probe F6	ABL	F6-CCTCCGAGAGCCGCTTCAAC-Q6	11	CL4
ABL probe F1	ABL	F1-CCTCCGAGAGCCGC (T-dabcyl) TCAAC-Q1	12	CL1
KRT20 For	KRT20	TTGAAGAGCTGCGAAGTCAGAT	13	CL3, CL4
KRT20 Rev	KRT20	TGAAGTCCTCAGCAGCCAGTT	14	CL3, CL4
KRT20 Probe (F3)	KRT20	F3-TCAACTGCAAAATGCTCGGTGTGTCC-Q3	15	CL3, CL4
IGF2 For_4	IGF2	CGCGGCTTCTACTTCAGCAG	16	CL3, CL4
IGF2 Rev_4	IGF2	GCGGAAACAGCACTCCTCAA	17	CL3, CL4
IGF2 Probe_2	IGF2	F5-TGTGAGCCGTCGCAGCCGTG-Q5	18	CL3, CL4
CRH_For	CRH	ACCCGGCTCACCTGCGAA	19	CL3, CL4, CL1
CRH_Rev	CRH	GGACTCCCGCGGACACAA	20	CL3, CL4,

				CL1
CRH_probe 3	CRH	F2-TCCTGGGAAGCGAGTGGCCCTAA-Q2	21	CL3, CL1
CRH_probe_F1	CRH	F1-CCTGGGAAGCGAG (T-dabcyl) GGGCCCTAA-Q1	22	CL4
Armored RNA [®] Fwd	exogenous control	GGCTATTCTCCTCTTGGCAGAT	23	CL1
Armored RNA [®] Rev	exogenous control	TGCTTGAGCTCCAGTCCCTAAG	24	CL1
Armored RNA [®] _Probe	exogenous control	F6-AGCCGAGAAGGCGGAGTCTGGC-Q6	25	CL1
ANXA10-FW	ANXA10	GTGAAACAAGTTTATGCAATCGATCAA	26	CL1
ANXA10-RV3	ANXA10	GATTGAAATTGGGAGCTGGGAA	27	CL1
ANXA10-F3	ANXA10	F3-TCATCCCTGAGGTTACAATTACCATCAA-Q3	28	CL1

F1 through F6 are detectably different dyes that can be detected and distinguished simultaneously in a multiplex reaction, and Q1 to Q6 are quenchers (in the present example, Q2, Q4, Q5, and Q6 are the same quencher and Q1 is dabcyl, as indicated in the sequences).

[00208] The final primer and probe compositions of three different reaction compositions are shown in Table 2.

Table 2: Primers and probes in TSR CL3, CL4, and CL1

TSR CL3					
Target	Label	Purpose	Final conc. Forw. Primer	Final conc. Rev primer	Final conc. Probe
ABL	F4	Normalization (endogenous control)	400 nM	400 nM	150 nM
KRT20	F3	Bladder cancer marker	400 nM	400 nM	75 nM
IGF2	F5	Bladder cancer marker	400 nM	400 nM	200 nM
CRH	F2	Bladder cancer marker	400 nM	400 nM	200 nM
TSR CL4					
Target	Label	Purpose	Final conc. Forw. Primer	Final conc. Rev primer	Final conc. Probe
ABL	F6	Normalization (endogenous control)	400 nM	400 nM	400 nM
KRT20	F3	Bladder cancer marker	400 nM	400 nM	75 nM
IGF2	F5	Bladder cancer marker	400 nM	400 nM	300 nM
CRH	F1	Bladder cancer marker	400 nM	400 nM	600 nM
TSR CL1					
Target	Label	Purpose	Final conc. Forw. Primer	Final conc. Rev primer	Final conc. Probe
ABL	F1	Normalization (endogenous control)	400 nM	400 nM	600 nM
Armored RNA [®]	F6	Exogenous control	400 nM	400 nM	400 nM
ANXA10	F3	Bladder cancer marker	400 nM	400 nM	75 nM
CRH	F2	Bladder cancer marker	400 nM	400 nM	300 nM

[00209] Each reaction contained 50-90 mM KCl, 3-5 mM MgCl₂, 400-825 μ M dNTPs, 20 mM Tris, pH 8.5, 0.01% sodium azide, and 0.9 units/ μ l of Rnase inhibitor. MMLV reverse transcriptase (0.375 units/ μ l) and AptaTaq (0.25 units/ μ l; Roche) were used for reverse transcription and amplification, respectively. TSR CL1 included an Armored RNA[®] exogenous control (SEQ ID NO: 47; Asuragen, Austin, TX).

[00210] For each sample to be tested, 5 mL of voided urine was added to 5 mL preservative (3.5M guanidine HCl, 1% N-acetyl-L-cysteine, 25 mM sodium citrate, and 2.5% Tween-20, pH 3.2), preferably within 1 hour of sample collection. The preserved samples were transported on ice and stored at 4 °C. Clinical information for each sample was provided by the collection sites. The number of red blood cells per millilitre was determined by microscopic evaluation.

[00211] Prior to use, the preserved urine was inverted three times to mix. 1.2 mL of preserved urine was loaded into a GeneXpert cartridge for analysis. The cartridge contained a 0.8 μ m filter to capture urothelial cells. The captured cells were washed and lysed using sonication (2-15 seconds, 8-16 μ m at 36 kHz) within the cartridge. The lysate was then used to reconstitute the reagents used for real-time RT-PCR (described above). The reaction cycle used was: 10 minutes at 45°C, followed by 2 minutes at 95°C, and then 45 cycles of (a) 5 seconds at 95°C, 20 seconds at 60°C, and 20 seconds at 72°C, using a GeneXpert[®] cartridge in a GeneXpert[®] system. Delta Ct (Δ Ct) was calculated as Ct (ABL) – Ct (marker). The Δ Ct cutoff was set as the Δ Ct that gave at least 95% specificity with samples from patients not expected to have bladder cancer (data not shown). A Δ Ct above the Δ Ct cutoff for any one of the markers was considered a positive result, indicative of the presence of bladder cancer.

[00212] Some samples were also tested using UroVysion[®] (Abbott Laboratories, Abbott Park, IL). The results of that experiment are shown in Table 3 (high grade bladder cancer) and Table 4 (low grade bladder cancer). Δ Cts above the threshold, indicating a positive result, are highlighted. Each of the three TSR lots, CL3, CL4, and CL1, detected 100% of high grade bladder cancer samples, as did UroVysion[®].

[00213] For low grade bladder cancer, the detection rate was 37% (7/19), compared to only 16% (3/19) for UroVysion[®].

Table 3: Detection of high grade bladder cancer

Sample ID	stage	grade	UroVysion® Result	cytology	history of bladder cancer	TSR lot CL3			TSR lot CL4			CIC TSR lot CL1		GX result
						CRH	KRT20	IGF2	KRT20	IGF2	CRH	ANXA10	CRH	
						-10	2.2	-1	4	0.5	-5	-0.5	-3	
67001	pTa	high	positive	suspicious	yes	-20	4.9	-20	4.8	1.1	-20	-1.6	-3.6	positive
67006	pT1	high	positive	positive	no	-0.7	4.4	-6.2	5.7	-2.8	-0.6	-20	2.2	positive
67009	pT2	high	positive	positive	no	-20	4.9	-0.4	4.9	1.5	-20	1.1	-20	positive
75211	pT2	high	positive	negative	no	-0.8	1.4	-4.1						positive
75216	pTa, C1C	high	positive	suspicious	yes	-3.5	2	-0.8	0.9	-0.6	-3.4			positive
75218	pTa	high	positive	negative	no	0.9	3.8	-0.9	3.8	-1.6	0.5			positive
75245	pTa	high	positive	NA	no	20	1.6	6.7	0.4	6.2	20	1.9	8.2	positive
75247	C1S	high	positive	atypical	no	-1.4	3.4	2.7	3.6	3.7	-3.8	-0.2	0.4	positive
75248	pT1/C1S	high	positive	atypical	no	-20	4.3	0	4.8	1	-20	0.4	-5.1	positive
75249	pT1	high	positive	negative	no	-20	3.3	2.1	3.4	2.5	-20	3.7	-20	positive
75258	C1S, pTa	high	positive	atypical	no				4.2	-3.7	0.6	-2	1.9	positive
75246			positive	positive	no	-20	4.3	5.3	4.4	5.9	9.1	-1	3.4	positive

Table 4: Detection of low grade bladder cancer

Sample ID	stage	grade	UroVysion® Result	cytology	history of bladder cancer	TSR lot CL3			TSR lot CL4			CIC TSR lot CL1		GX result
						CRH	KRT20	IGF2	KRT20	IGF2	CRH	ANXA10	CRH	
67002	pTa	low	negative	atypical	yes	-10	2.2	-1	4	0.5	-5	-0.5	-3	positive
67003	pTa	low	negative	negative	no	-20	3.9	-3.8	3.3	-1.5	-20	-1.7	-20	positive
67004	pTa	low	negative	negative	no	-2	4.7	-2.3	-20	-20	-20	-20	-20	negative
67010	pTa	low	positive	atypical	no	-20	5.3	-8.8	5.2	0.1	-1.7	2.4	2.2	positive
67011	pTa	low	positive	atypical	no	-1.3	4.6	1.9	-3.8	-6.7	-20	-5	-20	negative
67018	pTa	low	negative	atypical	yes	-20	-20	-4.4	4	2.4	-2	-1.3	1	positive
67050	pTa	low	negative	atypical	yes	-20	-0.4	3.4	-20	-20	-20	-20	-20	negative
67100	pTa	low	borderline	atypical	yes				1.9	3.3	-20	-0.5	-20	positive
75161	pTa	low	negative	atypical	yes	-20	-3	-4.7	-2.9	-2.7	-20	-20	-20	negative
75183	pTa	low	positive	negative	yes	-1.5	3.1	-2	-3.6	-1.3	-20	-5.7	-20	negative
75184	pTa	low	inconclusive	negative	yes	-20	-0.6	1.3	3.4	-0.5	0.7	-4.1	2.3	positive
75185	pTa	low	negative	negative	no	-20	3	5	-1.5	1.8	-20	-20	-20	positive
75191	pTa	low	negative	negative	yes	-20	-0.4	-3.4	1.3	4.7	-20	1.2	-4.2	positive
75202	pTa	low	negative	negative	yes	-20	0.3	-2.3	1.1	-0.5	-20	-4.1	-20	negative
75236	pTa	low	negative	negative	no	-20	-5.7	-7.9	-0.4	-1.5	-20	-2.4	-20	negative
75251	pTa	low	negative	negative	yes	-20	-3.6	-5.1	-20	-8.9	-20	-3.2	-20	negative
75257	pTa	low	negative	negative	no				-1	-4.4	-20	-20	-20	negative
75265	pTa	low	negative	negative	yes				-2.1	-0.9	-20	-20	-20	negative
									1.4	-20	-20	-20	-20	negative

[00214] A summary of the sensitivity for high grade bladder cancer and low grade bladder cancer, and the specificity in patients with a low risk of bladder cancer, is shown in Table 5 for each of the individual markers tested in Tables 3 and 4.

Table 5: Summary of sensitivity and specificity of individual markers

Marker	Sensitivity, high grade bladder cancer	Sensitivity, low grade bladder cancer	Specificity, low risk of bladder cancer
CRH	15/30 (50%)	9/53 (17%)	220/221 (99%)
KRT20	11/21 (52%)	6/34 (18%)	144/145 (99%)
IGF2	13/21 (62%)	8/34 (24%)	144/145 (99%)
ANXA10	5/9 (56%)	2/19 (11%)	74/76 (97%)
4 marker combo	12/12 (100%)	7/19 (37%)	83/88 (94%)

[00215] Exemplary alternative primers and probes for detecting the four markers, KRT20, IGF2, CRH, and ANXA10, are shown in Table 6. Table 6 also shows an exemplary set of primers and probes for detecting an exogenous control and an endogenous control, ABL. The dyes and quenchers shown in Table 6 are generic, and two or more of quenchers Q1 to Q6 may be the same (Q1 is dabcy1 as indicated in the sequence). One skilled in the art could select a suitable set, for example, a set of detectably different dyes for use in a multiplex assay. The predicted amplicon length for each set of primers is also shown, as well as the length of any intervening intron(s) between the primer sites on the genomic copy of the target.

Table 6: Primer and probe sequences

name	5' mod	sequence	3' mod	SEQ ID NO	amplicon length (bp)	intron length (bp)
KRT20 For_3		CGACTACAGTGCATATTACAGACAA		29	113	2142
KRT20 Rev_2		CAGCAGCCAGTTTAGCATTATCAA		30		
KRT20 Probe	F1	TCAACTGCAAAA (T-dabcy1) GCTCGGTGTGTCC	Q1	31		
IGF2 For_5		GGACCGCGGCTTCTACTTCA		32	95	1701
IGF2 Rev_5		CCAGGTCACAGCTGCGGAA		33		
IGF2 Probe_2_F4	F4	TGTGAGCCGTGCGAGCCGTG	Q4	34		
CRH_For 4		TGCGAAGCGCCTGGGAAGC		35	66	801
CRH_Rev		GGACTCCCGCGGACACAA		36		
CRH_probe_F2	F2	TGCCCTAACATGCGGCTGCC	Q2	37		
ANXA10_For_3		TCAGCGCTGCAATGCACAA		38	122	22,947

ANXA10_For_4		CTGCAATGCACAAAGGATGA		48	117	22,947
ANXA10_Rev_5		GGCCAGCCATCACATCTTTGAA		39		
ANXA10_Probe_3	F3	TAGAGCATGTATGGCCGGGACCT	Q3	40		
ABLa3a4 PrmrFwd4		GATCAACACTGCTTCTGATGGCAA		41	92	7666
ABLa3a4 PrmrRev1		CCACCGTTGAATGATGATGAACCAA		42		
ABL Probe F5	F5	CCTCCGAGAGCCGCTTCAAC	Q5	43		
Armored RNA [®] Fwd		GGCTATTCTCCTCTTGGCAGAT		44	101	NA
Armored RNA [®] Rev		TGCTTGAGCTCCAGTCCCTAAG		45		
Armored RNA [®] Probe	F6	AGCCGAGAAGGCGGAGTCTGGC	Q6	46		

5.2. Example 2: Assay sensitivity for detecting bladder cancer using marker panel KRT20, IGF2, CRH, and ANXA10 in a larger cohort

[00216] Urine samples were collected from subjects at seven different sites.

Eligibility criteria for inclusion in the study included:

- 18 years or older;
- Documented informed consent as required by the reviewing IRB or HREC, and a signed Experimental Subjects Bill of Rights for patients in California;
- At least one of the following criteria:
 - A history or recurrence of bladder cancer;
 - A referral for cystoscopy evaluation due to micro- or gross-hematuria in urine;
 - A referral for urology evaluation, but no previous history of bladder cancer or clinical evidence of bladder cancer;
- Consent to provide at least 15 ml voided urine in addition to that required for standard of care;
- Consent to allow pathology results for any biopsy specimens taken during cystoscopy procedure and other medical records to be reported.

Exclusion criteria included only under 18 years of age and first voided urine. Patients currently or previously treated with Bacillus Calmette-Guerin (BCG) and patients currently or previously treated with intravesical therapy or transurethral resection of bladder or radiation therapy for bladder cancer were eligible for the study. In addition, repeat enrollment during the course of the study was also permitted.

[00217] Two of the collection sites provided the results of UroVysion[®] analysis on the urine samples. For each sample to be tested 15 mL of voided urine was added to 15 mL of preservative (3.5M guanidine HCl, 1% N-acetyl-L-cysteine, 25 mM sodium citrate, and 2.5% Tween-20, pH 3.2), preferably within 1 hour of sample collection.

The preserved samples were transported on ice and stored at 4°C. Clinical information for each sample was provided by the collection sites.

[00218] Prior to use, the preserved urine was inverted three times to mix. 4 mL of preserved urine was loaded into a GeneXpert cartridge for analysis. The cartridge contained a 0.8 µm filter to capture urothelial cells. The captured cells were washed and lysed using sonication (2-15 seconds, 8-16 µm at 36 kHz) within the cartridge. The lysate was then used to reconstitute the reagents used for real-time RT-PCR (described above). The reaction cycle used was: 10 minutes at 45°C, followed by 2 minutes at 95°C, and then 45 cycles of (a) 5 seconds at 95°C, 20 seconds at 60°C, and 20 seconds at 72°C, using a GeneXpert[®] cartridge in a GeneXpert[®] system. For ANXA10, KRT20 and IGF2, delta Ct (Δ Ct) was calculated as Ct (ABL) – Ct (marker). The Δ Ct cutoff was set as the Δ Ct that gave high (>90%) specificity with samples from patients not expected to have bladder cancer (data not shown). A Δ Ct above the Δ Ct cutoff for any one of the markers was considered a positive result, indicative of the presence of bladder cancer. For CRH, Ct values were used instead of Δ Ct to determine positivity for the CRH marker. A CRH Ct value < 45 was considered a positive result, indicative of the presence of bladder cancer. In addition to the four bladder cancer markers (KRT20, IGF2, CRH, and ANXA10), the GeneXpert[®] bladder cancer assay included two controls: primers and probe for detecting ABL mRNA in the samples, and primers and probe for detecting an Armored RNA[®] exogenous control RNA.

[00219] In the first analysis, 132 samples collected from patients who had positive cystoscopy results for bladder cancer were tested with the GeneXpert[®] bladder cancer assay. Sixty of those samples had also been tested using UroVysion[®]. Table 7 shows the results for those 132 samples.

Table 7: Assay sensitivity by bladder cancer stage and grade

	Xpert Bladder				UroVysion			
	POS	NEG	Invalid/ Error**	Sensitivity	POS	NEG	inconclusive	Sensitivity
Stage :								
All	94	35	3	72.9%	29	26	5	52.7%
Ta, Grade Low	29	23	2	55.8%	5	18	4	21.7%
Ta, Grade High	20	2		90.9%	4	6	1	40.0%
T1	13	2		86.7%	6	1		85.7%
T2	11	2		84.6%	4	0		100.0%
T3	3	0		100.0%	1	0		100.0%
T4	2	0		100.0%				
CIS	11	1		91.7%	7	0		100.0%
UNK	5	5	1	50.0%	2	1		66.7%
Grade:								
All	94	35	3	72.9%	29	26	5	52.7%
Low Grade	33	28	2	54.1%	6	19	4	24.0%
High Grade	61	7	1	89.7%	23	7	1	76.7%

** Two invalid results were due to low ABL Ct, suggesting a low number of cells in the sample, and one of the invalid results was due to poor sample quality.

[00220] As shown in Table 7, for these samples, the GeneXpert® bladder cancer assay had a sensitivity of 54.1% for low grade bladder cancer and a sensitivity of 89.7% for high grade bladder cancer. In contrast, UroVysion® had a sensitivity of just 24% for low grade bladder cancer and a sensitivity of 76.6% for high grade bladder cancer. Further, the GeneXpert® bladder cancer assay was able to detect all grades and stages of bladder cancer.

[00221] The same data set was then divided according to three patient groups: (A) patients with a history of bladder cancer who were currently being monitored for recurrence of bladder cancer, (B) patients who had been treated with Bacillus Calmette-Guerin (BCG) within the three months prior to sample collection, and (C) patients who were symptomatic for bladder cancer and had no prior history of bladder cancer. The results for those patient groups are shown in Table 8.

Table 8: Assay sensitivity by patient population

Monitoring (Population A)								
Xpert Bladder*					UroVysion			
	POS	NEG	Invalid/ Error**	Sensitivity	POS	NEG	inconclusive	Sensitivity
Grade:								
All	50	20	1	71.4%	11	12		47.8%
Low Grade	21	18		53.8%	2	11	1	15.4%
High Grade	29	2	1	93.5%	9	1		90.0%

Treated with BCG in last 3 months (Population B)								
Xpert Bladder*					UroVysion			
	POS	NEG	Invalid/ Error**	Sensitivity	POS	NEG	inconclusive	Sensitivity
Grade:								
All	4	2		66.7%	1			100.0%
Low Grade								
High Grade	4	2		66.7%	1			100.0%

Symptomatic (Population C)								
Xpert Bladder*					UroVysion			
	POS	NEG	Invalid/ Error**	Sensitivity	POS	NEG	inconclusive	Sensitivity
Grade:								
All	40	13	2	75.5%	17	14	4	54.8%
Low Grade	12	10	2	54.5%	4	8	3	33.3%
High Grade	28	3		90.3%	13	6	1	68.4%

** Two invalid results were due to low ABL Ct, suggesting a low number of cells in the sample, and one of the invalid results was due to poor sample quality.

[00222] As shown in Table 8, the GeneXpert® bladder cancer assay had a similar sensitivity for low grade and high grade bladder cancer in patients being monitored for bladder cancer and in patients who were symptomatic of bladder cancer as in the patient group as a whole (*see* Table 7). In patients who had been treated with BCG within the last three months, the GeneXpert® bladder cancer assay had a sensitivity of 66.7%, although the sample size was too small (6 samples) to draw any conclusions from that result.

[00223] In order to have a direct comparison of the GeneXpert® bladder cancer assay and UroVysion®, a dataset was selected that included only samples that had been tested with both assays. Table 9 shows the results for that dataset, with the patients separated into two groups: (A&B) patients with a history of bladder cancer who were currently being monitored for recurrence of bladder cancer, combined with patients who had been treated with Bacillus Calmette-Guerin (BCG) within the three months prior to sample collection (these groups were combined because only one sample from a BCG-treated

patient had been tested with both assays), and © patients who were symptomatic for bladder cancer and had no prior history of bladder cancer.

Table 9: Assay sensitivity for samples tested with both GeneXpert® and UroVysion®

Monitoring and BCG treated (Populations A&B)								
	Xpert Bladder				UroVysion			
	POS	NEG	Invalid/Error**	Sensitivity	POS	NEG	inconclusive	Sensitivity
Grade:								
All	18	7	1	72.0%	12	13	1	48.0%
Low Grade	6	7	1	46.2%	2	12		14.3%
High Grade	12	0		100.0%	10	1	1	90.9%
Symptomatic (Population C)								
	Xpert Bladder				UroVysion			
	POS	NEG	Invalid/Error**	Sensitivity	POS	NEG	inconclusive	Sensitivity
Grade:								
All	26	8	1	76.5%	17	14	4	54.8%
Low Grade	7	7	1	50.0%	4	8	3	33.3%
High Grade	19	1		95.0%	13	6	1	68.4%

[00224] As shown in Table 9, for those samples that have been tested with both GeneXpert® bladder cancer assay and UroVysion®, the GeneXpert® bladder cancer assay showed greater sensitivity than UroVysion® for detecting low grade and high grade cancer in both patient groups.

[00225] Next, the data set was divided into samples that had been archived, meaning they were tested more than one week after collection (the samples ranged from 8 days old up to nine months old), and samples that were fresh, meaning they were tested within one week of collection. The results of that analysis are shown in Table 10.

Table 10: Assay sensitivity in archived and fresh samples

	Archived Samples				
	Xpert Bladder				
	n	POS	NEG	Invalid/Error**	Sensitivity
Grade:					
All	89	61	25	3	70.9%
Low Grade	46	23	21	2	52.3%
High Grade	43	38	4	1	90.5%
	Fresh Samples				
	Xpert Bladder				
	n	POS	NEG	Invalid/Error**	Sensitivity
Grade:					
All	43	33	10		76.7%
Low Grade	17	10	7		58.8%
High Grade	26	23	3		88.5%

** Two invalid results were due to low ABL Ct, suggesting a low number of cells in the sample, and one of the invalid results was due to poor sample quality.

[00226] As shown in Table 10, the GeneXpert® bladder cancer assay had a similar sensitivity for detecting low grade and high grade bladder cancer in fresh and archived samples.

5.3. Example 3: Assay specificity for detecting bladder cancer using marker panel KRT20, IGF2, CRH, and ANXA10 in a larger cohort

[00227] In addition to the samples from patients with positive cystoscopy results for bladder cancer, urine samples were collected at the seven sites from patients with negative cystoscopy results for bladder cancer, but who were being monitored for recurrence of bladder cancer, had received BCG within the three months prior to sample collection, and who appeared to be symptomatic for bladder cancer but had no prior history of bladder cancer. In addition, urine samples were collected from patients with urology referrals for other suspected conditions, such as kidney stones. Finally, urine samples were collected from healthy individuals. Urine samples were preserved and analyzed using the GeneXpert® bladder cancer assay as described in Example 2.

[00228] For the samples from patients with negative cystoscopy results for bladder cancer, the assay results were divided according to the three patient groups: (A) patients with a history of bladder cancer who were currently being monitored for recurrence of bladder cancer, (B) patients who had been treated with Bacillus Calmette-Guerin (BCG)

within the three months prior to sample collection, and (C) patients who were symptomatic for bladder cancer and had no prior history of bladder cancer. The results for those patient groups are shown in Table 11.

Table 11: Assay specificity in cystoscopy negative patients by population

Monitoring (Population A)			
Xpert Bladder			
POS	NEG	Invalid/Error	Specificity
55	156	17	73.9%
BCG Treated (Population B)			
Xpert Bladder			
POS	NEG	Invalid/Error	Specificity
1	6	0	85.7%
Symptomatic (Population C)			
Xpert Bladder			
POS	NEG	Invalid/Error	Specificity
16	86	10	84.3%

[00229] As shown in Table 11, the GeneXpert® bladder cancer assay had a specificity of 73.9%, 85.7%, and 84.3% for patient groups (A), (B), and (C), respectively.

[00230] Next, the specificity of the GeneXpert® bladder cancer assay in patients who were suspected of having other urological conditions, but not bladder cancer, was determined. Seventy patient samples were collected in this category. The results with the GeneXpert® bladder cancer assay were 14 positives, 50 negatives, and 6 invalid results. The specificity of the GeneXpert® bladder cancer assay for this patient population was therefore 78.1%.

[00231] Finally, the specificity of the GeneXpert® bladder cancer assay in healthy individuals was determined. Fifty-five samples were collected in this category. The results were 4 positives and 51 negatives, indicating a specificity of 92.7% for this subject category.

5.4. Example 4: Detection of bladder cancer in samples that were weakly positive or false negative using marker panel KRT20, IGF2, CRH, and ANXA10

[00232] A collection of samples, including many samples that were weakly positive or false negative from the cohorts above, were analyzed using two additional markers, androgen receptor (AR) and uroplakin 1B (UPK1B).

[00233] Two milliliters of preserved urine was loaded onto a GeneXpert® cartridge and the urine was run through the filter. Cells caught on the filter were removed

using a 3M guanidinium thiocyanate and 0.1% sarkosyl. RNA from the cells was purified using RNeasy mini kit (Qiagen). RNA samples were then analyzed on a SmartCycler® (Cepheid, Sunnyvale, CA). Each reaction contained 5 µl of purified RNA, 400 nM forward primer for ABL, 400 nM forward primer for each target RNA (AR and/or UPK1B), 400 nM reverse primer for ABL, 400 nM reverse primer for each target RNA, 200 nM ABL probe, and 300 nM probe for each target RNA, 50-90 mM KCl, 3-5 mM MgCl₂, 400-825 µM dNTPs, 20 mM Tris, pH 8.5, 0.01% sodium azide, and 0.125 units/µl of Rnase inhibitor. MMLV reverse transcriptase (0.375 units/µl) and AptaTaq (0.25 units/µl; Roche) were used for reverse transcription and amplification, respectively. The cycle used was 45°C for 600 seconds, 95°C for 120 seconds, and 45 cycles of: 95°C for five seconds, 60°C for 20 seconds, and 72°C for 20 seconds. Primer and probe sequences for detecting AR and UPK1B are shown in Table 12.

Table 12: Primers and probes

<u>Name</u>	<u>Sequence</u>	<u>SEQ ID NO</u>	<u>Amplicon length</u>	<u>Intron length</u>
UPK1B For	CGGACTGAGAATAATGATGCTGA	51	141	4658
UPK1B Rev	GATCAGTTCATAGCAGCCCTG	52		
UPK1B Probe	TCTCAACCTGGAGGCTTGTAAGCTAGG	53		
AR For	GGACTCCGTGCAGCCTATT	54	136	701
AR Rev	AGAAAGGATCTTGGGCACTTG	55		
AR Probe	TCAGTTCACCTTTTGACCTGCTAATCAAGTC	56		

[00234] If the ABL Ct was greater than 39, the sample was removed from the analysis. Delta Ct (Δ Ct) was calculated as Ct (ABL) – Ct (marker). The Δ Ct cutoff for AR was set as -4.5 and the Δ Ct cutoff for UPK1B was set at 1.5. A Δ Ct above the Δ Ct cutoff for any one of the markers was considered a positive result, indicative of the presence of bladder cancer. If no target was detected in the sample, the Ct was set at 0, and the Δ Ct was set at -20. The results of the experiment are shown in Table 13.

Table 13: Detection of bladder cancer using AR and UPK1B

	ABL	AR		UPK1B					
<u>Sample ID</u>	<u>Ct</u>	<u>Ct</u>	<u>ΔCt</u>	<u>Ct</u>	<u>ΔCt</u>	<u>Status</u>	<u>AR Result</u>	<u>UPK1B Result</u>	<u>Prior result</u>
14230	34.9	0	-20	35.4	-0.5	healthy	Neg	Neg	
14231	37.1	0	-20	0	-20	healthy	Neg	Neg	
14234	37.9	0	-20	0	-20	healthy	Neg	Neg	
14235	37.1	0	-20	0	-20	healthy	Neg	Neg	

14236	35.6	0	-20	0	-20	healthy	Neg	Neg	
14237	37.3	0	-20	0	-20	healthy	Neg	Neg	
14238	35.9	0	-20	0	-20	healthy	Neg	Neg	
165010	36.6	0	-20	0	-20	healthy	Neg	Neg	Neg
165017	38.1	0	-20	0	-20	healthy	Neg	Neg	Neg
165026	38.8			0	-20	healthy		Neg	Neg
165044	38.2			0	-20	healthy		Neg	Neg
165046	37.4	38.1	-0.7	0	-20	healthy	Pos	Neg	Neg
CPHD-021	36.6	0	-20	0	-20	healthy	Neg	Neg	Neg
CPHD-031	38.5	0	-20	0	-20	healthy	Neg	Neg	Neg
75171	34.4	36.3	-1.9	0	-20	SCI	Pos	Neg	
75178	35.8	40.5	-4.7	34.3	1.5	SCI	Neg	Neg	
75179	35.3	0	-20	34.3	1	SCI	Neg	Neg	
75194	36.7	0	-20	0	-20	SCI	Neg	Neg	
75210	37	37.2	-0.2	35.3	1.7	SCI	Pos	Pos	
75217	33.5	44.7	-11	27.7	5.8	SCI	Neg	Pos	
75225	34	0	-20	0	-20	SCI	Neg	Neg	
75230	34.1	0	-20	33.8	0.3	SCI, UTI	Neg	Neg	
67054	37.2	40.3	-3.1	35	2.2	HG	Pos	Pos	Pos
67111	34.1	0	-20	30.6	3.5	HG	Neg	Pos	Pos
75144	34.7	38.8	-4.1			HG	Pos		
75144	35.2			34.4	0.8	HG		Neg	
75151	31.6	33.6	-2			HG	Pos		
75151	31.8			26.4	5.4	HG		Pos	
75170	29.4	30.4	-1			HG	Pos		
75170	29.6			25	4.6	HG		Pos	
75203	32.1	35.9	-3.8			HG	Pos		
75203	32.4			27.1	5.3	HG		Pos	
75417	32.6	32.9	-0.3			HG	Pos		False Neg

75417	33.8			31.7	2.1	HG		Pos	False Neg
75462	36.9	0	-20			HG	Neg		Weak pos
75462	35.7			34	1.7	HG		Pos	Weak pos
75470	33.2	33.5	-0.3			HG	Pos		Weak pos
75470	34.5			30.5	4	HG		Pos	Weak pos
75540	32.5	32.7	-0.2	25.9	6.6	HG	Pos		Pos
165013	37.1	38.9	-1.8			HG	Pos		Weak pos
165013	38.7			36.5	2.2	HG		Pos	Weak pos
165099	37.5	0	-20			HG	Neg		False Neg
67010	34.1	0	-20			LG	Neg		False Neg
67010	34.5			0	-20	LG		Neg	False Neg
67011	35	39.8	-4.8			LG	Neg		Pos
67011	35.3			30.2	5.1	LG		Pos	
75089	37.1	0	-20			LG	Neg		
75089	36.2			30.5	5.7	LG		Pos	
75101	37.7	41.6	-3.9			LG	Pos		
75101	37.2			33	4.2	LG		Pos	
75245	32.6			29	3.6	LG		Pos	
75444	35.9	37	-1.1			LG	Pos		Weak pos
75444	37.3			33.6	3.7	LG		Pos	Weak pos
75475	33.9	34.4	-0.5	30.4	3.5	LG	Pos	Pos	False Neg
75483	34.6	34.5	0.1	31.5	3.1	LG	Pos	Pos	False Neg
75485	34.3	34.6	-0.3	30.5	3.8	LG	Pos	Pos	False Neg
75519	35.1	35.1	0	31.8	3.3	LG	Pos	Pos	False Neg
75539	37.6	36.8	0.8	31.2	6.4	LG	Pos	Pos	False Neg
165049	37.9	0	-20	0	-20	LG	Neg	Neg	False Neg
165062	38.7			0	-20	LG		Neg	False Neg
165098	37.8	0	-20	0	-20	LG	Neg	Neg	False Neg

SCI = spinal cord injury

UTI = urinary tract infection

HG = high-grade bladder cancer

LG = low grade bladder cancer

[00235] Using either AR or UPK1B as a bladder cancer marker, at least six samples were identified as positive for bladder cancer that had been previously been identified as false negatives using the four marker panel described above.

[00236] The sensitivity and specificity for detecting bladder cancer using AR or UPK1B is shown in Table 14.

Table 14: Sensitivity and specificity

AR		UPK1B	
<u>Sensitivity</u>	<u>Specificity</u>	<u>Sensitivity</u>	<u>Specificity</u>
n = 24 bladder cancer	n = 20 healthy	n = 25 bladder cancer	n = 22 healthy
67%	85%	80%	90.9%

[00237] Twenty-three high grade and low grade bladder cancer samples were tested with both AR and UPK1B. The sensitivity of the combination of AR and UPK1B (considering both single positives and double positives as positive results) for the tested samples is 87%.

[00238] Some of the samples had lower ABL levels, indicating that there may be a low number of cells in the sample. In samples with an ABL Ct of less than 36, the sensitivity of both markers increases, as shown in Table 15.

Table 15: Sensitivity and specificity in samples with ABL Ct < 36

AR		UPK1B	
<u>Sensitivity</u>	<u>Specificity</u>	<u>Sensitivity</u>	<u>Specificity</u>
n = 15 bladder cancer	n = 9 healthy	n = 16 bladder cancer	n = 9 healthy
80%	88.9%	87.5%	88.9%

5.5. Example 5: Detection of bladder cancer using additional markers

[00239] To determine the combination of markers that would give the best sensitivity for bladder cancer while keeping the specificity with normal samples as close to 95% as reasonable, the Xpert Bladder Assay was adapted to evaluate the expression levels of additional markers. A new reagent bead containing primers and probes for AR, ABL, PIK3CA, UPK1B, UPK2 and MGEA5 was formulated and cartridges were assembled so that each urine sample could be tested in each of the two assays and expression information on a total of 10 markers was collected.

[00240] The primers and probe sequences are shown in Table 16.

Table 16: Primer and probe sequences

oligo name	target	sequence	Final conc. in assay	SEQ ID NO
ABLa3a4 PrmrFwd4	ABL	GATCAACACTGCTTCTGATGGCAA	400 nM	8
ABLa3a4 PrmrRev1	ABL	CCACCGTTGAATGATGATGAACCAA	400 nM	9
ABL_F5	ABL	F5-CCTCCGAGAGCCGCTTCAAC-Q5	200 nM	10
MGEA5_For2	MGEA5	CATCTATGCGATCTCACCTGGA	400 nM	62
MGEA5_Rev2	MGEA5	CACCCAAACTGAGAAACCTGG	400 nM	63
MGEA5_Probe2_F4	MGEA5	F4-TCTAACCCCAAGGAAGTATCCACATTGA-Q4	200 nM	64
PIK3A_For	PIK3A	GTCTCTGCAAAAAGGCCACTG	400 nM	65
PIK3A_Rev	PIK3A	CTTGCCGTAAATCATCCCCAT	400 nM	66
PIK3A_Probe_F3	PIK3A	F3-AGAACCAGACATCATGTCAGAGTTACTGT-Q3	60 nM	67
AR_For4	AR	TCGAATGAACTACATCAAGGAACT	400 nM	68
AR_Rev3	AR	GCAGCTCTCTCGCAATAGG	400 nM	69
AR_Probe4_F6	AR	F6-TGCTCAAGACGCTTCTACCAGCT-Q6	400 nM	70
UPK1B_For	UPK1B	CGGACTGAGAATAATGATGCTGA	400 nM	71
UPK1B_Rev	UPK1B	GATCAGTTTCATAGCAGCCCTG	400 nM	72
UPK1B_F1(3)	UPK1B	F1-TCTCAACCTGGAGGCTTGTAAGTAGG-Q1	400 nM	73
UPK2_For2	UPK2	CAATGTCCACACTCCCTCGAAG	400 nM	74
UPK2_Rev2	UPK2	AGGAACATGGCGACAGAGAG	400 nM	75
UPK2_Probe2_F2	UPK2	F2-TGGGTATGGCCCGCACAGG-Q2	300 nM	76

F1 through F6 are detectably different dyes that can be detected and distinguished simultaneously in a multiplex reaction, and Q1 to Q6 are quenchers (in the present example, Q3 and Q4 are the same quencher and Q4 and Q5 are the same quencher).

[00241] Each reaction contained 50-90 mM KCl, 3-5 mM MgCl₂, 400-825 μM dNTPs, 20 mM Tris, pH 8.5, 0.01% sodium azide, and 0.9 units/μl of Rnase inhibitor. MMLV reverse transcriptase (0.375 units/μl) and AptaTaq (0.25 units/μl; Roche) were used for reverse transcription and amplification, respectively.

[00242] For each sample to be tested, 4.5 mL of voided urine was added to 4.5 mL preservative (3.5M guanidine HCl, 1% N-acetyl-L-cysteine, 25 mM sodium citrate, and 2.5% Tween-20, pH 3.2), preferably within 1 hour of sample collection. The container was inverted three times to mix. The preserved samples were transported on ice and stored at 2-8°C. Clinical information for each sample was provided by the collection sites.

[00243] A summary of the samples tested is shown in Table 17.

Table 17: Summary of samples

Population	Patient Status	Number of samples
Detection	Biopsy Positive High Grade	25

	Biopsy Positive Low Grade	9
	Biopsy Positive CIS	2
	Cystoscopy Negative	57
	Erythematous patch (included in negative cysto group)	3
Monitoring	Biopsy Positive High Grade	30
	Biopsy Positive Low Grade	18
	Biopsy Positive CIS	7
	Cystoscopy Negative	47
	Erythematous patch (included in negative cysto group)	3
Normal	Normal	45
	UTI (included in normal group)	2

Detection population = subjects with hematuria and without a history of bladder cancer

Monitoring population = subjects with a history of bladder cancer

Normal = subjects without hematuria and without a history of bladder cancer

[00244] Prior to use, the preserved urine was inverted three times to mix. 4 mL of preserved urine was loaded into a GeneXpert cartridge for analysis. The cartridge contained a 0.8 µm filter to capture urothelial cells. The captured cells were washed and lysed using sonication (2-15 seconds, 8-16 µm at 36 kHz) within the cartridge. The lysate was then used to reconstitute the reagents used for real-time RT-PCR (described above). The reaction cycle used was: 10 minutes at 45°C, followed by 2 minutes at 95°C, and then 45 cycles of (a) 5 seconds at 95°C, 20 seconds at 60°C, and 20 seconds at 72°C, using a GeneXpert® cartridge in a GeneXpert® system. Delta Ct (ΔCt) was calculated as Ct (ABL) – Ct (marker). The ΔCt cutoff was set as the ΔCt that gave at least 95% specificity with samples from patients not expected to have bladder cancer. A ΔCt above the ΔCt cutoff for any one of the markers was considered a positive result, indicative of the presence of bladder cancer.

[00245] ABL Ct > 36 in the four marker assay shown in Table 20 were excluded from the study. ABL Ct > 37 in the five marker assay shown in Table 21 and in the four marker assay shown in Table 22 were excluded from the study. The cutoff values for positivity were chosen to allow about 95% specificity with the normal samples. For MGEA5 a positive call is a target delta Ct value that is less than the cutoff as this marker was found to be down regulated in urine specimens from patients with bladder cancer.

[00246] UPK2 was found to have a similar expression pattern as ABL, indicating that it could be used as an endogenous control in place of ABL (data not shown). Since UPK2 is primarily urothelial cell specific, samples with few or no urothelial cells should have low levels of UPK2 RNA, so it should be a useful sample adequacy control.

[00247] Table 18 summarizes the sensitivity and specificity for each of the nine markers and Table 19 indicates how many samples were positive by one marker only.

Table 18: Sensitivity and specificity by individual marker

Patient Status	N=	AR	PIK3CA	UPK1B	UPK2	MGEA5	ANXA10	KRT20	CRH	IGF2
Cysto Neg Specificity	110	109 (99%)	109 (99%)	102 (93%)	105 (95%)	94 (85%)	103 (94%)	99 (90%)	85 (77%)	102 (93%)
High Grade Sensitivity	55	7 (13%)	0	17 (31%)	1 (2%)	10 (18%)	17 (31%)	27 (49%)	43 (78%)	30 (55%)
Low Grade Sensitivity	27	8 (30%)	0	10 (37%)	2 (7%)	5 (19%)	6 (22%)	8 (30%)	12 (44%)	12 (44%)
CIS Sensitivity	9	3 (33%)	0	3 (33%)	0	2 (22%)	1 (11%)	2 (22%)	8 (89%)	2 (22%)
Normal Specificity	47	46 (98%)	45 (96%)	46 (98%)	46 (98%)	43 (92%)	46 (98%)	46 (98%)	46 (98%)	47 (100%)

Table 19: Number of samples positive by one marker only

Patient Status	N=	AR	PIK3CA	UPK1B	UPK2	MGEA5	ANXA10	KRT20	CRH	IGF2
High Grade Sensitivity	55	0	0	0	0	0	0	0	1 (2%)	3 (5%)
Low Grade Sensitivity	27	0	0	0	0	2 (7%)	1 (4%)	0	1 (4%)	0
CIS Sensitivity	9	0	0	0	0	0	0	0	2 (22%)	0

[00248] Table 20 summarizes the sensitivities and specificities for a four-marker combination assay using KRT20, ANXA10, CRH, and IGF2. Table 21 summarizes the sensitivities and specificities for a five-marker combination assay using KRT20, ANXA10, CRH, IGF2, and UPK1B. Table 22 summarizes the sensitivities and specificities for a four-marker combination assay using UPK1B, ANXA10, CRH, and IGF2.

Table 20: Sensitivity and specificity of 4 marker combination of KRT20, ANXA10, CRH and IGF2

		By this marker Alone in 4 Marker Combo				
Patient Status	N=	KRT20	ANXA10	CRH	IGF2	4 Marker Combo
Cysto Neg Specificity	110	105 (95%)	106 (96%)	93 (85%)	109 (99%)	74 (67%)
High Grade Sensitivity	55	0	0	7 (13%)	4 (7%)	51 (93%)
Low Grade Sensitivity	27	0	1 (4%)	4 (15%)	1 (4%)	19 (70%)
CIS Sensitivity	9	0	0	5 (56%)	0	8 (89%)
Normal Specificity	47	46 (98%)	46 (98%)	46 (98%)	47 (100%)	44 (94%)

Table 21: Sensitivity and specificity of 5 marker combination of KRT20, ANXA10, CRH, IGF2 and UPK1B

		By this marker Alone in 5 Marker Combo					
Patient Status	N=	KRT20	UPK1B	ANXA10	CRH	IGF2	5 Marker Combo
Cysto Neg Specificity	110	106 (96%)	108 (98%)	106 (96%)	94 (85%)	109 (99%)	72 (65%)
High Grade Sensitivity	55	0	0	0	4 (7%)	4 (7%)	51 (93%)
Low Grade Sensitivity	27	0	3 (11%)	1 (4%)	2 (7%)	0	22 (81%)
CIS Sensitivity	9	0	1 (11%)	0	4 (44%)	0	9 (100%)
Normal Specificity	47	46 (98%)	46 (98%)	46 (98%)	46 (98%)	47 (100%)	44 (94%)

Table 22: Sensitivity and specificity 4 marker combination of ANXA10, CRH, IGF2 and UPK1B

		By this marker Alone in 4 Marker Combo				
Patient Status	N=	UPK1B	ANXA10	CRH	IGF2	4 Marker Combo
Cysto Neg Specificity	110	107 (97%)	107 (97%)	95 (86%)	109 (99%)	76 (69%)
High Grade Sensitivity	55	0	0	8 (15%)	4 (7%)	51 (93%)
Low Grade Sensitivity	27	3 (11%)	1 (4%)	4 (15%)	1 (4%)	22 (81%)
CIS Sensitivity	9	1 (11%)	0	4 (44%)	0	9 (100%)
Normal Specificity	47	46 (98%)	46 (98%)	46 (98%)	47 (100%)	44 (94%)

[00249] The addition of UPK1B, or replacing KRT20 with UPK1B, improved the assay sensitivity for low grade and CIS. Replacing KRT20 with UPK1B also improved the specificity with patients with negative cystoscopies from 67% to 69% compared to the 4-marker combination assay with KRT20.

[00250] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

[00251] While various specific embodiments have been illustrated and described, it will be appreciated that changes can be made without departing from the spirit and scope of the invention(s).

TABLE OF CERTAIN SEQUENCES

SEQ ID NO	Description	Sequence							
1	Human CRH mRNA	TCGTTCCCTTG	GCAGGGCCCT	ATGATTTATG	CAGGAGCAGA	GGCAGCACGC	AATCGAGCTG		
		TCAAGAGAGC	GTCAGCTTAT	TAGGCAAAATG	CTGCGTGGTT	TTTGAAGAGG	GTCGACACTA		
		TAAAAATCCCA	CTCCAGGCTC	TGGAGTGGAG	AAACTCAGAG	ACCAAGTCCA	TTGAGAGACT		
		GAGGGGAAAG	AGAGGAGAGA	AAGAAAAAGA	GAGTGGGAAC	AGTAAAGAGA	AAGGAAGACA		
		ACCTCCAGAG	AAAGCCCCCG	GAGACGTCTC	TCTGCAGAGA	GGCGGCAGCA	CCCGGCTCAC		
		CTGCGAAGCG	CCTGGGAAGC	GAGTGCCCCC	AACATGCGGC	TGCCGCTGCT	TGTGTCCGCG		
		GGAGTCCTGC	TGGTGGCTCT	CCTGCCCTGC	CCGCCATGCA	GGGCGCTCCT	GAGCCGCGGG		
		CCGGTCCCGG	GAGCTCGGCA	GGCGCCGCAG	CACCCCTCAGC	CCTTGGATTT	CTTCCAGCCG		
		CCGCCGCAGT	CCGAGCAGCC	CCAGCAGCCG	CAGGCTCGGC	CGGTCTCTGT	CCGCATGGGA		
		GAGGAGTACT	TCCTCCGCCT	GGGGAACCTC	AACAAGAGCC	CGGCCGCTCC	CCTTTCGCCC		
		GCCTCCTCGC	TCCTCGCCGG	AGGCAGCGGC	AGCCGCCCTT	CGCCGGAACA	GGCGACCGCC		
		AACTTTTTCC	GCGTGTGTGT	GCAGCAGCTG	CTGCTGGCTC	GGCGCTCGCT	CGACAGCCCC		
		GCGGCTCTCG	CGGAGCGCGG	CGCTAGGAAT	GCCCTCGGCG	GCCACCAGGA	GGCACC GGAG		
		AGAGAAAGGC	GGTCCGAGGA	GCCTCCCATC	TCCCTGGATC	TCACCTTCCA	CCTCCTCCGG		
		GAAGTCTTGG	AAATGGCCAG	GGCCGAGCAG	TTAGCACAGC	AAGCTCACAG	CAACAGGAAA		
		CTCATGGAGA	TTATTGGGAA	ATAAAACGGT	GCGTTTGGCC	AAAAAGAATC	TGCATTTAGC		
		ACAAAAAATA	TTTAAAAATA	TACAGTATTC	TGTACCATAG	CGCTGCTCTT	ATGCCATTTG		
		TTTATTTTAA	TATAGCTTGA	AACATAGAGG	GAGAGAGGGA	GAGAGCCTAT	ACCCCTTACT		
		TAGCATGCAC	AAAGTGTATT	CACGTGCAGC	AGCAACACAA	TGTTATTCTG	TTTGTCTACG		
		TTTAGTTTCC	GTTTCCAGGT	GTTTATAGTG	GTGTTTAA	GAGAATGTAG	ACCTGTGAGA		
		AAACGTTTTG	TTTGAAAAAG	CAGACAGAAG	TCACTCAATT	GTTTTTGTG	TGGTCTGAGC		
		CAAAGAGAAAT	GCCATTCTCT	TGGGTGGGTA	AGACTAAATC	TGTAAGCTCT	TTGAAACAAC		
		TTTCTCTTGT	AAACGTTTCA	GTAATAAAAC	ATCTTTCCAG	TCCTTGGTCA	GTTTGGTTGT		
		GTAAGAGAAAT	GTTGAATACT	TATATTTTTA	ATAAAAGTTG	CAAAGGTAAT	CATG		
2	Human IGF2 mRNA, transcript variant 2	CCGCTAATGT	ACCATGCCCT	GGTGCTGGAA	AGTGCCTGAG	CCAGCTGCCC	CAGCGGCCTC		
		AGCACTACCA	AGTTGGCACA	AAGCTCCCCA	AATTCGGAGG	GGCTCAGGGA	AACGAGTGGA		
		GGGGATGAGG	AGGTGAGGGG	TAAACCCATC	ATTTCACTTG	GCATTTGAGC	AGGTGCCATG		
		CTCAGCGGAG	ATGAGGCTCT	CCCATCTGTA	GGGGCCGTAT	TAACATGCAC	ACTCTAAAAG		
		TGCCCTTCGT	TTCTCCAGCC	TCAGCTTTGT	CCCTCTCCTC	CTCCACGTCA	ACCTGGCCAG		
		AGGGTCTGGA	CGCCACAGCC	AGGGCACCCC	CTGCTTTGGT	GGTGACTGCT			
		AATATTGGCC	AGGCCGGCGG	ATCATCGTCC	AGGCAGTTTC	GGCAGAGAGC	CTTGGGCACC		
		AGTGACTCCC	CGGTCCCTCT	TATCCACTGT	CCAGGAGCTG	CGGGGACTGC	GCAGGGACTA		
		GAGTACAGGG	GCCGAAGAGT	CACCACCGAG	CTTGTGTGGG	AGGAGGTGGA	TTCCAGCCCC		
		CAGCCCCAGG	GCTCTGAATC	GCTGCCAGCT	CAGCCCCCTG	CCCAGCCTGC	CCCACAGCCT		
		GAGCCCCAGC	AGGCCAGAGA	GCCCAGTCCT	GAGGTGAGCT	GCTGTGGCCT	GTGGCCAGG		
		CGACCCCAGC	GCTCCAGAA	CTGAGGCTGG	CAGCCAGCCC	CAGCCTCAGC	CCCAACTCGG		
		AGGCAGAGAG	ACACCAATGG	GAATCCCAAT	GGGGAAGTCG	ATGCTGGTGC	TTCTCACCTT		
		CTTGGCCTTC	GCCTCGTGCT	GCATTGCTGC	TTACCGCCCC	AGTGAGACCC	TGTGCGGCGG		
		GGAGCTGGTG	GACACCCTCC	AGTTCGTCTG	TGGGGACCGC	GGCTTCTACT	TCAGCAGGCC		
		CGCAAGCCGT	GTGAGCCGTC	GCAGCCGTGG	CATCGTTGAG	GAGTGCTGTT	TCCGCAGCTG		
		TGACCTGGCC	CTCCTGGAGA	CGTACTGTGC	TACCCCGCCG	AAGTCCGAGA	GGGACGTGTC		
		GACCCCTCCG	ACCGTGCTTC	CGGACAACCT	CCCCAGATA	CCCCGAGGCA			
		AGTTCTTCCA	ATATGACACC	TGGAAGCAGT	CCACCCAGCG	CCTGCGCAGG	GGCCTGCCTG		
		CCCTCCTGCG	TGCCCGCCGG	GGTCACGTGC	TCGCCAAGGA	GCTCGAGGCG	TTCAGGGAGG		
		CCAAACGTCA	CCGTCCCTTG	ATTGCTCTAC	CCACCCAAGA	CCCCGCCAC	GGGGGCGCCC		
		CCCCAGAGAT	GGCCAGCAAT	CGGAAGTGAG	CAAACTGCC	GCAAGTCTGC	AGCCCGCGCG		
		CACCATCCTG	CAGCCTCCTC	CTGACCACGG	ACGTTTCCAT	CAGGTTCCAT	CCCGAAAATC		
		TCTCGGTTCC	ACGTCCCCCT	GGGGCTTCTC	CTGACCCAGT	CCCCGTGCCC	CGCCTCCCCG		
		AAACAGGCTA	CTCTCCTCGG	CCCCCTCCAT	CGGGCTGAGG	AAGCACAGCA	GCATCTTCAA		
		ACATGTACAA	AATCGATTGG	CTTTAAACAC	CCTTCACATA	CCCTCCCCCC	AAATTATCCC		
		CAATTATCCC	CACACATAAA	AAATCAAAAC	ATTAACTAA	CCCCCTTCCC	CCCCCCCCAC		
		AACAACCCTC	TTAAAACTAA	TTGGCTTTTT	AGAAACACCC	CACAAAAGCT	CAGAAATTGG		
		CTTTAAAAAA	AACAACCACC	AAAAAAAATC	AATTGGCTAA	AAAAAAAAG	TATTA AAAAC		
		GAATTGGCTG	AGAAACAATT	GGCAAAATAA	AGGAATTTGG	CACTCCCCAC	CCCCCTCTTT		
		CTCTTCTCCC	TTGGACTTTG	AGTCAAATTG	GCCTGGACTT	GAGTCCCTGA	ACCAGCAAAG		
		AGAAAAGAAG	GACCCAGAA	ATCACAGGTG	GGCACGTGCG	TGCTACCGCC	ATCTCCCTTC		
		TCACGGGAAT	TTTCAGGGTA	AACTGGCCAT	CCGAAAATAG	CAACAACCCA	GACTGGCTCC		
		TCACTCCCTT	TTCCATCACT	AAAAATCACA	GAGCAGTCAG	AGGGACCCAG	TAAGACCAAA		
		GGAGGGGAGG	ACAGAGCATG	AAAACCAAAA	TCCATGCAAA	TGAAATGTAA	TTGGCACGAC		
		CCTCACCCCC	AAATCTTACA	TCTCAATTCC	CATCTAAAA	AGCACTCATA	CTTTATGCAT		
		CCCCGCAGCT	ACACACACAC	AACACACAGC	ACACGCATGA	ACACAGCACA	CACACGAGCA		
		CAGCACACAC	ACAAACGCAC	AGCACACACA	GCACACAGAT	GAGCACACAG	CACACACACA		
		AACGCACAGC	ACACACACGC	ACACACATGC	ACACACAGCA	CACAAACGCA	CGGCACACAC		

		ACGCACACAC ATGCACACAC AGCACACACA CAAACGCACA GCACACACAA ACGCACAGCA CACACGCACA CACAGCACAC ACACGAGCAC ACAGCACACA AACGCACAGC ACACGCACAC ACATGCACAC ACAGCACACA CACTAGCACACA CAGCACACAC ACAAAGACAC AGGCACACACA TGCACACACA GCACACACAC GCGAACACAG CACACACGAA CACAGCACAC ACAGCACACA CACAAACACA GCACACACAT GCACACAGCA CACGCACACA CAGCACACAC ATGAACACAG CACACAGCAC ACACATGCAC ACACAGCACACA CACGCATGCA CAGCACACAT GAACACAGCA CACACACAAA CACACAGCAC ACACATGCAC ACACAGCACACA CACACTCATG CGCAGCACAT ACATGAACAC AGCTCACAGC ACACAAACAC GCAGCACACA CGTTGCACAC GCAAGCACCC ACCTGCACAC ACACATGCGC ACACACACGC ACACCCCCAC AAAATTGGAT GAAAAAATA AGCATATCTA AGCAACTACG ATATCTGTAT GGATCAGGCC AAAGTCCCGC TAAGATTCTC CAATGTTTTT ATGGTCTGAG CCCGCTCCT GTTCCCATCT CCACTGCCCC TCGGCCCTGT CTGTGCCCTG CCTCTCAGAG GAGGGGGCTC AGATGGTGC GCGTGGTGT GCGGCCGGCG GCATTGGGA TACACCCGTA GGGTGGGCGG GGTGTGTCCC AGGCCTAATT CCATCTTTCC ACCATGACAG AGATGCCCTT GTGAGGCTGG CCTCCTTGGC GCGTGTCCCC ACGGCCCCCG CAGCGTGAGC CACGATGCTC CCCATACCCC ACCCATTTCC GATACACCTT ACTTACTGTG TGTGGCCCCA GCCAGAGTGA GGAAGGAGTT TGGCCACATT GGAGATGGCG GTAGCTGAGC AGACATGCCC CCACGAGTAG CCTGACTCCC TGGTGTGCTC TGGAAGGAA GATCTTGGG ACCCCCCAG CGGAGGCACAC CTAGGGATCA TCTTTGCCCG TCTCCTGGGG ACCCCCCAAG AAATGTGGAG TCCTCGGGGG CCGTGCCTG ATGCGGGGAG TGTGGGAAGT CTGGCGGTTG GAGGGGTGGG TGGGGGGCAG TGGGGGCTGG GCGGGGGGAG TTCTGGGGTA GGAAGTGGTC CCGGGAGATT TTGGATGGAA AAGTCAGGAG GATTGACAGC AGACTGACG AATTACATAG AGAAATTAGG AACCCCCAAA TTTCATGTCA ATTGATCTAT TCCCCCTCTT TGTTCCTTGG GGCATTTTTC CTTTTTTTTT TTTTTTTTGT TTTTTTTTAC CCCTCCTTAG CTTTATGCGC TCAGAAACCA AATTAAACCC CCCCCCATG TAACAGGGGG GCAGTGACAA AAGCAAGAAC GCACGAAGCC AGCCTGGAGA CCACCACGTC CTGCCCCCGC CCATTTATCG CCCTGATTGG ATTTTGTGTT TCATCTGTCC CTGTTGCTTG GGTGAGTTG AGGGTGGAGC CTCCTGGGGG GCACTGGCCA CTGAGCCCCC TTGGAGAAGT CAGAGGGGAG TGGAGAAGGC CACTGTCCGG CCTGGCTTCT GGGGACAGTG GCTGGTCCCC AGAAGTCCCT AGGGCGGAGG GGGGGGTTGG GCAGGGTCTC CTCAGGTGTC AGGAGGGTGC TCGGAGGCCA CAGGAGGGGG CTCCTGGCTG GCCTGAGGCT GGCCGGAGGG GAAGGGGCTA GCAGGTGTGT AAACAGAGGG TTCCATCAGG CTGGGGCAGG GTGGCCGCTT TCCGCACACT TGAGGAACCC TCCCCTCTCC CTCGGTGACA TCTTGCCCGC CCCTCAGCAC CCTGCCTTGT CTCCAGGAGG TCCGAAGCTC TGTGGGACCT CTTGGGGGCA AGGTGGGGTG AGGCCGGGGA GTAGGGAGGT CAGGCGGGTC TGAGCCACA GAGCAGGAGA GCTGCCAGGT CTGCCCATCG ACCAGGTTGC TTGGGCCCCG GAGCCACGG GTCTGGTGAT GCCATAGCAG CCACCACCGC GCGCGCTAGG GCTGCGGCAG GGAATCGGCC TCTGGGAGGT TTACCTCGCC CCCACTTGTG CCCCCAGCTC AGCCCCCTG CACGCAGCCC GACTAGCAGT CTAGAGGCTT GAGGCTTCTG GGTCTTGGTG ACGGGGCTGG CATGACCCCG GGGGTCGTCC ATGCCAGTCC GCCTCAGTCG CAGAGGGTCC CTCGGCAAGC GCCCTGTGAG TGGGCCATTG GGAACATTGG ACAGAAGCCC AAAGAGCCAA ATTGTACAA TTGTGGAACC CACATTGGCC TGAGATCCAA AACGCTTCGA GGCACCCCAA ATTACCTGCC CATTCGTGAG GACACCCACC CACCCAGTGT TATATTCTGC CTCGCGGAG TGGGTGTTCC CGGGGGCACT TGCCGACCAG CCCCTTGCCT CCCAGGTTT GCAGCTCTCC COTGGGCCAC TAACCATCCT GGCCCGGGCT GCCTGTCTGA CCTCCGTGCC TAGTCGTGGC TCTCCATCTT GTCTCCTCCC CGTGTCCCCA ATGTCTTCAG TGGGGGGCCC CCTCTTGGGT CCCCTCCTCT GCCATCACCT GAAGACCCCC ACGCCAAACA CTGAATGTCA CCTGTGCCTG CCGCCTCGGT CCACCTTGCG GCCCGTGTGTT GACTCAACTC AACTCCTTTA ACGCTAATAT TTCCGGCAAA ATCCCATGCT TGGGTTTTGT CTTTAACCTT GTAACGCTTG CAATCCCAAT AAAGCATTAA AAGTCATGAA AAAAAAAAAA AAAAAA
3	Human IGF2 mRNA, transcript variant 1	CGCCTGTCCC CCTCCCGAGG CCGGGGCTCG CGACGGCAGA GGGCTCCGTC GGCCCAAACC GAGCTGGGCG CCCGCGGTCC GGGTGCAGCC TCCACTCCGC CCCCCAGTCA CCGCTCCTCC CGGCCCCCTCG ACGTGGCGCC CTTCCCTCCG CTTCTCTGTG CTCCCCGCGC CCTCTTGGC GTCTGGCCCC GGGCCCCGCT CTTTCTCCCG CAACCTTCCC TTCGCTCCCT CCCGCCCCC CCAGCTCCTA GCCTCCGACT CCTCCCCCCC CTCACGCCCC CCTCTCGCC TTCGCCAAC CAAAGTGGAT TAATTACAG CTTTCTGTTT CTCTCCGTGC TGTCTCTCC CGCTGTGCGC CTGCCCGCCT CTCGCTGTCC TCTCTCCCC TCGCCCTCTC TCGGGCCCC CCTTTACAG TTCACTCTGT CTCTCCCACT ATCTCTGCCC CCTCTATCC TTGATAACA AGCTGACCTC ATTTCCCGAT ACCTTTTCCC CCCCAGAAAAG TACAACATCT GGCCCGCCCC AGCCGAAGA CAGCCCGTCC TCCCTGGACA ATCAGACGAA TTCTCCCCC CCCCCAAAA AAAAGCCATC CCCCCGCTCT GCCCCGTGCG ACATTCGGCC CCCGCGACTC GGCCAGAGCG GCGCTGGCAG AGGAGTGTCC GGCAGGAGGG CCAACGCCCC CTGTTGCGGT TCGGACACGC AGCAGGGAGG TGGGCGGCAG CGTCGCGGGC TTCCAGACAC CAATGGGAAT CCCAATGGGG AAGTCGATGC TGGTGTCTCT CACCTTCTTG GCCTTCGCCT CGTGTGTCAT TGTGCTTAC CGCCCAAGTG AGACCCTGTG CGGCGGGGAG CTGGTGGACA CCCTCCAGTT CGTCTGTGGG GACCGCGGCT TCTACTTCAG CAGGCCCGCA AGCCGTGTGA GCCGTGCGAG CCGTGGCATC GTTGAGGAGT GCTGTTTCCG CAGCTGTGAC CTGGCCCTCC TGGAGACGTA CTGTGCTACC CCGCCCAAGT CCGAGAGGGA CGTGTGACAC CCTCCGACCG TGCTTCCGGA CAACTTCCCC AGATAACCCG TGGGCAAGTT CTTCCAATAT GACACCTGGA AGCAGTCCAC CCAGCGCCTG CGCAGGGGCC TGCTGTCCCT CCTGCGTGCC CGCCGGGGTC ACGTGCTCGC CAAGGAGCTC GAGGCGTTCA

		GGGAGGCCAA ACGTCACCGT CCCCTGATTG CTCTACCCAC CCAAGACCCC GCCCACGGGG GCGCCCCCCC AGAGATGGCC AGCAATCGGA AGTGAGCAAA ACTGCCGCAA GTCTGCAGCC CGGCGCCACC ATCCTGCAGC CTCCTCCTGA CCACGGACGT TTCCATCAGG TTCCATCCCG AAAATCTCTC GGTTCACGT CCCCTGGGG CTTCTCCTGA CCCAGTCCCC GTGCCCCGCC TCCCCGAAAC AGGCTACTCT CCTCGGCCCC CTCCATCGGG CTGAGGAAGC ACAGCAGCAT CTTCAAACAT GTACAAAATC GATTGGCTTT AAACACCCCTT CACATACCCCT CCCCCAAAT TATCCCCAAT TATCCCCACA CATAAAAAAT CAAAACATTA AACTAACCCC CTCCCCCCC CCCCACAACA ACCCTCTTAA AACTAATTGG CTTTTTAGAA ACACCCACA AAAGCTCAGA AATTGGCTTT AAAAAAACA ACCACCAAAA AAAATCAATT GGCTAAAAAA AAAAAGTATT AAAAACGAAT TGGCTGAGAA ACAATTGGCA AAATAAAGGA ATTTGGCACT CCCCACCCCC CTCTTTCTCT TCTCCCTTGG ACTTTGAGTC AAATTGGCCT GGACTTGAGT CCCTGAACCA GCAAAGAGAA AAGAAGGACC CCAGAAATCA CAGGTGGGCA CGTCGTGCT ACCGCCATCT CCCTTCTCAC GGGAAATTTT AGGGTAAACT GGCCATCCGA AAATAGCAAC AACCCAGACT GGCTCCTCAC TCCCTTTTCC ATCACTAAAA ATCACAGAGC AGTCAGAGGG ACCCAGTAAG ACCAAAGGAG GGGAGGACAG AGCATGAAAA CCAAATCCCA TGCAAATGAA ATGTAATTGG CACGACCCCT ACCCCCAAAT CTTACATCTC AATTCCCATC TAAAAAGCA CTCATACTTT ATGCATCCCC GCAGCTACAC ACACACAACA CACAGCACAC GCATGAACAC AGCACACACA CGAGCACAGC ACACACAAA ACGCACAGCA CACACAGCAC ACAGATGAGC ACACAGACACA CACACAAACG CACAGCACAC ACACGCACAC ACATGCACAC ACAGCACACA AACGCACGGC ACACACACGC ACACACATGC ACACACAGCA CACACACAAA CGCACAGCAC ACACAAACGC ACAGCACACA CGCACACAGA GCACACACAC GAGCACACAG CACACAAACG CACAGCACAC GCACACACAT GCACACACAG CACACACACT AGCACACAGC ACACACACA AGACACAGCA CACACATGCA CACACAGCAC ACACACGCGA ACACAGCACA CACGAACAA GCACACACAG CACACACACA AACACAGCAC ACACATGCAC ACAGCACACG CACACACAGC ACACACATGA ACACAGCACA CAGCACACAC ATGCACACAC AGCACACACG CATGCACAGC ACACATGAAC ACAGCACACA CACAAACACA CAGCACACAC ATGCACACAC AGCACACACA CTCATGCGCA GCACATACAT GAACACAGCT CACAGCACAC AAACACGCAG CACACACGTT GCACACGCAA GCACCCACCT GCACACACAC ATGCGCACAC ACACGCACAC CCCCACAAA TTGGATGAAA ACAATAAGCA TATCTAAGCA ACTACGATAT CTGTATGGAT CAGGCCAAAG TCCCGCTAAG ATTCTCCAAT GTTTTCATGG TCTGAGCCCC GCTCCTGTTC CCATCTCCAC TGCCCTCGG CCCTGTCTGT GCCCTGCCTC TCAGAGGAGG GGGCTCAGAT GGTGCGGCT GAGTGTGCGG CCGGCGGCAT TTGGGATACA CCGTAGGGT GGGCGGGTG TGTCCCAGCT CTAATTCCAT CTTCCACCA TGACAGAGAT GCCCTGTGA GGCTGGCCTC CTTGGCGCCT GTCCCCACGG CCCCCGCAGC GTGAGCCACG ATGCTCCCCA TACCCACCC ATTCCCGATA CACCTTACTT ACTGTGTGTT GGCCAGCCCA GAGTGAGGAA GGAGTTTGGC CACATTGGAG ATGGCGGTAG CTGAGCAGAC ATGCCCCAC GAGTAGCCTG ACTCCCTGGT GTGCTCCTGG AAGGAAGATC TTGGGGACCC CCCCACGGA GCACACCTAG GGATCATCTT TGCCGCTC CTGGGGACCC CCCAAGAAAT GTGAGTCCT CGGGGGCCGT GCACTGATGC GGGGAGTGTG GGAAGTCTGG CGGTGAGG GGTGGGTGG GGGCAGTGG GGCTGGGCG GGGGAGTTCT GGGGTAGGAA GTGGTCCCGG GAGATTTTGG ATGGAAAAGT CAGGAGGATT GACAGCAGAC TTGCAGAATT ACATAGAGAA ATTAGGAACC CCCAAATTTT ATGTCAATTG ATCTATTCCC CCTCTTTGTT TCTTGGGCA TTTTTCCTTT TTTTTTTTTT TTTGTTTTTT TTTTACCCTT CTTAGCTTT ATGCGCTCAG AAACCAAATT AAACCCCCC CCCATGTAAC AGGGGGGCG TGACAAAAGC AAGAACGCAC GAAGCCAGCC TGGAGACCAC CACGTCCTGC CCCCAGCCAT TTATCGCCCT GATTGGATTT TGTTTTTCAT CTGTCCCTGT TGCTTGGGTT GAGTTGAGGG TGGAGCCTCC TGGGGGGCAC TGGCCACTGA GCCCCCTTGG AGAAGTCAGA GGGGAGTGGG GAAGGCCACT GTCCGGCCTG GCTTCTGGGG ACAGTGGCTG GTCCCAGAA GTCCTGAGG CGGAGGGGGG GGTGGGCGAG GGTCTCCTCA GGTGTCAGGA GGGTGCTCG AGGCCACAGG AGGGGGCTCC TGGCTGGCCT GAGGCTGGCC GGAGGGGAAG GGGCTAGCAG GTGTGTAAAC AGAGGGTTCC ATCAGGCTGG GGCAGGGTGG CCGCCTTCCG CACACTTGAG GAACCCCTCC CTCTCCCTCG GTGACATCTT GCCCGCCCT CAGCACCTG CCTTGCTCCT AGGAGGTCCG AAGCTCTGTG GGACCTCTTG GGGCAAGGT GGGGTGAGG CGGGGAGTAG GGAGGTGAG CGGGTCTGAG CCCACAGAGC AGGAGAGCTG CCAGGTCTGC CCATCGACCA GGTGCTTGG GCCCGGAGC CCACGGTCT GGTGATGCCA TAGCAGCCAC CACCGGCGG CCTAGGGCTG CGGCAGGGAC TCGGCCTCTG GAGAGTTTAC CTCGCCCCCA CTTGTGCCCC CAGCTCAGCC CCCCAGCAG CAGCCGACT AGCAGTCTAG AGGCCTGAG CTTCTGGGTC CTGCTGACGG GGCTGGCATG ACCCCGGGGG TCGTCCATGC CAGTCCGCC CAGTCGAGA GGGTCCCTCG GCAAGCGCCC TGTGAGTGG CCATTGCGAA CATTGGACAG AAGCCCAAAG AGCCAAATTG TCACAATTGT GGAACCCACA TTGGCCTGAG ATCCAAAACG CTTGAGGCA CCCCAAATTA CCTGCCATT CGTCAGGACA CCCACCCACC CAGTGTTATA TTCTGCCTCG CCGGAGTGGG TGTCCCGGG GGCACTTGCC GACCAGCCCC TTGCGTCCCC AGGTTTGAG CTCTCCCTG GGGCCTAAC CATCCTGGCC CGGGCTGCCT GTCTGACCTC CGTGGCTCTC CATCTGTCT CCTCCCCGTG TCCCCAATGT CTTAGTGGG GGGCCCCCTC TTGGGTCCCC TCCTCTGCCA TCACCTGAAG ACCCCACGC CAAACACTGA ATGTCACCTG TGCCTGCCGC CTCGGTCCAC CTTGGGCCCC GTGTTTGA CTCACTCACT CTTTAAACGC TAATATTTCC GGCAAAATCC CATGCTTGGG TTTTGTCTTT AACCTTGTA CGCTTGCAAT CCCAATAAAG CATTAAAGT CATGAAAAAA AAAAAAATAA AA
4	Human IGF2	GGCCGCGCGC CCTCAGGACG TGGACAGGGA GGGCTTCCCC GTGTCCAGGA AAGCGACCGG

mRNA, transcript variant 3	GCATTGCCCC CAGTCTCCCC CAAATTTGGG CATTGTCCCC GGGTCTTCCA ACGGACTGGG
	CGTTGCTCCC GGACACTGAG GACTGGCCCC GGGGTCTCGC TCACCTTCAG CAGCGTCCAC
	CGCCTGCCAC AGAGCGTTCC ATCGCTCGCT GCCTGAGCTC CTGGTGCGCC CGCGGACGCA
	GCCTCCAGCT TCGCGGAGAT GGTTCCTCCA GACCCCAAAA TTATCGTGGT GGCCCCGAG
	ACCGAACTCG CGTCTATGCA AGTCCAACGC ACTGAGGACG GGGTAACCAT TATCCAGATA
	TTTGGGTGG GCCGCAAAGG CGAGCTACTT AGACGCACCC CGGTGAGCTC GGCCATGCAG
	ACACCAATGG GAATCCCAAT GGGGAAGTCG ATGCTGGTGC TTCTACCTT CTTGCCCTTC
	GCCTCGTGCT GCATTGTGCT TTACCGCCCC AGTGAGACCC TGTGCGGCGG GGAGCTGGTG
	GACACCTCC AGTTCGTCTG TGGGGACCGC GGCTTCTACT TCAGCAGGCC CGCAAGCCGT
	GTGAGCCGTC GCAGCCGTGG CATCGTTGAG GAGTGCTGTT TCCGCAGCTG TGACCTGGCC
	CTCCTGGAGA CGTACTGTGC TACCCCGGCC AAGTCCGAGA GGGACGTGTC GACCCCTCCG
	ACCGTGCTTC CGGACAACTT CCCAGATAC CCCGTGGGCA AGTTCTTCCA ATATGACACC
	TGGAAGCAGT CCACCCAGCG CCTGCGCAGG GGCTTGCCTG CCTCCTCGG TGCCCGCCGG
	GGTCACGTGC TCGCCAAGGA GCTCGAGGCG TTCAGGGAGG CCAAACGTCA CCGTCCCCTG
	ATTGCTCTAC CCACCCAAGA CCCGCGCCAC GGGGGCGCCC CCCAGAGAT GGCCAGCAAT
	CGGAAGTGAG CAAAACTGCC GCAAGTCTGC AGCCCGGCGC CACCATCTCG CAGCCTCCTC
	CTGACCACGG ACGTTTCCAT CAGGTTCCAT CCCGAAAATC TCTCGGTTCC ACGTCCCCCT
	GGGGCTTCTC CTGACCCAGT CCCCGTGCCC CGCCTCCCCG AAACAGTCTC CTCTCCTCGG
	CCCCCTCCAT CGGGCTGAGG AAGCACAGCA GCATCTTCAA ACATGTACAA AATCGATTGG
	CTTTAAACAC CCTTCACATA CCTCCCCCCC AAATTATCCC CAATTATCCC CACACATAAA
	AAATCAAAAC ATTAACTAA CCCCTTCCC CCCCCCCAC AACAACCTC TTAATACTAA
	TTGGCTTTTT AGAAACACCC CACAAAAGCT CAGAAATTGG CTTTAAAAA AACAAACCAC
	AAAAAAATC AATTGGCTAA AAAAAAAG TATTAAAAA GAATTGGCTG AGAAACAATT
	GGCAAAATAA AGGAATTTGG CACTCCCCAC CCCCCTCTTT CTCTTCTCCC TTGGACTTTG
	AGTCAAATTG GCCTGGACTT GAGTCCCTGA ACCAGCAAAG AGAAAAAGAG GACCCAGAA
	ATCACAGGTG GGCACGTGCG TGCTACCGCC ATCTCCCTTC TCACGGGAAT TTTCAGGGTA
	AACTGGCCAT CCGAAAATAG CAACAACCCA GACTGGCTCC TCAGTCCCTT TTCCATCACT
	AAAAATCACA GAGCAGTCAG AGGGACCCAG TAAGACCAAA GAGGGGAGT ACAGAGCATG
	AAAACCAAAA TCCATGCAAA TGAAATGTAA TTGGCACGAC CCTCACCCCC AAATCTTACA
	TCTCAATTCC CATCCTAAAA AGCACTCATA CTTTATGCAT CCCCAGAGCT ACACACACAC
	AACACACAGC ACACGCATGA ACACAGCACA CACACGAGCA CAGCACACAC ACAAACGCAC
	AGCACACACA GCACACAGAT GAGCACACAG CACACACACA AACGCACAGC ACACACACGC
	ACACACATGC ACACACAGCA CACAAACGCA CGGCACACAC ACGCACACAC ATGCACACAC
	AGCACACACA CAAACGCACA GCACACACAA ACGCACAGCA CACACGCACA CACAGCACAC
	ACACGAGCAC ACAGCACACA AACGCACAGC ACACGCACAC ACATGCACAC ACAGCACACA
	CACTAGCACA CAGCACACAC ACAAAGACAC AGCACACACA TGCACACACA GCACACACAC
	GCGAACACAG CACACACGAA CACAGCACAC ACAGCACACA CACAAACACA GCACACAT
	GCACACAGCA CACGCACACA CAGCACACAC ATGAACACAG CACACAGCAC ACACATGCAC
	ACACAGCACA CACGCATGCA CAGCACACAT GAACACAGCA CACACACAAA CACACAGCAC
	ACACATGCAC ACACAGCACA CACACTCATG CGCAGCACAT ACATGAACAC AGCTCACAGC
	ACACAAACAC GCAGCACACA CGTTGCACAC GCAAGCACCC ACCTGCACAC ACACATGCGC
	ACACACACGC ACACCCCCAC AAAATTGGAT GAAAAACAATA AGCATATCTA AGCAACTACG
	ATATCTGTAT GGATCAGGCC AAAGTCCCGC TAAGATTCTC CAATGTTTTC ATGGTCTGAG
	CCCCGCTCCT GTTCCCATCT CCACTGCCCC TCGGCCCTGT CTGTGCCCTG CCTCTCAGAG
	GAGGGGGCTC AGATGGTGCG GCCTGAGTGT GCGGCCGGCG GCATTTGGGA TACACCCGTA
	GGGTGGGCGG GGTGTGTCCC AGGCCTAATT CCATCTTTCC ACCATGACAG AGATGCCCTT
	GTGAGGCTGG CCTCCTTGGC GCCTGTCCCC ACGGCCCCCG CAGCGTGAGC CAGCATGCTC
	CCCATACCCC ACCCATTTCC GATACACCTT ACTTACTGTG TGTTGGCCCA GCCAGAGTGA
	GGAAGGAGTT TGGCCACATT GGAGATGGCG GTAGCTGAGC AGACATGCCC CCACGAGTAG
	CCTGACTCCC TGGTGTGCTC CTGGAAGGAA GATCTTGGGG ACCCCCCCAC CGGAGCACAC
	CTAGGGATCA TCTTTGCCCG TCTCCTGGGG ACCCCCCAAG AAATGTGGAG TCCTCGGGGG
	CCGTGCACTG ATGCGGGGAG TGTGGGAAGT CTGGCGGTTG GAGGGGTGGG TGGGGGCGAG
	TGGGGGCTGG GCGGGGGGAG TTCTGGGGTA GGAAGTGGTC CCGGGAGATT TTGGATGGAA
	AAGTCAGGAG GATTGACAGC AGACTTGCAG AATTACATAG AGAAATTAGG AACCCCCAAA
	TTTCATGTCA ATTGATCTAT TCCCCCTCTT TGTTTCTTGG GGCATTTTTC CTTTTTTTTT
	TTTTTTTGT TTTTTTTTAC CCTCCTTAG CTTTATGCGC TCAGAAACCA AATTAAACCC
	CCCCCCATG TAACAGGGGG GCAGTGACAA AAGCAAGAAC GCACGAAGCC AGCCTGGAGA
	CCACCACGTC CTGCCCCCG CCATTTATCG CCCTGATTGG ATTTTGTTTT TCATCTGTCC
	CTGTGCTTG GGTGAGTTG AGGGTGGAGC CTCCTGGGG GCCTGGCCA CTGAGCCCC
	TTGAGAAAGT CAGAGGGGAG TGAGAAAGGC CACTGTCCGG CCTGGCTTCT GGGGACAGTG
	GCTGGTCCCC AGAAGTCTCG AGGGCGGAGG GGGGGGTTGG GCAGGGTCTC CTCAGGTGTC
	AGGAGGGTGC TCGGAGGCCA CAGGAGGGGG CTCCTGGCTG GCTGAGGCT GGCCGGAGGG
	GAAGGGGCTA GCAGGTGTGT AAACAGAGGG TTCCATCAGG CTGGGGCAGG GTGGCCGCCT
	TCCGCACACT TGAGGAACCC TCCCCTCTCC CTCGGTGACA TCTTGCCGCG CCCTCAGCAC
	CCTGCCTTGT CTCCAGGAGG TCCGAAGCTC TGTGGGACCT CTTGGGGGCA AGGTGGGGTG
	AGGCCGGGGA GTAGGGAGGT CAGGCGGGTC TGAGCCCACA GAGCAGGAGA GCTGCCAGGT
	CTGCCCATCG ACCAGGTTGC TTGGGCCCCG GAGCCACGG GTCTGGTGAT GCCATAGCAG
	CCACCACCGC GCGCCTAGG GCTGCGGCAG GGACTCGGCC TCTGGGAGGT TTACCTCGCC

		CCCACTTGTG CCCCCAGCTC AGCCCCCCTG CACGCAGCCC GACTAGCAGT CTAGAGGCCT GAGGCTTCTG GGTCTTGGTG ACGGGGCTGG CATGACCCCG GGGGTCTGTC ATGCCAGTCC GCCTCAGTCG CAGAGGGTCC CTCGGCAAGC GCCCTGTGAG TGGGCCATTTC GGAAACATTGG ACAGAAGCCC AAAGAGCCAA ATTGTCACAA TTGTGGAACC CACATTGGCC TGAGATCCAA AACGCTTCGA GGCACCCCAA ATTACCTGCC CATTCTGTCAG GACACCCACC CACCAGTGT TATATTCTGC CTCGCCGAG TGGGTGTTCC CGGGGGCACT TGCCGACCAG CCCCTTGCGT CCCCAGGTTT GCAGCTCTCC CCTGGGCCAC TAACCATCCT GGCCCGGGCT GCCTGTCTGA CCTCCGTGCC TAGTCGTGGC TCTCCATCTT GTCTCTCCCG CGTGTCCCA ATGTCTTCAG TGGGGGGCCC CCTCTTGGGT CCCCTCCTCT GCCATCACCT GAAGACCCCC ACGCCAAACA CTGAATGTCA CCTGTGCCTG CCGCCTCGGT CCACCTTGCG GCCCGTGTTC GACTCAACTC AACTCCTTTA ACGCTAATAT TTCCGGCAAA ATCCCATGCT TGGGTTTTGT CTTTAACTTT GTAACGCTTG CAATCCCAAT AAAGCATTA AAGTCATGAA AAAAAAAAAA AAAAAA
5	Human KRT20 mRNA	GAGACACACT CTGCCCCAAC CATCCTGAAG CTACAGGTGC TCCCTCCTGG AATCTCCAAT GGATTTCAGT CGCAGAAGCT TCCACAGAAG CCTGAGCTCC TCCTTGCAGG CCCCTGTAGT CAGTACAGTG GGCATGCAGC GCCTCGGGAC GACACCCAGC GTTTATGGGG GTGCTGGAGG CCGGGGCATC CGCATCTCCA ACTCCAGACA CACGGTGAAC TATGGGAGCG ATCTCACAGG CGGCGGGGAC CTGTTTGTG GCAATGAGAA AATGGCCATG CAGAACCTAA ATGACCGTCT AGCGAGCTAC CTAGAAAAGG TCGGGACCCCT GGAGCAGTCC AACTCCAAAC TTGAAGTGCA AATCAAGCAG TGGTACGAAA CCAACGCCCC GAGGGCTGGT CGCGACTACA GTGCATATTA CAGACAAATT GAAGAGCTGC GAAGTCAGAT TAAGGATGCT CAACTGCAAA ATGCTCGGTG TGTCCTGCAA ATTGATAATG CTAAACTGGC TGCTGAGGAC TTCAGACTGA AGTATGAGAC TGAGAGAGGA ATACGTCTAA CAGTGAAGC TGATCTCAA GGCCTGAATA AGGTCTTTGA TGACCTAACC CTACATAAAA CAGATTTGGA GATTCAAATT GAAGAAGTGA ATAAAGACCT AGCTCTCCTC AAAAAGGAGC ATCAGGAGGA AGTCGATGGC CTACACAAGC ATCTGGGCAA CACTGTCAAT GTGGAGGTTG ATGCTGCTCC AGGCCGTAAC CTTGGCGTCA TCATGAATGA AATGAGGCAG AAGTATGAAG TCATGGCCCA GAAGAACCTT CAAGAGGCCA AAGAACAGTT TGAGAGACAG ACTGCAGTTC TGCAGCAACA GGTACAGTG AATACTGAAG AATTAAGAGG AACTGAGGTT CAACTAACGG AGCTGAGACG CACCTCCAG AGCCTTGAGA TAGAACTCCA GTCCCATCTC AGCATGAAAG AGTCTTTGGA GCACACTCTA GAGGAGACCA AGGCCCGTTA CAGCAGCCAG TTAGCCAACC TCCAGTCGCT GTTGAGCTCT CTGGAGGCC AACTGATGCA GATTCGGAGT AACATGGAAC GCCAGAACAA CGAATACCAT ATCCTTCTTG ACATAAAGAC TCGACTTGAA CAGGAAATTG CTACTTACCG CCGCCTTCTG GAAGGAGAA ACGTAAAAAC TACAGAATAT CAGTTAAGCA CCCTGGAAGA GAGAGATATA AAGAAAAACA GGAAGATTAA GACAGTCGTG CAAGAAGTAG TGGATGGCAA GGTCTGTGTA TCTGAAGTCA AAGAGGTGGA AGAAAATATC TAAATAGCTA CCAGAAGGAG ATGCTGCTGA GGTTTTGAAA GAAATTTGGC TATAATCTTA TCTTTGCTCC CTGCAAGAAA TCAGCCATAA GAAAGCACTA TTAATACTCT GCAGTGATTA GAAGGGGTGG GGTGGCGGGA ATCCTATTTA TCAGACTCTG TAATTGAATA TAAATGTTTT ACTCAGAGGA GCTGCAAAAT GCCTGCAAAA ATGAAATCCA GTGAGCACTA GAATATTTAA AACATCATT CTGCCATCTT TATCATGAAG CACATCAATT ACAAGCTGTA GACCACCTAA TATCAATTG TAGGTAATGT TCCTGAAAAT TGCAATACAT TTCAATTATA CTAAACCTCA CAAAGTAGAG GAATCCATGT AAATTGCAAA TAAACCACTT TCTAATTTTT TCCTGTTTCT GAATTGTAAA ACCCCCTTTG GGAGTCCCTG GTTTCTTATT GAGCCAATTT CTGGG
6	Human ANXA10 mRNA	ATCCAGATTT GCTTTTACAT TTTCTTGCCT GAGTCTGAGG TGAACAGTGA ACATATTTAC ATTTGATTTA ACAGTGAACC TTAATCTTT CTGGCTTCAC AGTGAAACAA GTTTATGCAA TCGATCAAAT ATTTTCATCC CTGAGGTTAA CAATTACCAT CAAAATGTTT TGTGGAGACT ATGTGCAAGG AACCATCTTC CCAGCTCCCA ATTTCAATCC CATAATGGAT GCCCAATGTC TAGGAGGAGC ACTCCAAGGA TTTGACTGTG ACAAAGACAT GCTGATCAAC ATTTGACTC AGCGCTGCAA TGCACAAAGG ATGATGATTG CAGAGGCATA CCAGAGCATG TATGGCCGGG ACCTGATTGG GGATATGAGG GAGCAGCTTT CGGATCACTT CAAAGATGTG ATGGCTGGCC TCATGTACCC ACCACCACTG TATGATGCTC ATGAGCTCTG GCATGCCATG AAGGGAGTAG GCACTGATGA GAATTGCCTC ATTGAAATAC TAGCTTCAAG AACAAATGGA GAAATTTTCC AGATGCGAGA AGCCTACTGC TTGCAATACA GCAATAACCT CCAAGAGGAC ATTTATTCAG AGACCTCAGG ACACCTCAGA GATACTCTCA TGAACCTGGT CCAGGGGACC AGAGAGGAAG GATATACAGA CCCTGCGATG GCTGCTCAGG ATGCAATGGT CCTATGGGAA GCCTGTCAGC AGAAGACGGG GGAGCACAAA ACCATGCTGC AAATGATCCT GTGCAACAAG AGCTACCAGC AGCTGCGGCT GGTTTTCCAG GAATTTCAAA ATATTTCTGG CCAAGATATG GTAGATGCCA TTAATGAATG TTATGATGGA TACTTTCAGG AGCTGCTGGT TGCAATTGTT CTCTGTGTTT GAGACAAACC AGCCTATTTT GCTTATAGAT TATATAGTGC AATTATGAC TTTGGTTTCC ATAATAAAAC TGTAATCAGG ATTCTCATTG CCAGAAGTGA AATAGACCTG CTGACCATAA GGAACGATA CAAAGAGCGA TATGGAAAAT CCCTATTTCA TGATATCAGA AATTTTGCTT CAGGGCATT TAAGAAAAGCA CTGCTTGCCA TCTGTGCTGG TGATGCTGAG GACTACTAAA ATGAAGAGGA CTTGGAGTAC TGTGCACTCC TCTTTCTAGA CACTTCCAAA TAGAGATTTT CTCACAATTT TGTACTGTTT ATGGCACTAT TAACAAAAC ATACAATCAT ATTTTCTCTT CTATCTTTGA AATTATTTCT AGCCAAAAGAA AACTATGAAT GAAAGTATAT GATACTGAAT TTGCCTACTA TCCTGAATTT GCCTACTATC TAATCAGCAA TTAAATAAAT TGTGCATGAT GGAATAATAG AAAAATTGCA TTGGAATAGA TTTTATTTAA ATGTGAACCA TCAACAACCT ACAACAA

7	Human ABL mRNA	GGTTGGTGAC TTCCACAGGA AAAGTTCTGG AGGAGTAGCC AAAGACCATC AGCGTTTCCT TTATGTGTGA GAATTGAAAT GACTAGCATT ATTGACCCTT TTCAGCATCC CCTGTGAATA TTTCTGTTTA GGTTTTTCTT CTTGAAAAGA AATTGTTATT CAGCCCGTTT AAAACAAATC AAGAACTTTT TGGGTAACAT TGCAATTACA TGAAATTGAT AACC CGCGAAA ATAATTGGAA CTCCTGCTTG CAAGTGTCAA CCTAAAAAAA GTGCTTCCTT TTGTTATGGA AGATGTCTTT CTGTGATTGA CTTCAATTGC TGA CTGTGTGG AGATGCAGCG AATGTGAAAT CCCACGTATA TGCCATTTCC CTCTACGCTC GCTGACCGTT CTGGAAGATC TTGAACCTC TTCTGGAAAG GGGTACCTAT TATTACTTTA TGGGGCAGCA GCCTGGAAAA GTACTTGGGG ACCAAAAGAAG GCCAAGCTTG CCTGCCCCTGC ATTTTATCAA AGGAGCAGGG AAGAAGGAAT CATCGAGGCA TGGGGGTCCA CACTGCAATG TTTTGTGGA ACATGAAGCC CTTCAGCGGC CAGTAGCATC TGA CTTTGAG CCTCAGGGTC TGAGTGAAGC CGCTCGTTGG AACTCCAAGG AAAACCTTCT CGCTGGACCC AGTGAAAATG ACCCCAACCT TTTCTGTGCA CTGTATGATT TTGTGGCCAG TGGAGATAAC ACTCTAAGCA TAACTAAAGG TGAAAAGCTC CGGGTCTTAG GCTATAATCA CAATGGGGAA TGGTGTGAAG CCCAAACCAA AAATGGCCAA GGCTGGGTCC CAAGCAACTA CATCACGCCA GTCAACAGTC TGGAGAAACA CTCCTGGTAC CATGGGCTG TGTCGCCCAA TGCCGCTGAG TATCTGCTGA GCAGCGGGAT CAATGGCAGC TTCTTGGTGC GTGAGAGTGA GAGCAGTCCT GGCCAGAGGT CCATCTCGCT GAGATACGAA GGGAGGGTGT ACCATTACAG GATCAACACT GCTTCTGATG GCAAGCTCTA CGTCTCCTCC GAGAGCCGCT TCAACACCCT GGCCGAGTTG GTTCATCATC ATTCAACGGT GGCCGACGGG CTCATCACC CGCTCCATTA TCCAGCCCCA AAGCGCAACA AGCCCACTGT CTATGGTGTG TCCCCCACT ACGACAAGTG GGAGATGGAA CGCACGGACA TCACCATGAA GCACAAGCTG GCGGGGGGCC AGTACGGGGA GGGTACGAG GCGGTGTGGA AGAAATACAG CCTGACGGTG GCGCTGAAGA CCTTGAAGGA GGACACCATG GAGGTGGAAG AGTTCTTGAA AGAAGCTGCA GTCATGAAAG AGATCAAACA CCCTAACCTG GTGCAGCTCC TTGGGGTCTG CACCCGGGAG CCCCCGTCT ATATCATCAC TGAGTTTCATG ACCTACGGGA ACCTCCTGGA CTACCTGAGG GAGTGCAACC GGCAGGAGGT GAACGCCGTG GTGCTGCTGT ACATGGCCAC TCAGATCTCG TCAGCCATGG AGTACCTGGA GAAGAAAAAC TTCATCCACA GAGATCTTGC TGCCCCGAAAC TGCTGTGATG GGGAGAACCA CTTGGTGAAG GTAGCTGATT TTGGCCTGAG CAGGTTGATG ACAGGGGACA CCTACACAGC CCATGCTGGA GCCAAGTCC CCATCAAATG GACTGCACCC GAGAGCCCTG CCTACAACAA GTTCTCCATC AAGTCCGACG TCTGGGCATT TGGAGTATTG CTTTGGGAAA TTGTACCTA TGGCATGTCC CCTTACCCGG GAATTGACCT GTCCCAGGTG TATGAGCTGC TAGAGAAGGA CTACCGCATG GAGCGCCCG AGGGCTGCCC AGAGAAGGTC ATGAACTCA TGGGAGCATG TTGGCAGTGG AATCCCTCTG ACCGGCCCTC CTTTGCTGAA ATCCACCAAG CCTTTGAAAC AATGTTCAG GAATCCAGTA TCTCAGACGA AGTGGAAGAG GAGCTGGGGA AACAGGCGT CCGTGGGGCT GTGAGTACCT TGCTGCAGGC CCCAGAGCTG CCCACCAAGA CGAGGACCTC CAGGAGAGCT GCAGAGCACA GAGACACCAC TGACGTGCCT GAGTGCCTC ACTCCAAGGG CCAGGGAGAG AGCGATCCTC TGGACCATGA GCCTGCGGTG TCTCCATTGC TCCCTCGAAA AGAGCGAGGT CCCCCGAGG GCGGCCTGAA TGAAGATGAG CGCCTTCTCC CCAAAGACAA AAAGACCAAC TTGTTACGCG CCTTGATCAA GAAGAAGAAAG AAGACAGCCC CAACCCCTCC CAAACGCAGC AGCTCCTTCC GGGAGATGGA CGGCCAGCCG GAGCGCAGAG GGGCCGGCGA GGAAGAGGGG CGAGACATCA GCAACGGGGC ACTGGCTTTC ACCCCTTGG ACACAGCTGA CCCAGCCAAG TCCCCAAGC CCAGCAATGG GGCTGGGGTC CCCAATGGAG CCCTCCGGGA GTCCGGGGGC TCAGGCTTCC GGTCTCCCA CCTGTGGAAG AAGTCCAGCA CGCTGACCAG CAGCCGCCTA GCCACCGCG AGGAGGAGGG CGGTGGCAGC TCCAGCAAGC GCTTCTGCG CTCTTGCTCC GCCTCCTGCG TTCCCCATGG GGCCAAGGAC ACGGAGTGGG GGTCACTCAG GCTGCCTCGG GACTTGCACT CCACGGGAAG ACAGTTTGAC TCGTCCACAT TTGGAGGGCA CAAAAGTGAG AAGCCGCTC TGCTCGGAA GAGGGCAGGG GAGAACAGGT CTGACCAGGT GACCCGAGGC ACAGTAACGC CTCCCCCAG GCTGGTGAAA AAGAATGAGG AAGCTGCTGA TGAGGTCTTC AAAGACATCA TGGAGTCCAG CCCGGGCTCC AGCCCCGCCA ACCTGACTCC AAAACCCCTC CGGCGGCAGG TCACCGTGGC CCCTGCCTCG GGCTTCCCC ACAAGGAAGA AGCTGGAAG GGCAGTGCCT TAGGGACCCC TGCTGCAGCT GAGCAGTGA CCCCACCAG CAAAGCAGGC TCAGGTGCAC CAGGGGGCAC CAGCAAGGGC CCCGCCGAGG AGTCCAGAGT GAGGAGGCAC AAGCACTCCT CTGAGTCGCC AGGGAGGGAC AAGGGGAAAT TGTCCAGGCT CAAACCTGCC CCGCCGCCCC CACCAGCAGC CTCTGCAGGG AAGGCTGGAG GAAAGCCCTC GCAGAGCCCC AGCCAGGAG CGGCCGGGA GGCAGTCTTG GCGCAAAAG CAAAAGCCAC GAGTCTGGTT GATGCTGTGA ACAGTGACGC TGCCAAGCCC AGCCAGCCGG GAGAGGGCCT CAAAAAGCCC GTGCTCCCG CCACTCCAAA GCCACAGTCC GCCAAGCCGT CGGGGACCCC CATCAGCCCA GCCCCGTTT CCTCCACGTT GCCATCAGCA TCCTCGGCCC TGGCAGGGGA CCAGCCGTCT TCCACCGCCT TCATCCCTCT CATATCAACC CGAGTGTCTC TTCGAAAAC CCGCCAGCCT CCAGAGCGGA TCGCCAGCGG CGCCATCACC AAGGGCGTGG TCCTGGACAG CACCAGGCG CTGTGCCTCG CCATCTCTAG GAACCTCGAG CAGATGGCCA GCCACAGCGC AGTGCTGGAG GCGGGCAAAA ACCTCTACAC GTTCTGCGTG AGCTATGTGG ATTCCATCCA GCAAATGAGG AACAAGTTT CTTCCGAGA GGCCATCAAC AAAGTGGAGA ATAATCTCCG GGAGCTTCAG ATCTGCCCG CGACAGCAGG CAGTGGTCCA GCGGCCACTC AGGACTTCAG CAAGCTCCTC AGTTCGGTGA AGGAAATCAG TGACATAGTG CAGAGGTAGC AGCAGTCAGG GGTCAGGTGT CAGGCCCCGT GGAGCTGCCT GCAGCACATG CCGGCTGCCT CATACCCGTG ACAGTGGCTG ACAAGGGACT AGTGAGTCAG CACCTTGGCC CAGGAGCTCT GCGCCAGGCA
---	----------------	--

		GAGCTGAGGG CCTGTGGAG TCCAGTCTA CTACCTACGT TTGCACCGCC TGCCCTCCCG CACCTTCCTC CTCCCCGCTC CGTCTCTGTC CTCGAATTTT ATCTGTGGAG TTCTGTCTCC GTGGACTGCA GTCGGCATGC CAGGACCCGC CAGCCCGCT CCCACCTAGT GCCCCAGACT GAGCTCTCCA GGCCAGGTGG GAACGGCTGA TGTGGACTGT CTTTTTCATT TTTTCTCTC TGGAGCCCCCT CCTCCCCGG CTGGGCCTCC TTCTTCCACT TCTCCAAGAA TGAAGCCTG AACTGAGGCC TTGTGTGTCA GGCCCTCTGC CTGCACTCCC TGGCCTTGCC CGTCGTGTGC TGAAGACATG TTCAAGAAC CGCATTTCCG GAAGGGCATG CACGGGCATG CACACGGCTG GTCACCTGTC CCTCTGTGC TGCCCGGGGT GGGGTGCAT CGCCATTTC TCACGTGCAG GACAGCTCTT GATTTGGGTG GAAAACAGGG TGCTAAAGCC AACCAGCCTT TGGGTCTCTG GCAGGTGGGA GCTGAAAAG ATCGAGGCAT GGGGCATGTC CTTTCCATCT GTCCACATCC CCAGAGCCCA GCTCTTGCTC TCTGTGACG TGCATGTGA ATCCTGGCAA GAAAGCTTGA GTCTCAAGGG TGGCAGGTCA CTGTCACTGC CGACATCCCT CCCCCAGCA AATGGAGGCA GGGACAAAG GAGGCAGTGG CTAGTGGGGT GAACAGCTGG TGCCAAATAG CCCCAGACTG GGCCAGGCA GGTCTGCAAG GGCCAGAGT GAACCGTCCT TTCACACATC TGGGTGCCCT GAAAGGGCCC TTCCCCCTCC CCACTCCTCT AAGACAAAGT AGATTCTTAC AAGGCCCTTT CCTTTGGAAC AAGACAGCCT TCACTTTTCT GAGTTCTTGA AGCATTTCAA AGCCCTGCCT CTGTGTAGCG GCCCTGAGAG AGAATAGAGC TGCCACTGGG CACCTGCGCA CAGGTGGGAG GAAAGGGCCT GGCCAGTCCT GGTCTGGCT GCACCTTGA ACTGGGGCAA TGTCTTATTT AATTACCGTG AGTGACATAG CCTCATGTTT TGTGGGGGTC ATCAGGGAGG GTTAGGAAAA CCACAAACGG AGCCCCGAA AGCCTCACGT ATTTACAGA GCACGCCTGC CATCTTCTCC CCGAGGCTGC CCCAGGCCGG AGCCAGATA CGGGGCTGT GACTCTGGGC AGGGACCCGG GGTCTCCTGG ACCTTGACAG AGCAGCTAAC TCCGAGAGCA GTGGCCAGGT GGCCGCCCT GAGGCTTCAC GCCGGGAGAA GCCACCTTCC CACCCCTTCA TACCGCTCG TGCCAGCAGC CTCGCACAGG CCCTAGCTTT ACGCTCATCA CCTAACTTG TACTTTATTT TTCTGATAGA AATGTTTCC TCTGGATCGT TTTATGCGGT TCTTACAGA CATCACCTCT TTGCCCCGA CGGCTGTGAC GCAGCCGGAG GGAGGCACTA GTCACCGACA GCGGCCTTGA AGACAGAGCA AAGCGCCAC CCAGGTCCCC CGACTGCCTG TCTCCATGAG GTACTGGTCC TTCTCTTTG TTAACGTGAT GTGCCACTAT ATTTTACAG TATCTCTTG TATGACTCT TTATAGACGC TCTTTTCTAA GTGGCGTGTG CATAGCGTCC TGCCCTGCC CCTCGGGGC CTGTGGTGGC TCCCCCTCTG CTTCTCGGG TCCAGTGCAT TTTGTTCTG TATATGATTC TCTGTGGTTT TTTTTGAATC CAAATCTGTC CTCTGTAGTA TTTTTTAAAT AAATCAGTGT TTACATTAGA A
47	Armored RNA [®] sequence	GAUCUCACU UCAUCUAUGG UUACCCUGGG ACUUUUACAC CAACAGAACU AGCAUCAUCC UCUGCAUGGU CAGGUCAUGG AUCGGCAUCC UGACAGUUUC GGGAAUUAGG CAUCUGCAGU CUUACUGCUC AUCGGCUGAU GAUGCUGCUG UAAUCCCCAU CCAAGCAAGC UUGUGAUCCU CCGCCAUUUA CCAAAUUGGU AUAACAUUUA GGACUUAAAG CUAUGCAAUU AUCACCUUGU UUUUCACAG CAAGACCUAA UAUUUUCUUU UCAUCAUUAA UGCCUUUGU UGGAUAGGC AACCAUUUUA AAAUAUGUUC ACCAGCCGAA GUCAGUAGUG AUUGGGUGGU UCCUGGCUUG GGAUCAUGCC GCUGCAGAGG CUUUCUCCU CUUGGCAGAU UGUCUGUAGC CGAGAAGGCG GAGUCUGGCA AUGAUGAUGC AUACAGUGUA CGACAGCCUU AGGGACUGGA GCUCAAGCAG UGUUUCCUCA ACCAGUCACA
49	Human androgen receptor (AR) mRNA, transcript variant 1 (GenBank NM_000044.3)	CGAGATCCCC GGGAGCCAGC TTGCTGGGAG AGCGGGACGG TCCGGAGCAA GCCCAGAGGC AGAGGAGGCG ACAGAGGGAA AAAGGGCCGA GCTAGCCGCT CCAGTGCTGT ACAGGAGCCG AAGGGACGCA CCACGCCAGC CCCAGCCCGG CTCCAGCGAC AGCCAACGCC TCTTGCAGCG CGGCGGCTTC GAAGCCGCG CCCGGAGCTG CCCTTTCCTC TTCGGTGAAG TTTTAAAG CTGCTAAAGA CTCGGAGGAA GCAAGGAAAG TGCCTGGTAG GACTGACGGC TGCCTTTGTC CTCCTCCTCT CCACCCCGCC TCCCCCACC CTGCCTTCCC CCCCCTCCCC GTCTTCTCTC CCGAGCTGTC CTCAGTCGGC TACTCTCAGC CAACCCCTC CACCACCTT CTCCCACCC GCCCCCGCG CCCCCTCGGC CCAGCGCTGC CAGCCCGAGT TTGCAGAGAG GTAACCTCCT TTGGCTGCGA GCGGGCGAGC TAGCTGCACA TTGCAAAGAA GGCTCTTAGG AGCCAGGCGA CTGGGGAGCG GCTTCAGCAC TGCAAGCACG ACCCGCCTGG TTAGGCTGCA CGCGGAGAGA ACCCTCTGTT TTCCCCACT CTCTCTCCAC CTCTCCTGC CTCCCCACC CCAGTCCGG AGCCAGAGAT CAAAAGATGA AAAGGCAGTC AGGTCTTCAG TAGCCAAAAA ACAAAGCAAA CAAAAACAAA AAAGCCGAAA TAAAAGAAAA AGATAATAAC TCAGTTCTTA TTTGCACCTA CTTCAGTGGA CACTGAATTT GGAAGGTGGA GGATTTGT TTTTCTTTT AAGATCTGGG CATCTTTTGA ATCTACCTT CAAGTATTAA GAGACAGACT GTGAGCCTAG CAGGGCAGAT CTTGTCCACC GTGTGTCTT TCTGACAGC GACTTTGAGG CTGTGAGAGC GTTTTGTGCG TGGTTGCTCC CGCAAGTTTCT CTCTCTGGA GCTTCCGCA GGTGGGAGC TAGCTGCAGC GACTACCGCA TCATCACAGC CTGTGAACT CTTCTGAGCA AGAGAAGGGG AGGCGGGGTA AGGGAAGTAG GTGGAAGATT CAGCCAAGCT CAAGGATGGA AGTGACGTTA GGGCTGGGAA GGGTCTACCC TCGGCCGCG TCCAAGACCT ACCGAGGAGC TTTCCAGAA CTGTTCAGA GCGTGCGCGA AGTATCCAG AACCCGGGCC CCAGGCACCC AGAGCCGCG AGCGCAGCAC CTCCGCGCGC CAGTTTGTCT CTGCTGCAGC AGCAGCAGCA GCAGCAGCAG CAGCAGCAGC AGCAGCAGCA GCAGCAGCAG CAGCAGCAGC AGCAAGAGAC TAGCCCCAGG CAGCAGCAGC AGCAGCAGGG TGAGGATGGT TCTCCCAAG CCCATCGTAG AGGCCCCACA GGCTACCTGG TCCTGGATGA GGAACAGCAA CCTTCACAGC CGCAGTCGGC CCTGGAGTGC CACCCCGAGA GAGGTTGCGT CCCAGAGCCT GGAGCCGCGG TGGCCGCCAG CAAGGGGTCT CCGCAGCAGC TGCCAGCACC TCCGAGCAG GATGACTCAG CTGCCCCATC CACGTTGTCT CTGCTGGGCC

		CCACTTTCCC	CGGCTTAAGC	AGCTGCTCCG	CTGACCTTAA	AGACATCCTG	AGCGAGGCCA
		GCACCATGCA	ACTCCTTCAG	CAACAGCAGC	AGGAAGCAGT	ATCCGAAGGC	AGCAGCAGCG
		GGAGAGCGAG	GGAGGCCTCG	GGGGCTCCCA	CTTCCTCCAA	GGACAATTAC	TTAGGGGGCA
		CTTCGACCAT	TTCTGACAAC	GCCAAGGAGT	TGTGTAAGGC	AGTGTCCGGT	TCCATGGGCC
		TGGGTGTGGA	GGCGTTGGAG	CATCTGAGTC	CAGGGGAACA	GCTTCGGGGG	GATTGCATGT
		ACGCCCCACT	TTTGGGAGTT	CCACCCGCTG	TGCGTCCAC	TCCTTGTGCC	CCATTGGCCG
		AATGCAAAGG	TTCTCTGCTA	GACGACAGCG	CAGGCAAGAG	CAGTGAAGAT	ACTGCTGAGT
		ATTCCCCTTT	CAAGGGAGGT	TACACCAAAG	GGCTAGAAGG	CGAGAGCCTA	GGCTGCTCTG
		GCAGCGCTGC	AGCAGGGAGC	TCCGGGACAC	TTGAACTGCC	GTCTACCTTG	TCTCTCTACA
		AGTCCGGAGC	ACTGGACGAG	GCAGCTGCGT	ACCAGAGTCG	CGACTACTAC	AACTTTCCAC
		TGGCTCTGGC	CGGACCGCCG	CCCCCTCCGC	CGCCTCCCCA	TCCCCACGCT	CGCATCAAGC
		TGGAGAACCC	GCTGGACTAC	GGCAGCGCCT	GGGCGGCTGC	GGCGGCGCAG	TGCCGCTATG
		GGGACCTGGC	GAGCCTGCAT	GGCGCGGGTG	CAGCGGGACC	CGGTTCTGGG	TCACCGCTCAG
		CCGCCGCTTC	CTCATCCTGG	CACACTCTCT	TCACAGCCGA	AGAAGGCCAG	TTGTATGGAC
		CGTGTGGTGG	TGGTGGGGGT	GGTGGCGGCG	GCGGCGGCGG	CGGCGGCGGC	GGCGGCGGCG
		GCGGCGGCGG	CGGCGAGGCG	GGAGCTGTAG	CCCCCTACGG	CTACACTCGG	CCCCCTCAGG
		GGCTGGCGGG	CCAGGAAAGC	GACTTCACCG	CACCTGATGT	TGGGTACCCT	GGCGGCATGG
		TGAGCAGAGT	GCCCTATCCC	AGTCCCACCT	GTGTCAAAAG	CGAAATGGGC	CCCTGGCATGG
		ATAGCTACTC	CGGACCTTAC	GGGGACATGC	GTTTGGAGAC	TGCCAGGGAC	CATGTTTTGC
		CCATTGACTA	TTACTTTCCA	CCCCAGAAGA	CCTGCCTGAT	CTGTGGAGAT	GAAGCTTCTG
		GGTGTCACTA	TGGAGCTCTC	ACATGTGGAA	GCTGCAAGGT	CTTCTTCAAA	AGAGCCGCTG
		AAGGGAAACA	GAAGTACCTG	TGCGCCAGCA	GAAATGATTG	CACATATTAT	AAATTCGGAA
		GGAAAAATTG	TCCATCTTGT	CGTCTTCGGA	AATGTTATGA	AGCAGGGATG	ACTCTGGGAG
		CCCGGAAGCT	GAAGAACTT	GGTAATCTGA	AACTACAGGA	GGAAGGAGAG	GCTTCCAGCA
		CCACCAGCCC	CACTGAGGAG	ACAACCCAGA	AGCTGACAGT	GTCACACATT	GAAGGCTATG
		AATGTCAGCC	CATCTTTCTG	AATGTCCTGG	AAGCCATTGA	GCCAGGTGTA	GTGTGTGCTG
		GACACGACAA	CAACCAGCCC	GACTCCTTTG	CAGCCTTGCT	CTCTAGCCTC	AATGAACCTG
		GAGAGAGACA	GCTTGTACAC	GTGGTCAAGT	GGGCCAAGGC	CTTGCTTGGC	TTCCGCAACT
		TACACGTGGA	CGACCAGATG	GCTGTCAATC	AGTACTCCTG	GATGGGGCTC	ATGGTGTGTT
		CCATGGGCTG	GCGATCCTTC	ACCAATGTCA	ACTCCAGGAT	GCTCTACTTC	GCCCCTGATC
		TGGTTTTCAA	TGAGTACCGC	ATGCACAAGT	CCCGGATGTA	CAGCCAGTGT	GTCCGAATGA
		GGCACCTCTC	TCAAGAGTTT	GGATGGCTCC	AAATCACCCC	CCAGGAATTC	CTGTGCATGA
		AAGCACTGCT	ACTCTTCAGC	ATTATTCCAG	TGGATGGGCT	GAAAAATCAA	AAATTCTTTG
		ATGAACTTCG	AATGAACTAC	ATCAAGGAAC	TCGATCGTAT	CATTGCATGC	AAAAGAAAAA
		ATCCACATC	CTGCTCAAGA	CGCTTCTACC	AGCTCACCAA	GCTCCTGGAC	TCCGTGCAGC
		CTATTGCGAG	AGAGCTGCAT	CAGTTCACCT	TTGACCTGCT	AATCAAGTCA	CACATGGTGA
		GCGTGGACCT	TCCGGAATG	ATGGCAGAGA	TCATCTCTGT	GCAAGTGCCT	AAGATCCTTT
		CTGGGAAAGT	CAAGCCCATC	TATTTCCACA	CCCAGTGAAG	CATTGGAAAC	CCTATTTCCC
		CACCCAGCT	CATGCCCCCT	TTCAGATGTC	TTCTGCCTGT	TATAACTCTG	CACTACTCCT
		CTGCAGTGCC	TTGGGGAATT	TCCTCTATTG	ATGTACAGTC	TGTCATGAAC	ATGTTCCCTG
		ATTCTATTTG	CTGGGCTTTT	TTTTTCTCTT	TCTCTCCTTT	CTTTTTCTTC	TTCCCTCCCT
		ATCTAACCCCT	CCCATGGCAC	CTTCAGACTT	TGCTTCCCCT	TGTGGCTCCT	ATCTGTGTTT
		TGAATGGTGT	TGTATGCCTT	TAAATCTGTG	ATGATCCTCA	TATGGCCCAG	TGTCAAAGTT
		TGCTTGTTTA	CAGCACTACT	CTGTGCCAGC	CACACAAACG	TTTACTTATC	TTATGCCACG
		GGAAAGTTTAG	AGAGCTAAGA	TTATCTGGGG	AAATCAAAAC	AAAAACAAGC	AAACAAAAAA
		AAAAAGCAAA	AACAAAACAA	AAAAATAAGC	AAAAAACCTT	GCTAGTGTTC	TTTCTCTCAA
		AATAAATAAA	TAAATAAATA	AATACGTACA	TACATACACA	CATACATACA	AACATATAGA
		AATCCCCAAA	GAGGCCAATA	GTGACGAGAA	GGTGAAAATT	GCAGGCCCAT	GGGGAGTTAC
		TGATTTTTTC	ATCTCCTCCC	TCCACGGGAG	ACTTTATTTT	CTGCCAATGG	CTATTGCCAT
		TAGAGGGCAG	AGTGACCCCA	GAGCTGAGTT	GGGCAGGGGG	GTGGACAGAG	AGGAGAGGAC
		AAGGAGGGCA	ATGGAGCATC	AGTACCTGCC	CACAGCCTTG	GTCCCTGGGG	GCTAGACTGC
		TCAACTGTGG	AGCAATTATC	TATACTGAAA	ATGTGCTTGT	TGTTGAAAAT	TTGTCTGCAT
		GTTAATGCCT	CACCCCCAAA	CCCTTTTCTC	TCTCACTCTC	TGCCTCCAAC	TTCAGATTGA
		CTTTCAATAG	TTTTTCTAAG	ACCTTTGAAC	TGAATGTTCT	CTTCAGCCAA	AACTTGGCGA
		CTTCCACAGA	AAAGTCTGAC	CAGTGAAGA	AAGGAGAGCA	GAGATTTTAA	CCTTTGTAAG
		GCCCCATTG	GATCCAGGTC	TGCTTTCTCA	TGTGTGAGTC	AGGGAGGAGC	TGGAGCCAGA
		GGAGAAGAAA	ATGATAGCTT	GGCTGTTCTC	CTGCTTAGGA	CAGTACTGTA	ATAGTTAAAC
		TCTCACTGCC	ACTACCTTTT	CCCCACCTTT	AAAAGACCTG	AATGAAGTTT	TCTGCCAAAC
		TCCGTGAAGC	CACAAGCACC	TTATGTCCTC	CCTTCAGTGT	TTTGTGGGCC	TGAATTTTCAT
		CACACTGCAT	TTCAGCCATG	GTCAATCAAG	CTGTTTGCTT	CTTTTGGGCA	TGTTACACAGA
		TTCTCTGTTA	AGAGCCCCCA	CCACCAAGAA	GGTTAGCAGG	CCAACAGCTC	TGACATCTAT
		CTGTAGATGC	CAGTAGTCAC	AAAGATTTCT	TACCAACTCT	CAGATCTGCT	GAGCCCTTAG
		ACAAACTGGA	AAGAAGGCAT	CAAAGGGATC	AGGCAAGCTG	GGCGTCTTGC	CCTTGTCCCC
		CAGAGATGAT	ACCCTCCCAG	CAAGTGGAGA	AGTTCTCACT	TCCTTCTTTA	GAGCAGCTAA
		AGGGGCTACC	CAGATCAGGG	TTGAAGAGAA	AACTCAATTA	CCAGGGTGGG	AAGAATGAAG
		GCACTAGAAC	CAGAAACCTT	GCAAAATGCT	TTCTTGTGTC	CCAGCATATC	CACCTGCAGA
		AGTCATGAGA	AGAGAGAAGG	AACAAAGAGG	AGACTCTGAC	TACTGAATTA	AAATCTTCAG
		CGGCAAAAGC	TAAAGCCAGA	TGGACACCAT	CTGGTGAGTT	TACTCATCAT	CCTCCTCTGC

		TGCTGATTCT GGGCTCTGAC ATTGCCATA CTCACTCAGA TTCCCCACCT TTGTTGCTGC CTCTTAGTCA GAGGGAGGCC AAACCATTGA GACTTCTAC AGAACCATGG CTTCCTTCGG AAAGGTCTGG TTGGTGTGGC TCCAATACTT TGCCACCCAT GAACTCAGGG TGTGCCCTGG GACACTGGTT TTATATAGTC TTTTGGCACA CCTGTGTTCT GTTGACTTCG TTCTTCAAGC CCAAGTGCAA GGGAAAAATGT CCACCTACTT TCTCATCTTG GCCTCTGCCT CCTTACTTAG CTCTTAATCT CATCTGTTGA ACTCAAGAAA TCAAGGGCCA GTCATCAAGC TGCCCATTTT AATTGATTCA CTCTGTTTGT TGAGAGGATA GTTCTGAGT GACATGATAT GATCCACAAG GGTTTCCTTC CCTGATTCT GCATTGATAT TAATAGCCAA ACGAACTCA AAACAGCTTT AAATAACAAG GGAGAGGGGA ACCTAAGATG AGTAATATGC CAATCCAAGA CTGCTGGAGA AAACTAAAGC TGACAGGTTC CCTTTTGGG GTGGGATAGA CATGTTCTGG TTTTCTTTAT TATTACACAA TCTGGCTCAT GTACAGGATC ACTTTTAGCT GTTTTAAACA GAAAAAATA TCCACCACTC TTTTCAGTTA CACTAGGTTA CATTTTAATA GGTCCCTTAC ATCTGTTTTG GAATGATTTT CATCTTTTGT GATACACAGA TTGAATTATA TCATTTTCTAT ATCTCTCCTT GTAAATACTA GAAGCTCTCC TTTACATTTT TCTATCAAAT TTTTCTCTT TATGGGTTTC CCAAATTGTGA CTCTTGCTCT CATGAATATA TGTTTTTCAT TTGCAAAAGC CAAAAATCAG TGAAACAGCA GTGTAATTAA AAGCAACAAC TGGATTACTC CAAATTTCCA AATGACAAAA CTAGGGAAAA ATAGCCTACA CAAGCCTTTA GGCCTACTCT TTCTGTGCTT GGGTTTGAGT GAACAAAGGA GATTTTAGCT TGGCTCTGTT CTCCCATGGA AGGATTTTTT AGGATTTTTT TTTTCTTTTG GCCATTGATG TTCTAGCCAA TGTAATTGAC AGAAGTCTCA TTTTGCATGC GCTCTGCTCT ACAACAGAG TTGGTATGGT TGGTATACTG TACTCACCTG TGAGGGACTG GCCACTCAGA CCCACTTAGT TGGTGAGCTA GAAGATGAGG ATCACTCACT GGAAAGTCA CAAGGACCAT CTCCAACAA GTTGGCAGTG CTCGATGTGG ACGAAGAGTG AGGAAGAGAA AAAGAAGGAG CACCAAGGAG AAGGCTCCGT CTGTGCTGGG CAGCAGACAG CTGCCAGGAT CACGAACTCT GTAGTCAAAG AAAAGAGTCG TGTGGCAGTT TCAGCTCTCG TTCATTGGGC AGCTCGCCTA GGCCACGCT CTGAGCTGAC ATGGGAGTTG TTGGATTCTT TGTTTCATAG CTTTTCTAT GCCATAGGCA ATATTGTTGT TCTTGAAAG TTTATTATTT TTTTAACTCC CTTACTCTGA GAAAGGATA TTTTGAAGGA CTGTCATATA TCTTTGAAA AAGAAAATCT GTAATACATA TATTTTATG TATGTTCACT GGCCTAATAA AATATAGAGA GCTTCATCTT GTCCTTTGGG TAGTTGCTGA GGTAATTGTC CAGGTTGAAA AATAATGTGC TGATGCTAGA GTCCCTCTCT GTCCATACTC TACTTCTAAA TACATATAGG CATACATAGC AAGTTTTATT TGACTTGTAC TTTAAGAGAA AATATGTCCA CCATCCACAT GATGCACAAA TGAGCTAACA TTGAGCTTCA AGTAGCTCT AAGTGTTTGT TTCATTAGGC ACAGCAGGAA TGGGCTCTTT CCCCCTTCT CTCCCTTGAT ATCTGGCAGG GCATAAAGGC CCAGGCCACT TCCTCTGCCC CTTCCAGGCC CTGCACCAA GCTGCATTTT AGGAGACTCT CTCCAGACAG CCCAGTAACT ACCCGAGCAT GGCCCTGCA TAGCCCTGGA AAAATAAGAG GCTGACTGTC TACGAATTAT CTTGTTCCAG TTGCCCAGGT GAGAGGGCAC TGGGCCAAGG GAGTGGTTTT CATGTTTGAC CCACTACAAG GGGTCATGGG AATCAGGAAT GCCAAAGCAC CAGATCAAA CTCAAACCTTA AAGTCAAAAT AAGCCATTCA GCATGTTTCA TTTCTTGAA AAGGAAGTTT CTACCCCTGA TGCCTTTGTA GGCAGATCTG TTCTACCAT TAATCTTTT GAAAATCTTT TAAAGCAGTT TTTAAAAAGA GAGATGAAAG CATCACATTA TATAACCAA GATTACATTG TACCTGCTAA GATACAAAA TTCATAAGGG CAGGGGGGGA GCAAGCATTA GTGCCTCTTT GATAAGCTGT CCAAAGACAG ACTAAAGGAC TCTGCTGGTG ACTGACTTAT AAGAGCTTGT TGGGTTTTTT TTTCCCTAAT AATATACATG TTTAGAAGAA TTGAAAATAA TTTGCGGAAA ATGGGATTAT GGGTCCTTCA CTAAGTGATT TTATAAGCAG AACTGGCTTT CCTTTTCTCT AGTAGTTGCT GAGCAAATTG TTGAAGCTCC ATCATTGCAT GGTGGAAT GGAGCTGTTC TTAGCCACTG TGTTTGCTAG TGCCCATGTT AGCTTATCTG AAGATGTGAA ACCCTTGCTG ATAAGGGAGC ATTTAAAGTA CTAGATTTG CACTAGAGGG ACAGCAGGCA GAAATCCTTA TTTCTGCCCA CTTTGGATGG CACAAAAAGT TATCTGCAGT TGAAGGCAGA AAGTTGAAAT ACATTGTAAA TGAATATTTG TATCCATGTT TCAAAATTGA AATATATATA TATATATATA TATATATATA TATATATATA TAGTGTGTGT GTGTGTTCTG ATAGCTTTAA CTTTCTCTGC ATCTTTATAT TTGGTTCCAG ATCACACCTG ATGCCATGTA CTTGTGAGAG AGGATGCAGT TTTGTTTTGG AAGCTCTCTC AGAACAACA AGACACCTGG ATTGATCAGT TAACATAAAG TTTTCTCCCC TATTGGGTTT GACCCACAGG TCCTGTGAAG GAGCAGAGGG ATAAAAAGAG TAGAGGACAT GATACATTGT ACTTTACTAG TTCAAGACAG ATGAATGTGG AAAGCATAAA AACTCAATGG AACTGACTGA GATTTACCAC AGGGAAGGCC CAAACTTGGG GCCAAAAGCC TACCCAAGTG ATTGACCAGT GGCCCCCTAA TGGGACCTGA GCTGTTGGAA GAAGAGAAGT GTTCTTGGT CTTCAACATC CTGTGTGAGG AAGGGCAGTT TCCTGCATTG GAACCTGGAG CAAGCGCTCT ATCTTTCACA CAAATTCCCT CACCTGAGAT TGAGGTGCTC TTGTTACTGG GTGTCTGTGT GCTGTAATTC TGGTTTTGGA TATGTTCTGT AAAGATTTTG ACAAATGAAA ATGTGTTTTT CTCTGTTAAA ACTTGTGAGA GTAGTAGAAG TTGTATCTCT GTAGGTGCAG GTCCATTTCT GCCCACAGGT AGGGTGTTTT TCTTTGATTA AGAGATTGAC ACTTCTGTTG CCTAGGACCT CCCAACTCAA CCATTTCTAG GTGAAGGCAG AAAAAATCCAC ATTAGTTACT CCTTCTCAGA CATTTCAGCT GAGATAACAA ATCTTTTGGG ATTTTTCAC CCATAGAAAG AGTGGTAGAT ATTTGAATTT AGCAGGTGGA GTTTCATAGT AAAACAGCT TTTGACTCAG CTTTGATTTA TCCTCATTTG ATTTGGCCAG AAAGTAGGTA ATATGCATTG ATTTGGCTCT GATTCCAATT CAGTATAGCA AGGTGCTAGG TTTTTCCTT TCCCCACCTG TCTCTTAGCC TGGGGAATTA AATGAGAAGC CTTAGAAATGG GTGGCCCTTG TGACCTGAAA CACTTCCAC ATAAGCTACT TAACAAGATT GTCATGGAGC TGCAGATTCC ATTGCCACC AAAGACTAGA ACACACACAT
--	--	---

		ATCCATACAC CAAAGGAAAG ACAATTCTGA AATGCTGTTT CTCTGGTGGT TCCCTCTCTG GCTGCTGCCT CACAGTATGG GAACCTGTAC TCTGCAGAGG TGACAGGCCA GATTTCGATT ATCTCACAAC CTTAGCCCTT GGTGCTAACT GTCCCTACAGT GAAAGTGCCCTG GGGGGTTGTC CTATCCCATAGCCACTTGG ATGCTGACAG CAGCCACCAT CAGAATGACC CACGCAAAAA AAAGAAAAAA AAAATTAAAA AGTCCCCTCA CAACCCAGTG ACACCTTTCT GCTTTCCTCT AGACTGGAAC ATTGATTAGG GAGTGCCTCA GACATGACAT TCTTGTGCTG TCCTTGGAAAT TAATCTGGCA GCAGGAGGGA GCAGACTATG TAAACAGAGA TAAAAATTAA TTTTCAATAT TGAAGGAAAA AAGAAATAAG AAGAGAGAGA GAAAGAAAGC ATCACACAAA GATTTCTTTA AAAGAAACAA TTTTGCTTGA AATCTCTTTA GATGGGGCTC ATTTCTCAGC GTGGCACTTG GCCTCCACTG GGCAGCAGGA CCAGCTCCAA GCGCTAGTGT TCTGTTCTCT TTTTGTAAATC TTGGAATCTT TTGTTGCTCT AAATACAATT AAAAAATGGCA GAAACTTGTT TGTGACTA CATGTGTGAC TTTGGGTCTG TCTCTGCCTC TGCTTTCAGA AATGTCATCC ATTGTGTAAA ATATTGGCTT ACTGGTCTGC CAGCTAAAAAC TTGGCCACAT CCCCTGTTAT GGCTGCAGGA TCGAGTTATT GTTAACAAAG AGACCAAGA AAAGCTGCTA ATGTCCTCTT ATCATTGTTG TTAATTTGTT AAAACATAAA GAAATCTAAA ATTTCAAAAA
57	Human androgen receptor (AR) mRNA, transcript variant 2 (GenBank NM_001011645 .2)	GCTGCGAGCA GAGAGGGATT CCTCGGAGGT CATCTGTTC ATCTTCTTGC CTATGCAAAAT GCCTGCCTGA AGCTGTCTGA GGCTGGCTTT GTACCGGACT TTGTACAGGG AACAGGGAA ACGAATGCAG AGTGCTCCTG ACATTGCCTG TCACCTTTTC CCATGATACT CTGGCTTCAC AGTTTGAGAGA CTGCCAGGGA CCATGTTTTG CCCATTGACT ATTACTTTCC ACCCCAGAAG ACCTGCCTGA TCTGTGGAGA TGAAGCTTCT GGGTGTCACT ATGGAGCTCT CACATGTGGA AGCTGCAAGG TCTTCTTCAA AAGAGCCGCT GAAGGGAAAC AGAAGTACCT GTGCCGCCAG AGAAATGATT GCACATTTGA TAAATCCGA AGGAAAAATT GTCCATCTTG TCGTCTTCGG AAATGTTATG AAGCAGGGAT GACTCTGGGA GCCCGGAAGC TGAAGAACT TGGTAATCTG AACTACAGG AGGAAGGAGA GGCTTCCAGC ACCACCAGCC CCACTGAGGA GACAACCCAG AAGCTGACAG TGTCACACAT TGAAGGCTAT GAATGTCAGC CCATCTTTCT GAATGTCCTG GAAGCCATTG AGCCAGGTGT AGTGTGTGCT GGACACGACA ACAACCAGCC CGACTCCTTT GCAGCCTTGC TCTCTAGCCT CAATGAACG GGAGAGAGAC AGCTTGTATA CGTGGTCAAG TGGGCCAAGG CTTGCCTGG CTTCCGCAAC TTACACGTGG ACAGCAGAT GCCTGTCTATT CAGTACTCCT GGATGGGGCT CATGGTGTTC GCCATGGGCT GGCGATCCTT CACCAATGTC AACTCCAGGA TGCTCTACTT CGCCCTGAT CTGGTTTTCA ATGAGTACCG CATGCACAAG TCCCGGATGT ACAGCCAGTG TGCCGAATG AGGCACCTCT CTCAAGAGTT TGGATGGCTC CAAAATCACC CCCAGGAATT CCTGTGCATG AAAGCACTGC TACTCTTAC CATTATTCCA GTGGATGGG TGAAAAATCA AAAATTCTTT GATGAACTTC GAATGAACATA CATCAAGGAA CTCGATCGTA TCATTGCATG CAAAAGAAAA AATCCCACAT CCTGCTCAAG ACGCTTCTAC CAGCTCACCA AGCTCCTGGA CTCCGTGCAG CCTATTGCGA GAGAGCTGCA TCAGTTCACT TTTGACCTGC TAATCAAGTC ACACATGGTG AGCGTGGACT TTCCGGAAT GATGGCAGAG ATCATCTCTG TGCAAGTGCC CAAGATCCTT TCTGGGAAAG TCAAGCCCAT CTATTTCAC ACCCAGTGAA GCATTGGAAA CCCTATTTCC CCACCCAGC TCAGTCCCTT TTTTCAGATG CTTCTGCCTG TTATAACTCT GCACTACTCC TCTGCAGTGC CTTGGGGAAT TTCTCTATT GATGTACAGT CTGTTCATGAA CATGTTCCCTG AATTCTATTT GCTGGGCTTT TTTTCTCTCT TTCTCTCCTT TCTTTTCTT CTTCCTCCC TATCTAACC TCCCATGGCA CCTTCAGACT TTGCTTCCCA TTGTGGCTCT TATCTGTGTT TTGAATGGTG TTGATGCTT TTAATCTGT GATGATCCTC ATATGGCCCA GTGTCAAGTT GTGCTTGTTC ACAGCACTAC TCTGTGCCAG CCACACAAAC GTTTACTTAT CTTATGCCAC GGGAAAGTTA GAGAGCTAAG ATTATCTGGG GAAATCAAAA CAAAAACAAG CAAACAAAAA AAAAAAGCAA AAACAAAAACA AAAAATAAGC CAAAAAACCT TGCTAGTGT TTTTCTCAA AAATAAATAA ATAAATAAAT AAATACGTAC ATACATACAC ACATACATAC AAACATATAG AAATCCCCAA AGAGGCCAAT AGTGACGAGA AGGTGAAAAAT TGCAGGCCCA TGGGGAGTTA CTGATTTTTT CATCTCCTCC CTCCACGGGA GACTTTATTT TCTGCCAATG GCTATTGCCA TTAGAGGGCA GAGTGACCCC AGAGCTGAGT TGGGCAGGGG GGTGGACAGA GAGGAGAGGA CAAGGAGGGC AATGGAGCAT CAGTACCTGC CCACAGCCTT GGTCCCCTGG GGCTAGACTG CTCAACTGTG GAGCAATTCA TTATACTGAA AATGTGCTTG TTGTTGAAAA TTTGTCTGCA TGTTAATGCC TCACCCCAAC ACCCTTTCT CTCTCACTCT CTGCCTCAA CTTCAGATTG ACTTTCAATA GTTTTTCTAA GACCTTTGAA CTGAATGTTT TCTTCAGCCA AAACCTGGCG ACTTCCACAG AAAAGTCTGA CCACTGAGAA GAAGGAGAGC AGAGATTAA CCCTTTGTAA GGCCCATTT GGATCCAGGT CTGCTTTCTC ATGTGTGAGT CAGGGAGGAG CTGGAGCCAG AGGAGAAGAA AATGATAGCT TGGCTGTCTT CCTGCTTAGG ACACCTGACT AATAGTTAAA CTCTCACTGC CACTACCTTT TCCCACCTT TAAAAGACCT GAATGAAGTT TTCTGCCAAA CTCCGTGAAG CCACAAGCAC CTTATGTCCT CCCTTCAGTG TTTTGTGGG CTGAATTTCA TCACACTGCA TTTCAGCCAT GGTCAATCAAG CCTGTTTGCT TCTTTTGGG ATGTTACAG ATTCTCTGTT AAGAGCCCC ACCACCAAGA AGGTTAGCAG GCCAACAGCT CTGACATCTA TCTGTAGATG CCAGTAGTCA CAAAGATTTT TTACCAACTC TCAGATCGCT GGAGCCCTTA GACAACTGG AAAGAAGGCA TCAAGGGAT CAGGCAAGCT GGGCGTCTTG CCCTGTCCC CCAGAGATGA TACCCTCCCA GCAAGTGGAG AAGTTCTCAC TTCCTTCTT AGAGCAGCTA AAGGGGCTAC CCAGATCAGG GTTGAAGAGA AACTCAATT ACCAGGTGG GAAGAATGAA GGCACATGAA CCAGAAACCC TGCAATGCT CTTCTTGTC CCCAGCATAT CCACCTGCAG AAGTCATGAG AAGAGAGAAG GAACAAAGAG GAGACTCTGA CTACTGAATT AAAATCTTCA GCGGCAAGC CTAAAGCCAG ATGACACCA TCTGGTGAGT TTAATCATCA TCCTCTCTG CTGCTGATTC TGGGCTCTGA CATTGCCCAT

		ACTCACTCAG	ATTCCCCACC	TTTGTGCTG	CCTCTTAGTC	AGAGGGAGGC	CAAACCATG
		AGACTTTCTA	CAGAACCATG	GCTTCTTTTCG	GAAAGGTCTG	GTGGGTGTGG	CTCCAATACT
		TTGCCACCCA	TGAACTCAGG	GTGTGCCCTG	GGACACTGGT	TTTATATAGT	CTTTTGGCAC
		ACCTGTGTTT	TGTTGACTTC	GTTCTTCAAG	CCCAAGTGCA	AGGGAAAAATG	TCCACCTACT
		TTCTCATCTT	GGCCTCTGCC	TCCTTACTTA	GCTCTTAATC	TCATCTGTG	AACTCAAGAA
		ATCAAGGGCC	AGTCATCAAG	CTGCCCATTT	TAATTGATTC	ACTCTGTTTG	TTGAGAGGAT
		AGTTTCTGAG	TGACATGATA	TGATCCACAA	GGGTTTCCTT	CCCTGATTTC	TGCATTGATA
		TTAATAGCCA	AACGAACTTC	AAAACAGCTT	TAAATAACAA	GGGAGAGGGG	AACCTAAGAT
		GAGTAATATG	CCAATCCAAG	ACTGCTGGAG	AAAACATAAG	CTGACAGGTT	CCCTTTTTTG
		GGTGGGATAG	ACATGTTCTG	GTTTCTTTTA	TTATTACACA	ATCTGGCTCA	TGTACAGGAT
		CACTTTTAGC	TGTTTTAAAC	AGAAAAAAT	ATCCACCACT	CTTTTCAGTT	ACACTAGGTT
		ACATTTTAAT	AGGTCCTTTA	CATCTGTTTT	GGAATGATTT	TCATCTTTTG	TGATACACAG
		ATTGAATTAT	ATCATTTTCA	TATCTCTCCT	TGTAATAACT	AGAAGCTGTC	CTTTACATTT
		CTCTATCAAA	TTTTTCATCT	TTATGGGTTT	CCCAATTGTG	ACTCTTGCTC	TCATGAATAT
		ATGTTTTTCA	TTTGCAAAAG	CCAAAAATCA	GTGAAACAGC	AGTGTAATTA	AAAGCAACAA
		CTGGATTACT	CCAAATTTCC	AAATGACAAA	ACTAGGGAAA	AATAGCCTAC	ACAAGCCTTT
		AGGCCTACTC	TTTCTGTGCT	TGGGTTTGAG	TGAACAAAGG	AGATTTTAGC	TTGGCTCTGT
		TCTCCCATGG	ATGAAAGGAG	GAGGATTTTT	TTTTTCTTTT	GGCCATTGTC	GTTCTAGCCA
		ATGTAATTGA	CAGAAGTCTC	ATTTTGCATG	CGCTCTGCTC	TACAAACAGA	GTTGGTATGG
		TTGTATACT	GTACTCACCT	GTGAGGGACT	GGCCACTCAG	ACCCACTTAG	CTGGTGAGCT
		AGAAGATGAG	GATCACTCAC	TGGAAAAGTC	ACAAGGACCA	TCTCCAAACA	AGTTGGCAGT
		GCTCGATGTG	GACGAAGAGT	GAGGAAGAGA	AAAAGAAGGA	GCACCAAGGA	GAAGGCTCCG
		TCTGTGCTGG	GCAGCAGACA	GCTGCCAGGA	TCACGAACCTC	TGTAGTCAAA	GAAAGAGATC
		GTGTGGCAGT	TTCAGCTCTC	GTTCAATTGGG	CAGCTCGCCT	AGGCCAGGCC	TCTGAGCTGA
		CATGGGAGTT	GTTGGATTCT	TTGTTTCATA	GCTTTTCTTA	TGCCATAGGC	AATATTGTTG
		TTCTTGGA	GTTTATTATT	TTTTTAACTC	CCTTACTCTG	AGAAAGGGAT	ATTTTGAAGG
		ACTGTCATAT	ATCTTTGAAA	AAAGAAAATC	TGTAATACAT	ATATTTTTAT	GTATGTTTAC
		TGGCACTAAA	AAATATAGAG	AGCTTTCATT	TGTCCTTTGG	GTAGTTGTG	AGGTAATTGT
		CCAGGTTGAA	AAATAATGTG	CTGATGCTAG	AGTCCCTCTC	TGTCCATACT	CTACTTCTAA
		ATACATATAG	GCATACATAG	CAAGTTTTAT	TTGACTTGTA	CTTTAAGAGA	AAATATGTCC
		ACCATCCACA	TGATGCACAA	ATGAGCTAAC	ATTGAGCTTC	AAGTAGCTTC	TAAGTGTTTG
		TTTCATTAGG	CACAGCACAG	ATGTGGCCTT	TCCCCCTTTC	TCTCCCTTGA	TATCTGGCAG
		GGCATAAAGG	CCCAGGCCAC	TTCTCTGCCC	CCTTCCCAGC	CCTGCACCAA	AGCTGCATTT
		CAGGAGACTC	TCTCCAGACA	GCCCAGTAAC	TACCCGAGCA	TGGCCCCCTG	ATAGCCCTGG
		AAAAATAAGA	GGCTGACTGT	CTACGAATTA	TCTTGTGCCA	GTTGCCCAAG	TGAGAGGGCA
		CTGGGCCAAG	GGAGTGGTTT	TCATGTTTGA	CCCACTACAA	GGGGTCATGG	GAATCAGGAA
		TGCCAAAGCA	CCAGATCAAA	TCCAAAACCT	AAAGTCAAAA	TAAGCCATTC	AGCATGTTCA
		GTTTCTTGGA	AAAGGAAGTT	TCTACCCCTG	ATGCCTTTGT	AGGCAGATCT	GTTCTCACCA
		TTAATCTTTT	TGAAAATCTT	TTAAAGCAGT	TTTTAAAAAG	AGAGATGAAA	GCATCACATT
		ATATAACCAA	AGATTACATT	GTACCTGCTA	AGATACCAAA	ATTCATAAGG	GCAGGGGGGG
		AGCAAGCATT	AGTGCCCTCT	TGATAAGCTG	TCCAAAGACA	GACTAAAGGA	CTCTGCTGGT
		GACTGACTTA	TAAGAGCTTT	GTGGGTTTTT	TTTTCCCTAA	TAATATACAT	GTTTAGAAGA
		ATTGAAAATA	ATTTCCGGAA	AATGGGATTA	TGGGTCCTTC	ACTAAGTGAT	TTTATAAGCA
		GAACTGGCTT	TCCTTTTCTC	TAGTAGTTGC	TGAGCAAATT	GTTGAAGCTC	CATCATTGCA
		TGGTTGGAAA	TGGAGCTGTT	CTTAGCCACT	GTGTTTGCTA	GTGCCCATGT	TAGCTTATCT
		GAAGATGTGA	AACCCTTGCT	GATAAGGGAG	CATTTAAAGT	ACTAGATTTT	GCACTAGAGG
		GACAGCAGGC	AGAAAATCCT	ATTTCTGCCC	ACTTTTGATG	GCACAAAAAG	TTATCTGCAG
		TTGAAGGCAG	AAAGTTGAAA	TACATTGTAA	ATGAATATTT	GTATCCATGT	TTCAAAATTG
		AAATATATAT	ATATATATAT	ATATATATAT	ATATATATAT	ATAGTGTGTG	TGTGTGTTCT
		GATAGCTTTA	ACTTTCTCTG	CATCTTTTATA	TTTGGTTCCA	GATCACACCT	GATGCCATGT
		ACTTGTGAGA	GAGGATGCAG	TTTTGTTTTG	GAAGCTCTCT	CAGAACAAAC	AAGACACCTG
		GATTGATCAG	TTAACTAAAA	GTTTTCTCCC	CTATTGGGTT	TGACCCACAG	GTCTGTGAA
		GGAGCAGAGG	GATAAAAAAG	GTAGAGGACA	TGATACATTG	TACTTTACTA	GTTCAAGACA
		GATGAATGTG	GAAAGCATAA	AAACTCAATG	GAACTGACTG	AGATTTTACCA	CAGGGAAGGC
		CCAAACTTGG	GGCCAAAAGC	CTACCCAAGT	GATTGACCAG	TGGCCCCCTA	ATGGGACCTG
		AGCTGTTGGA	AGAAGAGAAC	TGTTCTTTGG	TCTTCACCAT	CCTTGTGAGA	GAAGGGCAGT
		TTCTGTCATT	GGAACCTGGA	GCAAGCGCTC	TATCTTTCAC	ACAAATTTCC	TCACCTGAGA
		TTGAGGTGCT	CTTGTTACTG	GGTGTCTGTG	TGCTGTAATT	CTGGTTTTGG	ATATGTTCTG
		TAAAGATTTT	GACAAATGAA	AATGTGTTTT	TCTCTGTTAA	AACTTGTGAG	AGTACTAGAA
		GTTGTATCTC	TGTAGGTGCA	GGTCCATTTT	TGCCCACAGG	TAGGGTGTGT	TTCTTTGATT
		AAGAGATTGA	CACCTTCTGT	GCCTAGGACC	TCCCAACTCA	ACCATTCTTA	GGTGAAGGCA
		GAAAAATCCA	CATTAGTTAC	TCCTCTTCAG	ACATTTTCAGC	TGAGATAACA	AATCTTTTGG
		AATTTTTTCA	CCCATAGAAA	GAGTGGTAGA	TATTTGAATT	TAGCAGGTGG	AGTTTCATAG
		TAAAAACAGC	TTTTGACTCA	GCTTTGATTT	ATCCTCATTT	GATTTGGCCA	GAAAGTAGGT
		AATATGCATT	GATTGGCTTC	TGATTCCAAT	TCAGTATAGC	AAGGTGCTAG	GTTTTTTCCT
		TTCCCCACCT	GTCTCTTAGC	CTGGGGAATT	AAATGAGAAG	CCTTAGAATG	GGTGGCCCTT
		GTGACCTGAA	ACACTTCCCA	CATAAGCTAC	TTAAACAAGAT	TGCTATGGAG	CTGCAGATTC
		CATTGCCCCA	CAAAGACTAG	AACACACACA	TATCCATACA	CCAAAGGAAA	GACAATTCTG

		AAATGCTGTT TCTCTGGTGG TTCCCTCTCT GGCTGCTGCC TCACAGTATG GGAACCTGTA CTCTGCAGAG GTGACAGGCC AGATTTCAT TATCTCACA CCTTAGCCCT TGGTGCTAAC TGTCCTACAG TGAAGTGCCCT GGGGGGTTGT CCTATCCCAT AAGCCACTTG GATGCTGACA GCAGCCACCA TCAGAATGAC CCACGCAAAA AAAAGAAAA AAAAATTAAA AAGTCCCCTC ACAACCCAGT GACACCTTTC TGCTTTCCCTC TAGACTGGAA CATTGATTAG GGAGTGCCTC AGACATGACA TTCTTGTGCT GTCCTTGGAA TTAATCTGGC AGCAGGAGGG AGCAGACTAT GTAAACAGAG ATAAAAATTA ATTTTCAATA TTGAAGGAAA AAAGAAATAA GAAGAGAGAG AGAAAGAAAAG CATCACACAA AGATTTTCTT AAAAGAAAACA ATTTTGCTTG AAATCTCTTT AGATGGGGCT CATTCTCTAC GGTGGCACTT GGCCCTCCACT GGGCAGCAGG ACCAGCTCCA AGCGCTAGTG TTCTGTTCTC TTTTGTGAAT CTTGGAATCT TTTGTTGCTC TAAATACAAT TAAAAATGGC AGAAACTTGT TTGTTGGACT ACATGTGTGA CTTTGGGTCT GTCTCTGCCT CTGCTTTCAG AAATGTCATC CATGTGTGTA AATATTGGCT TACTGGTCTG CCAGCTAAAA CTTGCCACA TCCCTGTTA TGGCTGCAGG ATCGAGTTAT TGTTAACAAA GAGACCCAAG AAAAGCTGCT AATGTCCTCT TATCATTGTT GTTAATTGTT TAAACATAA AGAAATCTAA AATTTCAAAA AA
50	Human uroplakin 1B (UPK1B) mRNA	ACCTGGGTCG GGTGCAGACT GCGGAGCGGG CCCTACCGTG TGCGCAGAAA GAGGAGGCGC TTGCCTTCAG CTTGTGGGAA ATCCCGAAGA TGGCCAAAGA CAACTCAACT GTTCGTTGCT TCCAGGGCCT GCTGATTTT GGAATGTGA TTATTGGTTG TTGCGGCATT GCCCTGACTG CGGAGTGCAT CTTCTTTGTA TCTGACCAAC ACAGCCTCTA CCCACTGCTT GAAGCCACCG ACAACGATGA CATCTATGGG GCTGCCTGGA TCGGCATATT TGTGGGCATC TGCTCTTCT GCCTGTCTGT TCTAGGCATT GTAGGCATCA TGAAGTCCAG CAGGAAAAAT CTCTGGCGT ATTTCACTCT GATGTTTATA GTATATGCCCT TTGAAGTGGC ATCTTGTATC ACAGCAGCAA CACACAAGA CTTTTTACA CCCAACCTCT TCCTGAAGCA GATGCTAGAG AGGTACCAAA ACAACAGCCC TCCAAACAAT GATGACCAGT GGA AAAACA TGGAGTCACC AAAACCTGGG ACAGGCTCAT GCTCCAGGAC AATTGCTGTG GCGTAAATGG TCCATCAGAC TGGCAAAAAT ACACATCTGC CTTCCGGACT GAGAATAATG ATGCTGACTA TCCCTGGCCT CGTCAATGCT GTGTTATGAA CAATCTTAAA GAACCTCTCA ACCTGGAGGC TTGTAAACTA GCGTGCCTG GTTTTATCA CAATCAGGCG TGCTATGAAC TGATCTCTGG TCCAATGAAC CGACACGCT GGGGGGTTGC CTGGTTTGA TTTGCCATT CTTGCTGGAC TTTTGGGT CTCTGGGTA CCATGTTCTA CTGGAGCAGA ATTGAATATT AAGCATAAAG TGTGGCCACC ATACCTCCTT CCCCGAGTGA CTCTGGATTT GGTGCTGGAA CCAGCTCTCT CTAATATTC CACGTTTGTG CCCCACACTA ACGTGTGTGT CTTACATTGC CAAGTCAGAT GGTACGGAT TCCTTTAGGA TCTCAGGCTT CTGAGTTCT CATGACTCCT ACTTTTCATC CTAGTCTAGC ATTTGCAAC ATTTATATAG ACTGTTGAAA GGAGAATTTG AAAAATGCAT AATAACTACT TCCATCCCTG CTTATTTTTA ATTTGGGAAA ATAAATACAT TCGAAGGAAC CTGTGTTATC ACAGTAACCC AGAGCTGTAT TTGGCTAGCA ATCTGCCTGT ATCTCTCACT ATTATCTAAA AGAAACCTTC CAATGCTTCT GTTGATCTCA GTATTGTCAG GGAACAGAG AAGTTGGGA AAGATTACTG AAATATACCT TTTGCATTTC TTTCTAGAGT AGCTCCATA TATGGAGATG GGTGATCTC TTGATGCCAC CTTCAGATCC TTTTATTCTC CAGAATAATT CTTAACAGTG GTTCAAATTT CCTTTCATAC CTTGAAGTAT GTGTTTAGTA GCCTCAATTC TCCATTAAT AAAAGTGTGG GCTGGGCGTG GGGGCTCATG CCTGTAATCC CAGCACTTTG GGAGGCCGAG GTGGGCAGAT CACCTGAGGT CAGGAGTTCA AGACCAGCCT GGCCAACATG GTGAAACCCC CTCTCTACAA AAATACAAAA ATTAGCCAGG CGTGATGGCA GGTGCCTGTA ATCCTAGCTA CTTGGCAGGC TAACGCAGGA GAATCACTTG ACCGGGAGAC AGAGGTGCA GTGAGCTGAG ATCGTACCTA TTGCACTCCA TCCTGGATGA AAGAGCCAGA CTCTGTCTCA AAACAAACAA AAAAGCGTGG GGACTTCTGG GGACAGACAA GGTGCCTGTT ATATATTTAC TCAGTCTTTG CCTGAATGG TCTCAGCTTG AGACCATTTT AAACGGAGA GAAGCAAGCC AGCCAATGAT ATGGGGTGAT TTACAGGGAT TTCTGTTTAC TGTCAAAAATA TTTCTCATCT GCACATATGT TCCATTTGTG GTCTGAAGG AAATCTTAT AACTCAACAT TTGTCTGGTC TTATAAGTAA AGACAGCTTT AAAACTGTT CACTTTCAAA
58	Human uroplakin 2 (UPK2) mRNA	ACTTGCTCA GGAACCCAG CCTGCCAGCA CCTATTCCAC CTCCCAGCCC AGCATGGCAC CCCTGCTGCC CATCCGACC TTGCCCTTGA TCCTGATTCT GCTGGCTGCG CTGTCCCAG GGGCTGCAGA CTTCAACATC TCAAGCCTCT CTGGTCTGCT GTCCCCGTCG CTAACGCAGA GCCTGCTGGT TGCCTTGCCC CCCTGTCACC TCACAGGAGG CAATGCCACA CTGATGGTCC GGAGAGCCAA TGACAGCAA GTGGTGACGT CCAGCTTGT GGTGCCTCCG TGCCGTGGGC GCAGGGAAC TGTGAGTGT GTGGACAGTG GTGCTGGCTT CACAGTCACT CGGCTCAGTG CATACCAGG GACAAACCTC GTGCCAGGAA CCAAATCTA CATTTCTACT CTAGTGAAGA AGGGGACAG CACTGAGTCC AGCAGAGAGA TCCCAATGTC CACACTCCCT CGAAGGAACA TGGAATCCAT TGGGCTGGGT ATGGCCCGCA CAGGGGGCAT GGTGGTCACT ACGGTGCTGC TCTCTGTCGC CATGTTCTG CTGGTGCTGG GCTTCATCAT TGCCCTGGCA CTGGGCTCCC GCAAGTAAGG AGGTCTGCCC GGAGCAGCAG CTTCTCCAGG AAGCCCAGGG CACCATCCAG CTCCCAGCC CACTGCTCC CAGGCCCCAG GCCTGTGGCT CCCTTGGTGC CTTGCTCTCC TCCTCCTGCC CTCCTCTCCC CTAGAGCCCT CTCCTCCCTC TGTCCCTCTC CTTGCCCCCA GTGCTCACC TTCCAACACT CCATTATTCC TCTCACCCCA CTCCTGTGAG AGTTGACTTT CCTCCATTT TACCACCTTA AACACCCCA TAACAATTC CCCATCCTTC AGTGAACATA GTCCCTATAA TAAAGGCTGA GGCTGCATCT GCCAAAAAA AAAAAA
59	Human	GGTCTGCAGC GCAAGCGCAG TGCGGATAAA CAGGAAGCGG GCGGTGGAGG CAGCAGCAGA

meningioma expressed antigen 5 (MGEA5) mRNA, transcript variant 1	GGGAGAGCTC	GGGGCTTGGA	GGGGAACACG	CGGAAGACCT	AAGATTATCG	GGAGGGCAGC
	AGAGGCAGAG	AACGAGGACA	GGACCCTTGG	CCGTCTTCTT	CCAGGGAACG	AGAGGTACAA
	GCCTCGCTCT	CCGCTTAGGC	TTCTGGCGCC	CCAGCTTAAA	GCCGAGGTG	CCGGTGACAA
	AGGGCTCGCG	CCGGTGCCGC	CGCCCTTCTC	ATCCGGGCAT	TCGGGTCCCT	GCGGAGAGGG
	AGGGGGAAGG	GCAGAGGGGG	AGGGGAAGGA	GCCGAGGGGG	CGCACACTTG	GAGCTGAAGC
	CCTCTCCAGG	GCTCCGGGCC	GGTGCCCCAA	CGGACAGAGG	TCGAGGAGGA	CCCGCAGAGG
	TGGCAGCGGC	CGGGGGCAGG	AGGATGGTGC	AGAAGGAGAG	TCAAGCGACG	TTGGAGGAGC
	GGGAGAGCGA	GCTCAGCTCC	AACCCCTGCCG	CCTCTGCGGG	GGCATCGGTG	GAGCCGCGCG
	CAGCTCCGGC	ACCCGGGAGAA	GACAACCCCG	CCGGGGCTGG	GGGAGCGGCG	GTGGCCGGGG
	CTGCAGGAGG	GGCTCGGCGG	TTCTCTGCGG	GTGTGGTGGA	AGGATTTTAT	GGAAAGACCTT
	GGGTTATGGA	ACAGAGAAAA	GAACCTTTTA	GAAGGCTCCA	GAAATGGGAA	TTAAATACAT
	ACTTGTATGC	CCCAAAAGAT	GACTACAAAC	ATAGGATGTT	TTGGCGAGAG	ATGTATTACG
	TGGAGGAAGC	TGAGCAACTT	ATGACTCTCA	TCTCTGCTGC	ACGAGAATAT	GAGATAGAGT
	TCATCTATGC	GATCTCACCT	GGATTGGATA	TCACCTTTTC	TAACCCCAAG	GAAGTATCCA
	CATTGAAACG	TAAATTGGAC	CAGGTTTCTC	AGTTTGGGTG	CAGATCATTT	GCTTTGCTTT
	TTGATGATAT	AGACCATAAT	ATGTGTGCAG	CAGACAAAGA	GGTATTCAGT	TCTTTTGCTC
	ATGCCCAAGT	CTCCATCACA	AATGAAATCT	ATCAGTACCT	AGGAGGCCA	GAACCTTTCC
	TCTTCTGTCC	CACAGAAATAC	TGTGGCACCT	TCTGTATATC	AAATGTGTCT	CAGTCTCCAT
	ATTTAAGGAC	TGTGGGTGAA	AAGCTTCTAC	CTGGAATTGA	AGTGCTTTGG	ACAGGTCCCA
	AAGTTGTTTT	TAAAGAAATT	CCAGTAGAGT	CCATCGAAGA	GGTTTCTAAG	ATTATTAAGA
	GAGCTCCAGT	AATCTGGGAT	AACATTCATG	CTAATGATTA	TGATCAGAAG	AGACTGTTTC
	TGGGCCCGTA	CAAAGGAAGA	TCCACAGAAC	TCATCCCACG	GTTAAAGGA	GTCTCTACTA
	ATCCAAATTG	TGAATTTGAA	GCCAACTACG	TTGCTATCCA	CACCCCTGCC	ACCTGGTACA
	AATCAAACAT	GAATGGAGTG	AGAAAAGATG	TAGTGATGAC	TGACAGTGAA	GATAGTACTG
	TGTCCATCCA	GATAAAATTA	GAAAATGAAG	GCAGTGATGA	AGATATTGAA	ACTGATGTAC
	TCTATAGTCC	ACAGATGGCT	CTAAAGCTAG	CATTAACAGA	ATGGTTGCAA	GAGTTTGGTG
	TGCCTCATCA	ATACAGCAGT	AGGCAAGTTG	CACACAGTGG	AGCTAAAGCA	AGTGTAGTTG
	ATGGGACTCC	TTTAGTTGCA	GCACCTCTTT	TAAATGCCAC	AACCGTAGTA	ACAACAGTTT
	ATCAGGAGCC	CATTATGAGC	CAGGGAGCAG	CCTTGAGTGG	TGAGCCTACT	ACTCTGACCA
	AGGAAGAAGA	AAAGAAACAG	CCTGATGAAG	AACCCATGGA	CATGGTGTG	GAAAAACAAG
	AAGAAACGGA	CCACAAGAA	GACAATCAAA	TACTGAGTGA	AATTGTTGAA	GCGAAAAATGG
	CAGAGGAATT	GAAACCAATG	GACACTGATA	AAGAGAGCAT	AGCTGAATCA	AAATCCCCAG
	AGATGTCCAT	GCAAGAAGAT	TGTATTAGTG	ACATTGCCCC	CATGCAAACT	GATGAACAGA
	CAAACAAGGA	GCAGTTTGTG	CCAGGTCCAA	ATGAAAAGCC	TTTGTACACT	GCGGAACCAG
	TGACCCTGGA	GGATTTGCAG	TTACTTGCTG	ATCTATTCTA	CCTTCCTTAC	GAGCATGGAC
	CCAAAGGAGC	ACAGATGTTA	CGGGAATTTT	AATGGCTTCG	AGCAAAAGT	AGTGTGTCTA
	GTGTCAATTG	CAAAGGAAAA	GACTCTGAAA	AAATTGAAGA	ATGGCGGTCA	CAGACAGCCA
	AGTTTGAAGA	GATGTGTGGA	CTAGTGATGG	GAATGTTTAC	TCGGCTCTCC	AATTGTGCCA
	ACAGGACAAT	TCTTTATGAC	ATGTACTCCT	ATGTTTGGGA	TATCAAGAGT	ATAATGTCTA
	TGGTGAAGTC	TTTTGTACAG	TGGTTAGGGT	GTCGTAGTCA	TTCTTCAGCA	CAATTCCTTA
	TTGGAGACCA	AGAACCCTGG	GCCTTTAGAG	GTGGTCTAGC	AGGAGAGTTC	CAGCGTTTGG
	TGCCAATTGA	TGGGGCAAAT	GATCTCTTTT	TTCAGCCACC	TCCACTGACT	CCTTACCTCA
	AAGTTTATAC	TATCAGACCT	TATTTTCCTA	AGGATGAGGC	ATCCGTGTAC	AAGATTTGCA
	GAGAAATGTA	TGACGATGGA	GTGGGTTTAC	CCTTTCAAAG	TCAGCCTGAT	CTTATTGGAG
	ACAAGTTAGT	AGGAGGGCTG	CCTTCCCTCA	GCCTGGATTA	CTGCTTTTGC	CTAGAAGATG
	AAGATGGCAT	ATGTGGTTAT	GCCTTGGGCA	CTGTAGATGT	GACCCCTTTT	ATTAAAAAAT
	GTAAAATTTT	CTGGATCCCC	TTCATGCAAG	AGAAGTATAC	CAAGCCAAAT	GGTGACAAGG
	AACTCTCTGA	GGCTGAGAAA	ATAATGTTGA	GTTTCCATGA	AGAACAGGAA	GTACTGCCAG
	AACTTTTCTT	TGCTAATTTT	CCTTCTCTGA	TAAAGATGGA	CATTACAAAA	AAAGTAACTG
	ACCCAAGTGT	GGCCAAAAGC	ATGATGGCTT	GCCTCCTGTC	TTCACCTGAG	GCTAATGGCT
	CCCGGGGAGC	TTTCTGTGAA	GTGAGACCAG	ATGATAAAAG	AATTCTGGAA	TTTTACAGCA
	AGTTAGGATG	TTTTGAAATT	GCAAAAATGG	AAGGATTTCC	AAAGGATGTG	TTTACTATTG
	GTGCGAGCCT	GTGACATTGG	TTGACACTGT	GAACGTGTCC	AAAGTCTCTT	AACTGCACCT
	TGTGAATGGT	AGTTGAGGTC	TTCATACAGT	TCAGCCTCTA	GAATGGTAAC	AAATCAGCCA
	ATTGGATTCC	AAACAAAGAA	GACTATGTAA	AACTCACCCA	TCACACTTTG	AGACTACTCA
	CTGGTTGGAA	GAATATAGTA	TTGCAGCAAA	TCCTGTATGA	AAGAGAGATG	TGGGCTTCCT
	TTTTGAGTCT	TGTGTTAGGT	GCTGAGACCT	TTTACATGGG	CTTATACAGG	GAGAGAGTCT
	TCAATAAATG	TAGTCAGCAC	TATTTTCTGC	ATCCAGTGTG	GTTGCGTTTC	TCACCTGAGA
	GTAATCAAGA	TAACATCTGT	CATCTTCCTT	GGTTTATTGA	GTGAAATGCC	TCTCAGTCTT
	AGGGGACATG	GCAGAGATGA	AAGAAAAGAAA	GAGTGGGTTT	CAGAAGTGTG	AGGGTGGAGT
	GATTCCAAGT	GGGATGGTTG	TGGCATTAGT	TTAAGCTGAA	TAAATAATTT	CAATTTGGGG
	CAGTTATTCT	GCTTTTGTGA	AAGCCGTGGC	CAATTGTCTC	CTGTAATGAC	GTTGGTTTCA
	GGCATGTTGT	ACTTTGTAGG	GACAAATGTG	CATTTGTTTG	TGGCAAAAGC	CTACAATTGA
	CAAACCTGTA	AATTTCTTTG	TATATAAACT	AGCTGTAACC	TGACTATCCT	TTGTGTTTAC
	TGTTTTTGTG	AATTTTTTTC	CTCTATAAAT	GAAAGGGTGT	TGGTTTCAGAA	TGGCACTTTG
	AATAATGTAA	ACCAGTGAAA	AGTGGATTTT	CTTTACTTTT	GTCTTTGGGT	TTGGGGTTGT
	TTTTGTCTTT	TTTGAAGTTT	TATTATTTTT	AAAGTGCCCT	CCACCTAGGC	GTAGGCCATG
	ACCATTGGGG	GTACGAGAGC	CTAATTTTGT	AGGACTTAAT	CTGTTGAAAA	GTGCAGTTAC

		TTCTGGAAT TAACCTCAAT ATTAGGTCAG CATGTGAAAT GTTGGATTG ACATGTCAGG TAGGGTTCAG GGACTGATTG GTCCCATTTG CCCTCAGGTC AGTTGTTTAA TCTCAAGACC TGTTACTACT GATTTTATTA AATCAGAGTC TTTAATTCTT GCATGTTTGT ATCTAATTTT TGAACGAATG AGCACACTTT AACCAGTTAT TTACAGTTAC CTTTTTCCTT TAACCGGATT GTGAAAGCTT CATGTATTTT AATTAGATT CTGTGTTTTT AAGGGTCTG AGCATGAAGC TGGCAGATAG TCGGCAGGAC TCATTTTTTC ATCATGGCTG GCTGATTTCT CCATAGATTG ATAACAGTAT TTTGTTATCT TGCTTCTCTG TAGTTTTGCA TCAGCTGTTT AACTTTGAGC TGAGTGAGGG GAGAGGGGTA AAGAGAAAAGA AACTTAAGTT TCTTTTCACA GAACGCCACC ATTGTGGGCT TTGAGAGAGC CCTAAAGCAT TGTACCTAGT GGTACCTAGT GACTTCCAAC CAAAGCCTTT GAGTATGCAC TAAATAGGTG AGAAGAAAGG AGAGAAGGTT TTTAGGTTAG AAACCTTTAA CCGATAGAAG GATATGGTAT GTTGTAAAGC TGAACCAAG TTTGCATTTT TGAGGGCTTG AGATGAAGGG AAGACTCTTA CCAGATAGTA AGACAGCTGA GTTTTCCTCA GTTTTCTCGT CTTAACACTA GTGGACAATT CTAGCATTTT GTTTGGAGGA TTTGAGAGTT AACCTCATGG AATTTCAGGAT TTTTTCAGCA GTTTGCTTTT GGTTTTATCT TGGCTTTTAG TAATCATGTT GGCTGGTCTG GTCACAGGTG ACTGTGAAAC AGATGCCCTG GTCTTGCTTT CATCACTCTA GGATCATGAA GTGCTATGCT ATTTCTGCTT TATGAATATT AAGGTTGGAA TTACATTTTT ATTGATTGTT TGGATCAGAG CTCAGTTCCT GTAGAAAACG AACTGTAAAA GACCATGCAA GAGGCAAAAT AAAACTTGAA GTGAATGCTT GTAAAAAAA AAAAAAAAAA
60	Human meningioma expressed antigen 5 (MGEA5) mRNA, transcript variant 2	GGTCTGCAGC GCAAGCGCAG TCGGATAAAA CAGGAAGCGG GCGGTGGAGG CAGCAGCAGA GGGAGAGCTC GGGGCTTGGA GGGGAAACAG CCGAAGACCT AAGATTATCG GGAGGGCAGC AGAGGCAGAG AACGAGGACA GGAACCTTGG CCGTCTTCTT CCAGGGAACG AGAGGTCACA GCCTCGCTCT CCGCTTAGGC TTCTGGCGCC CCAGCTTAAA GCCGAGGCTG CCGCTGACAA AGGGCTCGCG CCGGTGCGCG CGCCTTCTC ATCCGGGCAT TCGGGTCCCT GCGGAGAGGG AGGGGAAGG GCAGAGGGGG AGGGGAAGGA GCCGGAGGGG CGCACACTTG GAGCTGAAGC CCTCTCCAGG GCTCCGGGCC GGTGCCCCAA CGGACAGAGG TCGAGGAGGA CCCGAGAGG TGGCAGCGCG CGGGGGCAGG AGGATGGTGC AGAAGGAGAG TCAAGCCAGC TTGGAGGAGC GGGAGAGCGA GCTCAGCTCC AACCTGCGG CCTCTGCGGG GGCATCGCTG GAGCCGCCGG CAGCTCCGGC ACCCGGAGAA GACAACCCCG CCGGGGCTGG GGGAGCGCGG GTGGCCGGGG CTGCAGGAGG GGCTCGGCGG TTCTCTGCG GTGTGGTGGA AGGATTTTAT GGAAGACCTT GGTTATGGA ACAGAGAAAA GAACTCTTTA GAAGGCTCCA GAAATGGGAA TTAATACAT ACTTGATGCG CCCAAAAGAT GACTACAAAC ATAGGATGTT TTGGCGAGAG ATGTATTCAG TGGAGGAAGC TGAGCAACTT ATGACTCTCA TCTCTGCTGC ACGAGAATAT GAGATAGAGT TCATCTATGC GATCTCACCT GGATTGGATA TCACTTTTTC TAACCCCAAG GAAGTATCCA CATTGAAACG TAAATTGGAC CAGGTTTCTC AGTTTGGGTG CAGATCATTT GCTTTGCTTT TTGATGATAT AGACCATAAT ATGTGTGCAG CAGACAAAGA GGTATTCAGT TCTTTTGCTC ATGCCCAAGT CTCCATCACA AATGAAATCT ATCAGTACCT AGGAGAGCCA GAAACTTTCC TCTTCTGTCC CACAGAATAC TGTGGCACTT TCTGTTATCC AAATGTGTCT CAGTCTCCAT ATTTAAGGAC TGTGGGTGAA AAGCTTCTAC CTGGAATTGA AGTGCTTTGG ACAGGTCCCA AAGTTGTTTC TAAAGAAATT CCAGTAGAGT CCATCGAAGA GGTTCCTAAG ATTATTAAGA GAGCTCCAGT AATCTGGGAT AACATTCATG CTAATGATTA TGATCAGAAG AGACTGTTT TGGGCCCGTA CAAAGGAAGA TCCACAGAAC TCATCCACG GTTAAAGGA GTCTCCTACTA ATCCAAATTG TGAATTTGAA GCCAACTACG TTGCTATCCA CACCCTTGCC ACCTGGTACA AATCAAACAT GAATGGAGTG AGAAAAGATG TAGTGATGAG TAGGCAAGTT GCACACAGTG GAGCTAAAGC AAGTGTAGTT GATGGGACTC CTTTAGTTGC AGCACCTCT TTAATGCCA CAACCGTAGT AACACAGTT TATCAGGAGC CCATTATGAG CCAGGAGCA GCCTGAGTG GTGAGCCTAC TACTCTGACC AAGGAAGAAG AAAAGAAACA GCCTGATGAA GAACCCATGG ACATGGTGGT GGAAAAACAA GAAGAAACGG ACCACAAGAA TGACAATCAA ATACTGAGTG AAATGTTTGA AGCGAAAATG GCAGAGGAAT TGAAACCAAT GGACACTGAT AAAGAGAGCA TAGCTGAATC AAAATCCCCA GAGATGTCCA TGCAAGAAGA TTGTATTAGT GACATTGCCC CCATGCAAAAC TGATGAACAG ACAACAAGG AGCAGTTTGT GCCAGGTCCA AATGAAAAGC CTTTGTACAC TCGGGAACCA GTGACCCTGG AGGATTTGCA GTTACTTGCT GATCTATTCT ACCTTCCTTA CGAGCATGGA CCCAAAGGAG CACAGATGTT ACGGGAATTT CAATGGCTTC GAGCAAATAG TAGTGTGTC AGTGTCATTT GCAAAGGAAA AGACTCTGAA AAAATTGAAG AATGGCGGTC ACGAGCAGCG AAGTTTGAAG AGATGTGTGG ACTAGTGATG GGAATGTTCA CTCGGCTCTC CAATTGTGCC AACAGGACAA TTCTTTATGA CATGTACTCC TATGTTTGGG ATATCAAGAG TATAATGTCT ATGGTGAAGT CTTTTGTACA GTGGTTAGGG TGTCGTAGTC ATTCTTCAGC ACAATTCTTA ATTGGAGACC AAGAACCCTG GGCCTTTAGA GGTGGTCTAG CAGGAGAGTT CCAGCGTTTG CTGCCAATTG ATGGGGCAAA TGATCTCTTT TTTGAGCCAC CTCCACTGAC TCCTACCTCC AAAGTTTATA CTATCAGACC TTATTTTCTT AAGGATGAGG CATCCGTGTA CAAGATTGTC AGAGAAATGT ATGACGATGG AGTGGGTTTA CCCTTCAAA GTCAGCCTGA TCTTATTGGA GACAAGTTAG TAGGAGGGCT GCTTTCCCTC AGCCTGGATT ACTGCTTTGT CCTAGAAGAT GAAGATGGCA TATGTGGTTA TGCCCTGGGC ACTGTAGATG TGACCCCTTT TATTAAAAA TGTAAAATTT CCTGGATCCC CTTTCATGAG GAGAAGTATA CCAAGCCAAA TGGTGACAA GAACTCTCTG AGGCTGAGAA AATAATGTT AGTTTCCATG AAGAACAGGA AGTACTGCCA GAACTTTTCC TTGCTAATTT CCTTCTCTG ATAAAGATGG ACATTCACAA AAAAGTAACT GACCCAAGTG TGGCCAAAAG CATGATGGCT TGCTCCTGT

		CTTCACTGAA GGCTAATGGC TCCCGGGGAG CTTTCTGTGA AGTGAGACCA GATGATAAAA GAATTCTGGA ATTTTACAGC AAGTTAGGAT GTTTTGAAAT TGCAAAAAATG GAAGGATTTT CAAAGGATGT GGTATATACTT GGTGCGGAGCC TGTGACATTT GTTGACACTG TGAACGTGCC AAAAGTCTCT TAAGTGCACC TTGTGAATGG TAGTTGAGGT CTTACATACAG TTCAGCCTCT AGAATGGTAA CAAATCAGCC AATTGGATTG GAAACAAAGA AGACTATGTA AAATCACCCT ATCACACTTT GAGACTACTC ACTGGTTGGA AGAATATAGT ATTCGAGCAA ATCCTGTATG AAAGAGAGAT GTGGGCTTCC TTTTGTAGTC TTGTGTAGG TGCTGAGACC TTTTACATGG GCTTATACAG GGAGAGAGTC TTCAATAAAAT GTAGTCAGCA CTATTTTCTG CATCCAGTGT GGTTGCGTTT CTCACCTGAG AGTAATCAAG ATAACATCTG TCATCTTCTT TGGTTTATTG AGTGAAATGC CTCTCAGTCT TAGGGGACAT GGCAGAGATG AAAGAAAGAA AGAGTGGGTT TCAGAAGTGT CAGGGTGGAG TGATTCCAAG TGGGATGGTT GTGGCATTAG TTTAAGCTGA ATAAATAATT TCAATTTGGG GCAGTTATTC TGCTTTTGT AAAGCCGTGG CCAATTGTCT CCTGTAATGA CTGTTGGTTC AGGCATGTTG TACTTTGTAG GGACAAATGT GCATTTGTTT GTGGCAAAAG CCTACAATTG ACAAACCTTGT AAATTTCTTT GTATATAAAC TAGCTGTAAC CTGACTATCC TTTGTGTTTA CTGTTTTTGT AAATTTTTTT CCTCTATAAA TGAAAGGGTG TTGGTTCAGA ATGGCACTTT GAATAATGTA AACCAGTGAA AAGTGGATTT TCTTTACTTT TGTCTTTGGG TTTGGGGTTG TTTTGTCTT TTTTGAAGTT TTATTTATTT TAAAGTGCCT CCCACCTAGG CGTAGGCCAT GACCATTTGG GGTACGAGAG CCTAATTTTG TAGGACTTAA TCTGTTGAAA AGTGCAGTTA CTTCTGAAA TTAACCTCAA TATTAGGTCA GCATGTGAAA TGTTGGATTT GACATGTCAG GTAGGGTTCA GGGACTGATT GGTCCCATT GCCCTCAGGT CAGTTGTTTA ATCTCAAGAC CTGTTACTAC TGATTTTATT AAATCAGAGT CTTTAATTCT TGCAATGTTT TATCTAATTT CTGAACGAAT GAGCACACTT TAACCAGTTA TTTACAGTTA CCTTTTCTCT TTAACCGGAT TGTGAAAGCT TCATGTATTT TAATTTAGAT TCTGTGTTTT TAAGGGTTCT GAGCATGAAG CTGGCAGATA GTCGGCAGGA CTCATTTTTT CATCATGGCT GGCTGATTTT TCCATAGATT GATAACAGTA TTTTGTATC TTGCTTCTCT GTAGTTTTGC ATCAGCTGTT TAACTTTGAG CTGAGTGAGG GGAGAGGGGT AAAGAGAAAG AAACTTAAGT TTTCTTTCAC AGAAGCTCCAC CATTTGTGGC TTTGAGAGAG CCCTAAAGCA TTGTACCTAG TGGTACCTAG TGACTTCCAA CCAAAGCCTT TGAGTATGCA CTAATTAGTT GAGAAGAAAG GAGAGAAGGT TTTTAGGTTA GAAACCTTTA ACCGATAGAA GGATATGGTA TGTGTAAAG CTGAACCAA GTTTGCATTT TTGAGGGCTT GAGATGAAGG GAAGACTCTT ACCAGATAGT AAGACAGCTG AGTTTTCTCT AGTTTTCTCG TCTTAACACT AGTGGACAAT TCTAGCATTT TGTTTGGAGG ATTTACAGAGT TAACCTCATG GAATTCAGGA TTTTITAGCA AGTTTGCTTT TGGTTTTATC TTGGCTTTTA GTAATCATGT TGGCTGGTCT GGTACAGAGT GACTGTGAAA CAGATGCCCT GGTCTTGCTT TCATCACTCT AGGATCATGA AGTGCTATGC TATTTCTTGG TTATGAATAT TAAGGTTGGA ATTACATTTT TATTGATTGT TTGGATCAGA GCTCAGTTCC TGTAGAAAAC GAACTGTAAA AGACCATGCA AGAGGCAAAA TAAACTTGA AGTGAATGCT TGTAATAAAA AAAAAAAAAA A
61	Human phosphatidylinosi tol-4,5- bisphosphate 3- kinase, catalytic subunit alpha (PIK3CA) mRNA	TCTCCCTCGG CGCCGCCGCC GCCGCCCGCG GGGCTGGGAC CCGATGCGGT TAGAGCCGCG GAGCCTGGAA GAGCCCGGAG CGTTTCTGCT TTGGGACAAC CATACATCTA ATTCCTTAAA GTAGTTTTAT ATGTAAAACT TGCAAGAAT CAGAACAATG CCTCCACGAC CATCATCAGG TGAAGTGTGG GGCATCCACT TGATGCCCCC AAGAATCCTA GTAGAATGTT TACTACCAAA TGGAATGATA GTGACTTTAG AATGCCTCCG TGAGGCTACA TTAATAACCA TAAAGCATGA ACTATTTAAA GAAGCAAGAA AATACCCCTT CCATCAACTT CTTCAAGATG AATCTTCTTA CATTTTCGTA AGTGTTACTC AAGAAGCAGA AAGGGAAGAA TTTTITGATG AAACAAGACG ACTTTGTGAC CTTCGGCTTT TTCAACCTT TTTAAAAGTA ATTGAACCAG TAGGCAACCG TGAAGAAAAG ATCCTCAATC GAGAAATTGG TTTTGCTATC GGCATGCCAG TGTGTGAATT TGATATGGTT AAAGATCCAG AAGTACAGGA CTTCCGAAGA AATATTCTGA AATATCAGGG AGAAGCTGTG GATCTTAGGG ACCTCAATTC ACCTCATAGT AGAGCAATGT ATGTCTATCC TCCAAATGTA GAATCTTCAC CAGAATTGCC AAAGCACATA TATAATAAAT TAGATAAAGG GCAAATAATA GTGGTGATCT GGGTAATAGT TTCTCCAAAT AATGACAAGC AGAAGTATAC TCTGAAAATC AACCATGACT GTGTACCAGA ACAAGTAATT GCTGAAGCAA TCAGGAAAAA AACTCGAAGT ATGTTGCTAT CCTCTGAACA ACTAAAACCT TGTGTTTTAG AATATCAGGG CAAGTATATT TTAAAAGTGT GTGGATGTGA TGAATACTTC CTAGAAAAAT ATCCTCTGAG TCAGTATAAG TATATAAGAA GCTGTATAAT GCTTGGGAGG ATGCCCCAAT TGATGTTGAT GGCTAAAGAA AGCCTTTTAT CTCAACTGCC AATGGACTGT TTTACAATGC CATCTTATTC CAGACGCATT TCCACAGCTA CACCATATAT GAATGGAGAA ACATCTACAA AATCCCTTTG GGTTATAAAT AGTGCACCTA GAATAAAAAAT TCTTTGTGCA ACCTACGTGA ATGTAAATAT TCGAGACATT GATAAGATCT ATGTTGCAAC AGGTATCTAC CATGGAGGAG AACCCCTATG TGACAATGTG AACACTCAAA GAGTACCTTG TTCCAATCCC AGGTGGAATG AATGGCTGAA TTATGATATA TACATTCTCG ATCTTCCTCG TGCTGCTCGA CTTTGCCTTT CCATTTGCTC TGTTAAAGGC CGAAAGGGTG CTAAAGAGGA ACACTGTCCA TTGGCATGGG GAAATATAAA CTTGTTTGAT TACACAGACA CTCTAGTATC TGGAAAAATG GCTTTGAATC TTTGGCCAGT ACCTCATGGA TTAGAAGATT TGCTGAACCC TATTGGTGTT ACTGGATCAA ATCCAAATAA AGAAACTCCA TGCTTAGAGT TGGAGTTTGA CTGGTTCAGC AGTGTGGTAA AGTTCCAGA TATGTCAGTG ATTTGAAGAGC ATGCCAATTG GTCTGTATCC CGAGAAGCAG GATTAGCTA TTCCACGCA GGAAGTGAAG ACAGACTAGC TAGAGACAAT GAATTAAGGG AAAATGACAA AGAACAGCTC AAAGCAATTT CTACACGAGA TCCTCTCTCT GAAATCAGG AGCAGGAGAA AGATTTTCTA TGGAGTCACA GACACTATTG TGTAACATAT CCCGAAATTC TACCCAAATT

		GCTTCTGTCT GTTAAATGGA ATTCTAGAGA TGAAGTAGCC CAGATGTATT GCTTGGTAAA
		AGATTGGCCT CCAATCAAAC CTGAACAGGC TATGGAACCT CTGGACTGTA ATTACCCAGA
		TCCTATGGTT CGAGGTTTTG CTGTTTCGGTG CTTGGAAAAA TATTTAACAG ATGACAAACT
		TTCTCAGTAT TTAATTCAGC TAGTACAGGT CCTAAAAATAT GAACAATATT TGGATAACTT
		GCTTGTGAGA TTTTACTGA AGAAAGCATT GACTAATCAA AGGATTGGGC ACTTTTCTT
		TTGGCATTTA AAATCTGAGA TGCACAATAA AACAGTTAGC CAGAGGTTTG GCCTGCTTTT
		GGAGTCCTAT TGTCGTGCAT GTGGGATGTA TTTGAAGCAC CTGAATAGGC AAGTCGAGGC
		AATGGAAAAG CTCATTAAC TAACTGACAT TCTCAAACAG GAGAAGAAGG ATGAAACACA
		AAAGGTACAG ATGAAGTTTT TAGTTGAGCA AATGAGGCGA CCAGATTTCA TGGATGCTCT
		ACAGGGCTTT CTGTCTCCTC TAAACCCTGC TCATCAACTA GGAAACCTCA GGCTGAAGA
		GTGTCGAATT ATGTCTCTG CAAAAAGGCC ACTGTGGTTG AATTGGGAGA ACCCAGACAT
		CATGTCAGAG TTACTGTTT AGAACAATGA GATCATCTTT AAAAATGGGG ATGATTTACG
		GCAAGATATG CTAACACTTC AAATTATTTCG TATTATGGAA AATATCTGGC AAAATCAAGG
		TCTTGATCTT CGAATGTTAC CTTATGGTTG TCTGTCAATC GGTGACTGTG TGGGACTTAT
		TGAGGTGGTG GAAATTCTC AACTATTAT GCAAATTCAG TGCAAAGCGG GCTTGAAAGG
		TGCACTGCAG TTCAACAGCC ACACACTACA TCAGTGGCTC AAAGACAAGA ACAAGGAGA
		AATATATGAT GCAGCCATTG ACCTGTTTAC ACGTTCATGT GCTGGATACT GTGTAGCTAC
		CTTCATTTTG GGAATTGGAG ATCGTCACAA TAGTAACATC ATGGTGAAAG ACGATGGACA
		ACTGTTTCAT ATAGATTTTG GACACTTTTT GGATCACAAG AAGAAAAAAT TTGGTTATAA
		ACGAGAACGT GTGCCATTG TTTTGACACA GGATTCTTA ATAGTGATTA GTAAAGGAGC
		CCAAGAATGC ACAAGACAA GAGAATTTGA GAGGTTTCAG GAGATGTGTT ACAAGGCTTA
		TCTAGCTATT CGACAGCATG CCAATCTCTT CATAAATCTT TTCTCAATGA TGCTGGCTC
		TGGAATGCCA GAACTACAAT CTTTGTATGA CATTGCATAC ATTCGAAAGA CCCTAGCCTT
		AGATAAACT GAGCAAGAGG CTTTGGAGTA TTTTCATGAA CAAATGAATG ATGCACATCA
		TGGTGGCTGG ACAACAAAAA TGGAATTGGAT CTTCCACACA ATTAACAGC ATGCATTGAA
		CTGAAAAGAT AACTGAGAAA ATGAAAGCTC ACTCTGGATT CCACACTGCA CTGTTAATAA
		CTCTCAGCAG GCAAAGACCG ATTGCATAGG AATTGCACAA TCCATGAACA GCATTAGAAT
		TTACAGCAAG AACAGAAATA AAATACTATA TAATTTAAAT AATGTAAACG CAAACAGGGT
		TTGATAGCAC TTAACTAGT TCATTTCAAA ATTAAGCTTT AGAATAATGC GCAATTTTCAT
		GTTATGCCTT AAGTCCAAAA AGGTAAACTT TGAAGATTGT TTGTATCTTT TTTTAAAAAA
		CAAAACAAAA CAAAAATCCC CAAAATATAT AGAAATGATG GAGAAGGAAA AAAAAAAAAA
		AAAA

WHAT IS CLAIMED IS:

1. A method for detecting the presence of bladder cancer in a subject comprising detecting the levels of each marker of a set of bladder cancer markers in a sample from the subject, wherein the set of bladder cancer markers comprises uroplakin 1B (UPK1B), or androgen receptor (AR), or both UPK1B and AR, and wherein detection of an elevated level of at least one marker selected from UPK1B and AR indicates the presence of bladder cancer in the subject.
2. A method of monitoring anti-androgen therapy in a subject with bladder cancer comprising detecting the levels of each marker of a set of bladder cancer markers in a sample from the subject at a first time point, wherein the set of bladder cancer markers comprises uroplakin 1B (UPK1B), or androgen receptor (AR), or both UPK1B and AR, wherein a decrease in the level of the at least one marker at the first time point relative to a second time point indicates that the subject is responding to anti-androgen therapy.
3. The method of claim 2, wherein the method comprises comparing the level of the at least one marker at the first time point to the level of the at least one marker at the second time point.
4. The method of claim 2 or claim 3, wherein the subject is undergoing anti-androgen therapy at the first time point.
5. The method of any one of claims 2 to 4, wherein the second time point is prior to the subject beginning anti-androgen therapy.
6. The method of any one of claim 2 to 4, wherein the subject is undergoing anti-androgen therapy at the second time point.
7. The method of any one of claims 1 to 6, wherein the set of bladder cancer markers comprises at least one marker selected from corticotrophin releasing hormone (CRH), insulin-like growth factor 2 (IGF2), keratin 20 (KRT20) and annexin 10 (ANXA10).
8. The method of claim 7, wherein the set of bladder cancer markers comprises:
 - a) AR, CRH, IGF2, KRT20, and ANXA10;
 - b) AR, CRH, IGF2, and KRT20;
 - c) UPK1B, CRH, IGF2, KRT20, and ANXA10;
 - d) UPK1B, CRH, IGF2, and KRT20;
 - e) AR, UPK1B, CRH, IGF2, KRT20, and ANXA10;
 - f) AR, UPK1B, CRH, IGF2, and KRT20;
 - g) UPK1B, CRH, IGF2, and ANXA10; or

h) UPK1B, CRH, KRT20, and ANXA10.

9. The method of any one of claims 1 to 8, wherein the method further comprises detecting an endogenous control.

10. The method of claim 9, wherein the endogenous control is selected from ABL, GUSB, GAPDH, TUBB, and UPK1a.

11. The method of any one of the preceding claims, wherein the method further comprises detecting an exogenous control.

12. The method of claim 11, wherein the exogenous control is an RNA.

13. The method of any one of the preceding claims, wherein the detecting comprises RT-PCR.

14. The method of claim 13, wherein the method comprises quantitative RT-PCR.

15. The method of claim 14, wherein the method comprises comparing a Ct value or a Δ Ct value to a threshold Ct value or Δ Ct value.

16. The method of claim 15, wherein Δ Ct is the Ct value for the endogenous control minus the Ct value for the marker.

17. The method of any one of claims 13 to 16, wherein the RT-PCR reaction takes less than 2 hours from an initial denaturation step through a final extension step.

18. The method of any one of the preceding claims, wherein the method comprises contacting RNA from the sample with a set of bladder cancer marker primer pairs, wherein the set of bladder cancer marker primer pairs comprises a first primer pair for detecting AR, or a first primer pair for detecting UPK1B, or a first primer pair for detecting AR and a second primer pair for detecting UPK1B.

19. The method of claim 18, wherein the set of bladder cancer marker primer pairs comprises a third primer pair for detecting a marker selected from CRH, IGF2, KRT20, and ANXA10.

20. The method of claim 19, wherein the set of bladder cancer marker primer pairs comprises a fourth primer pair for detecting a marker selected from CRH, IGF2, KRT20, and ANXA10, wherein the third primer pair and the fourth primer pair detect different markers.

21. The method of claim 20, wherein the set of bladder cancer marker primer pairs comprises a fifth primer pair for detecting a marker selected from CRH, IGF2, KRT20, and ANXA10, wherein the third primer pair, the fourth primer pair, and the fifth primer pair detect different markers.

22. The method of claim 18, wherein the set of bladder cancer marker primer pairs comprises:

- a) a first primer pair for detecting AR, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, a fourth primer pair for detecting KRT20, and a fifth primer pair for detecting ANXA10;
- b) a first primer pair for detecting AR, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, and a fourth primer pair for detecting KRT20;
- c) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, a fourth primer pair for detecting KRT20, and a fifth primer pair for detecting ANXA10;
- d) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, and a fourth primer pair for detecting KRT20;
- e) a first primer pair for detecting AR, a second primer pair for detecting UPK1B, a third primer pair for detecting CRH, a fourth primer pair for detecting IGF2, a fifth primer pair for detecting KRT20, and a sixth primer pair for detecting ANXA10;
- f) a first primer pair for detecting AR, a second primer pair for detecting UPK1B, a third primer pair for detecting CRH, a fourth primer pair for detecting IGF2, and a fifth primer pair for detecting KRT20;
- g) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, and a fourth primer pair for detecting ANXA10; or
- h) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting KRT20, and a fourth primer pair for detecting ANXA10.

23. The method of any one of claims 18 to 22, wherein each bladder cancer marker primer pair produces an amplicon that is 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, or 50 to 150 nucleotides long.

24. The method of any one of claims 18 to 23, wherein at least one, at least two, or at least three bladder cancer marker primer pair spans an intron in the genomic sequence.

25. The method of any one of claims 18 to 24, wherein each bladder cancer marker primer pair spans an intron in the genomic sequence.

26. The method of any one of claims 18 to 25, wherein the primer pair for detecting AR comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 54 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 55, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

27. The method of any one of claims 18 to 26, wherein the primer pair for detecting UPK1B comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 51 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 52, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

28. The method of any one of claims 18 to 27, wherein the method further comprises contacting RNA from the sample with an endogenous control primer pair.

29. The method of claim 28, wherein the endogenous control primer pair is for detecting ABL.

30. The method of any one of claims 18 to 29, wherein the method further comprises contacting RNA from the sample with an exogenous control primer pair.

31. The method of claim 30, wherein the exogenous control primer pair is for detecting an exogenous RNA.

32. The method of any one of claims 18 to 31, wherein the method further comprises forming a set of bladder cancer marker amplicons, wherein the set of bladder cancer marker amplicons comprises an AR amplicon, or a UPK1B amplicon, or both an AR amplicon and a UPK1B amplicon, and contacting the bladder cancer marker amplicons with a set of bladder cancer marker probes, wherein the set of bladder cancer marker probes comprises a first probe for detecting the AR amplicon, or a first probe for detecting the UPK1B amplicon, or a first probe for detecting the AR amplicon and a second probe for detecting the UPK1B amplicon.

33. The method of claim 32, wherein the set of bladder cancer marker amplicons comprises a third amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, and a ANXA10 amplicon, and wherein the set of bladder cancer marker probes comprises a third probe for detecting the third amplicon.

34. The method of claim 33, wherein the set of bladder cancer marker amplicons comprises a fourth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, and a ANXA10 amplicon, wherein the set of bladder cancer marker probes comprises a fourth probe for detecting the fourth amplicon, and wherein the third amplicon and the fourth amplicon are different.

35. The method of claim 34, wherein the set of bladder cancer marker amplicons comprises a fifth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20

amplicon, and a ANXA10 amplicon, wherein the set of bladder cancer marker probes comprises a fifth probe for detecting the fifth amplicon, and wherein the third amplicon, the fourth amplicon, and the fifth amplicon are different.

36. The method of claim 32, wherein the set of bladder cancer amplicons and the set of bladder cancer marker probes comprise:

- a) an AR amplicon, a CRH amplicon, an IGF2 amplicon, a KRT20 amplicon, and an ANXA10 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the AR amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, a fourth probe for detecting the KRT20 amplicon, and a fifth probe for detecting the ANXA10 amplicon;
- b) an AR amplicon, a CRH amplicon, an IGF2 amplicon, and a KRT20 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the AR amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, and a fourth probe for detecting the KRT20 amplicon;
- c) a UPK1B amplicon, a CRH amplicon, an IGF2 amplicon, a KRT20 amplicon, and an ANXA10 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, a fourth probe for detecting the KRT20 amplicon, and a fifth probe for detecting the ANXA10 amplicon;
- d) a UPK1B amplicon, a CRH amplicon, an IGF2 amplicon, and a KRT20 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, and a fourth probe for detecting the KRT20 amplicon;
- e) an AR amplicon, a UPK1B amplicon, a CRH amplicon, an IGF2 amplicon, a KRT20 amplicon, and an ANXA10 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the AR amplicon, a second probe for detecting the UPK1B amplicon, a third probe for detecting the CRH amplicon, a fourth probe for detecting the IGF2 amplicon, and a fifth probe for detecting the KRT20 amplicon, and a sixth probe for detecting the ANXA10 amplicon;
- f) an AR amplicon, a UPK1B amplicon, a CRH amplicon, an IGF2 amplicon, and a KRT20 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the AR amplicon, a second probe for detecting the UPK1B amplicon, a third probe for detecting the CRH amplicon, a fourth probe for detecting the IGF2 amplicon, and a fifth probe for detecting the KRT20 amplicon;

g) a UPK1B amplicon, a CRH amplicon, an IGF2 amplicon, and an ANXA10 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, and a fourth probe for detecting the ANXA10 amplicon; or
h) a UPK1B amplicon, a CRH amplicon, an KRT20 amplicon, and an ANXA10 amplicon, and a set of bladder cancer marker probes comprising a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the KRT20 amplicon, and a fourth probe for detecting the ANXA10 amplicon.

37. The method of any one of claims 32 to 36, wherein the probe for detecting AR comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 56, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

38. The method of any one of claims 32 to 37, wherein the probe for detecting UPK1B comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 53, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

39. The method of any one of claims 32 to 38, wherein each bladder cancer marker probe comprises a dye, and wherein each dye is detectably different from the other three dyes.

40. The method of claim 39, wherein each probe comprises a fluorescent dye and a quencher molecule.

41. The method of any one of claims 32 to 40, wherein the method further comprises forming an endogenous control amplicon, and contacting the endogenous control amplicon with an endogenous control probe.

42. The method of claim 41, wherein the endogenous control probe comprises a dye that is detectably different from the dyes of the bladder cancer marker probes.

43. The method of any one of claims 32 to 42, wherein the method further comprises forming an exogenous control amplicon, and contacting the exogenous control amplicon with an exogenous control probe.

44. The method of claim 43, wherein the exogenous control probe comprises a dye that is detectably different from the dyes of the bladder cancer marker probes and the endogenous control probe.

45. The method of any one of the preceding claims, wherein the set of bladder cancer markers are detected in a single multiplex reaction.

46. The method of any one of the preceding claims, wherein the sample comprises urothelial cells.

47. The method of claim 46, wherein the sample is selected from a urine sample and a bladder washing sample.

48. The method of any one of the preceding claims, wherein the subject has at least one symptom of bladder cancer

49. The method of claim 48, wherein the at least one symptom is selected from abdominal pain, blood in the urine, painful urination, frequent urination, urinary urgency, and incontinence.

50. The method of any one of the preceding claims, wherein the subject has a history of bladder cancer.

51. The method of claim 50, wherein the subject is being monitored for recurrence of bladder cancer.

52. The method of claim 50 or claim 51, wherein the patient has been treated with Bacillus Calmette-Guerin (BCG) in the past three months.

53. The method of any one of the preceding claims, wherein the bladder cancer is low grade bladder cancer.

54. The method of any one of the preceding claims, wherein the method comprises using linear discriminant analysis (LDA) to combine two or more of the bladder cancer marker levels into a combined score.

55. The method of claim 54, wherein LDA is used to combine all of the bladder cancer marker levels into a combined score.

56. A composition comprising a set of bladder cancer marker primer pairs, wherein the set of bladder cancer marker primer pairs comprises a first primer pair for detecting AR, or a first primer pair for detecting UPK1B, or a first primer pair for detecting AR and a second primer pair for detecting UPK1B.

57. The composition of claim 56, wherein the set of bladder cancer marker primer pairs comprises a third primer pair for detecting a marker selected from CRH, IGF2, KRT20, and ANXA10.

58. The composition of claim 57, wherein the set of bladder cancer marker primer pairs comprises a fourth primer pair for detecting a marker selected from CRH, IGF2, KRT20, and ANXA10, wherein the third primer pair and the fourth primer pair detect different markers.

59. The composition of claim 58, wherein the set of bladder cancer marker primer pairs comprises a fifth primer pair for detecting a marker selected from CRH, IGF2, KRT20, and

ANXA10, wherein the third primer pair, the fourth primer pair, and the fifth primer pair detect different markers.

60. The composition of claim 56, wherein the set of bladder cancer marker primer pairs comprises:

- a) a first primer pair for detecting AR, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, a fourth primer pair for detecting KRT20, and a fifth primer pair for detecting ANXA10;
- b) a first primer pair for detecting AR, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, and a fourth primer pair for detecting KRT20;
- c) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, a fourth primer pair for detecting KRT20, and a fifth primer pair for detecting ANXA10;
- d) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, and a fourth primer pair for detecting KRT20;
- e) a first primer pair for detecting AR, a second primer pair for detecting UPK1B, a third primer pair for detecting CRH, a fourth primer pair for detecting IGF2, a fifth primer pair for detecting KRT20, and a sixth primer pair for detecting ANXA10;
- f) a first primer pair for detecting AR, a second primer pair for detecting UPK1B, a third primer pair for detecting CRH, a fourth primer pair for detecting IGF2, and a fifth primer pair for detecting KRT20;
- g) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting IGF2, and a fourth primer pair for detecting ANXA10; or
- h) a first primer pair for detecting UPK1B, a second primer pair for detecting CRH, a third primer pair for detecting KRT20, and a fourth primer pair for detecting ANXA10.

61. The composition of any one of claims 56 to 60, wherein each bladder cancer marker primer pair produces an amplicon that is 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, or 50 to 150 nucleotides long.

62. The composition of any one of claims 56 to 61, wherein at least one, at least two, or at least three bladder cancer marker primer pair spans an intron in the genomic sequence.

63. The composition of any one of claims 56 to 62, wherein each bladder cancer marker primer pair spans an intron in the genomic sequence.

64. The composition of any one of claims 56 to 63, wherein the the primer pair for detecting AR comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18

nucleotides of SEQ ID NO: 54 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 55, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

65. The composition of any one of claims 56 to 64, wherein the primer pair for detecting UPK1B comprises a first primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 51 and a second primer comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 52, wherein each primer is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

66. The composition of any one of claims 56 to 65, wherein the composition further comprises a set of bladder cancer marker probes, wherein the set of bladder cancer marker probes comprises a first probe for detecting an AR amplicon, or a first probe for detecting a UPK1B amplicon, or a first probe for detecting an AR amplicon and a second probe for detecting a UPK1B amplicon.

67. The composition of claim 66, wherein the set of bladder cancer marker probes comprises a third probe for detecting a third amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, and a ANXA10 amplicon.

68. The composition of claim 66, wherein the set of bladder cancer marker probes comprises a fourth probe for detecting a fourth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, and a ANXA10 amplicon, wherein the third amplicon and the fourth amplicon are different.

69. The composition of claim 66, wherein the set of bladder cancer marker probes comprises fourth probe for detecting a fifth amplicon selected from a CRH amplicon, a IGF2 amplicon, a KRT20 amplicon, and a ANXA10 amplicon, wherein the third amplicon, the fourth amplicon, and the fifth amplicon are different.

70. The composition of claim 66, wherein the set of bladder cancer marker probes comprises:

- a) a first probe for detecting the AR amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, a fourth probe for detecting the KRT20 amplicon, and a fifth probe for detecting the ANXA10 amplicon;

- b) a first probe for detecting the AR amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, and a fourth probe for detecting the KRT20 amplicon;
- c) a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, a fourth probe for detecting the KRT20 amplicon, and a fifth probe for detecting the ANXA10 amplicon;
- d) a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, and a fourth probe for detecting the KRT20 amplicon;
- e) a first probe for detecting the AR amplicon, a second probe for detecting the UPK1B amplicon, a third probe for detecting the CRH amplicon, a fourth probe for detecting the IGF2 amplicon, and a fifth probe for detecting the KRT20 amplicon, and a sixth probe for detecting the ANXA10 amplicon;
- f) a first probe for detecting the AR amplicon, a second probe for detecting the UPK1B amplicon, a third probe for detecting the CRH amplicon, a fourth probe for detecting the IGF2 amplicon, and a fifth probe for detecting the KRT20 amplicon;
- g) a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the IGF2 amplicon, and a fourth probe for detecting the ANXA10 amplicon; or
- h) a first probe for detecting the UPK1B amplicon, a second probe for detecting the CRH amplicon, a third probe for detecting the KRT20 amplicon, and a fourth probe for detecting the ANXA10 amplicon.

71. The composition of any one of claims 66 to 70, wherein the probe for detecting AR comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 56, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

72. The composition of any one of claims 66 to 71, wherein the probe for detecting UPK1B comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15, at least 16, at least 17, or at least 18 nucleotides of SEQ ID NO: 53, wherein the probe is less than 50, less than 45, less than 40, less than 35, or less than 30 nucleotides long.

73. The composition of any one of claims 56 to 72, further comprising an endogenous control primer pair.

74. The composition of claim 73, further comprising an endogenous control probe for detecting an endogenous control amplicon.

75. The composition of claim 73 or claim 74, wherein the endogenous control is ABL.

76. The composition of any one of claims 56 to 75, wherein the composition is a lyophilized composition.

77. The composition of any one of claims 56 to 75, wherein the composition is a solution.

78. The composition of claim 77, wherein the composition further comprises urothelial cells.

79. The composition of claim 78, wherein the urothelial cells are from a urine sample.

80. The composition of claim 77, wherein the composition further comprises lysed urothelial cells.

81. The composition of claim 80, wherein the urothelial cells are from a urine sample.

82. The composition of claim 77, wherein the composition further comprises RNAs from a human sample.

83. The composition of claim 80, wherein the RNAs are from cells of a urine sample.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021199

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G01N33/574
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOBBAN E D ET AL: "UROPLAKIN GENE EXPRESSION BY NORMAL AND NEOPLASTIC HUMAN UROTHELIUM", AMERICAN JOURNAL OF PATHOLOGY; [10640], ELSEVIER INC, US, vol. 153, no. 6, 1 December 1998 (1998-12-01), pages 1957-1967, XP000882577, ISSN: 0002-9440	1,9,10, 13,46, 48-53, 56, 61-66, 71-83
A	page 1962, right-hand column, paragraph 4; tables 1,3 ----- -/--	57-60, 67-70

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 May 2014

Date of mailing of the international search report

10/06/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Knudsen, Henrik

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/021199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JONATHON OLSBURGH ET AL: "Uroplakin gene expression in normal human tissues and locally advanced bladder cancer", THE JOURNAL OF PATHOLOGY, vol. 199, no. 1, 1 January 2003 (2003-01-01), pages 41-49, XP055028760, ISSN: 0022-3417, DOI: 10.1002/path.1252	1,14-18, 23-28, 32, 37-46, 48-53, 56, 61-66, 71-83
Y	page 43, right-hand column, lines 37-39; tables 1-2	11,12, 29-31,
A	page 47, left-hand column, lines 4-6	54,55 57-60, 67-70
X	----- PLESEA I A ET AL: "Dedection and morphological profile of early stages bladder carcinoma: preliminary study", VIRCHOWS ARCHIV, vol. 459, no. Suppl. 1, 066, August 2011 (2011-08), page S296, XP002724864, & 23RD ANNUAL EUROPEAN CONGRESS OF PATHOLOGY; HELSINKI, FINLAND; AUGUST 27 -SEPTEMBER 01, 2011 abstract	1,46, 48-53
X	----- WILLIAMS E ET AL: "Androgen Receptor Expression in Genitourinary Neoplasms", MODERN PATHOLOGY, vol. 26, no. Suppl. 2, February 2013 (2013-02), page 258A, XP002724865, & 102ND ANNUAL MEETING OF THE UNITED-STATES-AND-CANADIAN-ACADEMY-OF-PATH OLOGY (USCAP); BALTIMORE, MD, USA; MARCH 02 -08, 2013 abstract	1,46, 48-53
X	----- HIROSHI MIYAMOTO ET AL: "GATA binding protein 3 is down-regulated in bladder cancer yet strong expression is an independent predictor of poor prognosis in invasive tumor", HUMAN PATHOLOGY, vol. 43, no. 11, 1 November 2012 (2012-11-01), pages 2033-2040, XP055118819, ISSN: 0046-8177, DOI: 10.1016/j.humpath.2012.02.011 abstract	1,46, 48-53
	----- -/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Y. ZHENG ET AL: "Dihydrotestosterone upregulates the expression of epidermal growth factor receptor and ERBB2 in androgen receptor-positive bladder cancer cells", ENDOCRINE RELATED CANCER, vol. 18, no. 4, 4 July 2011 (2011-07-04), pages 451-464, XP055119386, ISSN: 1351-0088, DOI: 10.1530/ERC-11-0010 abstract	2-6
Y	----- LESTER M. SHULMAN ET AL: "Evaluation of Four Different Systems for Extraction of RNA from Stool Suspensions Using MS-2 Coliphage as an Exogenous Control for RT-PCR Inhibition", PLOS ONE, vol. 7, no. 7, 16 July 2012 (2012-07-16), page e39455, XP055119739, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0039455 abstract	11,12, 30,31
Y	----- D. W. LIU: "Choice of endogenous control for gene expression in nonsmall cell lung cancer", EUROPEAN RESPIRATORY JOURNAL, vol. 26, no. 6, 1 December 2005 (2005-12-01), pages 1002-1008, XP055119655, ISSN: 0903-1936, DOI: 10.1183/09031936.05.00050205 abstract	29
Y	----- J. BRABENDER: "The Molecular Signature of Normal Squamous Esophageal Epithelium Identifies the Presence of a Field Effect and Can Discriminate between Patients with Barrett's Esophagus and Patients with Barrett's-Associated Adenocarcinoma", CANCER EPIDEMIOLOGY, BIOMARKERS & PREVENTION, vol. 14, no. 9, 1 September 2005 (2005-09-01), pages 2113-2117, XP055119519, ISSN: 1055-9965, DOI: 10.1158/1055-9965.EPI-05-0014 abstract	54,55
	----- -/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOON H S ET AL: "Benzylidihydroxyoctenone, a novel anticancer agent, induces apoptosis via mitochondrial-mediated pathway in androgen-sensitive LNCaP prostate cancer cells", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 19, no. 3, 1 February 2009 (2009-02-01), pages 742-744, XP025925808, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2008.12.029 [retrieved on 2009-01-31]	56, 61-66, 71-83
A	abstract	57-60, 67-70
A	----- ANDREA E VARGA ET AL: "Methylation of a CpG Island within the Uroplakin Ib Promoter: A Possible Mechanism for Loss of Uroplakin Ib Expression in Bladder Carcinoma 1", NEOPLASIA, March 2004 (2004-03), pages 128-135, XP055118551, abstract	1
A	----- DAVID J. DEGRAFF ET AL: "Loss of the Urothelial Differentiation Marker FOXA1 Is Associated with High Grade, Late Stage Bladder Cancer and Increased Tumor Proliferation", PLOS ONE, vol. 7, no. 5, 10 May 2012 (2012-05-10), page e36669, XP055070192, DOI: 10.1371/journal.pone.0036669 abstract	1
A	----- SIRACUSANO S ET AL: "The Simultaneous Use of Telomerase, Cytokeratin 20 and CD4 for Bladder Cancer Detection in Urine", EUROPEAN UROLOGY, ELSEVIER BV, NL, vol. 47, no. 3, 1 March 2005 (2005-03-01), pages 327-333, XP027606339, ISSN: 0302-2838 [retrieved on 2005-03-01] abstract	1-55
A	----- EP 2 138 848 A1 (FINA BIOTECH S L U [ES]) 30 December 2009 (2009-12-30) abstract	1-83
A,P	----- WO 2013/158972 A1 (CEPHEID [US]; HIGUCHI RUSSELL [US]; WALLACE STACEY ELLEN [US]; LAI EDW) 24 October 2013 (2013-10-24) the whole document	1-83
	----- -/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	<p>WO 2014/018926 A1 (ARAGON PHARMACEUTICALS INC [US]) 30 January 2014 (2014-01-30) paragraph [0016]; claim 1 -----</p>	2-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/021199

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2138848	A1	30-12-2009	AT 487140 T 15-11-2010
		CA 2693847 A1	25-09-2008
		CN 101730848 A	09-06-2010
		DK 2138848 T3	21-02-2011
		EP 2138848 A1	30-12-2009
		ES 2304306 A1	01-10-2008
		ES 2357784 T3	29-04-2011
		JP 5314667 B2	16-10-2013
		JP 2010521981 A	01-07-2010
		PT 2138848 E	08-02-2011
		SI 2138848 T1	29-04-2011
		US 2010086932 A1	08-04-2010
		WO 2008113870 A1	25-09-2008

WO 2013158972	A1	24-10-2013	NONE

WO 2014018926	A1	30-01-2014	NONE
