CLAIMS

1. A cell-free assay method for detecting a ligand in a sample which ligand is capable of eliciting a steroid hormone genomic response, the assay method comprising the steps of:

   (i) contacting a sample with:

      (a) a steroid hormone receptor that forms a ligand-receptor complex with a ligand from the sample; and

      (b) a nucleic acid molecule comprising:

         (1) a polymerase promoter sequence;

         (2) a response element that is bound by the ligand-receptor complex; and

         (3) a reporter construct

         where the response element (2) is located between the promoter sequence (1) and the reporter construct (3), and (1), (2) and (3) are operably linked;

      (c) a single polypeptide polymerase; and

      (d) nucleoside triphosphates; and

   (ii) measuring a reduction or inhibition in transcription of the reporter construct caused by binding of the ligand-receptor complex to the response element,

   wherein, a measured reduction or inhibition in transcription of the reporter construct reflects detection of a ligand in the sample.

2. The assay method according to claim 1, further comprising contacting the sample with (e) a steroid hormone receptor cofactor selected from heat shock protein 90 (HSP90), a complex of HSP90 and heat shock protein 70 (HSP70), a complex of HSP90, HSP70 and heat shock protein 40 (HSP40), a complex of HSP90, HSP70, HSP40 and p23, a complex of HSP90, HSP70, HSP40, p23 and heat shock protein organizing protein (Hop), a complex of HSP90, HSP70, HSP40, p23, Hop and 48kD Hip protein (Hip), a complex of HSP90, HSP70, HSP40, p23, Hop, Hip and p60, and a complex of HSP90, HSP70, HSP40, p23, Hop, Hip, p60 and FKBP52.

3. The assay method according to claim 2, wherein the relative amount of HSP90 to steroid hormone receptor is x:1, where x is the amount of HSP90 and is defined as $[1.0 \leq x \leq 5.0]$.

4. The assay method according to any one of claims 1 to 3, wherein the relative amount of steroid hormone receptor to nucleic acid molecule is y:1, where y is the amount of steroid hormone receptor and is defined as [7.0 ≤ y ≤ 10.0].

5. The assay method according to any one of claims 1 to 4, wherein the polymerase is T7 RNA polymerase.

6. The assay method according to any one of claims 1 to 5, wherein the polymerase promoter sequence is defined by SEQ ID NO: 1.

7. The assay method according to any one of claims 1 to 6, wherein the reporter construct comprises a sequence encoding an RNA aptamer capable of binding to a fluorophore.

8. The assay method to claim 7, wherein the RNA aptamer is selected from Mango I, Mango II, Mango III and Mango IV, Spinach, iSpinach, Baby Spinach, Broccoli and Malachite Green.

9. The assay method according to claim 7 or claim 8 wherein the RNA aptamer is Mango II and comprises a F30 scaffold.

10. The assay method according to any one of claims 1 to 9, wherein the steroid hormone receptor is selected from the group consisting of androgen receptor (AR); estrogen receptor alpha (ER-α) and estrogen receptor beta (ER-β); progesterone receptor A (PRA) and progesterone receptor B (PRB); mineralocorticoid receptor (MR); and glucocorticoid receptor (GR).

11. The assay method according to any one of claims 1 to 10, wherein the response element is selected from:
    a. an androgen response element (ARE);
    b. an estrogen response element (ERE);
    c. a progesterone response element (PRE);
    d. a mineralocorticoid response element; and
    e. a glucocorticoid response element (GRE).

12. The assay method according to claim 11 wherein:
    a. the androgen response element (ARE) comprises 5'–AGAACAnnnTGTTCT–3' (SEQ ID NO: 4), wherein n is A, T, G or C;

b. an estrogen response element (ERE) comprises 5'–AGGTCAnnnTGACCT–3' (SEQ ID NO: 8), wherein n is A, T, G or C;
c. a progesterone response element (PRE) comprises 5'–GGTACAAACTGTTCT–3' (SEQ ID NO: 10);
d. a mineralocorticoid response element comprises 5'–AGAACAnAATGTTCT–3' (SEQ ID NO: 12), wherein n is A, T, G or C; and
e. a glucocorticoid response element (GRE) comprises 5'–AGAACAnAATGTTCT–3' (SEQ ID NO: 12), wherein n is A, T, G or C.

13. The assay method according to any one of claims 1 to 12, which is configured to:
a. detect a ligand that binds to an androgen receptor, and the nucleic acid molecule comprises a sequence defined by SEQ ID NO: 14;
b. detect a ligand that binds to an estrogen receptor, and the nucleic acid molecule comprises a sequence defined by SEQ ID NO: 15;
c. detect a ligand that binds to a progesterone receptor and the nucleic acid molecule comprises a sequence defined by SEQ ID NO: 16;
d. detect a ligand that binds to a mineralocorticoid receptor and the nucleic acid molecule comprises a sequence defined by SEQ ID NO: 17; and/or
e. detect a ligand that binds to a glucocorticoid receptor and the nucleic acid molecule comprises a sequence defined by SEQ ID NO: 18.

14. The assay method according to any one of claims 1 to 12, wherein the nucleic acid molecule comprises any one of the sequences defined by SEQ ID Nos: 19-73.

15. The assay method according to any one of claims 1 to 14, wherein the sample is a biological sample.

16. The assay method according to claim 15, wherein the biological sample is selected from blood, plasma, serum, saliva, interstitial fluid, semen and urine.

17. The assay method according to any one of claims 1 to 16, wherein the sample is a biological sample obtained from an athlete.

18. The assay method according to claim 17, wherein the athlete is selected from a human athlete, an equine athlete, a canine athlete and a camelid athlete.

19. The assay method according to claim 17 or claim 18, wherein the presence of a ligand in the biological sample reflects the doping status of the athlete.

20. The assay method according to any one of claims 1 to 14, wherein the sample is a water sample.