Reverse transcription-polymerase chain reaction (RT-PCR)

Objectives:

- To detect and quantify mRNA expression using a most sensitive technique
- Can be used to quantify mRNA levels from smaller samples
- Allows the determination of mRNA expression in various tissues
- Allows the comparison of mRNA expression between normal and pathophysiological, treated or untreated conditions

Summarized methodology:

Several steps are to be taken into account in order to optimize the results to be obtained:

- Tissue harvesting and homogenization in RNase free conditions
- Extraction of total RNA from tissue using the TRIzol® reagent method (checking of the absence of genomic DNA contamination)
- Amplification of cDNA using specific primers
- Loading of PCR product onto an agarose gel for electrophoresis

Endpoints:

- Measurement of changes in gene expression between different physiological or pathophysiological, treated or untreated conditions using relative quantification i.e. comparing mRNA expression levels between multiple samples, using an internal control (gene coding for ubiquitous protein expression i.e β2-microglobulin or GAPDH) for sample normalization.

NB: *Pelvipharm will gladly study the feasibility of evaluating the expression and activity of other proteins to meet its client's needs.*

Links to applicable therapeutic areas / targeted disorders:

- Sexual pharmacology
  * ED (Erectile Dysfunction)
  * Ejaculatory Disorders
  * FSD (Female Sexual Dysfunction)

- Lower urinary tract
  * BPH (Benign prostatic Hyperplasia)
  * SUI (Stress Urinary Incontinence)
  * SCI (Spinal Cord Injury)
  * NDO (Neurogenic Detrusor Overactivity)
  * OAB (Overactive Bladder)
  * IC (Interstitial Cystitis)

- Cardiovascular and metabolism pharmacology
  * Hypertension
  * Metabolic syndrome
  * Atherosclerosis
  * Diabetes Mellitus
  * Myocardial infarction