Supplementary Materials

Preparation of EBs for gene expression analysis

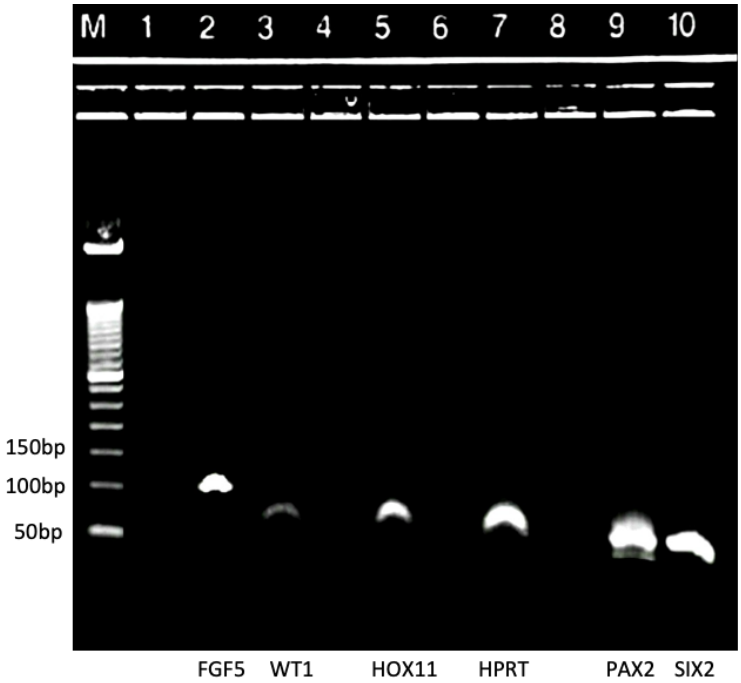
EBs from one well were transferred to a 1.5 mL eppendorf tube, washed and centrifuged at 1000 rpm with PBS twice for 5 minutes each. The supernatant was discarded, the EBs were suspended in 350µL lysis buffer prepared as stock solution beforehand containing RLT buffer from RNeasy mini kit (Qiagen) mixed with β-mercaptoethanol (Life Technologies) in 1:1 ratio. The samples were then stored at -80°C to be processed for gene expression analysis using RT-PCR later.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RNA extraction was carried out on EB samples preserved at -80°C with RNA lysis buffer (Qiagen) and β-mercaptoethanol (Sigma Aldrich) using an RNeasy mini RNA extraction kit (Qiagen) per manufacturer instructions. The yield and purity of RNA extracted was assessed in a Nanodrop Lyte spectrophotometer (ThermoFisher Scientific) using the 260: 280 nm absorbance ratio (A260/A280). Sample integrity was further assessed by gel electrophoresis. Extracted RNA was then amplified using a One-step RTPCR kit (Qiagen) and specific primers (Supplementary Table 1) in a thermal cycler. Amplified products were visualised by electrophoresis on 2% agarose SYBR Gold gels, imaged using the ChemiDoc™ XRS+ gel imaging system (Biorad). PCR product lengths were matched against a 50-2000 bp ladder (Invitrogen, ThermoFisher Scientific) sequence. Human Hypoxanthine-guanine Phospho Ribosyl Transferase (HPRT) was used as the housekeeping gene. All primer sequences were obtained using the Entrez Primer Blast primer designing tool. Amplicon lengths were confirmed using NCBI (National Centre for Biotechnology Information) Nucleotide Blast programme.

DEVELOPMENTAL STAGE	GENE	PRIMER SEQUENCES	T_m (°C)	T_A (°C)	AMPLICON LENGTH
Epiblast	hFGF5	**Fwd**: GGCTACACATCATCAATCAGTATTT	61.9	56	129bp
		Rev: AGGTGCTAGAGAGTGACTTGT	57.2		
Posterior Intermediate Mesoderm	hHOX11/TLX1	**Fwd**: AGGCAGCTCTCGGTTCATTG	61.7	65	60bp
		Rev: CGCTCCTCCGAAAGGATCAG	63.3		
	hWT1	**Fwd**: GTTTGAGGCCGGTCTTTGC	66.9	65	78bp
		Rev: AGAACTCGGGCCCAAGGATG	69.5		
Metanephric Mesenchyme	hPAX2	**Fwd**: CCCGCCTTACTAAGTTCCCC	65.5	62.9	70bp
		Rev: CTAGTGGCGGTCATAGGCAG	64.6		
	hSIX2	**Fwd**: TTCTGGTTGGCGATGCTCTC	67.7	62.9	67bp
		Rev: GCCTTCACCTAGCGACTGAC	63.9		

Supplementary Table S1: Information on primers used for RT-PCR gene expression in this study. Nephrogenic gene expression was analyzed against expression of constitutively expressed human HPRT (TA=65°C, 80 bp). All primer sequences were designed using Entrez Primer Blast programme and amplicon lengths were confirmed through NCBI Nucleotide Blast programme. NCBI=National Centre for Biotechnology Information; bp=Base pairs; Fwd=Forward; Rev=Reverse; h=Human; T_m= primer melting temperature; T_A= primer annealing temperature.



Supplementary Figure S1: Expression of renal genes measured by reverse transcriptase polymerase chain reaction (RT-PCR). HiPSC derived EBs were cultured in renal differentiation medium to drive development into metanephric mesenchyme. Gene expression was assessed at different developmental stages (Epiblast, Posterior Intermediate Mesoderm and Metanephric Mesenchyme) by reverse transcription polymerase chain reaction (RT-PCR). The image above was obtained with a ChemiDoc XRS+ gel imaging system following electrophoresis of the amplified cDNA product on 2% Agarose SYBR Gold E-Gels. Genes examined: FGF5 (Epiblast, 129bp amplicon); WT1 (posterior intermediate mesoderm, 78bp amplicon); HOX11 (posterior intermediate mesoderm, 80bp amplicon), PAX2 (metanephric mesenchyme, 70bp amplicon) and SIX2 (metanephric mesenchyme, 67bp amplicon). This is a representative image of n=3 experiments.