

Kidney Lineage & hPSC Differentiation Map

Below is a compact branch map of developmental origins for kidney cell types you asked about (glomerular components, proximal tubule, collecting duct) plus practical, commonly used hPSC differentiation cues and markers.

A. Differentiation Branch Map (developmental origins)

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hPSC (OCT4, NANOG, SOX2)
└─▶ Primitive streak / Mesendoderm (T/BRACHYURY, MIXL1, EOMES)
    └─▶ Intermediate mesoderm (OSR1, PAX2, LHX1)
        └─▶ Metanephric mesenchyme / Nephron progenitor cells (NPC) (SIX2,
CITED1, WT1, HOXA11)
            │   └─▶ Nephron epithelium (via MET; PAX2, LHX1, EPCAM)
            │       │   └─▶ Podocyte (WT1, NPHS1, NPHS2, PODXL, SYNPO)
            │       │   └─▶ Proximal tubule (LTL+, HNF4A, CUBN, SLC34A1, SLC22A6/OAT1)
            │       │   └─▶ Loop of Henle (UMOD, SLC12A1)
            │       │   └─▶ Distal tubule (SLC12A3, CALB1)
            │   └─▶ Cortical stroma (FOXD1, MEIS1)
            │       └─▶ Mesangial cell (PDGFRB, DESMIN, ACTA2(low), MEIS1)
            └─▶ Ureteric bud (RET, GATA3, HOXB7, SOX9, ETV5)
                └─▶ Collecting duct epithelium (KRT7, KRT8/18, GATA3, DBA+)
                    │   └─▶ Principal cell (AQP2, AQP3/4, SCNN1A/ENaCα, AVPR2)
                    │   └─▶ Intercalated cell (FOXI1, ATP6V1B1/B2, SLC4A1[α-IC],
SLC26A4[β-IC])

(Non-nephron, vascular influx)
└─▶ Endothelial lineage (hemangioblast → EC) (KDR/VEGFR2, PECAM1/CD31, CDH5/VE-
cadherin)
    └─▶ Glomerular capillary endothelium (PLVAP↓ with maturation; NOS3, EMCN)
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Notes - Podocytes and all nephron tubule segments derive from **nephron epithelium** (metanephric mesenchyme lineage), while **collecting duct** comes from the **ureteric bud** lineage. - **Glomerular capillary endothelium** is vascular (endothelial) in origin and immigrates into the glomerular cleft; **mesangial cells** are stromal (FOXD1+) derivatives.

B. Marker Panels (bench-friendly)

Early stages - hPSC: OCT4, NANOG, SOX2 - Primitive streak/mesendoderm: T/BRACHYURY, MIXL1, EOMES - Intermediate mesoderm: OSR1, PAX2, LHX1 (± GATA3 low)

Nephron progenitor (NPC / MM) - SIX2, CITED1, WT1, HOXA11; surface: ITGA8, PDGFRA(low)

Nephron epithelium & segments - Podocyte: WT1, NPHS1 (nephrin), NPHS2 (podocin), PODXL, SYNPO - Proximal tubule: LTL binding, HNF4A, CUBN, LRP2/megalin, SLC34A1, SLC22A6 (OAT1) - Loop of Henle (TAL): UMOD, SLC12A1 (NKCC2), KCNJ1 - Distal convoluted tubule: SLC12A3 (NCC), CALB1

Ureteric bud / Collecting duct - UB progenitor: RET, GATA3, HOXB7, SOX9, ETV5 - Collecting duct (generic): KRT7, KRT8/18, EPCAM, DBA lectin+ - Principal cell: AQP2 (apical), AQP3/4 (basolateral), AVPR2, SCNN1A (ENaC α) - Intercalated cell: FOXI1, ATP6V1B1/B2; α -IC: SLC4A1 (AE1); β -IC: SLC26A4 (Pendrin)

Vascular / Mesangial - Endothelium: KDR/VEGFR2, PECAM1/CD31, CDH5/VE-cadherin, vWF (mature), NOS3/eNOS - Glomerular EC maturation: EMCN, PLVAP \downarrow , response to shear - Mesangial: PDGFRB, DESMIN, ACTA2(α SMA, typically low to moderate), RGS5, MEIS1

Stroma - FOXD1, MEIS1, COL1A1/3A1

C. Practical hPSC Differentiation Cues (common, modular)

(Timings are representative; labs adapt per line. CHIR = CHIR99021.)

1) Toward Intermediate Mesoderm

- Days 0–2: PS induction with **CHIR** (WNT activation) \pm **BMP4/Activin A**
- Days 2–4: IM patterning with **FGF2/FGF9** + **Retinoic acid (RA)**; taper WNT
- Readouts: OSR1+, PAX2+, LHX1+

2) Nephron Progenitor / Metanephric Mesenchyme

- Maintain with **FGF9/20** + **BMP7**; brief **CHIR** pulse to sustain NPC
- Readouts: SIX2+, CITED1+, WT1+; epithelialization competence

3) Nephron Epithelium (Renal vesicle \rightarrow S-shaped body)

- **MET** via controlled **WNT (CHIR) pulse** + **FGF9**; 3D organoid aggregation helps
- Readouts: EPCAM+, PAX2+, LHX1+

3a) Podocyte maturation - Add **RA** + **Activin A** + **BMP7**; expose to **VEGF-A** (paracrine from UB/endothelium) - Optional: shear/flow, co-culture with ECs; - Readouts: WT1hi, NPHS1/2, PODXL, SYNPO; foot-process genes

3b) Proximal tubule maturation - Supplement **EGF**, **HGF**, **cAMP** (e.g., forskolin/IBMX) \pm **dexamethasone**; moderate **Notch activation** (Jagged1) can help - Readouts: LTL+, HNF4A, CUBN, LRP2, SLC34A1, OAT1/3 transport

3c) Loop of Henle / Distal - For TAL: maintain **cAMP**, longer culture; \pm **dDAVP** later - For DCT: **EGF** + partial **WNT inhibition** (e.g., IWR-1) - Readouts: UMOD, SLC12A1 (TAL); SLC12A3, CALB1 (DCT)

4) Ureteric Bud → Collecting Duct

- Specify from IM with **GDNF** + **FGF9/10** + **RA** (± **EGF**) to yield UB-like epithelium
- Branching/maturation with **GDNF** cycling, **HGF**, and 3D matrices
- Readouts: RET+, GATA3+, HOXB7+, SOX9+, ETV5+

4a) Principal cell fate - Elevate **cAMP** (forskolin) + **dDAVP** (vasopressin analog) ± **aldosterone** - Readouts: AQP2 apical trafficking (check with dDAVP), AQP3/4 basolateral, ENaCα

4b) Intercalated cell fate - Notch modulation (timed Jagged1 exposure or γ-secretase inhibition) + **EGF** - Bias to α-IC with **aldosterone**; to β-IC with **bicarbonate/alkali** - Readouts: FOXI1, ATP6V1B1/B2; α-IC (SLC4A1); β-IC (SLC26A4)

5) Endothelial (for glomerular capillaries)

- Mesodermal EC induction with **BMP4** + **FGF2** + **VEGF-A**; mature under **shear stress**
- Co-culture with podocytes ± **ANG1/ANGPT1**, **VEGF-A** gradients to promote a fenestrated glomerular phenotype
- Readouts: KDR/VEGFR2, PECAM1, CDH5 → NOS3, EMCN; PLVAP down with maturation

6) Mesangial (from FOXD1+ stroma)

- Pattern IM toward stroma with **PDGFR** signaling; then **PDGF-BB** + low **TGF-β1** for mesangiogenesis
- Readouts: PDGFRB, DESMIN, ACTA2(low-mod), MEIS1; matrix genes (COL4A5/6, FN1)

D. Assembly Tips (Organoids / Co-culture)

- Combine **NPC-derived nephron epithelium** with **UB-like epithelium** to improve segment identity; add **ECs** for vascularization.
- Use 3D matrices (Matrigel/ECM substitutes), apply **flow** where possible (microfluidics) to mature PT and ECs.
- Validate function: PT uptake (albumin, FITC-dextran), ENaC/AQP2 trafficking in CD, trans-endothelial resistance and fenestrations in ECs, slit diaphragm proteins in podocytes.

Quick Reference: Stage → Cues → Markers

- PS: CHIR ± BMP4/Activin → T, MIXL1
- IM: FGF2/9 + RA (taper WNT) → OSR1, PAX2, LHX1
- NPC/MM: FGF9/20 + BMP7 (± CHIR pulse) → SIX2, CITED1, WT1
- Nephron epith.: CHIR (pulse) + FGF9 → EPCAM, PAX2, LHX1
- Podocyte: RA + Activin + BMP7 + VEGF → WT1, NPHS1/2, PODXL
- PT: EGF + HGF + cAMP ± Dex; Notch mod. → LTL, HNF4A, CUBN, SLC34A1
- LOH/DCT: cAMP (± dDAVP) / WNT inhib. + EGF → UMOD/SLC12A1; SLC12A3/CALB1
- UB: GDNF + FGF9/10 + RA (± EGF) → RET, GATA3, HOXB7, SOX9
- CD Principal: cAMP + dDAVP ± Aldo → AQP2, AQP3/4, ENaCα

- CD Intercalated: Notch mod. \pm Aldo/alkali \rightarrow FOXI1, ATP6V1B1/B2, SLC4A1/SLC26A4
 - Endothelium: BMP4 + FGF2 + VEGF + shear \rightarrow KDR, CD31, CDH5 \rightarrow NOS3, EMCN
 - Mesangial: PDGF-BB + low TGF- β 1 \rightarrow PDGFRB, DESMIN, ACTA2(low)
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This map is designed to be practical at the bench: verify each stage with markers before moving on, and tune dose/time by line. If you want, I can adapt this into a one-page printable flowchart with reagent concentrations and day-by-day timing.