ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR RP25.UiPSC_mR5F_8 EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: RP25.UiPSC.mR5F.8

Annex 1: Morphology & exponential growth
Annex 2: Pluripotency markers by immunofluorescence and PCR
Annex 3: In vitro differentiation markers by Immunofluorescence
Annex 4: Karyotype
Annex 5: Authentication. Fingerprinting analysis
Annex 6: Mycoplasma test

Annex 1
Morphology & exponential growth

0 DIV

2 DIV

4 DIV

6 DIV

RP25_UiPSC_mR5F_8 Passage 8 Scale bars: 500μm
Annex 2
Pluripotency markers

Pluripotency associated markers OCT4, NANO<sub>G</sub>, and GAPDH (as a housekeeping gene) by PCR and SSEA<sub>3</sub>, OCT4, TRA181, SSEA4 and NANO<sub>G</sub> by immunofluorescence in RP25_UiPSC_mR5F_8 at passage 5. Scale bars: 50μm.
Annex 3

In vitro differentiation of embryoid bodies (EB) towards three germ layers and confocal images of immunofluorescence using specific antibodies against the ectodermal markers βIII-tubulin (TUJ1) and paired box protein 6 (PAX6), mesodermal marker α-sarcomeric actin (ASA) and endodermal markers α-fetoprotein (AFP) and SRY-box transcription factor 17 (SOX17), in RP25_UiPSC_mR5F_8 iPSC at passage 13. Nuclei stained with DAPI. Scale bars: 100 µm in a-c, d-e; 50 µm f-i
Case name: 21950499
NHC: CT0455
Name: PR25 p5
Date: 7/15/2022
Result: 46,XX
Annex 5
Authentication (microsatellites)

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<th>Loci STRs analizados</th>
<th>TH01</th>
<th>D21S11</th>
<th>D5S818</th>
<th>D13S317</th>
<th>D7S820</th>
<th>D16S539</th>
<th>CSF1PO</th>
<th>AMEL</th>
<th>vWA</th>
<th>TPOX</th>
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<tr>
<td>RP25 urine cells p2</td>
<td>6; 7</td>
<td>26; 30</td>
<td>12; 13</td>
<td>11; 12</td>
<td>11</td>
<td>9; 14</td>
<td>11; 13</td>
<td>X</td>
<td>16;</td>
<td>17; 8; 11</td>
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Microsatellite analysis authentication
Parental urine cells: RP25 urine cells p2
hiPS generated: RP25_UiPSC_mR5F p5
Annex 6 Mycoplasma test