

7. ANEXO

Tabla A1. Combinación de 12 primer forward con 8 reverse obteniendo 96 marcajes distintos.

| R ↓ | F → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|----|
| RA | RA;CGATGT F1;ATTGGC | RA;CGATGT F2;GCCAAT | RA;CGATGT F3;TGACCA | RA;CGATGT F3;TGACCA | RA;CGATGT F5;TAGCTT | RA;CGATGT F6;ACATCG | RA;CGATGT F7;GGCTAC | RA;CGATGT F8;AAGCTA | RA;CGATGT F9;CGTGAT | RA;CGATGT F10;TGGTCA | RA;CGATGT F11;CTGATC | RA;CGATGT F12;GATCTG | |
| RB | RB;GCCAAT F1;ATTGGC | RB;GCCAAT F2;GCCAAT | RB;GCCAAT F3;TGACCA | RB;GCCAAT F3;TGACCA | RB;GCCAAT F5;TAGCTT | RB;GCCAAT F6;ACATCG | RB;GCCAAT F7;GGCTAC | RB;GCCAAT F8;AAGCTA | RB;GCCAAT F9;CGTGAT | RB;GCCAAT F10;TGGTCA | RB;GCCAAT F11;CTGATC | RB;GCCAAT F12;GATCTG | |
| RC | RC;CAGATC F1;ATTGGC | RC;CAGATC F2;GCCAAT | RC;CAGATC F3;TGACCA | RC;CAGATC F3;TGACCA | RC;CAGATC F5;TAGCTT | RC;CAGATC F6;ACATCG | RC;CAGATC F7;GGCTAC | RC;CAGATC F8;AAGCTA | RC;CAGATC F9;CGTGAT | RC;CAGATC F10;TGGTCA | RC;CAGATC F11;CTGATC | RC;CAGATC F12;GATCTG | |
| RD | RD;GCCTAA F1;ATTGGC | RD;GCCTAA F2;GCCAAT | RD;GCCTAA F3;TGACCA | RD;GCCTAA F3;TGACCA | RD;GCCTAA F5;TAGCTT | RD;GCCTAA F6;ACATCG | RD;GCCTAA F7;GGCTAC | RD;GCCTAA F8;AAGCTA | RD;GCCTAA F9;CGTGAT | RD;GCCTAA F10;TGGTCA | RD;GCCTAA F11;CTGATC | RD;GCCTAA F12;GATCTG | |
| RE | RE;CACTGT F1;ATTGGC | RE;CACTGT F2;GCCAAT | RE;CACTGT F3;TGACCA | RE;CACTGT F3;TGACCA | RE;CACTGT F5;TAGCTT | RE;CACTGT F6;ACATCG | RE;CACTGT F7;GGCTAC | RE;CACTGT F8;AAGCTA | RE;CACTGT F9;CGTGAT | RE;CACTGT F10;TGGTCA | RE;CACTGT F11;CTGATC | RE;CACTGT F12;GATCTG | |
| RF | RF;ATTGGC F1;ATTGGC | RF;ATTGGC F2;GCCAAT | RF;ATTGGC F3;TGACCA | RF;ATTGGC F3;TGACCA | RF;ATTGGC F5;TAGCTT | RF;ATTGGC F6;ACATCG | RF;ATTGGC F7;GGCTAC | RF;ATTGGC F8;AAGCTA | RF;ATTGGC F9;CGTGAT | RF;ATTGGC F10;TGGTCA | RF;ATTGGC F11;CTGATC | RF;ATTGGC F12;GATCTG | |
| RG | RG;TCAAGT F1;ATTGGC | RG;TCAAGT F2;GCCAAT | RG;TCAAGT F3;TGACCA | RG;TCAAGT F3;TGACCA | RG;TCAAGT F5;TAGCTT | RG;TCAAGT F6;ACATCG | RG;TCAAGT F7;GGCTAC | RG;TCAAGT F8;AAGCTA | RG;TCAAGT F9;CGTGAT | RG;TCAAGT F10;TGGTCA | RG;TCAAGT F11;CTGATC | RG;TCAAGT F12;GATCTG | |
| RH | RH;CGTACG F1;ATTGGC | RH;CGTACG F2;GCCAAT | RH;CGTACG F3;TGACCA | RH;CGTACG F3;TGACCA | RH;CGTACG F5;TAGCTT | RH;CGTACG F6;ACATCG | RH;CGTACG F7;GGCTAC | RH;CGTACG F8;AAGCTA | RH;CGTACG F9;CGTGAT | RH;CGTACG F10;TGGTCA | RH;CGTACG F11;CTGATC | RH;CGTACG F12;GATCTG | |

Tabla A2. Primera librería obtenida tras la reacción en el procesador HTG con 10 muestras de CCM.

| # | Sample (3µl) | Sample Plate | Well | Forward primer (3µl) | Reverse primer (3µl) |
|----|--------------|--------------------|------|----------------------|----------------------|
| 1 | CM1 | 31012017_ASP_CM_IO | A1 | F1 | RA |
| 2 | CM2 | 31012017_ASP_CM_IO | B1 | F1 | RB |
| 3 | CM3 | 31012017_ASP_CM_IO | C1 | F1 | RC |
| 4 | CM4 | 31012017_ASP_CM_IO | D1 | F1 | RD |
| 5 | CM5 | 31012017_ASP_CM_IO | E1 | F1 | RE |
| 6 | CM6 | 31012017_ASP_CM_IO | F1 | F1 | RF |
| 7 | CM7 | 31012017_ASP_CM_IO | G1 | F1 | RG |
| 8 | CM8 | 31012017_ASP_CM_IO | H1 | F1 | RH |
| 9 | CM9 | 31012017_ASP_CM_IO | A2 | F2 | RA |
| 10 | CM10 | 31012017_ASP_CM_IO | B2 | F2 | RB |
| 11 | ASPS 001 | 31012017_ASP_CM_IO | C2 | F2 | RC |
| 12 | ASPS 003 | 31012017_ASP_CM_IO | D2 | F2 | RD |
| 13 | ASPS 004 | 31012017_ASP_CM_IO | E2 | F2 | RE |
| 14 | ASPS 005 | 31012017_ASP_CM_IO | F2 | F2 | RF |
| 15 | ASPS 006 | 31012017_ASP_CM_IO | G2 | F2 | RG |
| 16 | ASPS 007 | 31012017_ASP_CM_IO | H2 | F2 | RH |
| 17 | ASPS 008 | 31012017_ASP_CM_IO | A3 | F3 | RA |
| 18 | ASPS 009 | 31012017_ASP_CM_IO | B3 | F3 | RB |
| 19 | ASPS 010 | 31012017_ASP_CM_IO | C3 | F3 | RC |
| 20 | ASPS 011 | 31012017_ASP_CM_IO | D3 | F3 | RD |
| 21 | ASPS 012 | 31012017_ASP_CM_IO | E3 | F3 | RE |
| 22 | ASPS 013 | 31012017_ASP_CM_IO | F3 | F3 | RF |
| 23 | ASPS 015 | 31012017_ASP_CM_IO | G3 | F3 | RG |
| 24 | ASPS 016 | 31012017_ASP_CM_IO | H3 | F3 | RH |

Tabla A3. Segunda librería obtenida tras la reacción en el procesador HTG con 21 muestras de CCM.

| # | Sample (3µl) | Sample Plate | Well | Forward primer (3µl) | Reverse primer (3µl) |
|----|--------------|---------------------|------|----------------------|----------------------|
| 1 | ASPS18 | 02082017_ASPS_CM_IO | A1 | F1 | RA |
| 2 | ASPS19 | 02082017_ASPS_CM_IO | B1 | F1 | RB |
| 3 | ASPS20 | 02082017_ASPS_CM_IO | C1 | F1 | RC |
| 4 | CM11 | 02082017_ASPS_CM_IO | D1 | F1 | RD |
| 5 | CM12 | 02082017_ASPS_CM_IO | E1 | F1 | RE |
| 6 | CM13 | 02082017_ASPS_CM_IO | F1 | F1 | RF |
| 7 | CM14 | 02082017_ASPS_CM_IO | G1 | F1 | RG |
| 8 | CM15 | 02082017_ASPS_CM_IO | H1 | F1 | RH |
| 9 | CM16 | 02082017_ASPS_CM_IO | A2 | F2 | RA |
| 10 | CM17 | 02082017_ASPS_CM_IO | B2 | F2 | RB |
| 11 | CM18 | 02082017_ASPS_CM_IO | C2 | F2 | RC |
| 12 | CM19 | 02082017_ASPS_CM_IO | D2 | F2 | RD |
| 13 | CM20 | 02082017_ASPS_CM_IO | E2 | F2 | RE |
| 14 | CM21 | 02082017_ASPS_CM_IO | F2 | F2 | RF |
| 15 | CM22 | 02082017_ASPS_CM_IO | G2 | F2 | RG |
| 16 | CM23 | 02082017_ASPS_CM_IO | H2 | F2 | RH |
| 17 | CM24 | 02082017_ASPS_CM_IO | A3 | F3 | RA |
| 18 | CM25 | 02082017_ASPS_CM_IO | B3 | F3 | RB |
| 19 | CM26 | 02082017_ASPS_CM_IO | C3 | F3 | RC |
| 20 | CM27 | 02082017_ASPS_CM_IO | D3 | F3 | RD |
| 21 | CM28 | 02082017_ASPS_CM_IO | E3 | F3 | RE |
| 22 | CM29 | 02082017_ASPS_CM_IO | F3 | F3 | RF |
| 23 | CM30 | 02082017_ASPS_CM_IO | G3 | F3 | RG |
| 24 | CM31 | 02082017_ASPS_CM_IO | H3 | F3 | RH |

Tabla A4. Tercera librería obtenida tras la reacción en el procesador HTG con 17 muestras de CCM. Nótese la falta de la muestra 39

| # | Sample (3µl) | Sample Plate | Well | Forward primer (3µl) | Reverse primer (3µl) |
|----|--------------|----------------|------|----------------------|----------------------|
| 1 | CM32 | 21092017_CM_IO | A1 | F1 | RA |
| 2 | CM33 | 21092017_CM_IO | B1 | F1 | RB |
| 3 | CM34 | 21092017_CM_IO | C1 | F1 | RC |
| 4 | CM35 | 21092017_CM_IO | D1 | F1 | RD |
| 5 | CM36 | 21092017_CM_IO | E1 | F1 | RE |
| 6 | CM37 | 21092017_CM_IO | F1 | F1 | RF |
| 7 | CM38 | 21092017_CM_IO | G1 | F1 | RG |
| 8 | CM40 | 21092017_CM_IO | H1 | F1 | RH |
| 9 | CM41 | 21092017_CM_IO | A2 | F2 | RA |
| 10 | CM42 | 21092017_CM_IO | B2 | F2 | RB |
| 11 | CM43 | 21092017_CM_IO | C2 | F2 | RC |
| 12 | CM44 | 21092017_CM_IO | D2 | F2 | RD |
| 13 | CM45 | 21092017_CM_IO | E2 | F2 | RE |
| 14 | CM46 | 21092017_CM_IO | F2 | F2 | RF |
| 15 | CM47 | 21092017_CM_IO | G2 | F2 | RG |
| 16 | CM48 | 21092017_CM_IO | H2 | F2 | RH |
| 17 | CM49 | 21092017_CM_IO | A3 | F3 | RA |
| 18 | CM12rep | 21092017_CM_IO | B3 | F3 | RB |
| 19 | CM22rep | 21092017_CM_IO | C3 | F3 | RC |
| 20 | CM23rep | 21092017_CM_IO | D3 | F3 | RD |
| 21 | CM31rep | 21092017_CM_IO | E3 | F3 | RE |
| 22 | CM32rep | 21092017_CM_IO | F3 | F3 | RF |
| 23 | CM44rep | 21092017_CM_IO | G3 | F3 | RG |
| 24 | CM48rep | 21092017_CM_IO | H3 | F3 | RH |

Tabla A5. Primer *pool* de secuenciación con 24 muestras, de las cuales 10 son de este estudio.

| Assay Type: mRNA | | Loading Concentration: 20 pM | | | | | |
|--------------------------------|------------------------|------------------------------|-----------------|-------------------------|-------------------------------------|--|------------------------|
| Sample name | Sample dilution factor | Library concentration (pM) | % of total pool | Library dilution factor | Diluent (µL) (10 mM Tris pH 8.5) | Volume of diluted library to pool (µL) | Clean-up well location |
| CM1 | 1 : 10k | 530,10 | 4,2% | 1:5 | 20,00 | 4,72 | |
| CM2 | 1 : 10k | 2263,08 | 4,2% | 1:5 | 20,00 | 1,10 | |
| CM3 | 1 : 10k | 1623,49 | 4,2% | 1:5 | 20,00 | 1,54 | |
| CM4 | 1 : 10k | 1571,25 | 4,2% | 1:5 | 20,00 | 1,59 | |
| CM5 | 1 : 10k | 3079,98 | 4,2% | 1:10 | 45,00 | 1,62 | |
| CM6 | 1 : 10k | 1233,64 | 4,2% | 1:5 | 20,00 | 2,03 | |
| CM7 | 1 : 10k | 3599,78 | 4,2% | 1:10 | 45,00 | 1,39 | |
| CM8 | 1 : 10k | 6057,81 | 4,2% | 1:20 | 95,00 | 1,65 | |
| CM9 | 1 : 10k | 978,24 | 4,2% | 1:5 | 20,00 | 2,56 | |
| CM10 | 1 : 10k | 6021,92 | 4,2% | 1:20 | 95,00 | 1,66 | |
| ASPS001 | 1 : 10k | 723,70 | 4,2% | 1:5 | 20,00 | 3,45 | |
| ASPS003 | 1 : 10k | 157,13 | 4,2% | 1:1 | Use Neat | 3,18 | |
| ASPS004 | 1 : 10k | 1071,20 | 4,2% | 1:5 | 20,00 | 2,33 | |
| ASPS005 | 1 : 10k | 565,33 | 4,2% | 1:5 | 20,00 | 4,42 | |
| ASPS006 | 1 : 10k | 1705,33 | 4,2% | 1:5 | 20,00 | 1,47 | |
| ASPS007 | 1 : 10k | 1146,29 | 4,2% | 1:5 | 20,00 | 2,18 | |
| ASPS008 | 1 : 10k | 3083,64 | 4,2% | 1:10 | 45,00 | 1,62 | |
| ASPS009 | 1 : 10k | 1046,80 | 4,2% | 1:5 | 20,00 | 2,39 | |
| ASPS010 | 1 : 10k | 1502,81 | 4,2% | 1:5 | 20,00 | 1,66 | |
| ASPS011 | 1 : 10k | 625,46 | 4,2% | 1:5 | 20,00 | 4,00 | |
| ASPS012 | 1 : 10k | 411,26 | 4,2% | 1:1 | Use Neat | 1,22 | |
| ASPS013 | 1 : 10k | 1115,77 | 4,2% | 1:5 | 20,00 | 2,24 | |
| ASPS015 | 1 : 10k | 1014,63 | 4,2% | 1:5 | 20,00 | 2,46 | |
| ASPS016 | 1 : 10k | 1026,83 | 4,2% | 1:5 | 20,00 | 2,43 | |
| Total library vol (µL): | | | | | | 54,92 | |

Tabla A6. Segundo *pool* de secuenciación con 16 muestras, de las cuales 13 son de este estudio.

| Assay Type: mRNA | | Loading Concentration: 20 pM | | | | | |
|--------------------------------|------------------------|------------------------------|-----------------|-------------------------|-------------------------------------|--|------------------------|
| Sample name | Sample dilution factor | Library concentration (pM) | % of total pool | Library dilution factor | Diluent (µL) (10 mM Tris pH 8.5) | Volume of diluted library to pool (µL) | Clean-up well location |
| ASPS-018 | 1 : 10k | 857,61 | 6,3% | 1:5 | 20,00 | 4,37 | |
| ASPS-019 | 1 : 10k | 1967,14 | 6,3% | 1:5 | 20,00 | 1,91 | |
| ASPS-020 | 1 : 10k | 3331,58 | 6,3% | 1:5 | 20,00 | 1,13 | |
| CM11 | 1 : 10k | 3562,65 | 6,3% | 1:5 | 20,00 | 1,05 | |
| CM12 | 1 : 10k | 7287,35 | 6,3% | 1:10 | 45,00 | 1,03 | |
| CM13 | 1 : 10k | 13261,53 | 6,3% | 1:20 | 95,00 | 1,13 | |
| CM14 | 1 : 10k | 13333,11 | 6,3% | 1:20 | 95,00 | 1,13 | |
| CM15 | 1 : 10k | 3673,68 | 6,3% | 1:5 | 20,00 | 1,02 | |
| CM16 | 1 : 10k | 2641,17 | 6,3% | 1:5 | 20,00 | 1,42 | |
| CM17 | 1 : 10k | 1083,02 | 6,3% | 1:5 | 20,00 | 3,46 | |
| CM18 | 1 : 10k | 3825,02 | 6,3% | 1:10 | 45,00 | 1,96 | |
| CM19 | 1 : 10k | 1939,15 | 6,3% | 1:5 | 20,00 | 1,93 | |
| CM20 | 1 : 10k | 9951,22 | 6,3% | 1:20 | 95,00 | 1,51 | |
| CM21 | 1 : 10k | 7330,08 | 6,3% | 1:10 | 45,00 | 1,02 | |
| CM22 | 1 : 10k | 3080,45 | 6,3% | 1:5 | 20,00 | 1,22 | |
| CM23 | 1 : 10k | 9956,46 | 6,3% | 1:20 | 95,00 | 1,51 | |
| Total library vol (µL): | | | | | | 26,79 | |

Tabla A7. Tercer *pool* de secuenciación con 16 muestras, todas de CCM.

| Assay Type: mRNA | | Loading Concentration: 20 pM | | | | | |
|--------------------------------|------------------------|------------------------------|-----------------|-------------------------|-------------------------------------|--|------------------------|
| Sample name | Sample dilution factor | Library concentration (pM) | % of total pool | Library dilution factor | Diluent (µL) (10 mM Tris pH 8.5) | Volume of diluted library to pool (µL) | Clean-up well location |
| CM24 | 1 : 10k | 2627,51 | 6,3% | 1:5 | 20,00 | 1,43 | |
| CM25 | 1 : 10k | 2417,22 | 6,3% | 1:5 | 20,00 | 1,55 | |
| CM26 | 1 : 10k | 3189,08 | 6,3% | 1:5 | 20,00 | 1,18 | |
| CM27 | 1 : 10k | 1992,51 | 6,3% | 1:5 | 20,00 | 1,88 | |
| CM28 | 1 : 10k | 532,34 | 6,3% | 1:1 | Use Neat | 1,41 | |
| CM29 | 1 : 10k | 1621,14 | 6,3% | 1:5 | 20,00 | 2,31 | |
| CM30 | 1 : 10k | 1973,10 | 6,3% | 1:5 | 20,00 | 1,90 | |
| CM31 | 1 : 10k | 4397,66 | 6,3% | 1:10 | 45,00 | 1,71 | |
| CM32 | 1 : 10k | 2086,08 | 6,3% | 1:5 | 20,00 | 1,80 | |
| CM33 | 1 : 10k | 385,32 | 6,3% | 1:1 | Use Neat | 1,95 | |
| CM34 | 1 : 10k | 236,99 | 6,3% | 1:1 | Use Neat | 3,16 | |
| CM35 | 1 : 10k | 376,00 | 6,3% | 1:1 | Use Neat | 1,99 | |
| CM36 | 1 : 10k | 1087,32 | 6,3% | 1:5 | 20,00 | 3,45 | |
| CM37 | 1 : 10k | 1223,61 | 6,3% | 1:5 | 20,00 | 3,06 | |
| CM38 | 1 : 10k | 793,44 | 6,3% | 1:5 | 20,00 | 4,73 | |
| CM40 | 1 : 10k | 2277,25 | 6,3% | 1:5 | 20,00 | 1,65 | |
| Total library vol (µL): | | | | | | 35,15 | |

Tabla A8. Cuarto *pool* de secuenciación con 16 muestras, 9 de CCM. También se incluyen 7 repeticiones

| Assay Type: mRNA | | Loading Concentration: 20 pM | | | | | |
|--------------------------------|------------------------|------------------------------|-----------------|-------------------------|-------------------------------------|--|------------------------|
| Sample name | Sample dilution factor | Library concentration (pM) | % of total pool | Library dilution factor | Diluent (µL) (10 mM Tris pH 8.5) | Volume of diluted library to pool (µL) | Clean-up well location |
| CM41 | 1 : 10k | 373,06 | 6,3% | 1:1 | Use Neat | 2,01 | |
| CM42 | 1 : 10k | 138,46 | 6,3% | 1:1 | Use Neat | 5,42 | |
| CM43 | 1 : 10k | 421,49 | 6,3% | 1:1 | Use Neat | 1,78 | |
| CM44 | 1 : 10k | 1115,07 | 6,3% | 1:5 | 20,00 | 3,36 | |
| CM45 | 1 : 10k | 848,52 | 6,3% | 1:5 | 20,00 | 4,42 | |
| CM46 | 1 : 10k | 4865,14 | 6,3% | 1:10 | 45,00 | 1,54 | |
| CM47 | 1 : 10k | 3112,17 | 6,3% | 1:5 | 20,00 | 1,20 | |
| CM48 | 1 : 10k | 2267,51 | 6,3% | 1:5 | 20,00 | 1,65 | |
| CM49 | 1 : 10k | 189,81 | 6,3% | 1:1 | Use Neat | 3,95 | |
| CM12rep | 1 : 10k | 3749,36 | 6,3% | 1:5 | 20,00 | 1,00 | |
| CM22rep | 1 : 10k | 1724,34 | 6,3% | 1:5 | 20,00 | 2,17 | |
| CM23rep | 1 : 10k | 2192,50 | 6,3% | 1:5 | 20,00 | 1,71 | |
| CM31rep | 1 : 10k | 3237,87 | 6,3% | 1:5 | 20,00 | 1,16 | |
| CM32rep | 1 : 10k | 2586,69 | 6,3% | 1:5 | 20,00 | 1,45 | |
| CM44rep | 1 : 10k | 2214,05 | 6,3% | 1:5 | 20,00 | 1,69 | |
| CM48rep | 1 : 10k | 2169,67 | 6,3% | 1:5 | 20,00 | 1,73 | |
| Total library vol (µL): | | | | | | 36,26 | |