**Cycling conditions for PCR** :

10' [30",30",60"] 35c @ 54°C for Sine

3' [30", 30", 10"] 35c @ 55°C for Kdr\_w

**Details of all reagents and kits used for Sine and kdr\_w:**

Pool Master Mix: 5x FIREPol Master Mix Ready to load

Recommended PCR reaction mix

|  |  |  |
| --- | --- | --- |
| Component | Volume | Final Conc. |
| Pool Master Mix: 5x FIREPol Master Mix Ready to load | 4µl | 1x |
| Forward primer (10 pmol/µl) | 0.2-0.6 µl | 0.1-0.3 µM |
| Reverse primer (10 pmol/µl) | 0.2-0.6 µl | 0.1-0.3 µM |
| DNA template | Variable | Variable |
| H2O | Up to 20 µl |  |

Conc.of cDNA 0.01 pg/µl-0.1 ng/µl; gDNA 0.1 ng/µl-10ng/µl

**Details of the primers used :**

S200X 6.1F: TCG-CCT-TAG-ACC-TTG-CGT-TA

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| --- | --- | --- | --- |
| Synthesis scale: 0.04 µmol | No. Bases: 20 | Synthesised 4.5 OD | Purification: RP-Column |
| Dissolve in (µl): 222 µl | GC%: 50 | Delivered: 135 µg  22.2 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6059 g/mol | A3 C6 G4 T7 | TM: 51.8 °C |  |

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| --- | --- | --- | --- |
| Synthesis scale: 0.04 µmol | No. Bases: 20 | Synthesised 4.9 OD | Purification: RP-Column |
| Dissolve in (µl): 242 µl | GC%: 50 | Delivered: 147 µg  24.2 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6059 g/mol | A3 C6 G4 T7 | TM: 51.8 °C |  |

S200X 6.1R: CGC-TTC-AAG-AAT-TCG-AGA-TAC

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| Synthesis scale: 0.04 µmol | No. Bases: 21 | Synthesised 7.2 OD | Purification: RP-Column |
| Dissolve in (µl): 301 µl | GC%: 42.9 | Delivered: 193 µg  30.1 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6414 g/mol | A7 C5 G4 T5 | TM: 50.5°C |  |

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| Synthesis scale: 0.04 µmol | No. Bases: 21 | Synthesised 4.8 OD | Purification: RP-Column |
| Dissolve in (µl): 203 µl | GC%: 42.9 | Delivered: 130 µg  20.3 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6414 g/mol | A7 C5 G4 T5 | TM: 50.5°C |  |

Kdr\_w D1 : ATA-GAT-TCC-CCG-ACC-ATG

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| --- | --- | --- | --- |
| Synthesis scale: 0.04 µmol | No. Bases: 18 | Synthesised 6.8 OD | Purification: RP-Column |
| Dissolve in (µl): 351 µl | GC%: 50 | Delivered: 191 µg  35.1 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 5444 g/mol | A5 C6 G3 T4 | TM: 48.0 °C |  |

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| --- | --- | --- | --- |
| Synthesis scale: 0.04 µmol | No. Bases: 18 | Synthesised 5.4 OD | Purification: RP-Column |
| Dissolve in (µl): 278 µl | GC%: 50 | Delivered: 151 µg  27.8 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 5444 g/mol | A5 C6 G3 T4 | TM: 48.0 °C |  |

Kdr\_w D2 : AGA-CAA-GGA-TGA-TGA-ACC

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| --- | --- | --- | --- |
| Synthesis scale: 0.04 µmol | No. Bases: 18 | Synthesised 5.6 OD | Purification: RP-Column |
| Dissolve in (µl): 251 µl | GC%: 44.4 | Delivered: 140 µg  25.1 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 5566 g/mol | A8 C3 G5 T2 | TM: 45.7 °C |  |

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| --- | --- | --- | --- |
| Synthesis scale: 0.04 µmol | No. Bases: 18 | Synthesised 5.6 OD | Purification: RP-Column |
| Dissolve in (µl): 252 µl | GC%: 44.4 | Delivered: 140 µg  25.2 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 5566 g/mol | A8 C3 G5 T2 | TM: 45.7 °C |  |

Kdr\_w D3 : AAT-TTG-CAT-TAC-TTA-CGA-CA

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| Synthesis scale: 0.04 µmol | No. Bases: 20 | Synthesised 5.8 OD | Purification: RP-Column |
| Dissolve in (µl): 259 µl | GC%: 30 | Delivered: 157 µg  25.9 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6075 g/mol | A7 C4 G2 T7 | TM: 43.6 °C |  |

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| Synthesis scale: 0.04 µmol | No. Bases: 20 | Synthesised 5.1 OD | Purification: RP-Column |
| Dissolve in (µl): 226 µl | GC%: 30 | Delivered: 137 µg  22.6 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6075 g/mol | A7 C4 G2 T7 | TM: 43.6 °C |  |

Kdr\_w D4 : CTG-TAG-TGA-TAG-GAA-ATT-TA

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| Synthesis scale: 0.04 µmol | No. Bases: 20 | Synthesised 4.1 OD | Purification: RP-Column |
| Dissolve in (µl): 173 µl | GC%: 30 | Delivered: 107 µg  17.3 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6195 g/mol | A7 C1 G5 T7 | TM: 43.6 °C |  |

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| --- | --- | --- | --- |
| Synthesis scale: 0.04 µmol | No. Bases: 20 | Synthesised 4.5 OD | Purification: RP-Column |
| Dissolve in (µl): 190 µl | GC%: 30 | Delivered: 117 µg  19.0 nmol | Delivery Form: Solid |
| Conc: 100µM |  |  | QC: MALDI-TOF |
| MW: 6195 g/mol | A7 C1 G5 T7 | TM: 43.6 °C |  |

**Model of any equipment used:**

* **Thermocyclers:**
* Eppendorf Flexlid Mastercycler Nexus

SN: 6333GR016050

* Eppendorf Flexlid Mastercycler Nexus

SN: 6333GP315452

* Biorad S1000 Thermal Cycler

SN: SC008106

* AppliedBiosystems by life Technologies: 2720 Thermal cycler

SN: 272S4171502

* **Transluminator Fisher Bioblock Scientific: VILBER LOURMAT**
* SN: 04 15008
* SN: 04 15009
* SN: 04 15010
* **Migration cuve:**
* Apelex MIDIGEL

SN: 1703131

* Apelex MIDIGEL

SN: 1703137

* **Vortex tecnoKartell TK3S;**

SN: 2010450

* **centrifuges:**
* Eppendorf Centrifuge 5415D

SN: 5425 33708

* Eppendorf Centrifuge 5424

SN: 5424YP027418

* **Pipettes:**
* **Eppendorf (100-1000µl)**

SN: J52095H

* **Eppendorf (20-200µl)**

SN: K29861G

* **Eppendorf (10-100µl)**

SN: H29455H

* **Eppendorf (2-20µl)**

SN: J54731H

* **Eppendorf (0.5-10µl)**

SN: L11136G

* **Electrophoresis Power Supply:**
* E 815 CNSort:

SN: 67716

* E 844 CNSort:

SN: 63990

* **Autoclave NUVE NC 40M**
* **TissuelyserII QIAGEN;** SN: 1217141155E
* **Water bath: Grant Instruments (Cambridge) Ltd, SUB Aqua 18, Type VDB18EU**
* **Distillator: GFL Typ 2104;** SN: 10325004 J
* **Scale: DENVER INSTRUMENT;** SN: 17204501