

Sequencing reaction setup

Sequencing Reaction Diluent (SRDV2 - 4x buffer)

| | | |
|------------------------|-------|-------------------------------------|
| 1M Tris Base pH9.0 | 160ml | 0.317M |
| 1M MgCl ₂ | 3ml | 0.0059M |
| Tetramethylene Sulfone | 50ml | 9.89% v/v (Sigma: T2,220-9 – 250ml) |
| Tween-20 | 0.9ml | 0.178% v/v (Sigma: P-9416 – 100ml) |
| Glycerol 50% | 60ml | 5.9% v/v |
| Formamide | 5.5ml | 1.088% v/v (Sigma: F9037 – 100ml) |
| ddH ₂ O | 224ml | |

Add Tetramethylene Sulfone last of the ingredients above

Make the above first then add

| | | |
|----------------------|------|-------------------------------|
| Pottassium Glutamate | 0.8g | 0.0078M (Sigma: G1149 – 100g) |
| BSA (20mg/ml) | 2ml | 79µg/ml (Sigma: B8667 – 5ml) |

Total 505.4ml

Prepare day before use and store at 4°C

96 and 384 well plasmid sequencing (universal primers)

Solutions Required

SRD

BigDye™ V3.1 Terminator mix

dGTP BigDye™ V3.0 Terminator mix

Forward/Reverse Sequencing mix dilutions (store at 4°C for no more than 2 weeks)

1/64th

| | | |
|-----------------------------------|------------|------------|
| BigDye™ V3.1 Terminator mix | 0.125µl | 0.3125ml |
| SRD | 0.9225µl | 2.30625ml |
| Primer* | 0.01µl | 0.025ml |
| HPLC H ₂ O | 0.9225µl | 2.30625ml |
| dGTP Big Dye™ V3.0 Terminator mix | 0.02µl | 0.05ml |
| Total Vol | 2µl | 5ml |

1/128th

| | | |
|-----------------------------------|------------|------------|
| BigDye™ V3.1 Terminator mix | 0.0625µl | 0.15625ml |
| SRD | 0.95375µl | 2.384375ml |
| Primer* | 0.01µl | 0.025ml |
| HPLC H ₂ O | 0.95375µl | 2.384375ml |
| dGTP Big Dye™ V3.0 Terminator mix | 0.02µl | 0.05ml |
| Total Vol | 2µl | 5ml |

1/256th

| | | |
|-----------------------------------|------------|------------|
| BigDye™ V3.1 Terminator mix | 0.03125µl | 0.125ml |
| SRD | 0.471875µl | 1.875ml |
| Primer* | 0.005µl | 0.02ml |
| HPLC H ₂ O | 0.471875µl | 1.875ml |
| dGTP Big Dye™ V3.0 Terminator mix | 0.02µl | 0.08ml |
| Total Vol | 1µl | 4ml |

1/512th

| | | |
|-----------------------------------|-------------|-----------------|
| BigDye™ V3.1 Terminator mix | 0.015625µl | 0.0625ml |
| SRD | 0.4796875µl | 1.919ml |
| Primer* | 0.005µl | 0.02ml |
| HPLC H ₂ O | 0.4796875µl | 1.918ml |
| dGTP Big Dye™ V3.0 Terminator mix | 0.02µl | 0.08ml |
| Total Vol | 1µl | 3.9995ml |

* primer is resuspended using HPLC water to a concentration in each tube of 100pmoles/µl.

Robot reaction set-up 1/64th and 1/128th:

| | |
|-------|---|
| 2µl | Sequencing mix, dispense using the Multidrop™ Combi set to 384 well 10mm plates or 96 well 22mm plates and protocol 96_27, small cassette and volume 2µl. |
| 2-3µl | DNA - dispense using Evolution P ³ |

Robot reaction set-up 1/256th and 1/512th (384 well only).

| | |
|---------|--|
| 1µl | Sequencing mix - dispense using the Multidrop™ Combi set to 384 well 10mm plates, small cassette and volume 1µl. |
| 1-1.5µl | DNA - dispense using Evolution P ³ |

Unique primers**Solutions Required**

SRD

BigDye™ V3.1 Terminator mix

dGTP BigDye™ V3.0 Terminator mix

1/64th (store at 4°C for no more than 2 weeks)

| | | |
|-----------------------------------|------------|------------|
| BigDye™ V3.1 Terminator mix | 0.125µl | 0.3125ml |
| SRD | 0.9275µl | 2.31875ml |
| HPLC H ₂ O | 0.9275µl | 2.31875ml |
| dGTP Big Dye™ V3.0 Terminator mix | 0.02µl | 0.05ml |
| Total Vol | 2µl | 5ml |

Reaction set-up

| | |
|-----|---|
| 2µl | Sequencing mix - dispense using the Multidrop™ Combi set to 96 well 22mm plates, small cassette, volume 2µl and protocol 96_27mm. |
| 2µl | DNA - dispense using Evolution P ³ |
| 2µl | Primer* - dispense using a multichannel pipette or Evolution P ³ |

* Add 1ml HPLC water to the primer (already done for genotyping test) - stock. **To 2µl stock primer add 20µl HPLC water before use (prepare in an ABgene 96 well plate).**

Cycling Protocol

384 well

Lid temp set to 90°C constant

Lid touching top of plate (heat seal), pressing down on top of plate (plastic seal)

Mode set to calculated

Step 1 96°C 30sec

Step2 92°C 5sec

Step3 52°C 8sec

Step4 60°C 1min 50sec

Go to step2 another 54 times

Step5 10°C forever

End

96 well

Lid temp set to 95°C constant for 96 well plates

Mode set to calculated

Step 1 96°C 45sec

Step2 92°C 10sec

Step3 52°C 10sec

Step4 60°C 2min

Go to step2 another 59 times

Step5 10°C forever

End

Sequencing reaction ethanol precipitation procedure

Solutions Required

80% Ethanol -20°C

Precipitation mix:

| | |
|------------------------------------|--------------|
| 99.7-100% Analytical grade Ethanol | 770ml |
| 3M Sodium Acetate Soln. | 16ml |
| ddH ₂ O | 188ml |
| Total Vol | 974ml |

1. Add 30µl precipitation mix to each well using the Multidrop™ Combi set to 384 well 10mm plates, standard cassette and volume 30µl.
2. Centrifuge for 25min at 4000rpm and 4°C using an eppendorf centrifuge.
3. Invert plate onto a Whatman blotting pad and spin for 1 min at 400rpm using an Eppendorf centrifuge.
4. Add 30µl 80% Ethanol (-20°C) using the Multidrop™ Combi set to 384 well 10mm plates, standard cassette and volume 30µl.
5. Centrifuge for 10 min at 4000rpm and 4°C using an eppendorf centrifuge.
6. Invert plate onto a Whatman blotting pad and spin for 1 min at 400rpm using an Eppendorf centrifuge.
7. Dry plate overnight at room temperature or for 30 min at 50°C.