Capillary Separation
Quick Reference Guide

1. Quantify your purified DNA using gel electrophoresis
2. Combine the correct amount of DNA and primer in tubes (<48) / plates (>48) according to Table 1

Table 1: Recommended amounts of template and primer for sequencing reactions

Template	Recommended Quantity
PCR Product 100 – 200 bp	1 – 3 ng
PCR Product 200 – 400 bp	2 – 4 ng
PCR Product 400 – 600 bp	4 – 6 ng
PCR Product 600 – 800 bp	6 – 10 ng
PCR Product >800 bp	10 – 25 ng
Plasmid, Single-stranded	50 – 100 ng
Plasmid, Double-stranded	200 – 500 ng
Primer Quantity	**3.2 pmol**

3. Add Milli-Q, BDTv3.1 and 5 x Dilution Buffer according to the reaction volume and concentration outline in Table 2. AGRF recommends the "halfvolume 0.25x" protocol.

Table 2: Recommended amounts of template for sequencing reaction

Components	Half 0.125x	Half 0.25x	Full 0.5x	Full 1x
Template Primer (0.8pmol/ul) MilliQ Water	7.75µL	7.5µL	14µL	12µL
BDT v3.1	0.5µL	1µL	4µL	8µL
5x BDT Buffer	1.75µL	1.5µL	2µL	-
Total	**10µL**	**10µL**	**20µL**	**20µL**

4. Perform the BDT-labelling reaction in a thermocycler using the cycling conditions Table 3 (NOTE: use an annealing temperature 5 ºC -10ºC lower than your primer's T_m).

Table 3: General Cycling Conditions for BDT labeling reaction

Temperature and Time	Number of cycles
96ºC for 2 mins	1
96ºC for 10 secs (denaturing) 50ºC for 5 secs (annealing) 60ºC for 4 mins (extension)	25 – 30
4ºC	Hold

5. Perform the sequencing cleanup, and dry the samples if required
6. Log into the AGRF website and fill out your submission form, choosing "Capillary separation (CS)" as your sample type
7. Print off the sample sheet and deliver it along with your samples to AGRF

Purified DNA
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PCR Product 200 – 400 bp	6 – 12 ng
PCR Product 400 – 600 bp	12 – 18 ng
PCR Product 600 – 800 bp	18 – 30 ng
PCR Product >800 bp	30 – 75 ng
Plasmid, Single-stranded	150 – 300 ng
Plasmid, Double-stranded	600 – 1500 ng
Sequencing Primer (T_m=55- 60°C)	**10 pmol***
Total Volume	**12 µL**

**10pmol corresponds to 1uL of a 10uM primer solution. You may find it easier to make a more dilute primer solution and add in a greater volume of primer (e.g. 4uL of a 2.5uM primer solution)*

3. Log into the AGRF website and fill out your submission form, choosing "Purified DNA (PD)" as your sample type
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Email AGRF at: sequencing@agrf.org.au

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Plasmid, Double-stranded	600 – 1500 ng
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Total Volume	**12 µL**

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