Updated on 5/22/2009
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Protocol of BAC Fingerprinting with SNaPshot™ Kit

1. **Re-suspension:** BAC DNAs isolated by Qiagen R.E.A.L Prep 96 Plasmid kit were re-suspended in 42 µl of ddH_2O in deep well block, vortex gently, and keep at room temperature overnight or at 4 ^oC over weekend..

2. **Restriction enzyme digestion:** Add 8.0 µl of the restriction enzyme cocktail (see below); incubate at 37 ^oC, for 3 hrs or longer (overnight is OK).

   Enzyme Cocktail (1x) (NEB enzymes):

Bam HI	2.0 units (0.10 µl)
Eco RI	2.0 units (0.10 µl)
Xba I	2.0 units (0.10 µl)
Xho I	2.0 units (0.10 µl)
Hae III	2.0 units (0.20 µl)
NEBuffer 2	5.0 µl
100X BSA	0.5 µl
RNase A (0.5 µg/µl, DNase free)	1.0 µl
β-Mercaptoethanol (1%)	1.0 µl

3. Transfer 50.0 µl of the digested DNAs into 96-PCR plate.

4. **Labeling:** Add 10.0 µl of SNaPshot labeling cocktail (see below), briefly spin down; incubate at 65 ^oC for 60′.

   Labeling Cocktail (1x):

SNaPshot Multiplex Ready Reaction Mix (from ABI)	0.3 µl
NEBuffer 2	2.0 µl
100 mM Tris (*p*H = 9.0)	2.5 µl
ddH_2O	5.2 µl

5. **Precipitation:** Add 5.0 µl of 2.5M Sodium Acetate, 100 µl of pre-chilled ethanol (95%), and place at –80^oC for 10-15′. Spin at 4200 rpm for 30′; Wash with 70% ethanol and spin at 3500 rpm for 10′; spin upside down on paper towel at 300 rpm for 2′; air dry for 5′or longer.

6. **Re-suspension:** Re-suspend the pellet with mixture of 9.85 µl of Hi-Di formamide and 0.15 µl of GS1200Liz Size Standard, then vortex gently.

7. **ABI 3730XL:** Denature at 95 ^oC for 5′, and place on ice until ready to load on the ABI 3730.