

PROTOCOL OF BAC HICF-FINGERPRINTING

BAC DNA PREPARATION: CELL GROWTH AND PLASMID PURIFICATION

1. BAC DNAs isolated by AGENCOURT CosMCPREP KIT (see Manufacturer's protocol) were eluted in 40 µl of ddH₂O, then collected in two 384-well plates: 8 µl in the fingerprinting plate and 30 µl in the backup plate.

FINGERPRINTING REACTION: RESTRICTION ENZYME DIGESTION AND SNaPshot LABELLING

2. Add 2.0 µl of the reaction cocktail (see below) to the fingerprinting plate containing 8.0 µl of purified BAC DNA; briefly spin down; incubate at 37°C for 3 hrs and at 65°C for 60'.

Fingerprinting Cocktail (1x):

10X NEBuffer 2	1.00 µl (1X)
100X BSA	0.05 µl
10 µg/µl RNase A	0.05 µl
1% β-Mercaptoethanol	0.10 µl (0,01%)
BamHI	0.05 µl (1 U)
EcoRI	0.05 µl (1 U)
XbaI	0.05 µl (1 U)
XhoI	0.05 µl (1 U)
HaeIII	0.10 µl (1 U)
SNaPshot	0.20 µl
ddH ₂ O	0.30 µl

(NEB enzymes; ABI SNaPshot Multiplex Ready Reaction Mix)

POST EXTENSION TREATMENT: INTERNAL SIZE STANDARD ADDING, HEAT DENATURATION AND DYE CLEANUP

3. Add 0.02 µl of GeneScan 500Liz Size Standard and 4.98 µl of Hi-Di Formamide (premixed); briefly spin down.
4. Denature at 95°C for 3' and keep on ice for 10'; briefly spin down.
5. Add 4 µl of BigDye X terminator solution and 16 µl of SAM solution (premixed); seal the fingerprinting plate with heat sealing film (ClearSeal#3730) at 170°C for 1" using Thermo ALPS-50V; vortex at 2500 rpm for 30'; spin at 1200xg for 2'.
6. Assemble the fingerprinting plate in the appropriate retainer base; load on the ABI 3730XL; select the specific run module.