Easy UCD DNA Digests

This method was developed by Mike Miller's Lab at UCD and described in the citation below:

Ali, Omar A., et al. "RAD capture (Rapture): flexible and efficient sequence-based genotyping." Genetics 202.2 (2016): 389-400.

protocol

Samples: :

1. Make Liftons buffer (100 mM EDTA, 25 mM tris-HCl pH 7.5, 1% SDS).
    - 1X: 58 ul water, 16 ul 0.5 M EDTA pH 8.0, 2 ul 1 M tris-HCl pH 7.5, 4 ul 20% SDS
    - 110X: 110X: 6.38 ml water, 1.76 ml 0.5 M EDTA pH 8.0, 220 ul 1 M tris-HCl pH 7.5, 440 ul 20% SDS
    - can store at room temp
2. Into each 96 plate well, pipet 80 ul Liftons buffer Note: fully skirted plates are recommended.
3. Place strip caps on wells and place in freezer until needed.
4. Add fin clip sample to each well, we use a piece 2-25 mm2. Open one strip cap at a time and reseal when all eight wells are filled. This helps prevent cross contamination of samples. Successful weights used previous: .003-.005g of fin clip. Very high yields from this amount
5. Place in freezer until the next step.
6. Make digestion master mix- Fresh each time.
    - (Liftons buffer + 0.075 M DTT + 4.2 mg/ml Proteinase K).
    - 1X: 28.6 ul Liftons buffer, 8.4 ul 20 mg/ml Proteinase K, 3 ul 1 M DTT
    - 110X: 3.146 ml Liftons buffer, 0.924 ml 20 mg/ml Proteinase K, 330 ul 1 M DTT
7. Into each 96 plate well, pipet 40 ul of digestion master mix.
8. Seal plate with sealing foil and vortex to mix.
9. Incubate plate at 55°C overnight
10. Spin the plate quickly to collect any condensation, vortex the plate for 30 sec, spin the plate quickly again.
11. Transfer 80 ul of the lysate from the top of the wells to a new plate. Leave any solids behind.
12. Make Hybridization buffer in advance- Takes a long time to go into solution (2.5 M NaCl, 20% PEG 8000, 0.025 M DTT)
    - For 250 ml: 1 g DTT, 29 g NaCl, 50 g PEG 8000, water up to 250 ml (store Hybridization buffer at 4°C).
13. Into each 96 plate well containing 80 ul of digestion master mix, pipet 80 ul Hybridization buffer and 20 ul resuspended Ampure XP beads.
14. Mix by pipetting up and down.
15. Incubate plate at room temperature for 1 minute
16. Place the plate on a magnet
17. Aspirate and discard the supernatant

18. Remove the plate from the magnet and add 150 ul freshly prepared 80% ethanol, pipet up and down to resuspend the Ampure beads.
19. Place the plate back on the magnet
20. Aspirate and discard the supernatant.
21. Remove the plate from the magnet and add 150 ul freshly prepared 80% ethanol, pipet up and down to resuspend the Ampure beads.
22. Place the plate back on the magnet
23. Aspirate and discard the supernatant.
24. Remove the plate from the magnet and add 150 ul freshly prepared 80% ethanol, pipet up and down to resuspend the Ampure beads.
25. Place the plate back on the magnet
26. Aspirate and discard the supernatant.

    a total of three etoh washes

27. Allow the beads to air dry while on the magnet. It is very important that ethanol is not carried over.
28. Into each 96 plate well, pipet 100 ul of low TE (10 mM tris-HCl pH 7.5, 0.1 mM EDTA). (Can use 20-100ul low TE)
29. Remove plate from magnet and resuspend beads.
30. Incubate for 5 min at RT
31. Place the plate on the magnet, remove the supernatant containing the DNA to a new plate and quantitate yield.