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Sameday Oligopaint production using Lambda Exonuclease

1) 10 ml PCR
- 1 ml 10X Kapa Taq buffer
- 200 µl 10mM dNTP
- 50 µl 200uM 5'fluor-labeled Forward primer (~20-mer)
- 50 µl 200uM 5'Phosphorylated Reverse primer (~20-mer)
- 10 µl 1ng/µl template [Note: If using Secondaries, this will have to be a pre-touched up library]
- 100 µl Kapa Taq
- 8590 µl ddH20
    - Aliquot into strip tubes or plate
    - PCR Program: 95C-5min, (95C-30s, 60C-30s, 72C-15s)X40, 72C-5min

2) PCR purification by Zymo DNA clean & Concentrator-500 columns (D4031 & D4032)
- Pool PCR and add 5X (50ml) DNA Binding Buffer
- Place 2 columns in 2 50ml conical tubes
- Transfer 30mls of sample to columns (15ml each) and spin 5min 4,000rpm
- Discard flow-through and add final 30ml of sample to the columns (15ml each) and spin again
- Discard flow-through and add 10ml DNA wash buffer, and spin 5 min
- Transfer columns to new tubes and Elute each with 2800 µl ddH20
    - Should recover ~2200 µl per column, combine for 4400 µl (fill to this vol if necessary)

3) Lambda Exonuclease Reaction to make ssDNA probe
- 4400 µl DNA Eluant
- 500 µl 10X Lambda buffer
- 100 µl Lambda exonuclease 5000U/ml (if scaling up/down, keep enzyme to 5U/100 µl of PCR)
    - Aliquot into strip tubes
    - Set thermo-cycler to 37C-30 min, 75C-10 min

4) ppt ssDNA probe
- Combine Exo reactions ~5mls
- Add 1/50 (100µl) glycogen and mix
- Add 1/10 (500µl) 4M NH4OAc and mix
- Add 2.5X (12.5mls) cold 100% EtOH and mix
    - Aliquot into 2ml tubes (~9 tubes) and place at -80C for 30min
    - Spin max speed at 4C for 1 hour
    - Resuspend each pellet in 10µl ddH20 for 15min at 42C and combine
    - Spec based on fluorescence for concentration
        - Expect yield of ~3700pmol/10ml prep