Phylonests west 1 than regardent Gyserol Stock Label Manti ermymy JM19/Kan. 821615/ Frutting 111 x 470 pns 15 01-25 500 uL -> stock SOUL - cuttere Vertex hard DAL LB SPOR Tubes -80°C (Stere) Mini Prep redo BCDZ Spin (3000 rpm 5min) - adjust as needed + stre - 20 18 you went to step · P1 (40°C) (250µL) · Pz (cabnet) >don't mix hard (5000) - Lyse (2500) N3 W/m 5 mm (350 ML) Shake -> transfer to 1.5 micro hage 13,000 rpm for 7min > transtre superwrut to colour tube 93. 2 · spin 1 min, 1300mm · remove supernation+ 66.7 · add 500ml PB · sipin 4 min · remove superintent · PE buffer 750, al · spin 4 min · remae syperate/spin I min · 50 pl H20 (faw mn) -spm I min in microfuge · stre offoc

#1-5 = Repr- RHL (BOW OZHS/18) #6-10 = ceny GFP, XHL (BJW 2/21/18 FIGAL LB -> PCR Tubes (10:18am) I small colony inside Tube (shake) allow to sit. (10 x) 13 of Sample

0.5 x of PCR MI (Q5)

10 of Sample

0.5 x of F pnmer (UNSIF) > UIF

0.5 x of R pnmer (UXR R) UXR. 26 cycles (BJW Touch) > see image 3 grow @ 29°C shaking 16 hours inoculation Per tub 5ml -- if you can see through it then not good. (wait) Tomanow D Make gyserol stock 17 Mini Prep