High Yield Preparation of Genomic DNA from Streptomyces Summary
Modified 9.5.2016 by Keith Yamada

1. Culture in 30ml **GYM buffer** + **0.5% glycine** + 1.5ml stock culture
2. Incubate for 46-96 hrs with shaking at 30˚C
3. Centrifuge for 5 min at 4000g (optional: store pellet at -20˚C)
4. Wash 2 x 10ml of **10% sucrose**
5. Resuspend in 10ml of **lysis solution** (+RNase) in a 50ml Falcon tube
6. Add ~~10mg~~ 20mg **lysozyme** + ~~5mg~~ ~~**achromopeptidase**~~
7. Incubate for ~~20 min~~ 40 min at 37˚C
8. Add 1ml **10% SDS** + 5mg **proteinase K**
9. Incubate for 1.5 hrs at 55˚C
10. Add 3.6ml **5M NaCl** + 15ml **chloroform**
11. Rotate end-over-end for 20 min at 6 rpm
12. Centrifuge for 20 min at ~~5000g~~ 4000g
13. Transfer aqueous phase with wide pipet into a clean tube
14. Add 1 vol **isopropanol** to precipitate DNA
15. Spool using a sealed Pasteur pipet and transfer to a microcentrifuge tube
16. Rinse with 1ml cold **70% ethanol**
17. Air dry DNA
18. Dissolve with ~~minimal prewarmed (60˚C)~~ ~~**10mM Tris-HCl buffer**~~ ~~or~~ MQ H20
19. Store DNA at +4˚C

Lysis Solution

0.3M sucrose	20.54g	C1V1 = C2V2 == V1 = C2V2/C1
25mM EDTA	10ml (500mM)	
25mM Tris-HCl	10ml (500mM)	
pH 7.5	Fill to 200ml with H20	

2 U RNase (add immediately before use)

10% Sucrose Solution

20g / 200ml