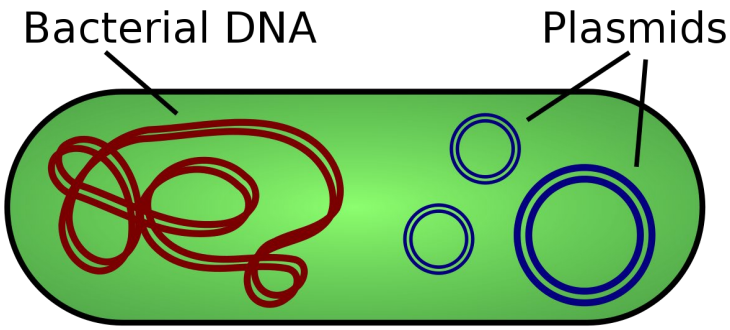
Plasmid Extraction – QIAGEN Plasmid **Mini Kit**



Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2 ml LB containing the appropriate selective antibiotic. Incubate for approximately 8 h at 37°C with vigorous shaking (~300 rpm)

Dilute the starter culture 1/500 (using 6 ul) to 1/1000 (using 3 ul) into 3 ml selective LB medium. Grow at 37°C for 12-16 h with vigorous shaking (~300 rpm)

Bacterial culture, harvest, and lysis

1. Pellet 3 ml overnight LB culture at 6000 X g for 15 min at 4°C
   - The bacterial cells are being forced to the bottom of the tube

2. Homogeneously resuspend the bacterial pellet in 0.3 ml Buffer P1
   - Buffer P1 = cell resuspension solution (with RNase A; store at 4 C freezer)
   - The bacterial cells are re-suspended in a small volume of buffer that keeps them from breaking open (lysing 溶解). It is important to make this suspension of cells homogeneous because cells trapped in clumps will be resistant to lysis reagents
   - LyseBlue is a color indicator which provides visual identification of optimum buffer mixing
     - Can be added to P1 (**shake P1 before use to resuspend LyseBlue particles**)
     - After adding P2 to P1, the color of the suspension changes to blue. Mixing should result in a homogeneously colored suspension. Upon addition of P3, LyseBlue turns colorless

3. Add 0.3 ml Buffer P2, mix thoroughly by inverting 4-6 times, and incubate at RT for 5 min
   - Buffer P2 = cell lysis buffer (contains detergent SDS, very alkaline)
   - Proteins in the cell membrane become denatured
   - **Mix gently; do NOT vertex, which will shear the chromosomal DNA**
   - All DNA becomes denatured to its single stranded form
     - Chromosomal DNA is long and is attached to broken pieces of the cell membrane

    - Plasmid DNA is linked, so it forms two attached circles
- All RNA is digested (RNase A in P1)

4. Add 0.3 ml chilled Buffer P3, mix thoroughly by vigorously inverting 4-6 times, and incubate on ice for 5 min (precipitation is enhanced)
- P3 = neutralization buffer/DNA binding buffer
- Contains potassium acetate; the KAc forms a precipitate when it interacts with SDS. At the same time, denatured proteins co-precipitate with SDS.
- At the neutral pH, all DNA tried to re-nature; plasmid can easily re-form to its double stranded form. Chromosomal DNA finds it difficult to re-nature b/c it has no reference point and homologous pieces of DNA may be blocked from finding each other by the cell debris present

5. Centrifuge at maximum speed for 10 min at 4°C
- Dense cell debris is pelleted to the bottom of the tube. Chromosomal DNA is also pelleted along with the cell debris

Bind, wash, and elute plasmid DNA on QIAGEN-tip
6. Equilibrate a QIAGEN-tip 20 by applying 1 ml Buffer QBT and allow column to empty by gravity flow

7. Apply the supernatant (step 5) to the QIAGEN-tip and allow it to enter the resin by gravity flow
- Plasmid DNA binds to the filter membrane

8. Wash the QIAGEN-tip with 2 X 2 ml Buffer QC. Allow Buffer QC to move through the QIAGEN-tip by gravity flow
- It is particularly important not to force out residual wash buffer; traces of wash buffers will not affect the elution step

9. Elute DNA with 0.8 ml Buffer QF into clean 2 ml vessel

Precipitate, wash, and re-dissolve plasmid DNA
10. Precipitate DNA by adding 0.56 ml RT isopropanol to the eluted DNA and mix. Centrifuge at maximum speed for 30 min at 4°C. Carefully decant supernatant

11. Wash DNA pellet with 1 ml RT 70% ethanol and centrifuge at maximum speed for 10 min. Carefully decant supernatant.

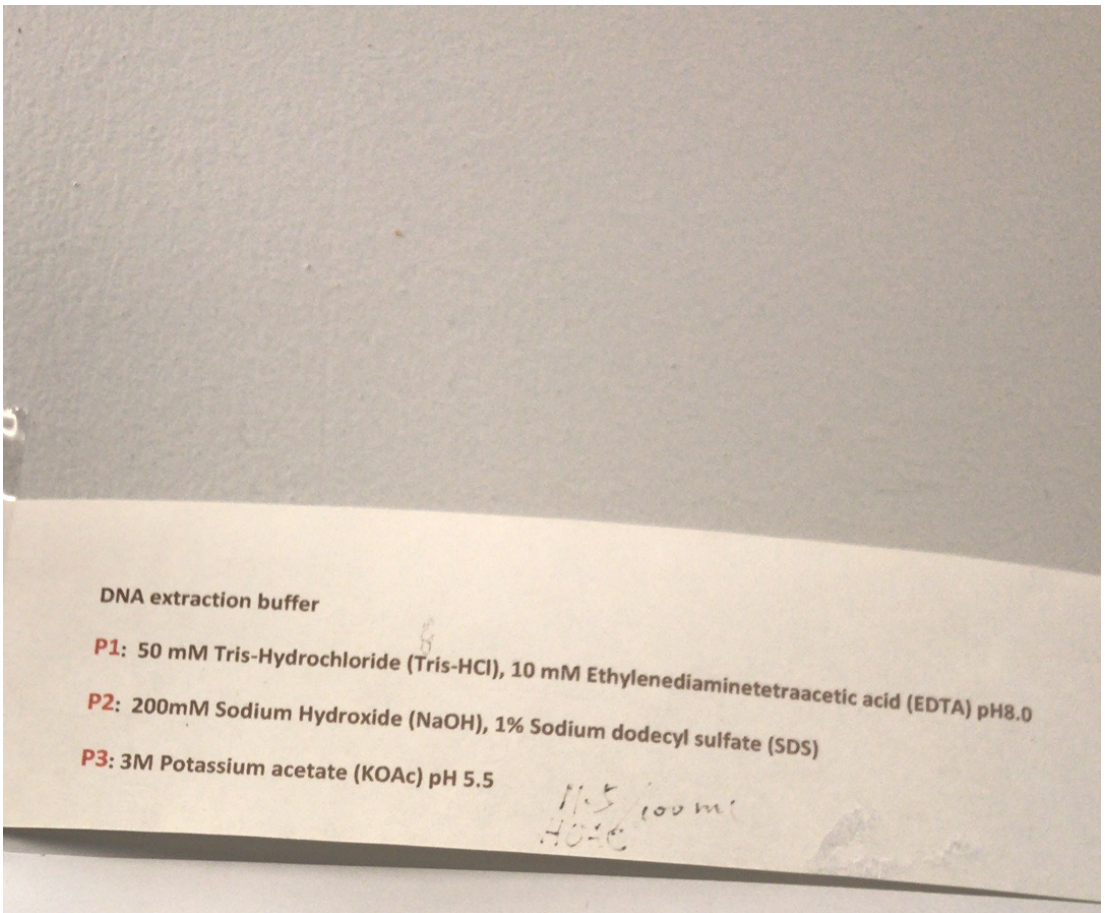
12. Air-dry pellet for 5-10 min and redissolve DNA in a suitable volume of buffer.

Buffer P1:
50 ml Tris•Cl, pH 8.0
10 mM EDTA
100 ug/ml RNase A
Storage at 2-8 °C, after addition of RNase A

Buffer P2:
200 mM NaOH
1% SDS (w/v)
Storage at RT

Buffer P3:
3.0M potassium acetate, pH 5.5
Storage at RT

From Bing's Lab:



DNA extraction buffer

P1: 50 mM Tris-Hydrochloride (Tris-HCl), 10 mM Ethylenediaminetetraacetic acid (EDTA) pH8.0

P2: 200mM Sodium Hydroxide (NaOH), 1% Sodium dodecyl sulfate (SDS)

P3: 3M Potassium acetate (KOAc) pH 5.5

ative Activity of OPTIZYME™ Restriction Enzymes in 1X Buffers

0X Buffer supplied with each restriction enzyme is optimized to give 100% activity at the 1X concentration. This t
gestions with multiple restriction enzymes. Enzyme activity is expressed as a percent of the activity obtained with
our digest.

rt ber	OPTIZYME* Restriction Enzyme	Recommended buffer for 100% activity	Enzyme activity in 1X OPTIZYME* buffers, %				
			Buffer 1	Buffer 2	Buffer 3	Buffer 4	
59	AarI	Unique	NR (+oligo)	NR (+oligo)	0-20 (+oligo)	NR (+oligo)	
41	AatII	Buffer 4	50-100	20-50	0-20	**100**	
5	AloI	Buffer 5	0-20	0-20	0-20	20-50	
5	AluI	Buffer 4	50-100	0-20	0-20	**100**	
9	Alw44I (ApaLI)	Buffer 4	50-100	100	0-20	**100**	
	ApaI	Buffer 1	**100**	20-50	0-20	20-50	
	AvaI (Eco88I)	Buffer 4	100	50-100	0-20	**100**	
	AvaII (Eco47I)	Buffer 5	0-20	50-100	50-100	50-100	
	BalI (MscI)	Buffer 5	0-20	20-50	0-20	20-50	
	BamHI	Unique	20-50*	100	20-50	100*	
	BclI	Buffer 2	20-50	**100**	20-50	100*	
	BglI	Buffer 3	0-20	50-100	**100**	0-20	
	BglII	Buffer 3	0-20	20-50	**100**	0-20	
	BpiI (BbsI)	Buffer 2	20-50	**100**	50-100	50-100	
	Bsh1236I (BstUI)	Buffer 5	0-20	0-20	50-100	20-50	
	BshTI (AgeI)	Buffer 3	0-20	20-50	**100**	20-50	
	BssHII (PauI)	Buffer 5	0-20	0-20	100	0-20	
	BstEII (Eco91I)	Buffer 3	20-50	20-50	**100**	NR	
	Cfr9I (XmaI)	Unique	0-20	0-20			