M1D4: Cloning

2/19/15

1. Upcoming office hours:
    - Friday, 3-4pm
    - No office hours on Monday, will try to respond to email

Announcements

- Please make sure your ligation spreadsheet works!
- Hand in up front in folder:
  1. Primer table + summary paragraph
  2. Schematic diagram
  3. Methods section

Schedule for today:

1. Leslie here to talk about figures (~2pm)
2. PCR purification + set-up gel (~2:30pm)
3. "Pre-lab" lecture while gel is running (~3:00 pm)
4. Djenet will help you image your gel — quick estimates (~3:30)
5. Set-up ligation —> transformations —> plating (~5pm)

Homework:

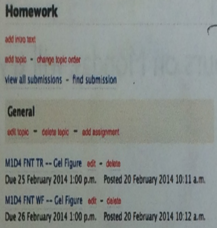
~~FNT~~ Assignment(s)

vector mass	bp·mol·vector	10 mole insert	660g insert	1900bp insert	μL	=
	660g	1 mole insert	bp·mol insert	3500 bp vector	X ng insert	

memo = primer design
Data Summary/Abstract ≡ microbiota

Chaotropic salt → chaos causing
→ dehydrate → disrupt protein
→ bind to column

Next time Part 1 due on Stellar — Figure of gel + Good caption



Homework

add intro text

add topic - change topic order

view all submissions - find submission

General

edit topic - delete topic - add assignment

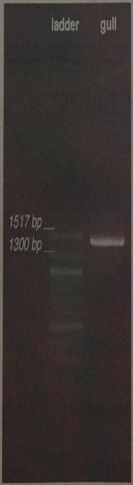
M1D4 FNT TR -- Gel Figure edit - delete
Due 25 February 2014 1:00 p.m. Posted 20 February 2014 10:11 a.m.

M1D4 FNT WF -- Gel Figure edit - delete
Due 26 February 2014 1:00 p.m. Posted 20 February 2014 10:12 a.m.

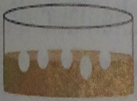
Bird Microbial Communities -- MID4 Overview

gel purify, clone and transform

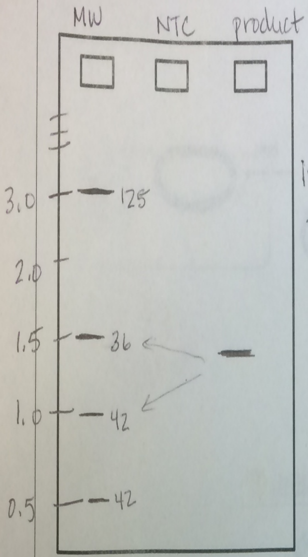
DAY 4


ladder
gull
1517 bp
1300 bp

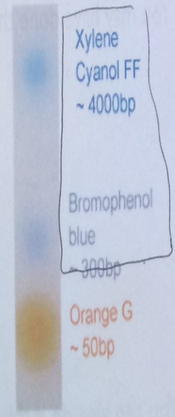
Individual 16S cell colonies



How do we visualize the DNA?


MW
NTC
product
3.0
125
2.0
1.5
36
1.0
42
0.5
42

Intensity due to mass of DNA

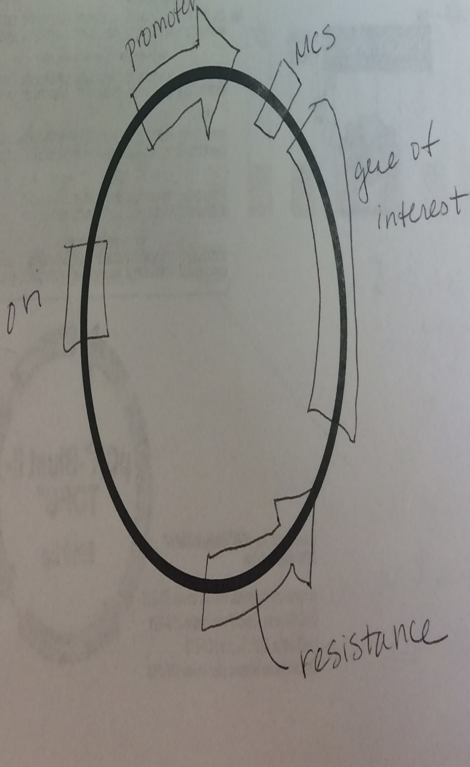

Xylene Cyanol FF ~ 4000bp
Bromophenol blue ~ 300bp
Orange G ~ 50bp

www.base-asia.com

http://en.wikibooks.org/wiki/Structural_Biochemistry/DNA_Gel_Electrophoresis

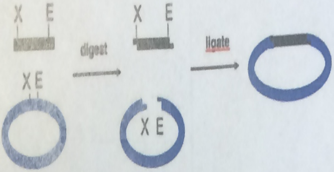
Cloning

Vector = Plasmid = Circular DNA

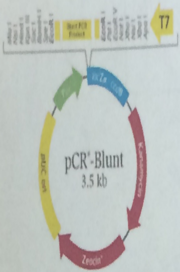

promoter
MCS
gene of interest
ori
resistance

Preview of M1D4: Cloning

You may have done this before:

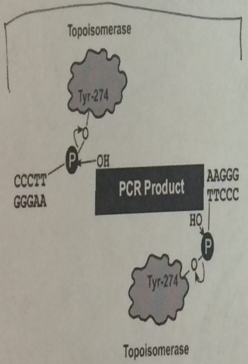

X
E
digest
ligate
X E
X E

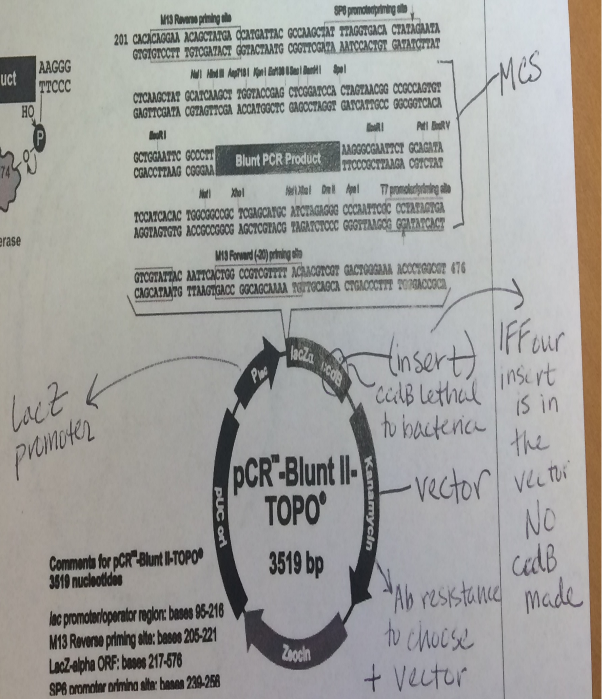
You can also do it this way:


Blunt PCR Product
T7
lacZα
ccdB
Kanamycin
Zeocin
pUC ori
pCR®-Blunt
3.5 kb

mechanism

Our System: TOPO CLONING


Topoisomerase
Tyr-274
O
P
OH
CCCTT
GGGAA
PCR Product
AAGGG
TTCCC
HO
P
O
Tyr-274
Topoisomerase


M13 Reverse priming site
SP6 promoter/priming site
201 CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTAT TTAGGTGACA CTATAGAATA
GTGTGTCCTT TGTCGATACT GGTACTAATG CGGTTCGATA AATCCACTGT GATATCTTAT
Nsi I
Hind III
Asp718 I
Kpn I
Eco136 II
Sac I
BamH I
Spe I
CTCAAGCTAT GCATCAAGCT TGGTACCGAG CTCGGATCCA CTAGTAACGG CCGCCAGTGT
GAGTTCGATA CGTAGTTCGA ACCATGGCTC GAGCCTAGGT GATCATTGCC GGCGGTCACA
EcoR I
EcoR I
Pst I
EcoR V
GCTGGAATTC GCCCTT
CGACCTTAAG CGGGAA
Blunt PCR Product
AAGGGCGAATTCT GCAGATA
TTCCCGCTTAAGA CGTCTAT
Not I
Xho I
Nsi I
Xba I
Dra II
Apa I
T7 promoter/priming site
TCCATCACAC TGGCGGCCGC TCGAGCATGC ATCTAGAGGG CCCAATTCGC CCTATAGTGA
AGGTAGTGTG ACCGCCGGCG AGCTCGTACG TAGATCTCCC GGGTTAAGCG GGATATCACT
M13 Forward (-20) priming site
GTCGTATTAC AATTCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT 476
CAGCATAATG TTAAGTGACC GGCAGCAAAA TGTTGCAGCA CTGACCCTTT TGGGACCGCA
MCS
lacZα
ccdB
Plac
Kanamycin
Zeocin
pUC ori
pCR™-Blunt II-TOPO®
3519 bp
LacZ promoter
(insert)
ccdB lethal to bacteria
vector
Ab resistance to choose + vector
IFF our insert is in the vector NO ccdB made

Comments for pCR™-Blunt II-TOPO®
3519 nucleotides

lac promoter/operator region: bases 95-216
M13 Reverse priming site: bases 205-221
LacZ-alpha ORF: bases 217-576
SP6 promoter priming site: bases 239-258