University of Zurich[UZH]

Group exercise

Gademann Group
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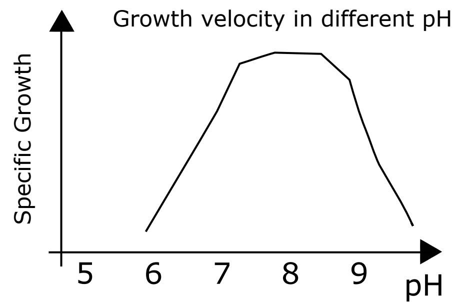
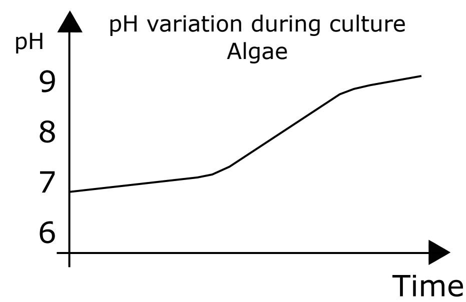
08.08.2019

1) Given the below information, which buffer is a good start point for *Chlamydomonas* media?





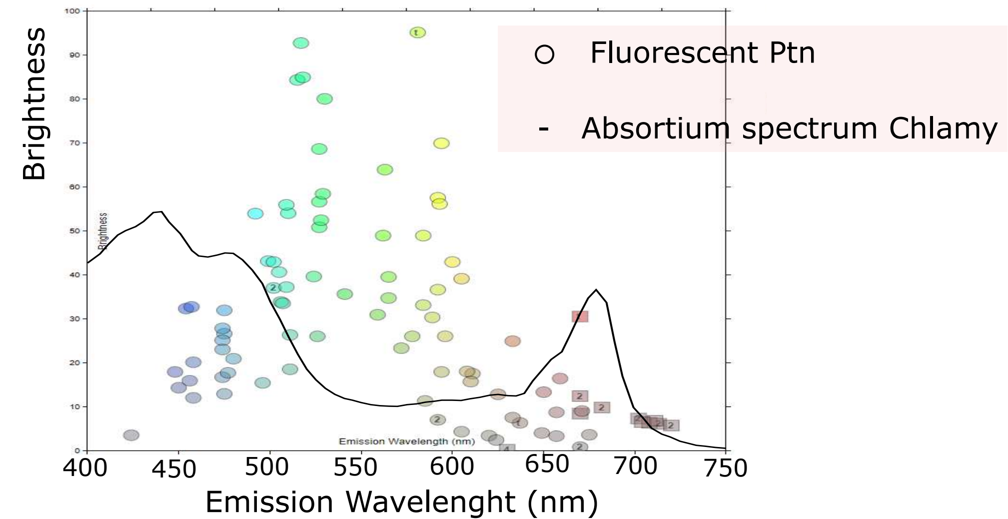
Buffer	pKa	Useful pH range
Citric Acid	3.13, 4.76, 6.40	2.1 - 7.4
TABS	8.49	8.2 - 9.1
Tris	8.06	7.0 - 9.0
KH2PO4	7.2	6.2 - 8.2
TAPS	8.4	7.7 - 9.1
Borate	9.24	8.25 - 10.25

2) Connect the materials with the suitable sterilization method:

A - Thermal sensible plastic
B - Eletronic equipment
C - Media and buffers
D - Media and buffer with thermo sensible component
E - Large bioreactors and tubes

1- Autoclavation
2-Filtration
3-Gamma irradiation
4-Steam flow sterilization
5-Ethylene Oxide gas

3) Which fluorescent proteins would be good reporters system for *Chlamydomonas reinhardtii*?



4) Translate the following sequence to obtain my protein:

5` ▬▬▬AGATGGAAATTAATGAGCCTAGACAAACGGAGATCAACTAA▬▬▬ 3`

	T		C		A		G	
T	TTT phe	F	TCT ser	S	TAT tyr	Y	TGT cys	C
	TTC phe		TCC ser		TAC tyr		TGC cys	
	TTA leu	L	TCA ser		TAA stop		TGA stop	
	TTG leu		TCG ser		TAG stop		TGG trp	W
C	CTT leu	L	CCT pro	P	CAT his	H	CGT arg	W
	CTC leu		CCC pro		CAC his		CGC arg	
	CTA leu		CCA pro		CAA gln	Q	CGA arg	
	CTG leu		CCG pro		CAG gln		CGG arg	
A	ATT ile	I	ACT thr	T	AAT asn	N	AGT ser	S
	ATC ile		ACC thr		AAC asn		AGC ser	
	ATA ile		ACA thr		AAA lys	K	AGA arg	W
	ATG met	M	ACG thr		AAG lys		AGG arg	
G	GTT val	V	GCT ala	A	GAT asp	D	GGT gly	G
	GTC val		GCC ala		GAC asp		GGC gly	
	GTA val		GCA ala		GAA glu	E	GGA gly	
	GTG val		GCG ala		GAG glu		GGG gly	

Ex:
AA ATG CAA AGT ACT
M Q S T

Video help

5) Design the primers to amplify the previous sequence and insert it on the plasmid below:

*Primers should be 10 nt

AGATGGAAATTAATGAGCCTAGACAAACGGAGATCAACTAA

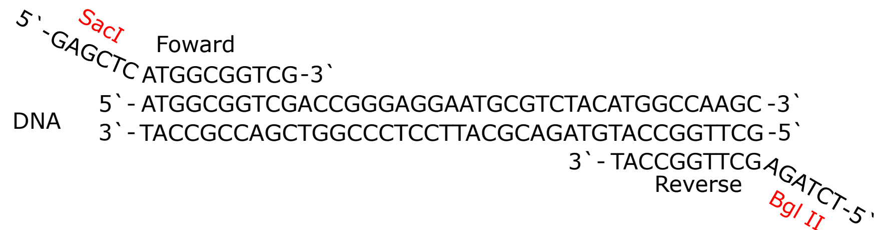
Foward

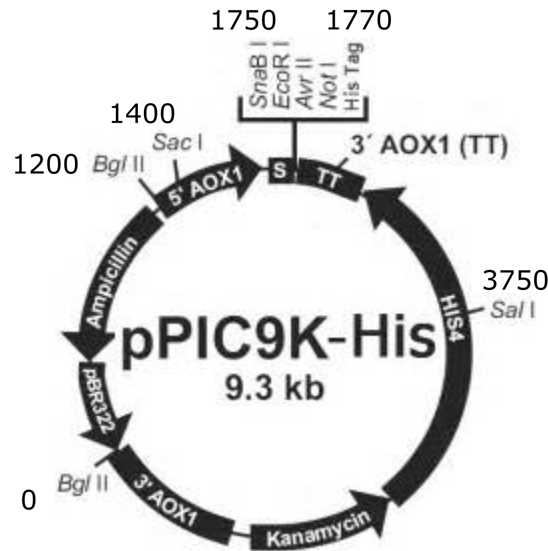
5` 3`

Reverse

5` 3`

Ex:





pPIC9K-His - Yeast expresion vector

Size - 9300 base pairs

Restriction Enzymes

SnaBI - TACGTA

EcoRI - GAATTC

Avr II - CCTAGG

Not I - GCGGCCGC

SacI - GAGCTC

Bgl II - AGATCT

Components

Ampicilin - Resist. gene Bacteria

5`AOX 1 - Promoter Yeast

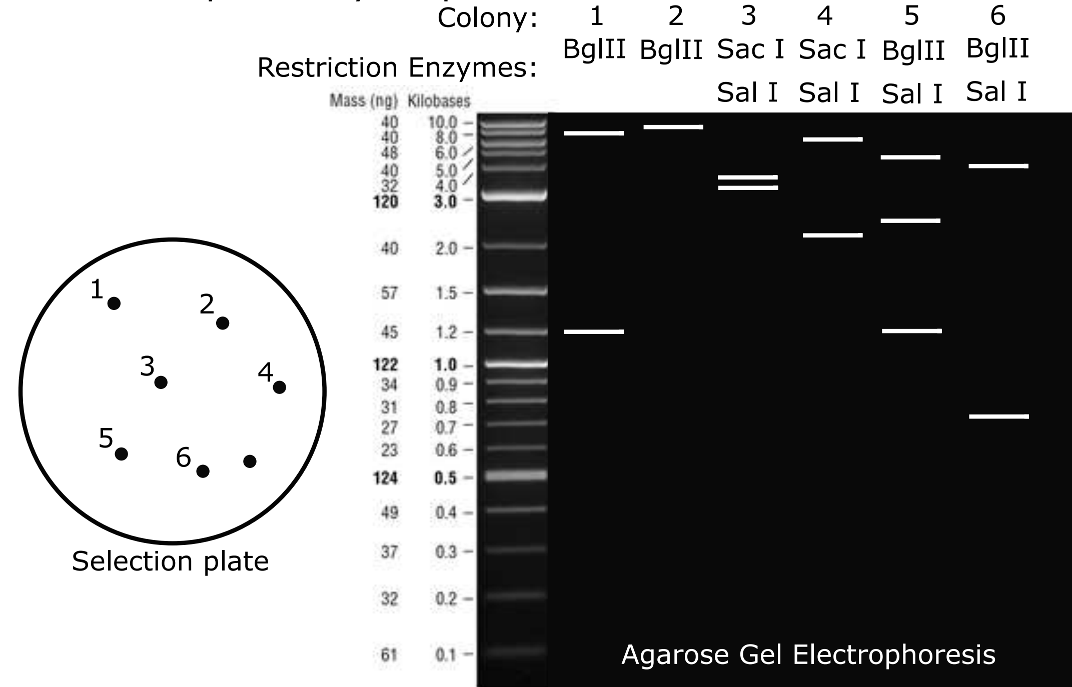
S - signal peptide

TT - Termination region

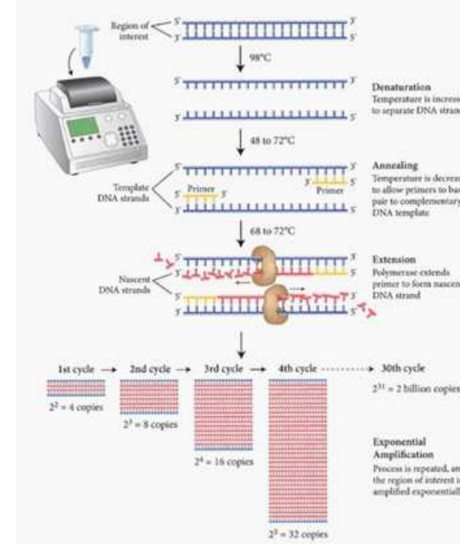
3` AOX1 - Promoter Yeast

Kanamycin - Resist. gene Yeast

6) After preparing the plasmid by ligation and transformation, several colonies appear. You extract the plasmid from them and digest with some restriction enzymes. Which gel lanes could correspond to your plasmid.



PCR info



PCR allows the amplification of a segment of DNA from a very small sample

You need a DNA template, primers, DNA polymerase, as well as dNTP

3 steps that repeat: denature, anneal, and extend

It is used to make many copies of DNA and this is useful in forensics