	Date	Topic	Content
Week 1	20.09.22	Introduction to PURE	Introduction
Week 2	27.09.22	Microbiology	Culturing bacteria and growth curve measurement
Week 3	04.10.22	Genetics	Recombinant DNA, plasmids, IPTG induction
Week 4	11.10.22	Genetics	DNA template, PCR, Agarose gel electrophoresis
Week 5	18.10.22	Onepot PURE protein production	Inoculate one pot PURE strains (teach how to inoculate all 36 strains)
Week 6	25.10.22	Onepot PURE protein purification	Column purification for one pot PURE proteins
Week 7	01.11.22	Onepot PURE protein adjustment	Buffer exchange and conc adjustment
Week 8	08.11.22	Ribosome production	His-tag ribosome RB1 culture and harvest. SDS PAGE for proteins
Week 9	15.11.22	Ribosome purification	His-tag ribosome column purification
Week 10	22.11.22	Ribosome adjustment	Buffer exchange and conc adjustment
Week 11	29.11.22	SDS PAGE and Biobits toehold experiment	SDS Page for ribosome. Energy soln preparation
Week 12	06.12.22	Onepot PURE reaction	PURE reaction for GFP
Week 13	13.12.22	Backup	backup
Week 14	20.12.22	Final Exam	Final exam

Introduction to PURE	**Introduction**	schedule							
2:00-3:00	Introduction (45 minutes)	Sebastian							
3:00-4:00	Introduction (45 minutes)	Sebastian							
4:00-4:10 4:10-4:40 around 5:00	Group allocation Media preparation Autocleave	TA							
5:00-5:30 5:30 - 6:00	Introduce pipette usage (2.5 ul to 1000 ul + Pipette gun) Excercise on pipette usage (Weigh water)	TA							
Preparation									
Before	**Task**	**Instruments**	**Chemicals**	**Cell**	**general stuffs**				
	Print the course plan	weighing tray	70% ethanol	x	Pipet				
		parafilm	LB powder		Pipet gun				
		Course plan			Counsuimables (Pipette tips, parafilm Gloves, tissue roll)				
		Glass ware (0.5Lx5)			waste container				
		Weigh machine							
		bunsen burner							
		Lighter							
After	Take out the autocleaved media								

Microbiology	**Culturing bacteria and growth curve measurement**	schedule			
2:10-2:30 2:30 - 3:15	Inoculate the E.coli culture Introduction - Typical growth curve, Optical Density, Buffers, pH, normality, molarity, moles				
3:00-4:00		aseptic technique: petri dish, liquid culture, test tube culture			
4:00-5:00	Measuring Optical Density (OD) every 30 mins	prepare glycerol stock			
5:00-6:00		Exercise: Plot OD curve	calculate growth curve		
Preparation	**Task**	**Instruments**	**Chemicals**	**Cell**	**general stuffs**
Before	Autocleave the cell culture glass ware (conical flask)	Glass ware for bacteria culture	LB media(auto cleaved)	E.colil	Pipet
	Bring the autocleaved media	bunsen burner	70% ethanol		Pipet gun
	Prepare stock solution:Magnesium Acetate, Potassium Chloride, Ammonium chloride, Imidazole and Tris Hcl	Lighter	50% Glycerol		Counsuimables (Pipette tips, parafilm Gloves, tissue roll)
	Prepare petri dish	cuvette			waste container
After	Clean up the glass wares				
					Experiment Results
Grading method					w2 Cell growth
Attendance	10%	Individual			w4 PCR
Log Book	30%	Individual			w7 PURE protein SDS
Experiment Results	20%	Group			w7 PURE protein activity
Excercise	20%	Individual			w10 Ribosome SDS
Presentation	20%	Group			w10 Ribosome protein activity
					w11 Toehold experiment
					w12 Complete PURE functionality

Genetics	**Recombinant DNA, plasmids, IPTG induction**		schedule					
2:00-3:00 2:30 - 2:45	Introduction - Central dogma, plasmids, Restriction enzymes, IPTG induction, fluorescent proteins (mcherry) Induce E.coli cells with IPTG							
3:00-4:00	Heat tranformation(E.coli /fluorescence plasmid)	Plasmid preparation (kit)						
4:00-5:00		Restriction Digest (1 hour)	Check the result from previous week (Agar plate)					
5:00-6:00	Compare plasmid expression with and without iPTG induction for mcherry							
	Spread plate after transformation							
Preparation	**Task**	**Instruments**	**Chemicals**	**Cell**	**general stuffs**			
	Inoculate E.coli culture for transformation	Glass ware for bacteria culture	IPTG	Overnight culture E.colil with pla	Pipet			
		bunsen burner	LB media	E.coli inducible gene	Pipet gun			
	O/N E.coli culture for Plasmid extraction	Lighter	Plasmid prep kit		Counsuimables (Pipette tips, parafilm Gloves, tissue roll)			
	Autocleave glassware	Streak loop			waste container			
	Agar Plate		Plasmid		70% ethanol			
			Restriction enzyme (BSAI)					
			Ampicillin					
Link								
Heat transformation protocol	https://www.protocols.io/view/e-coli-heat-shock-transformation-kqdg3q8zv25z/v1?step=8							
				pYTK095.gb plasmid contain BSAI				

Genetics	**DNA template, PCR, Agarose gel electrophoresis**		schedule		
2:00-2:30	Introduction : PCR, applications, gel electrophoresis	Heat transformation result: Check GFP expression in plate by UV radiation			
3:00-4:00	Run PCR for GFP Cast agarose gel				
4:00-5:00	Purify PCR product and conc measurement Run agaore gel for GFP DNA & digested plasmid				
5:00-6:00	Gel imaging Numerical exercises				
Preparation	**Task**	**Instruments**	**Cell**	**Chemicals**	**general stuffs**
	Question paper	PCR tubes	E.colil	PCR kits	Pipet
		electrophoresis device		Agarose gel	Pipet gun
		Microwave		Standard DNA template	Counsuimables (Pipette tips, parafilm Gloves, tissue roll)
				PCR product purification kit	waste container
				DNA stain	70% ethanol
				Loading dye	
				Parafilm wax	

Onepot PURE p	**Inoculate one pot PURE strains, induce and harvest cells (teach how to inoculate all 36 strains)**	schedule						
2:00-2:15 2:15-3:15	Induce the one pot PURE culture with IPTG Inoculate 36 strains							
3:15-3:45	Explain PURE system							
Break 15 mins								
4:00 - 5:00	Prepare columns for purification Demostrate how to charge the column							
5:00 - 6:00	Do first round of centrifugation							
5:20 - 7:45	Centrifuge cells and store cell pellet at -80 C							
Preparation	**Task**	**Instruments**	**Chemicals**	**Cell**	**general stuffs**			
	Autocleave glassware and media	Glass Wares for cell culture	LB media 3L	E.colil	Pipet			
	Prepare buffers for one pot PURE: Buffer A, Buffer B, Buffer HT, Stock buffer A, Stock buffer B		Agarose gel		Pipet gun			
	Prepare O/N culture for 36 strains		Standard DNA template		Counsuimables (Pipette tips, parafilm Gloves, tissue roll)			
	Inoculate 36 strains co-culture		Nickel sulfate solution		waste container			
			ampicillin		70% ethanol			
			IPTG					
			Column beads					

Onepot PURE protei	**Column purification for one pot PURE proteins**		schedule					
2:00-3:00	Sonicate resuspended cells Centrifuge cells Charge the column and equilibrate with Buffer A (in parallel)							
3:00-3:30	Loading supernatent onto column. Equilibrate for 30 mins at 4 C under rotation Explain centrifugal filter concept.							
3:30-4:00	Wash and elute proteins from column							
4:00-5:00	First round of buffer exchange using HT buffer Column restoration and washing (in parallel)							
5:00-6:00	Second round of buffer exchange using HT buffer Add stock buffer and store at -80 C							
Preparation	**Task**	**Instruments**	**Chemicals**	**Cell**	**general stuffs**			
	resuspend cells	Columns	Buffers	E.coli from students	Pipette			
	charge column with nickel sulfate	Column holder	Nickel sulfate		Pipet gun			
		Falcon tubes	column beads		Counsuimables (Pipette tips, parafilm Gloves, tissue roll)			
		Toxic waste container			waste container			
					70% ethanol			

Onepot PURE protei	**Concentration adjustment for PURE proteins**	schedule	
2:00-2:15 2:15 - 3:15	Introduction: Absorbance Centrifugal filter the protein and adjust concentration		
3:15-3:30	Break		
3:30-5:00	Setting up PURE reaction to check PURE frex functionality		
5:00-6:00	Introduce application for PURE		
Preparation	Instruments	Chemicals	
	Plate reader	Commercial PURE system	

Ribosome production	**His-tag ribosome RB1 culture and harvest. SDS PAGE for proteins**	schedule	
2:10-2:30	Inoculate the Rb1 strain (500 ml x 4)		
2:30-2:50	Introduce SDS PAGE		
2:50-4:00	SDS Page for PURE proteins		
4:00-5:00	Prepare buffers for ribosome purification: Lysis buffer, Elution buffer, Ribosome buffer Coomassie staining		
5:00-6:00	Destain the gel and result analysis		
6:00 - 7:30	Centrifuge cells and store cell pellet at -80 C		
Preparation	Instruments	Chemicals	Cell
	SDS PAGE electrophoresis device	TGS Buffer	RB1 E coli strain
	Glasswares	Commassie blue stain	
	PCR machine (for denaturation)	SDS Gel	
		LB media	
		Standard protein	
		Sample buffer	

Ribosome purifi	His-tag ribosome purification	schedule		For 500ml, thaw the cells at 7.5 ml lysis buffer and sonicate 4 * 20 on 20 off pulse				
2:00-3:00	Equilibrate the column with buffer Sonicate and centrifuge the cells							
3:00-5:30	Load cell lysate on column Wash using imidazole gradient buffers Elute the ribosomes							
5:30-6:00	Flash freeze ribosomes using liquid nitrogen Store at -80 C Column restoration							
6:00 - 7:00	Column restoration							
Preparation	Task	Instruments	Chemicals	Cell				
	Resuspend cells	Columns	Buffers	E.coli from stude	Pipet			
	Chare the column	Column holder	Nickel sulfate		Pipet gun			
		Falcon tubes	column beads		Counsuimables (Pipette tips, parafilm Gloves, tissue roll)			
		Toxic waste container			waste container			
					70% ethanol			
	First round of buffer exchange using ribosome buffer Prepare amino acid solution							
	Second round of buffer exchange using ribosome buffer							
	Third round of buffer exchange using ribosome buffer							
	Concentration adjustment for ribosomes							

Ribosome adjustment	Ribosome conc adjustment, functionality+ Toehold experiment 1	schedule	
2:00-3:00	Concentrate ribosome		
3:00-4:00	Setting up PURE reaction to check RB with PURE frex functionality		
4:00-5:00	Extract Banana DNA. Measure DNA concentration		
5:00-6:00			
Preparation	Instruments	Chemicals	Cell
	Freeze RPA tube		
Backup for redoing experiment			

SDS PAGE and	SDS Page for ribosome and Energy soln preparation	schedule				
2:00-3:00	SDS Page for ribomes Introduction about energy solution					
3:00-4:00	Comassie staining Prepare Potassium glutamate and Magnesium acetate stock solutions					
4:00-5:00	Destain gel and analysis Prepare energy solution					
5:00-6:00	Run Toehold Banana gene detection with PURE express					
Preparation	Instruments	Chemicals	Cell			

Onepot PURE re	One Pot PURE reaction for GFP	schedule	
2:00-2:30	Setting up the reaction - GFP expression		
2:30-3:00	Recombinase polymerase amplification (RPA) reaction		
4:00-5:00			
5:00-6:00	Result analysis		
Preparation	Instruments	Chemicals	Cell
	Plate reader	Liquid wax	

backup	backup	schedule	
2:00-3:00	Outline exam format and grading		
3:00-4:00	Hand out articles for presentation		
4:00-5:00			
5:00-6:00			
Preparation	Instruments	Chemicals	Cell

Examination	Final exam	schedule
2:00-3:00	Group presentation 30 minutes (20 minutes talk + 10 minutes discussion)	
3:00-4:00		
4:00-5:00		
5:00-6:00		
Preparation		
Question paper		

Module					
Microbiology	Prepare Media	Culturing Bacteria	Collecting bacteria		
Genetic	DNA Extraction	PCR	Gene induction	Run gel	
Protein	Purification	SDS PAGE			
Onepot	Cell culture	Protein purification	SDS-PAGE	energy solution	Functional test
Cell Free Expression system	Express GFP	Express simple gene circuit			
Useful link					
Bacterial painting	https://www.youtube.com/watch?v=LXwxU-nIcDY&ab_channel=AmericanSocietyforMicrobiology				
Biobits curriculum	https://drive.google.com/drive/folders/1Du6T0z1MtJgOTVkBiiIXwthlqGm9fzAl				