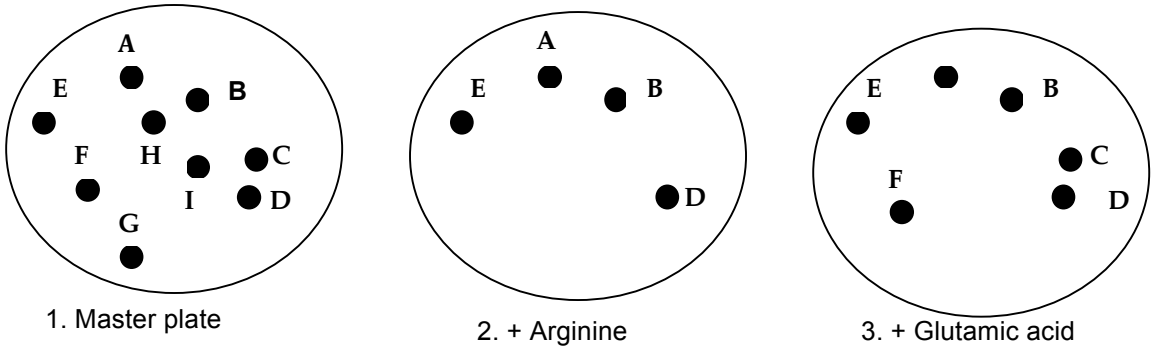
Practice Problems for Genetics, Session 4: Biochemical Genetics

Question 1

You are studying the synthesis of glutamic acid (Glu) in the fungi, *Neurospora crassa*. Synthesis of glutamic acid in *Neurospora* involves a multi-step pathway where each step of the pathway is catalyzed by a specific enzyme that is encoded by a specific gene. A cell missing any of these enzymes cannot synthesize glutamic acid. You identify the enzymes involved in catalyzing the different steps of the glutamic acid synthesis pathway through a mutant hunt. You start with a population of wild type cells (prototrophs), mutagenize them and isolate different auxotrophic mutants.

a) What media would you use to distinguish the Glu auxotrophs from the Glu prototrophs? Make a brief list of the important components is in each of the media you chose.

b) You plate the mutagenized cells on a master plate so that they form specific colonies (each represented by a solid dot in the diagram below). You then replica plate these colonies onto minimal media plates that contain either arginine or glutamic acid as supplements.



1. Master plate 2. + Arginine 3. + Glutamic acid

i) What type of medium would you use for your master plate? Make a brief list of the important components in the media you chose.

ii) Given only the data above, which colonies represent Glu auxotrophs?

iii) Colonies G, H and I are formed only on the master plate. How can you explain this observation?

c) You collect many mutants and select 4 to study further. Each mutant (1-4) is deficient in a single enzyme in the glutamic acid pathway. Mutant 1 is missing enzyme 1, mutant 2 is missing enzyme 2, etc. You grow each mutant on media supplemented with a compound that is an intermediate in the glutamic acid pathway. Each compound, when added to the growth media, can be taken up and used by the cells.

Mutant	Supplement added to growth media					
	None	Citrulline	Glutamic semialdehyde	Arginine	Ornithine	Glutamic acid
1	No growth	No growth	grows	No growth	No growth	grows
2	No growth	No growth	grows	grows	grows	grows
3	No growth	No growth	No growth	No growth	No growth	grows
4	No growth	No growth	grows	No growth	grows	grows

Below give the order of the intermediates in the glutamic acid pathway. Label each arrow with the enzyme (enzyme 1-4) that functions at that step.

_____ → _____ → _____ → _____ → _____

Question 1, continued

d) Explain how the pathway for glutamic acid synthesis outlined above, affects your answer to (b) part (ii) above.

e) Many fungi have both haploid and diploid life cycles. What type of cells would you have used in your original mutagenesis? Explain your answer.

Question 2

You do a second mutant hunt to screen for histidine mutants and isolate 15 mutants. You find that the his- phenotype is recessive in mutants 1-14, but dominant in mutant 15. You then organize the mutants based on complementation groups.

a) Describe the experiment that you performed and the results that you obtained which allowed you to determine that the his- phenotype is recessive in mutants 1-14, but dominant in mutant 15.

b) Describe how you would determine complementation groups for each of your mutants and explain what it means if two mutant are in the same complementation group as opposed to in different complementation groups.

c) The following is the result of a complementation assay. Here (-) represent no growth on minimal media and (+) represent growth on minimal media. Based on the information provided, arrange the mutants into complementation groups.

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
A1	-	+	-	+	-	+	+	+	+	+
A2		-	+	-	+	-	+	+	+	+
A3			-	+	-	+	+	+	+	+
A4				-	+	-	+	+	+	+
A5					-	+	+	+	+	+
A6						-	+	+	+	+
A7							-	-	+	+
A8								-	+	+
A9									-	-
A10										-

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