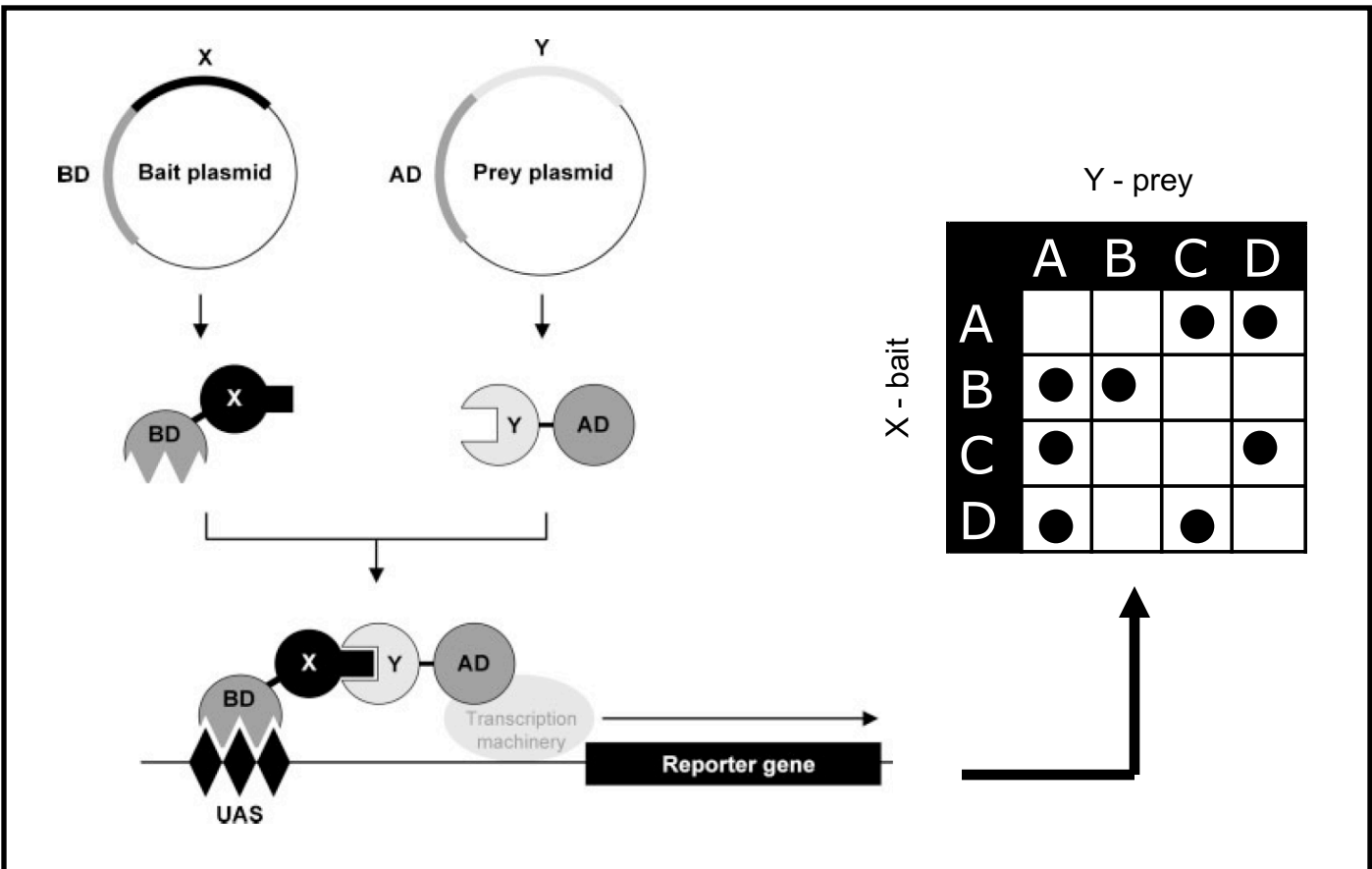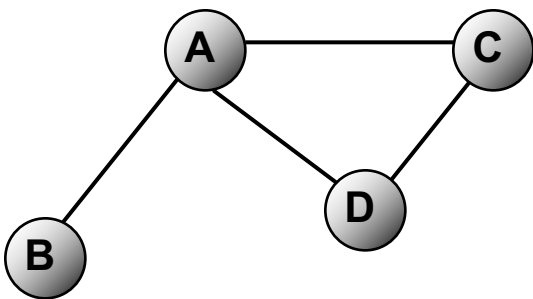# Protein interactions with yeast-two-hybrid (Y2H)
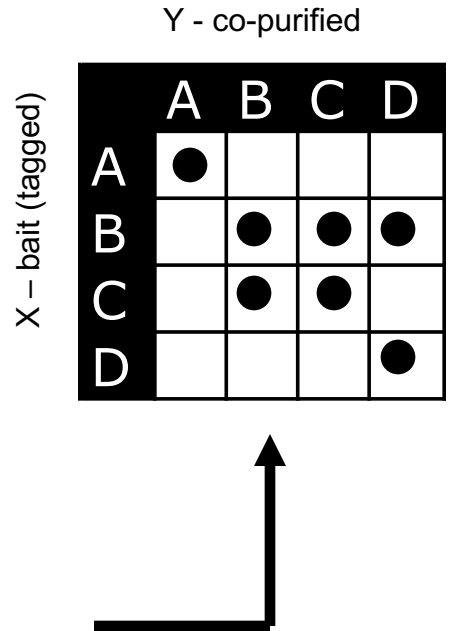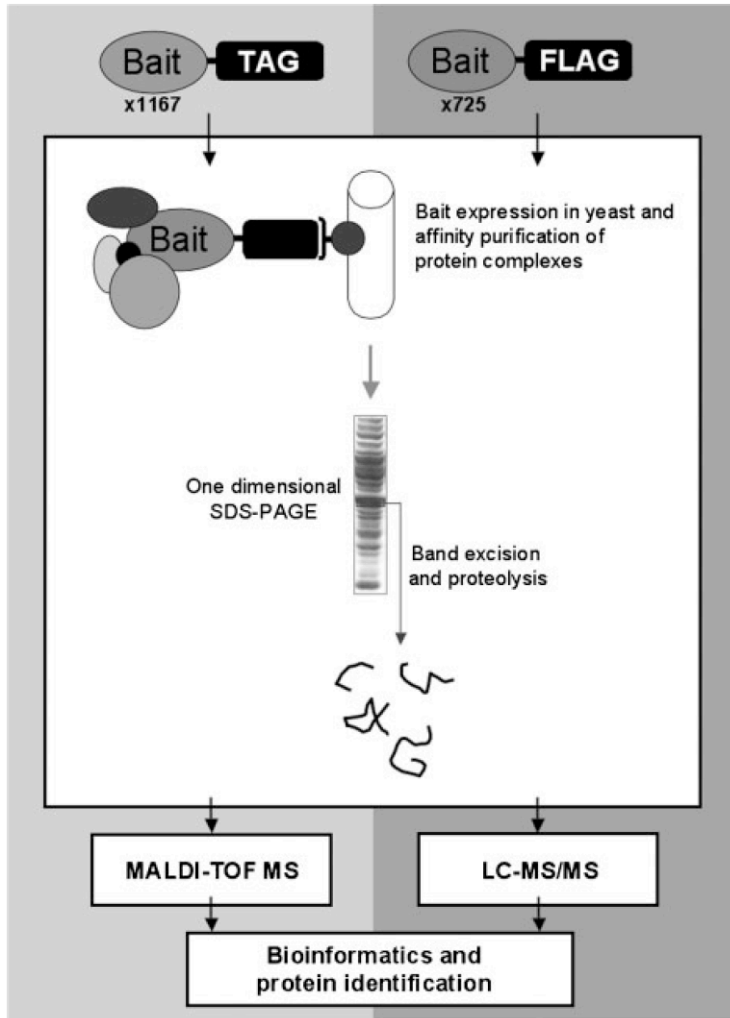

Exercise 1: *Based on the experiments above, draw a network below which represents the interactions between the proteins A, B, C and D. NOTE: In all cases in this exercise, interactions can be inferred even if they are not reciprocal.*

Comment: **Inconsistent results are often observed in Y2H screens (as for the B-A interaction). The B-B interaction could be an artifact of the experiment (auto-activation) or reflect dimerization. Therefore, negative controls (e.g. B as bait without prey) are always important.**

Exercise 2: *What is your confidence in the interaction between A and B? Can proteins, like B, interact with themselves? Are there proteins which do not interact?*

Protein interactions with APMS ("pull-downs")

Exercise 3: *Based on the experiments above, draw a network below which represents the interactions between the proteins A, B, C and D using the **matrix model** to interpret APMS based data.*
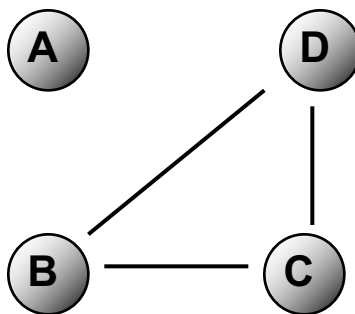
"matrix model"
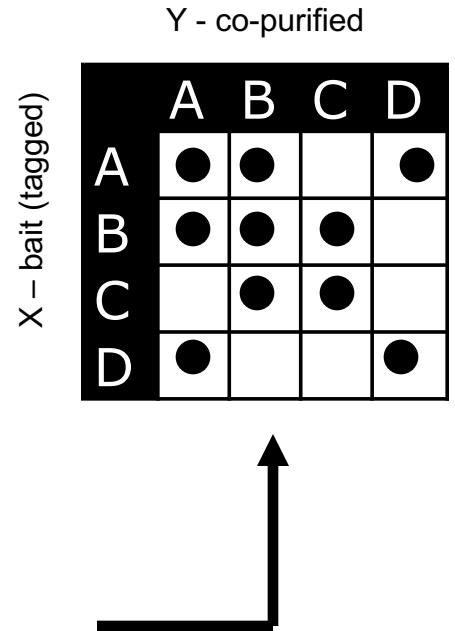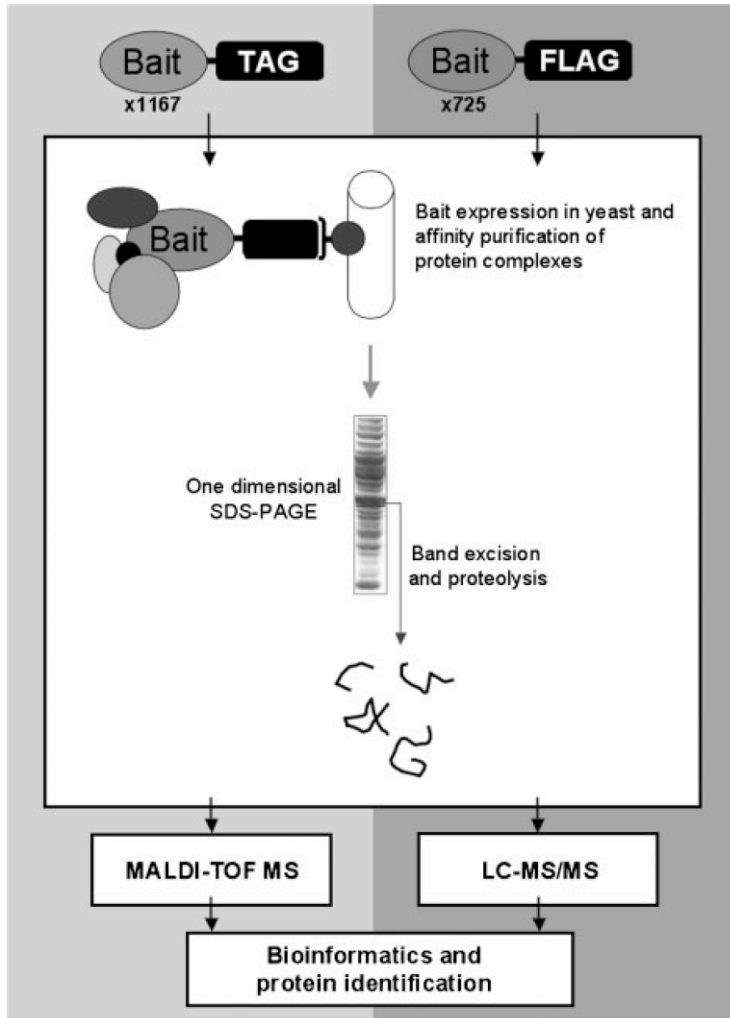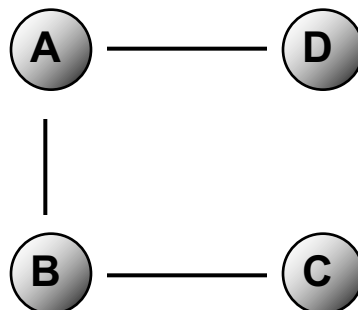
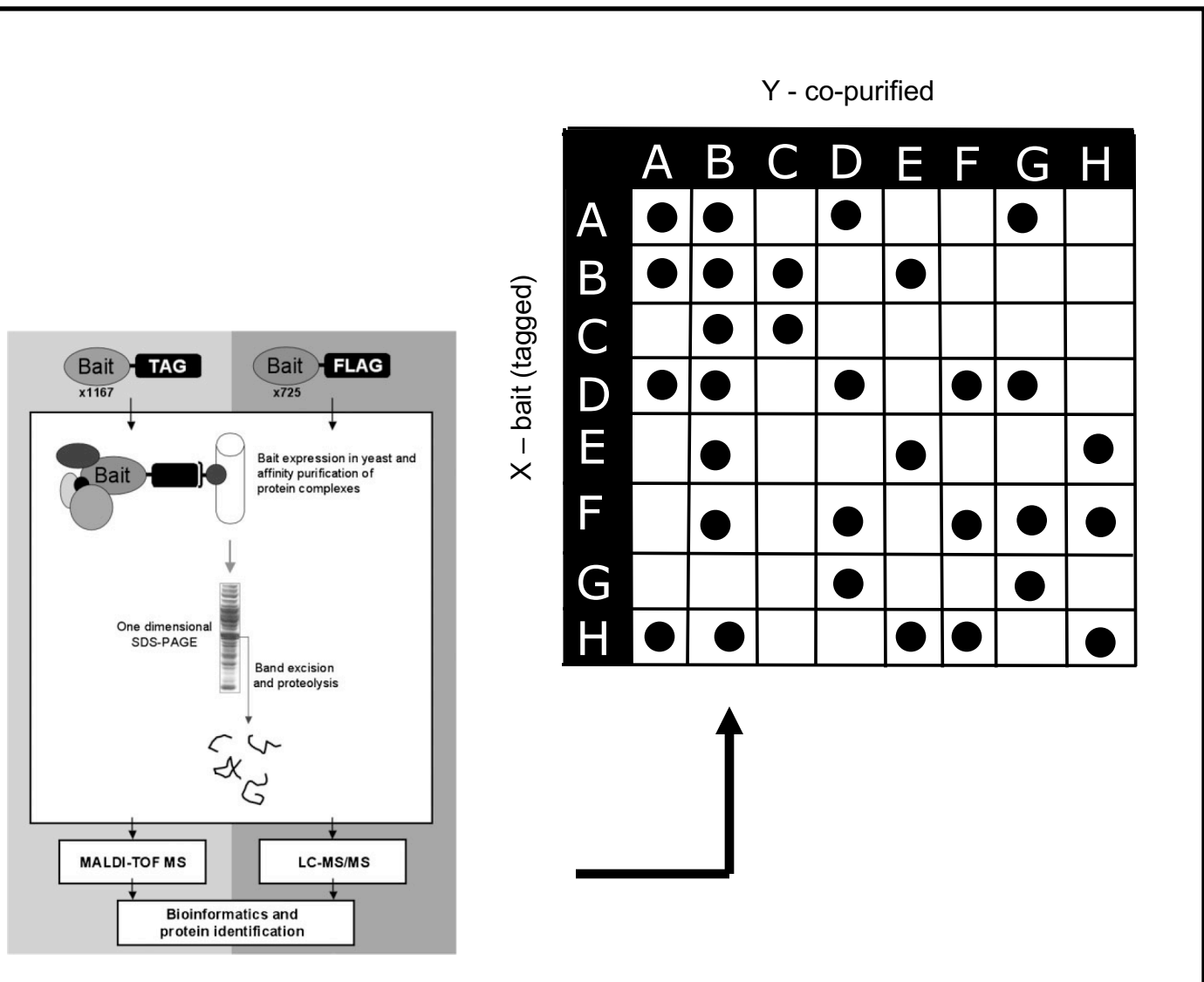# Protein interactions with APMS ("pull-downs")

Exercise 4: *Based on the experiments above, draw a network below which represents the interactions between the proteins A, B, C and D using the **spoke model** for interpreting APMS based data.*

"spoke model"

Protein interactions with APMS ("pull-downs")

Y - co-purified

X – bait (tagged)	A	B	C	D	E	F	G	H
A	●	●		●			●	
B	●	●	●		●			
C		●		●				
D	●	●		●		●	●	
E		●			●			●
F		●		●		●	●	●
G				●			●	
H	●	●			●	●		●

Bait — TAG x1167

Bait — FLAG x725

Bait expression in yeast and affinity purification of protein complexes

One dimensional SDS-PAGE

Band excision and proteolysis

MALDI-TOF MS

LC-MS/MS

Bioinformatics and protein identification

Exercise 5: *Based on the experiments above, draw a network which represents the interactions between the protein. (spoke model)*

Comment: Self-interactions are ignored.

Protein interactions with APMS ("pull-downs")

Y - co-purified

X – bait (tagged)

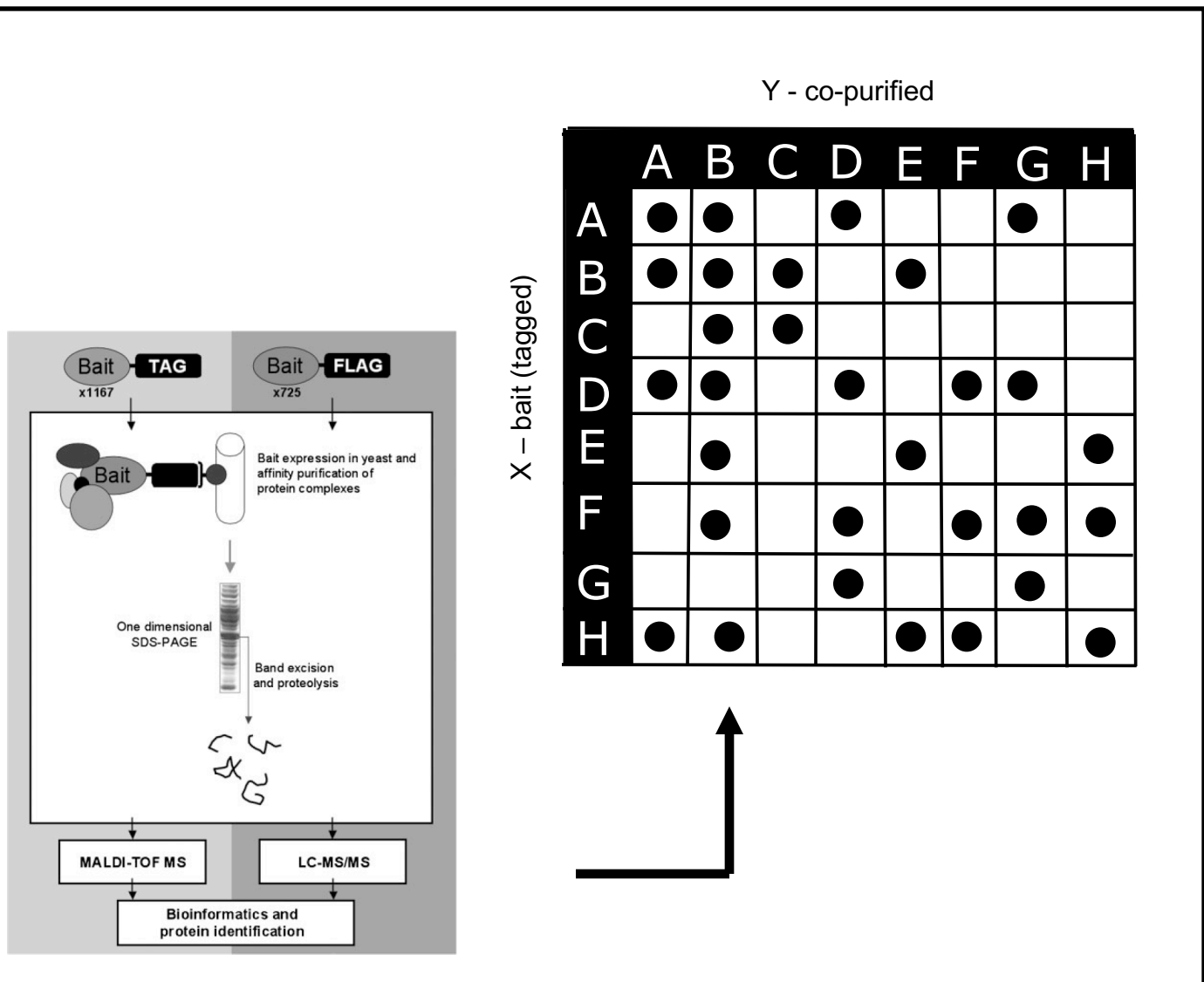|	A	B	C	D	E	F	G	H
A	●	●		●			●	
B	●	●	●		●			
C		●	●					
D	●	●		●		●	●	
E		●			●			●
F		●		●		●	●	●
G				●			●	
H	●	●			●	●		●

Exercise 5: *Based on the experiments above, draw a network which represents the interactions between the protein. (spoke model)*
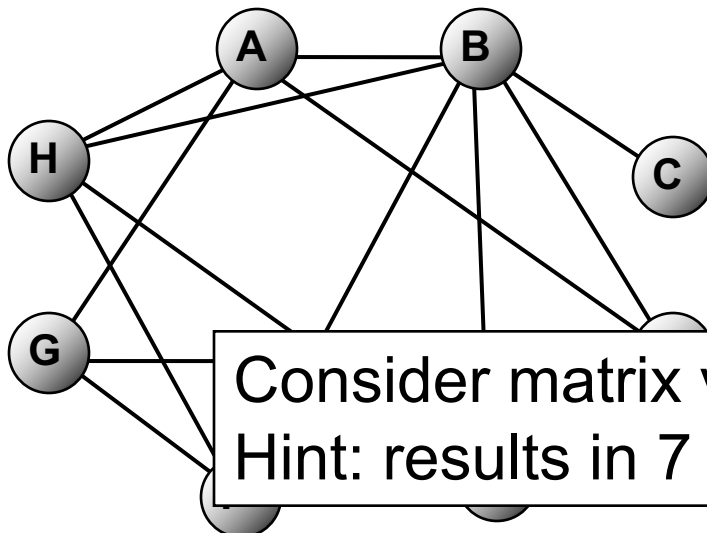
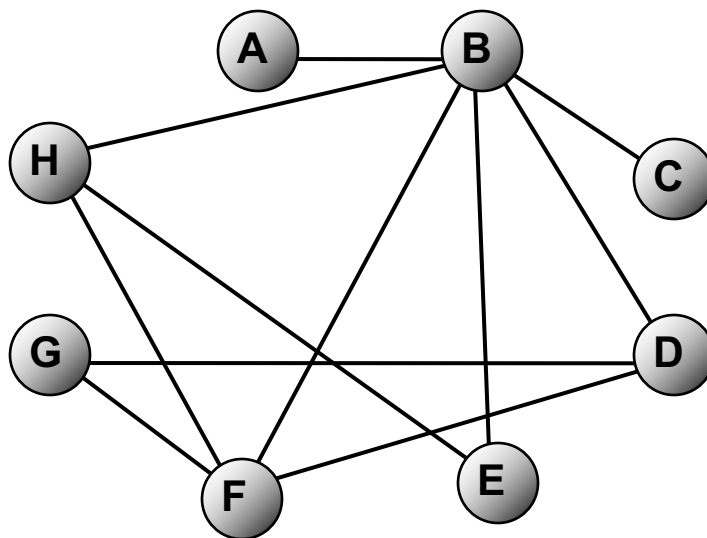**Comment: Self-interactions are ignored.**

Consider matrix version
Hint: results in 7 extra edges

Example: $S(A,B) = \log10((4*7)/(5*8))$

S(X,Y)	A	B	C	D	E	F	G	H
A		-0.155	na	-0.319	na	na	-0.319	-0.523
B			-0.234	-0.222	-0.183	-0.183	na	-0.183
C				na	na	na	na	na
D					na	-0.301	-0.194	-0.523
E						-0.505	na	-0.301
F							-0.301	-0.301
G								-0.523

Protein interactions with Y2H

Prey

	A	B	C	D	E	F	G	H
A		●		●			●	
B	●			●			●	
C		●				●		
D								
E								●
F							●	●
G						●		●
H					●	●	●	

Bait

X

BD | Bait plasmid

Y

AD | Prey plasmid

BD | X

Y | AD

BD | X | Y | AD

Transcription machinery

Reporter gene

UAS
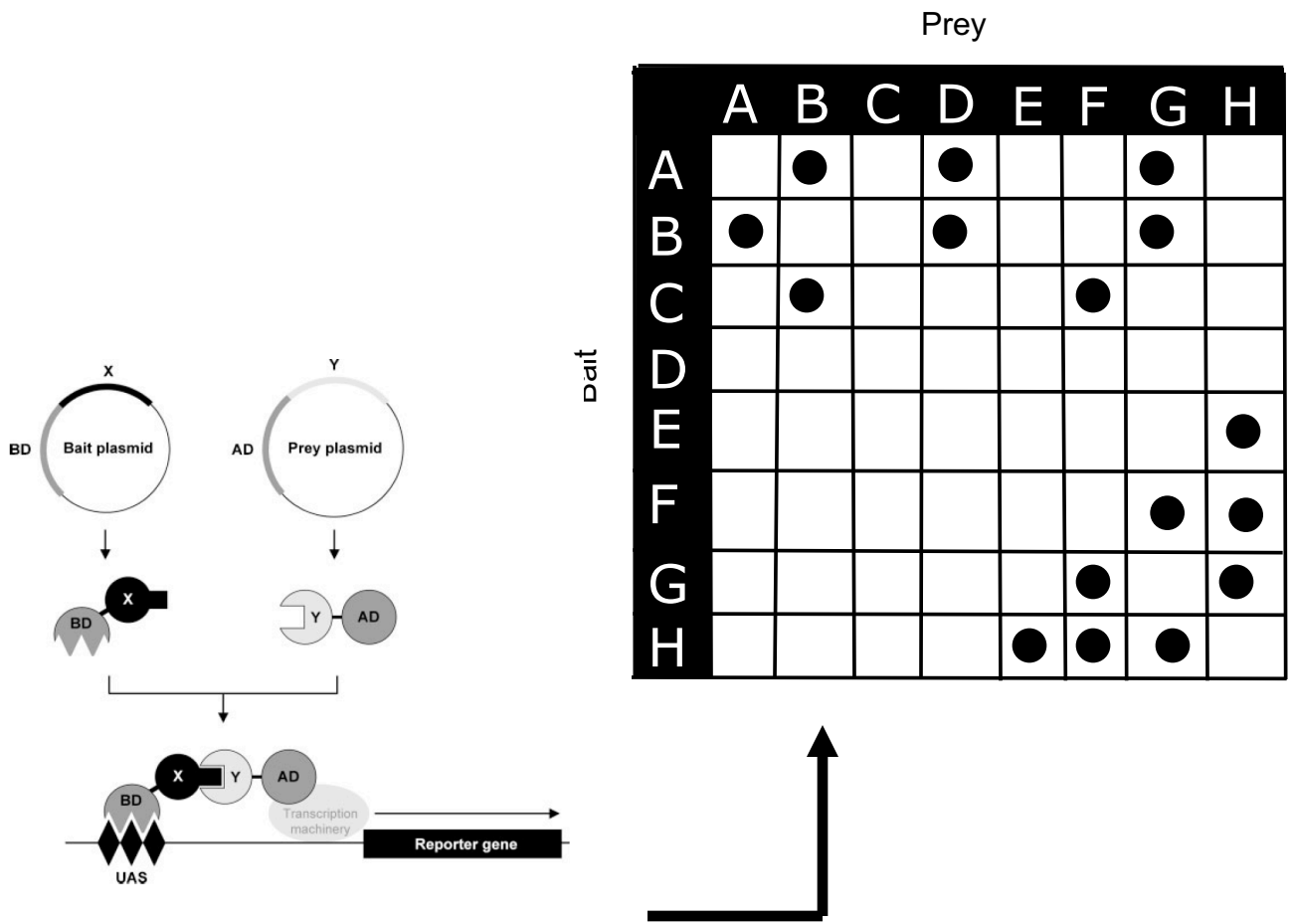
Exercise 7: *Based on the experiments above, draw a network which represents the interactions between the proteins.*
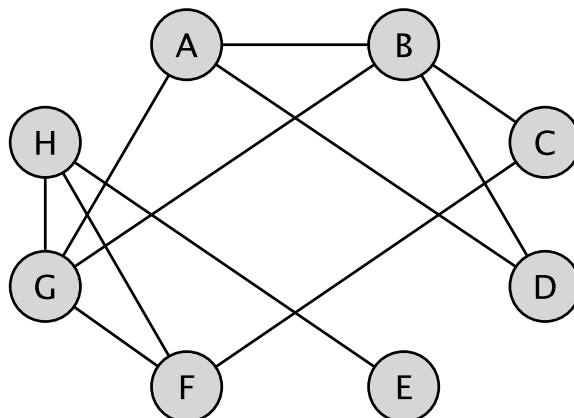
	A	B	C	D	E	F	G	H
A		-0.301		-0.301			-0.778	
B			-0.903	-0.477			-0.954	
C						-0.778		
D								
E								-0.477
F							-0.778	-0.602
G								-0.778
H								

Exercise 8: *Based on the experiments above, draw a network which represents the interactions between proteins with binary interaction scores above -0.40.*
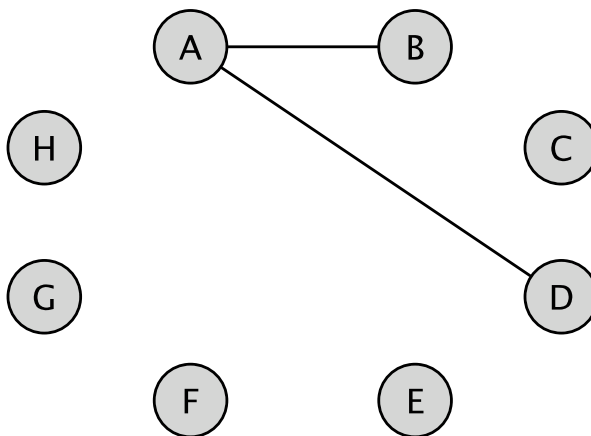
Exercise 9: *Based on the experiments above, draw a network which represents the interactions between proteins with binary interaction scores above -0.65.*