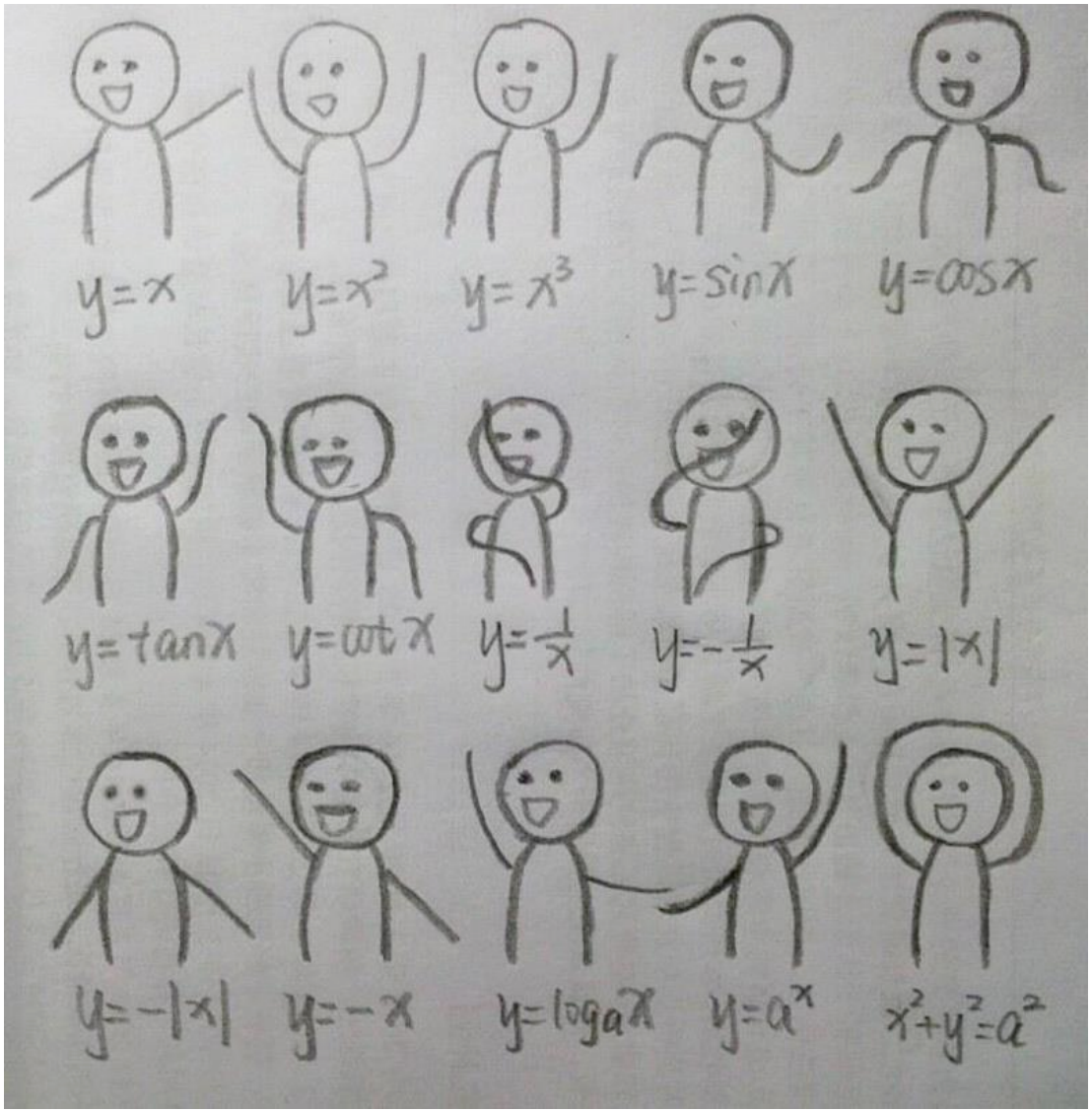
4/3/15

M2D5: DNA repair assays

Do the math dance!



Source: PhilipsShiu

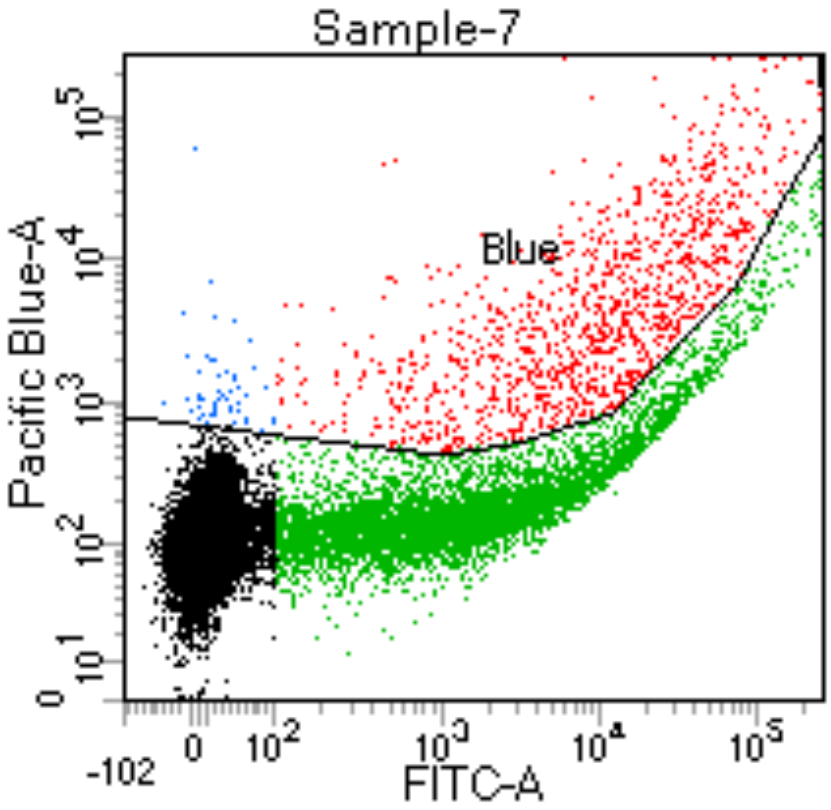
Today we do the tissue culture dance!

Announcements:

Mod1 Revision due **Sunday at 5pm**

Don't forget about your blog posts.

Flow cytometry: There are a lot of steps before we get to this plot!

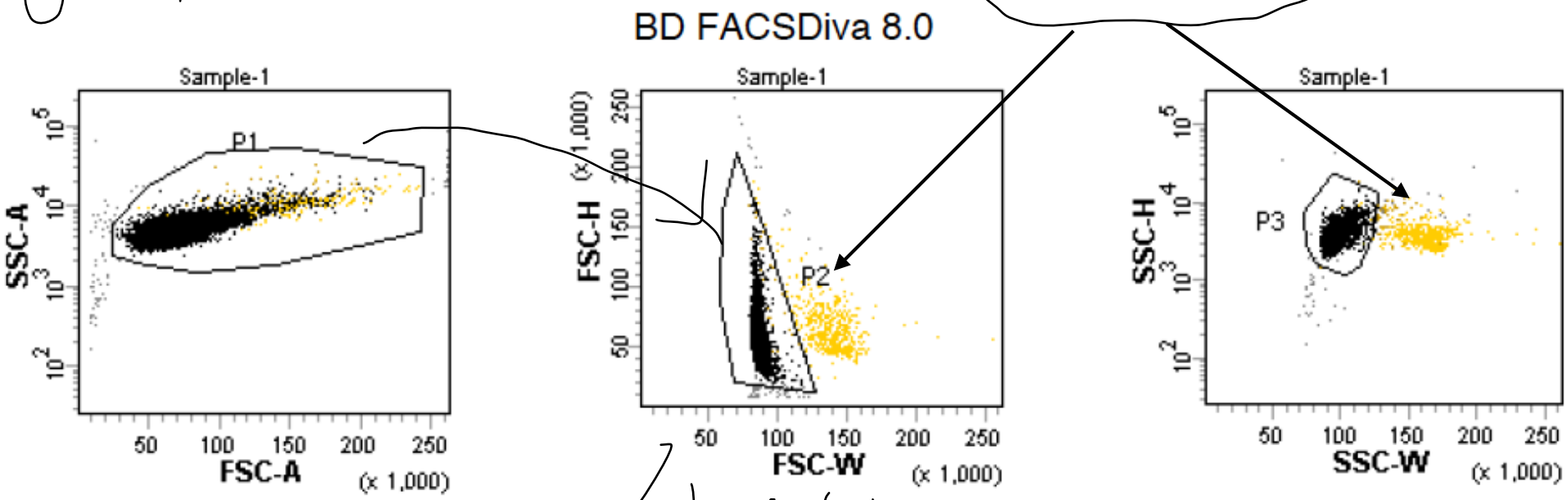


1) Determine the 'relevant' cell population

Unfluor cells

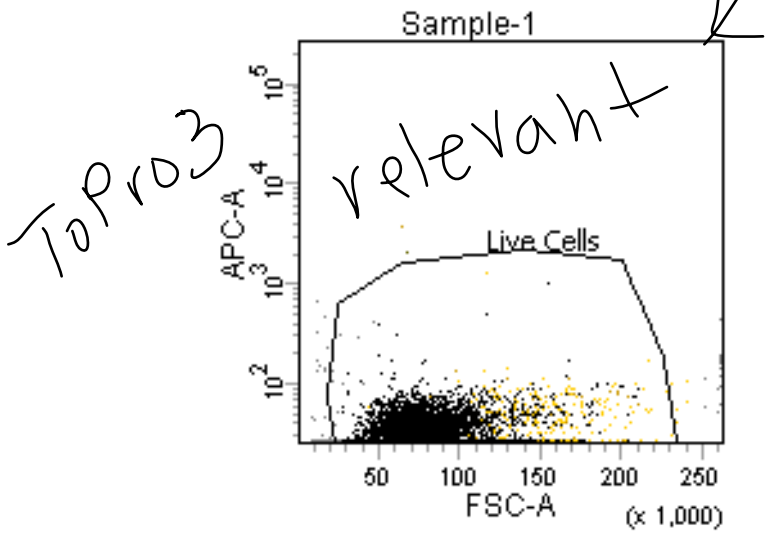
gate out debris, aggregates, dead cells

Doublets



height

width



ToPro3

relevant

Any cell with ToPro3 will have high far red fluorescence = **dead cells**

SSC = side = shape

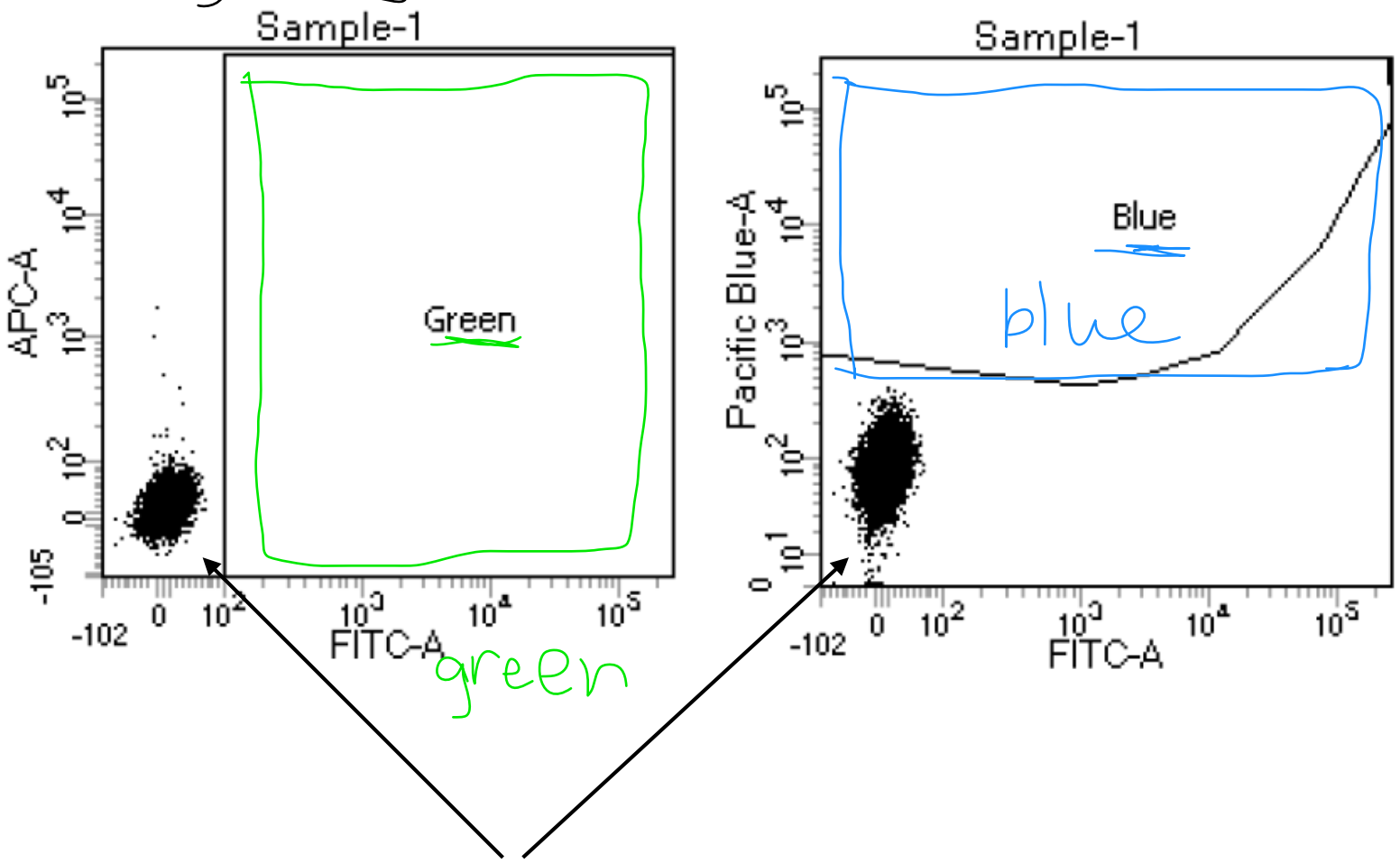
fsc = forward = size

OO] good :;] debris

aggregate

2) Set 'negative' gates with untransfected cells

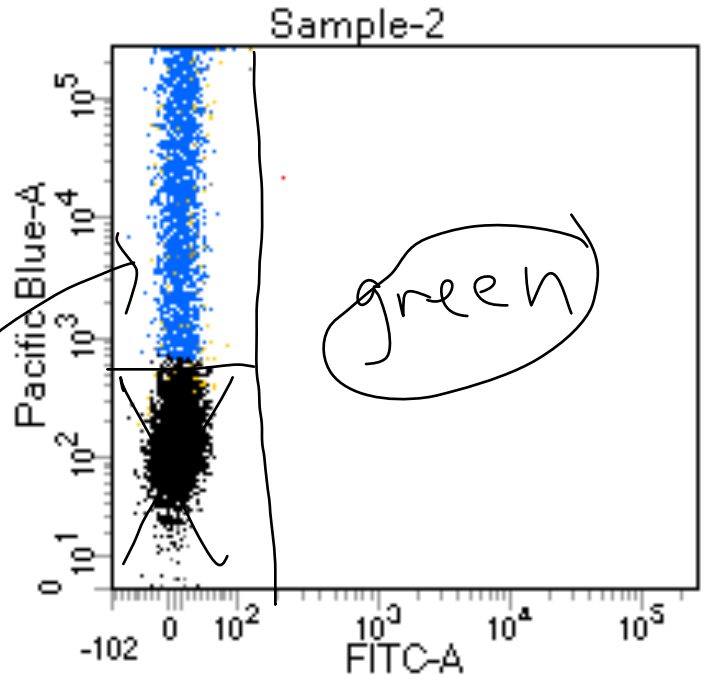
Unflvor } Set gates BFP + GFP



These are the "live cells"

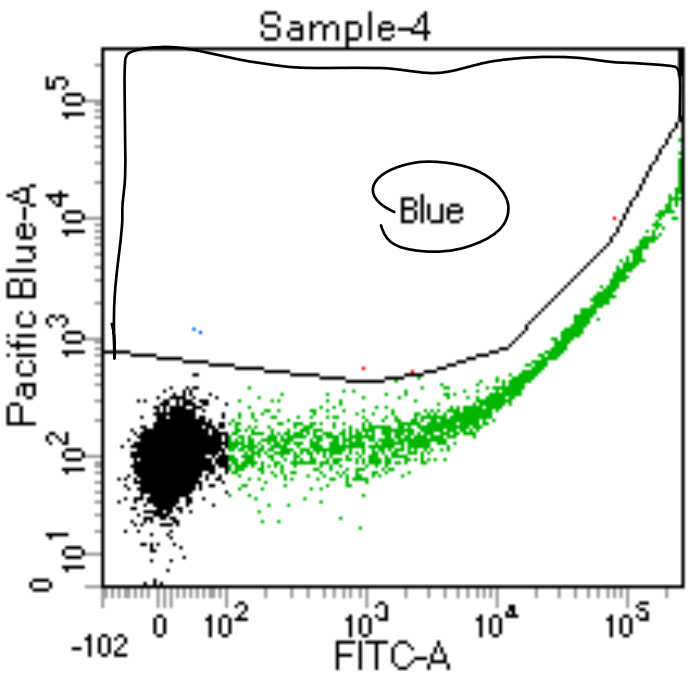
3) Set 'positive' gates with single color controls

BFP only



BFP (+)

GFP only

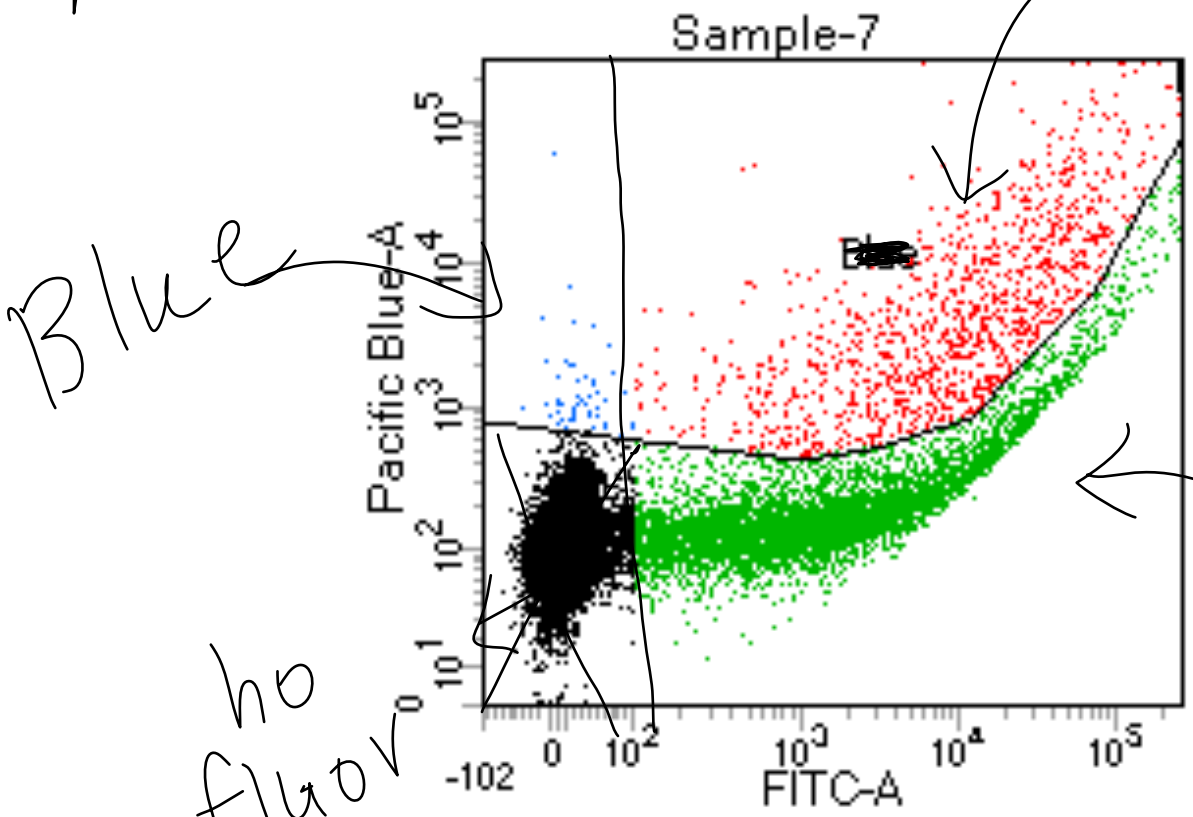


- In flow cytometry green bleeds into blue

4) Quantify experimental conditions

w expt condition

blue + green



Blue

green

no fluor

flow data readout: 1) % cells blu + green

2) mean 3) median fluor intensity ⇒ calculations

Part 1: Hints

1. Aspirating media — clean pipette tip between conditions
2. Correct tube labeling is key.

Condition	Red	Orange	Yellow	Green	Blue	Pink	Purple	Platinum
K1 Intact	10	22	34	46	58	70	82	94
K1 Intact	11	23	35	47	59	71	83	95
Drug Intact	12	24	36	48	60	72	84	96
Drug Intact	13	25	37	49	61	73	85	97
xrs6 Intact	14	26	38	50	62	74	86	98
xrs6 Intact	15	27	39	51	63	75	87	99
K1 damaged	16	28	40	52	64	76	88	100
K1 damaged	17	29	41	53	65	77	89	101
Drug damaged	18	30	42	54	66	78	90	102
Drug damaged	19	31	43	55	67	79	91	103
xrs6 damaged	20	32	44	56	68	80	92	104
xrs6 damaged	21	33	45	57	69	81	93	105

3. Mixing is very important — need single cell suspension!
4. Plan your workflow with partner BEFORE you start.

How will we know that the inhibitor works?

Protocol

1. We plated cells for you yesterday.
2. You make up drug dilutions (3ml/well)
3. You add drugs to cells
4. Marcus will irradiate cells with 4 Gy
5. After 7 days, stain colonies and count

NOTE: Teams using DMNB —
Vehicle = DMSO
The same volume of DMSO must be added to each well.

Today in the lab:

red/orange/yellow/green

1. ~~Orange, yellow, green~~ in TC first to prep flow. ~~Blue and purple~~ will follow once hoods open up.
2. Work at a purposeful pace.
3. The TC room has a rotating door policy today:
    1. Prep for flow —> leave
    2. Next team prep for flow —> leave
    3. Prep for 'kill curve' —> leave
    4. etc.