

## live - Dead staining

↳ no. of live cells >> no. of dead cells.

hence heat kill is carried out to increase no. of dead cells for better visualisation purposes.

Red stain  $\rightarrow$  Dead cells

Green stain  $\rightarrow$  Live cells

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Machine usually used for PCR  
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used to heat kill

$99^{\circ}\text{C}$   $\rightarrow$  2 mins

then to make PCR tube safe for touch! (can't handle  $99^{\circ}\text{C}$  tube).

$70^{\circ}\text{C} \rightarrow$  30 sec to make

# PCR tube manipulation

- Remove YPD from culture, because of undesired noise
- Instead 1x PBS is used, accounts for variable pH of media to maintain their life.
- Pellet down media to remove YPD media  
1<sup>st</sup> centrifugation removes YPD media → 5000 rpm (5 mins)

after YPD media is removed, reeth suspended culture in 1x PBS, and  
2<sup>nd</sup> centrifugation to wash clear 0g PBS solution  
diluted 10 times to obtain 1x PBS,  
(washing step → 3 min).  
Centrifuge

- Can't be exposed to light (will start photobleaching)

NAROTTAM

Ode to my self