WHERE THE WILD THINGS GROW

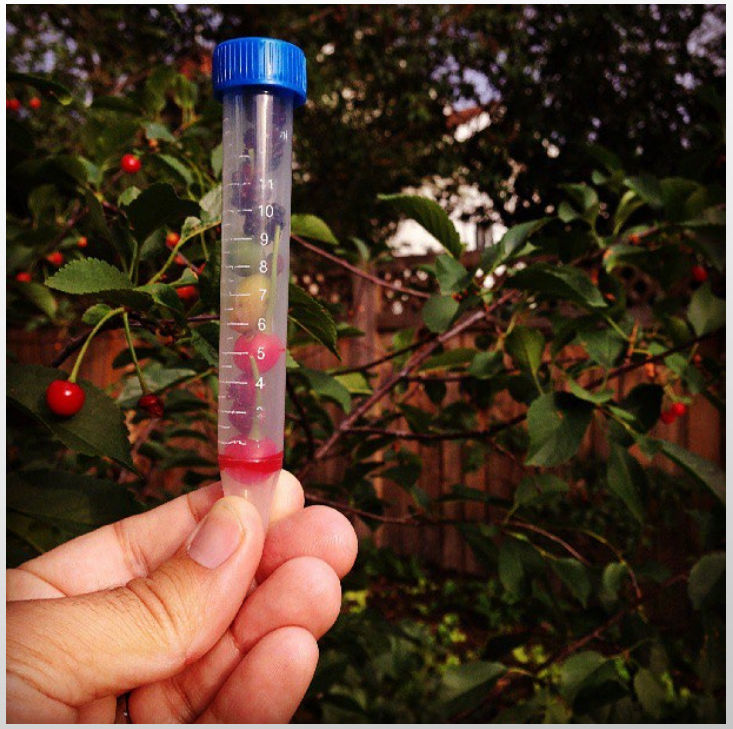
YEAST WRANGLING

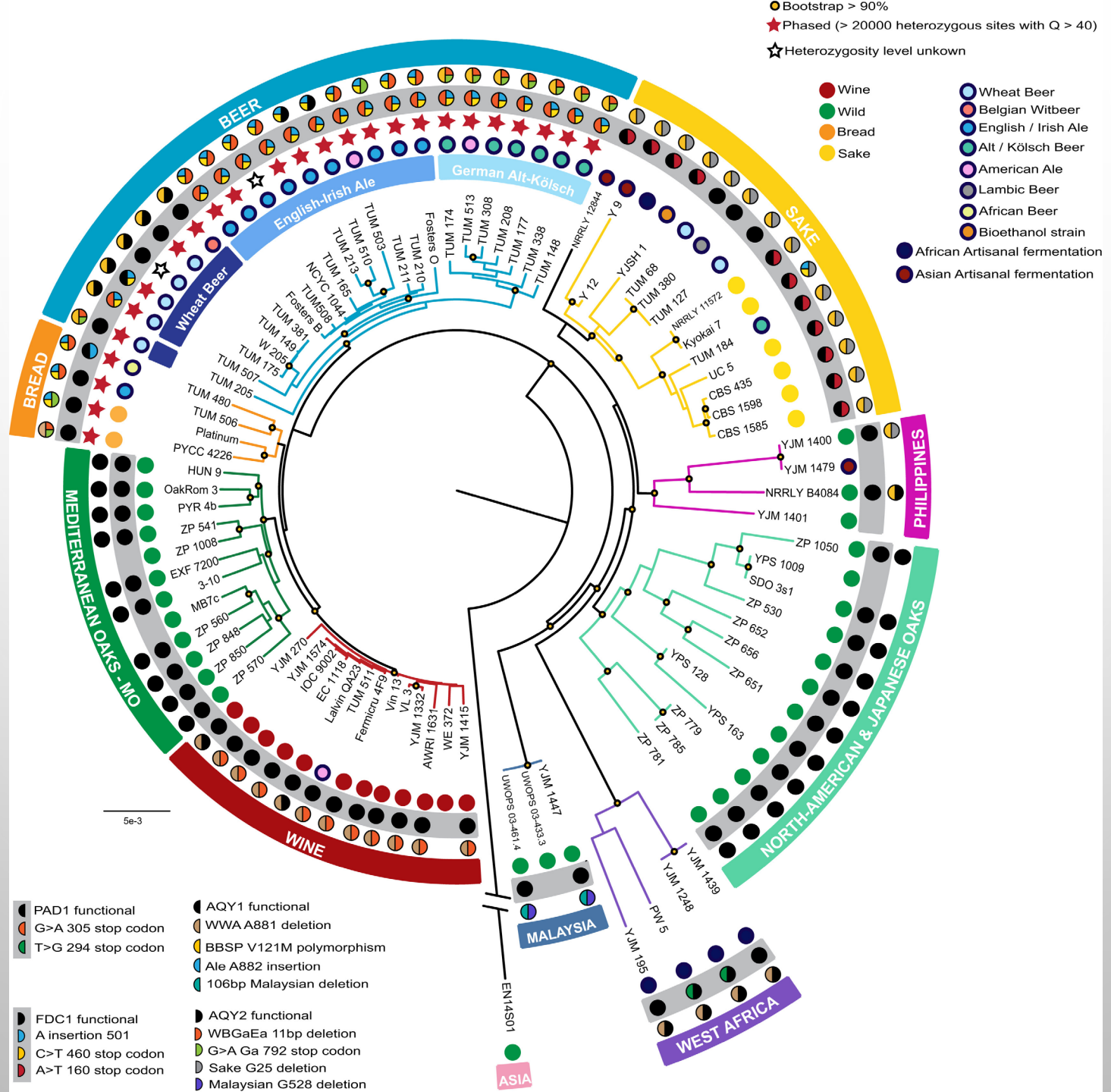
BIOPROSPECTING

- ISOLATING AUTOCHTHONOUS (WILD, NATIVE) YEASTS FROM VARIOUS SUBSTRATES, OR AIR
- YEASTS ARE UBIQUITOUS IN THE ENVIRONMENT
- LOCAL YEAST MAY PRODUCE DIFFERENT FLAVOR PROFILES THAN TRADITIONAL YEASTS

THE ECOLOGY OF WILD YEAST

- MOST COMMON PLACES
  - FRUIT
  - BARK
  - LEAF LITTER
- MOST COMMON APPEARS TO BE IN LOW SUGAR ENVIRONMENT OF LEAF LITTER (SEE KOWALLIK THESIS)




Bootstrap > 90%
Phased (> 20000 heterozygous sites with Q > 40)
Heterozygosity level unkown
Wine
Wild
Bread
Sake
Wheat Beer
Belgian Witbeer
English / Irish Ale
Alt / Kölsch Beer
American Ale
Lambic Beer
African Beer
Bioethanol strain
African Artisanal fermentation
Asian Artisanal fermentation
BEER
English-Irish Ale
German Alt-Kölsch
Wheat Beer
SAKE
BREAD
MEDITERRANEAN OAKS - MO
WINE
PHILIPPINES
NORTH-AMERICAN & JAPANESE OAKS
MALAYSIA
WEST AFRICA
ASIA
EN14S01
5e-3
TUM 503
TUM 510
TUM 213
TUM 165
NCYC 1044
TUM508
Fosters B
TUM 381
TUM 149
W 205
TUM 175
TUM 507
TUM 205
TUM 480
TUM 506
Platinum
PYCC 4226
HUN 9
OakRom 3
PYR 4b
ZP 541
ZP 1008
EXF 7200
3-10
MB7c
ZP 560
ZP 848
ZP 850
ZP 570
TUM 211
TUM 210
Fosters O
TUM 174
TUM 513
TUM 308
TUM 208
TUM 177
TUM 338
TUM 148
NRRLY 12844
Y 9
YJSH 1
TUM 68
TUM 380
TUM 127
NRRLY 11572
Kyokai 7
TUM 184
UC 5
CBS 435
CBS 1598
CBS 1585
YJM 1400
YJM 1479
NRRLY B4084
YJM 1401
ZP 1050
YPS 1009
SDO 3s1
ZP 530
ZP 652
ZP 656
ZP 651
YPS 128
YPS 163
ZP 779
ZP 785
ZP 781
YJM 270
YJM 1574
IOC 9002
EC 1118
Lalvin QA23
TUM 511
Fermicru 4F9
Vin 13
VL 3
YJM 1332
AWRI 1631
WE 372
YJM 1415
YJM 1447
UWOPS 03-433.3
UWOPS 03-461.4
YJM 1439
YJM 1248
PW 5
YJM 195
PAD1 functional
G>A 305 stop codon
T>G 294 stop codon
FDC1 functional
A insertion 501
C>T 460 stop codon
A>T 160 stop codon
AQY1 functional
WWA A881 deletion
BBSP V121M polymorphism
Ale A882 insertion
106bp Malaysian deletion
AQY2 functional
WBGaEa 11bp deletion
G>A Ga 792 stop codon
Sake G25 deletion
Malaysian G528 deletion

THE GOLDEN RULE

STERILITY, STERILITY, STERILITY IS NEXT TO GOOD TASTING BEER

STERILIZATION – REMOVAL OF ALL ORGANISMS

SANITIZATION – REDUCING THE AMOUNT OF "PROBLEM" ORGANISMS

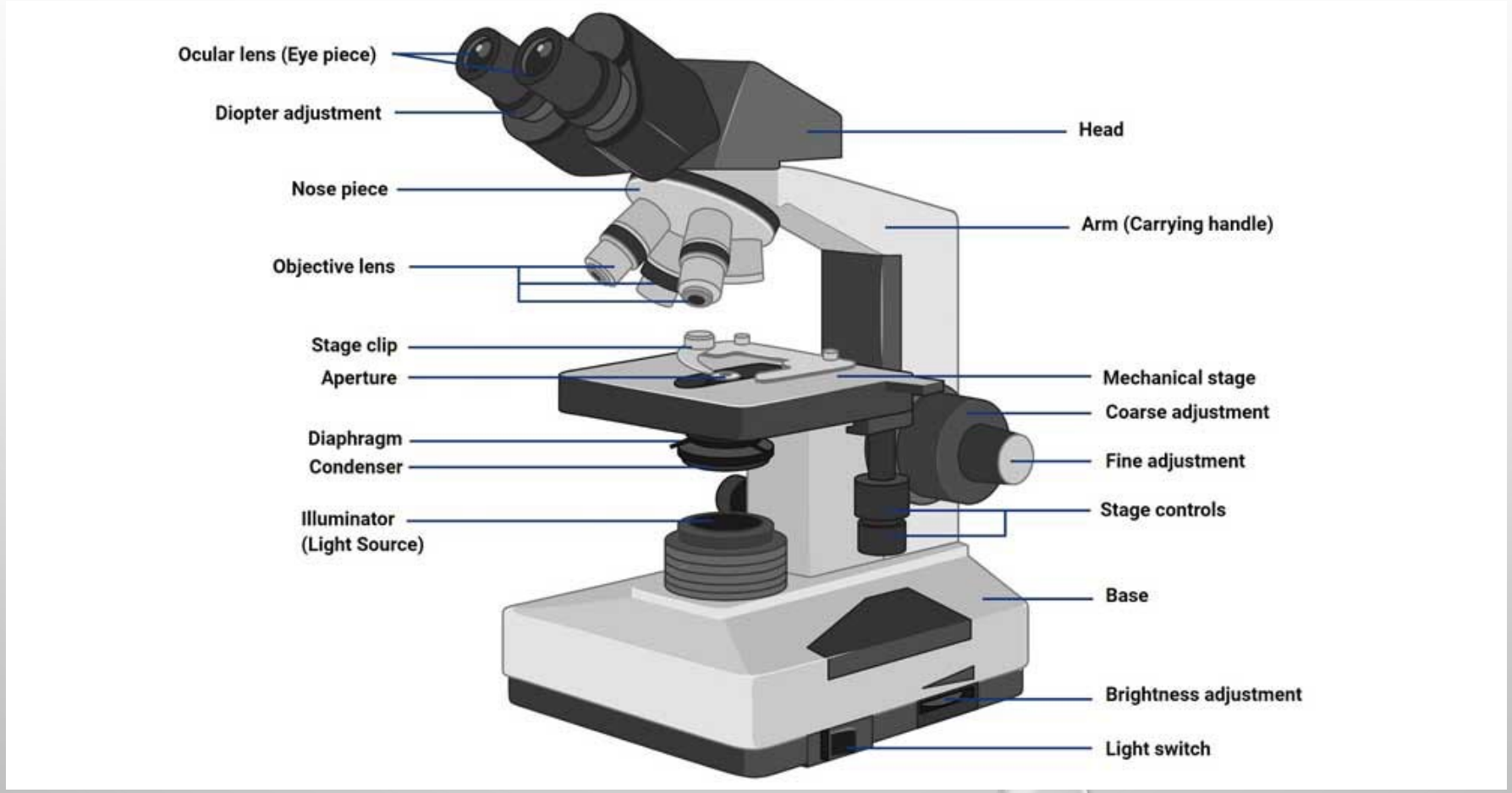
EQUIPMENT

- YEAST CAPTURING
  - DRY MALT EXTRACT
  - AGAR
  - STERILE PETRI DISHES
  - STERILE COTTON SWABS
  - MASON JARS
  - CENTRIFUGE TUBES
  - PARAFILM
- THE HOME LAB
  - MICROSCOPE
  - ALCOHOL BURNER
  - PRESSURE COOKER
  - LAMINAR FLOW HOOD
  - GLOVES
  - 70% ETHANOL
  - INOCULATION LOOP

MICROSCOPES

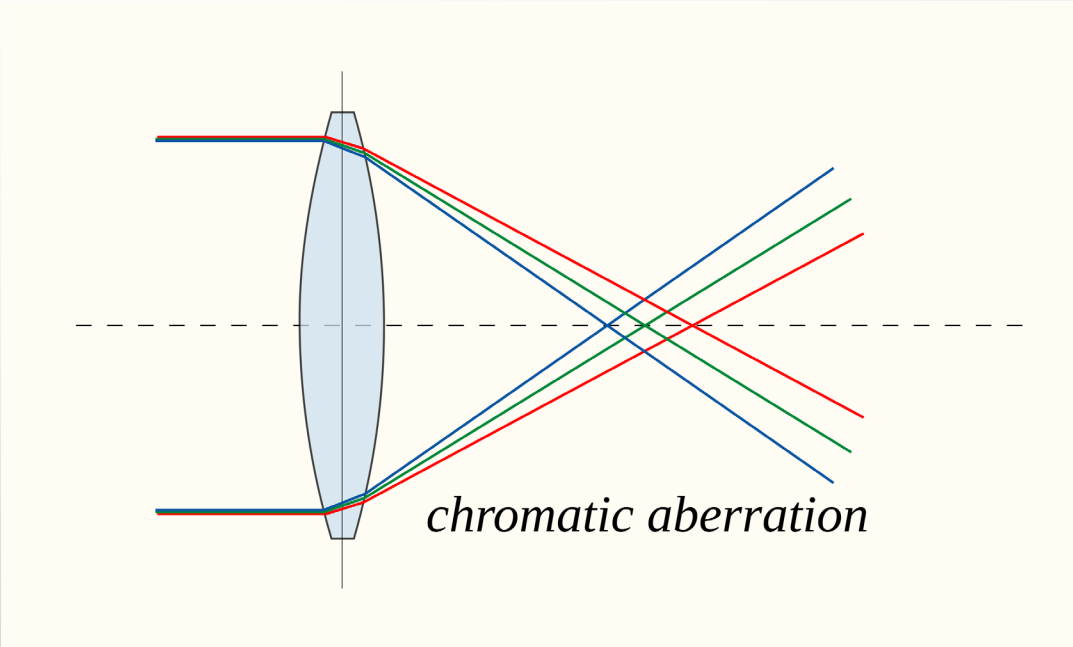
- USES
  - YEAST COUNTING
  - VIABILITY TESTING
  - CELLULAR MORPHOLOGY
  - LOOKING FOR WATER BEARS
- COSTS ~$100 - 300

MICROSCOPE PARTS



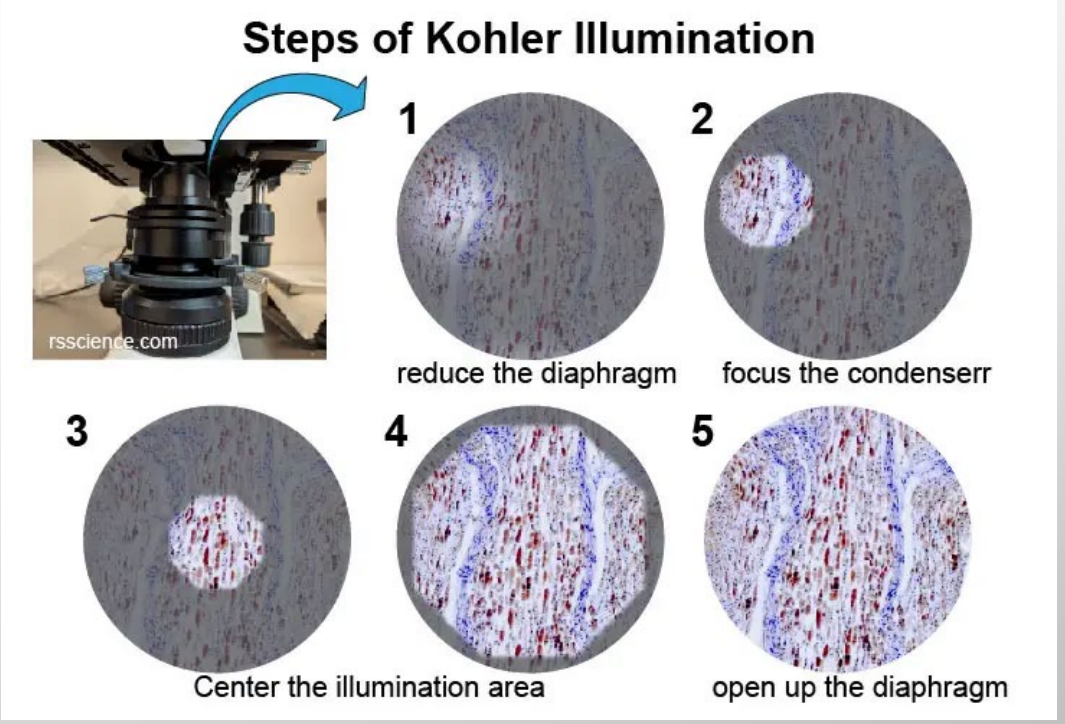
OPTICS

- MAGNIFICATION – OCULAR POWER X OBJECTIVE LENS POWER
  - 100X – BASIC VIEWING AND FOCUSING
  - 200X – CELL COUNTS
  - 400X – CELL COUNTS, MAYBE VIABILITY TESTING
  - 600X – VIABILITY TESTING
  - 1000X – GOOD FOR MORPHOLOGICAL VIEWING
- ABERRATION
  - CAUSES BLURRING OF IMAGE AND DISTORTION, AS WELL AS RAINBOWING AROUND OBJECTS
  - CAN BE CORRECTED FOR, BUT LENSES ARE EXPENSIVE
- PARFOCAL
  - ONCE FOCUSED, WILL STAY FOCUSED WHEN SWITCHING BETWEEN LENSES



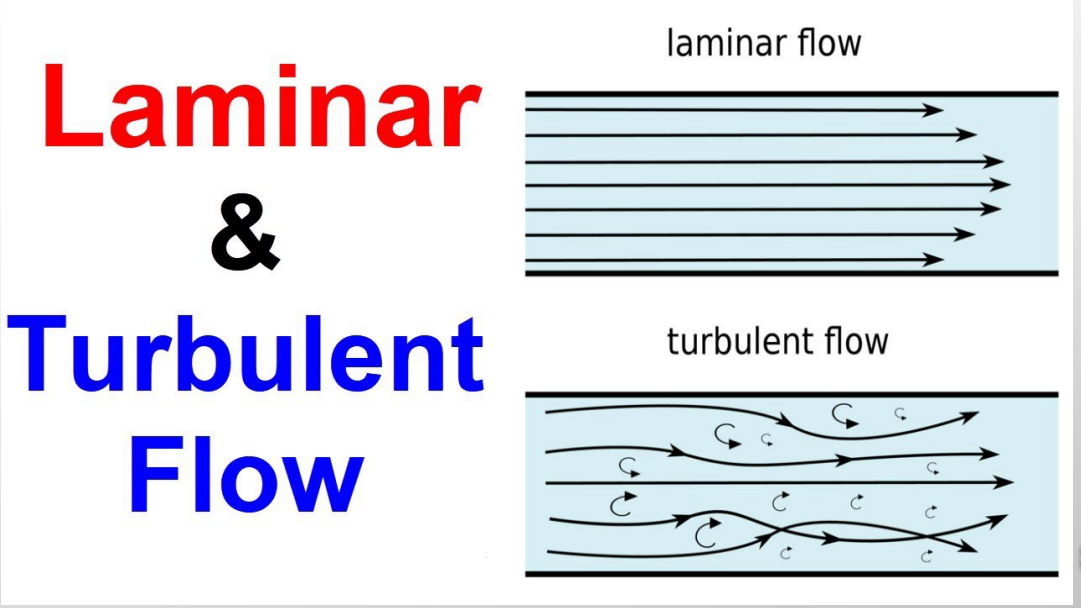
KÖHLER ILLUMINATION

- PRODUCES A UNIFORM ILLUMINATION OF THE SAMPLE



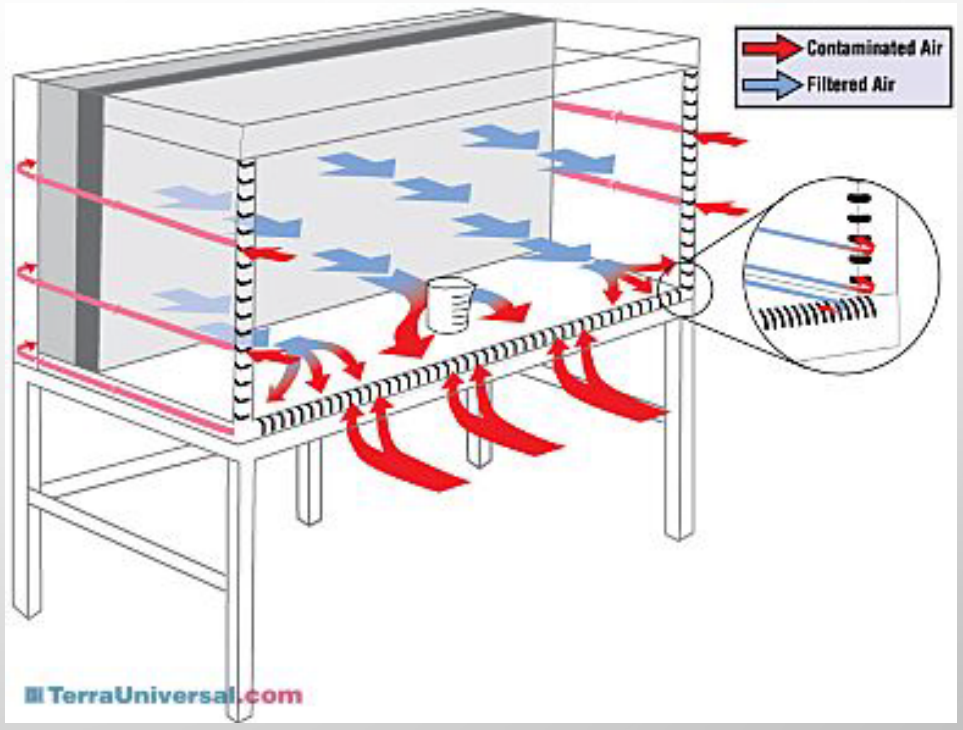
LAMINAR FLOW

- FLOW IN WHICH THE AIR TRAVELS SMOOTHLY OR IN REGULAR PATHS
- PREVENTS PARTICLES FROM MIXING
- COMBINE WITH HEPA FILTER, CAN PRODUCE VERY CLEAN ENVIRONMENTS



LAMINAR FLOW HOOD

- AIR BLOWS DIRECTLY AT THE USER.
- TYPICALLY, AREA IS PLASTIC AND STAINLESS STEEL, ANYTHING THAT CAN BE CLEANED
- CAN BE PURCHASED OR MADE FROM INEXPENSIVE PARTS
- WHILE NOT NEEDED, WILL UP THE GAME OF CLEANLINESS IN THE HOME LAB



LAMINAR FLOW HOOD

DIY LAMINAR FLOW HOOD

CLEANLINESS

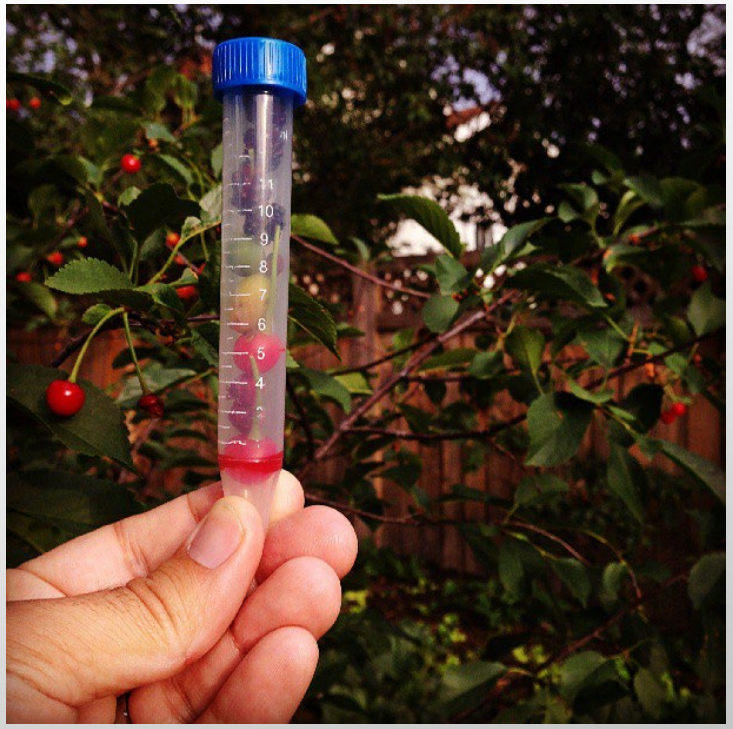
- IF MAKING PLATES YOURSELF, YOU CAN STERILIZE WITH A PRESSURE COOKER
  - ALL ITEMS THAT THE WORT IS PLACED INTO SHOULD BE OF GLASS
- PLASTIC ITEMS TYPICALLY COME PRESTERILIZED
- ABWG – ALWAYS BE WEARING GLOVES AND/OR ALWAYS BE SPRAYING HANDS WITH ETHANOL

CAPTURING - JARS

- CREATE A 1.040 STARTER WITH DME
- STERILIZE STARTER BY BOILING FOR 20 MINUTES WITH A SMALL AMOUNT OF HOPS
  - THE HOPS WILL INHIBIT *LACTOBACILLUS* GROWTH
- ONCE COOL, MEASURE PH AND DIVIDE INTO SANITIZED MASON JARS
- COVER WITH CHEESE CLOTH OR SIMILAR FINE MESH TO PREVENT FLIES
- PLACE JARS OUTSIDE OVER NIGHT
- TRANSFER TO LIQUID TO A SANITIZED FLASK OR JUG WITH AIRLOCK
- WATCH FOR FERMENTATION AND WAIT FOR COMPLETION
- ONCE COMPLETE MEASURE PH AGAIN, HIGH PH DISCARD
- ISOLATE ON AGAR PLATES

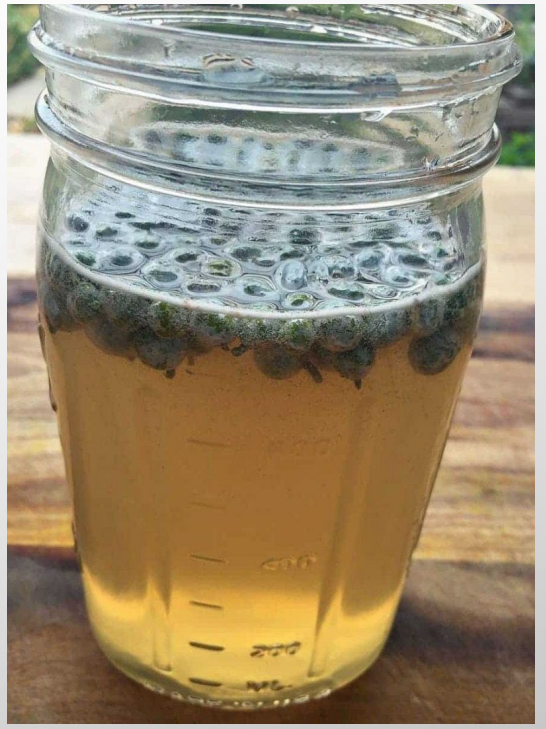
CAPTURING – FRUITS AND VEGS

- IF THE FRUIT IS SMALL
  - PLACE IN STERILE CENTRIFUGE TUBE
  - COVER WITH LOW GRAVITY WORT
  - SHAKE SHAKE SHAKE
  - SEAL TOP AND WAIT FOR FERMENTATION TO OCCUR
  - ONCE DONE STREAK ON AGAR PLATE



CAPTURING – FRUITS AND VEGS

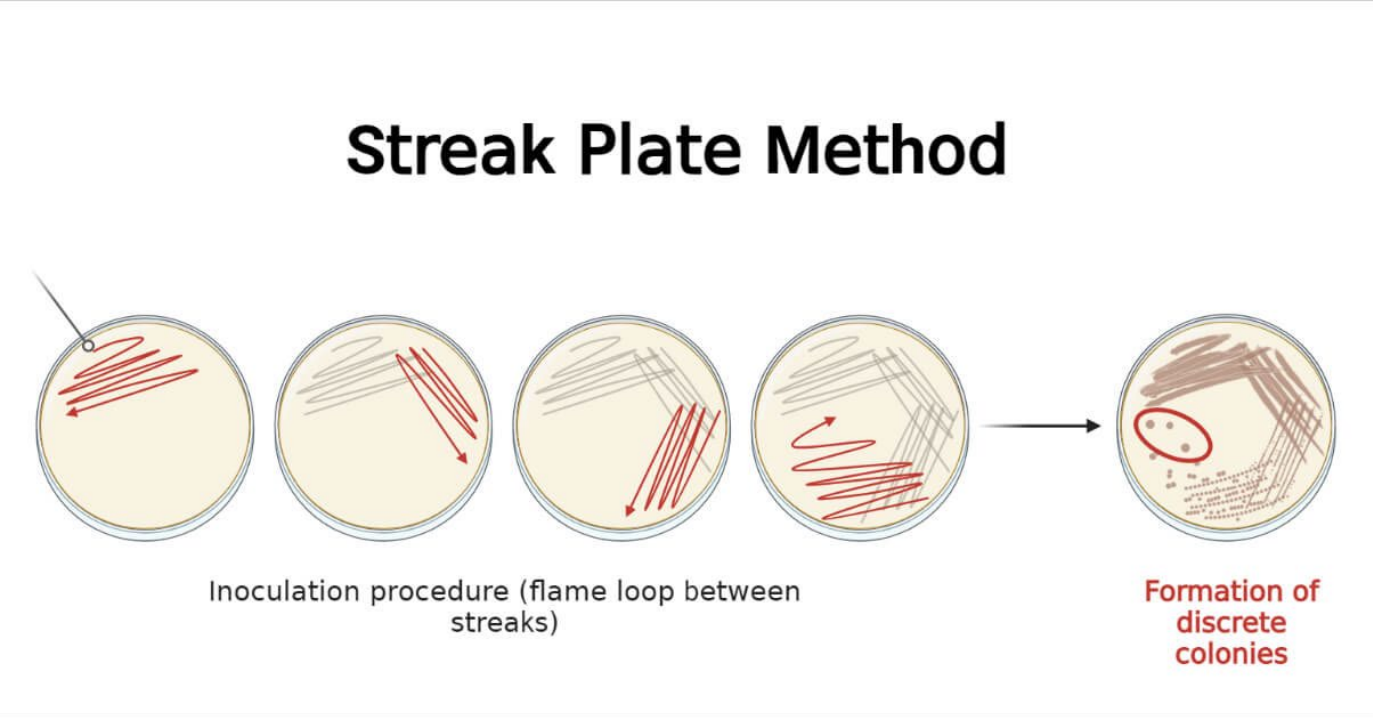
- IF THE FRUIT IS LARGE
  - PLACE IN SANITIZED JAR
  - COVER WITH LOW GRAVITY WORT
  - SHAKE SHAKE SHAKE
  - SEAL TOP AND WAIT FOR FERMENTATION TO OCCUR
  - ONCE DONE STREAK ON AGAR PLATE



CAPTURING - SWABBING

- PLACE LOW GRAVITY WORT IN STERILE CENTRIFUGE TUBE
- SWAB AREA OF INTEREST
- DUNK SWAB IN TUBE SEVERAL TIMES TO REMOVE ANY CELLS INTO WORT
- SEAL AND FERMENT FOR SEVERAL DAYS
- STREAK ONTO AGAR

AGAR STREAKING

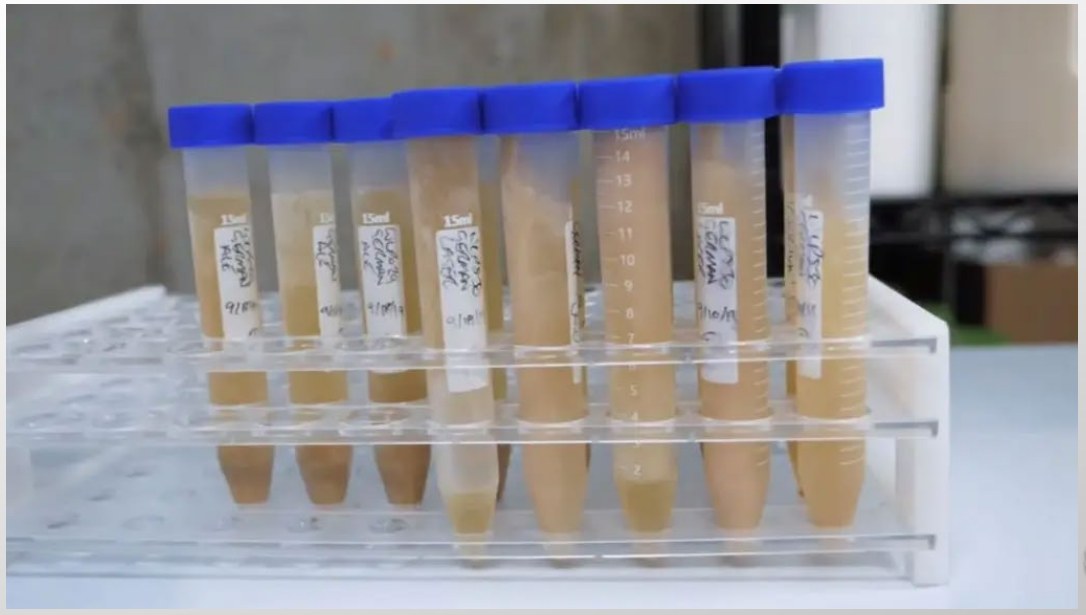


SLANTS

- GREAT FOR LONGER TERM STORAGE
- HOWEVER, DOES REQUIRE ROUTINE MAINTENANCE OF REGULAR TRANSFER
- PICK A SINGLE COLONY AND STREAK OVER THE ENTIRE SLANT

LONG TERM STORAGE - CRYOPRESERVATION

- MAKE AND STERILIZE A 25% **GLYCERIN** (USP GRADE) SOLUTION
- MAKE A YEAST STARTER WITH EITHER YOUR WILD YEAST OR YEAST OF INTEREST
- ADD 5 ML OF DILUTE GLYCERIN SOLUTION TO TUBE
- ADD 5 ML OF YEAST SLURRY TO SAME TUBE
- FREEZE – INDEFINITE STORAGE, AS LONG AS NO FREEZE/THAW CYCLE OCCURS
- THAW AND THEN PITCH INTO NEW WORT STARTER



INTERESTING LINKS

- DIY LAMINAR FLOW HOOD: HTTPS://WWW.YOUTUBE.COM/WATCH?V=CFXEKBORPAY
- ANOTHER HOOD: HTTPS://YOUTU.BE/LINFDAVVBTS
- THE NATURAL ECOLOGY OF SACCHAROMYCES YEASTS: HTTPS://MACAU.UNI-KIEL.DE/RECEIVE/DISS_MODS_00018537?LANG=EN
- BOOTLEG BIOLOGY: HTTPS://BOOTLEGBIOLOGY.COM/BACKYARD-YEAST-WRANGLING-TOOL-KIT/
- MILK THE FUNK: HTTPS://WWW.MILKTHEFUNK.COM/WIKI/WILD_YEAST_ISOLATION
- SURI GENERIS BREWING: HTTPS://SUIGENERISBREWING.COM