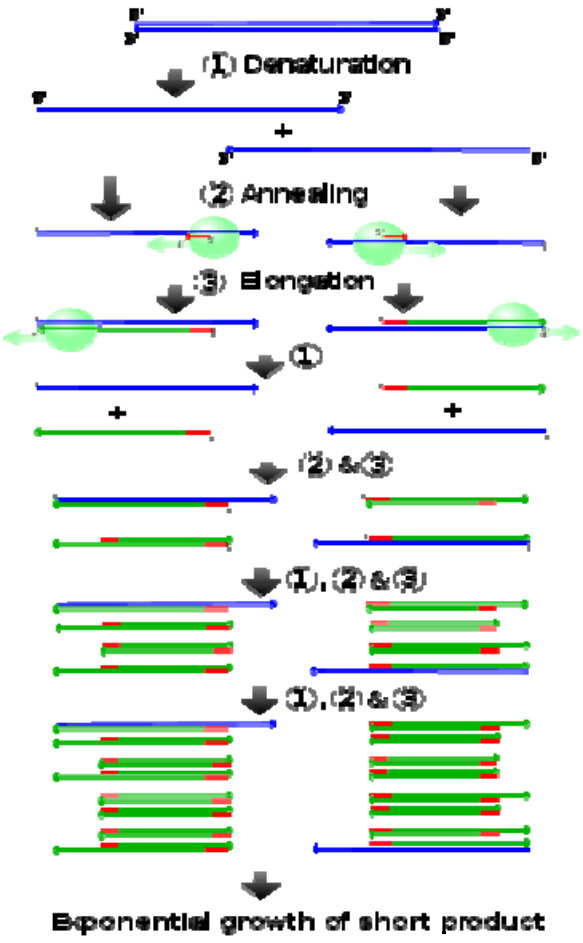
Experiment 3: Genotyping and Chemotaxis

(1) Genotyping

Last week you have observed several worm mutant phenotypes. However, in molecular biological research it is also important to know about the genotype, which means what happen to the mutated gene (for example, deletion, point-mutation etc.). The **polymerase chain reaction** (**PCR**) is a technique widely used in molecular biology. With PCR it is possible to amplify a single or few copies of a piece of DNA which contains particular nucleotide sequences across several orders of magnitude, generating its millions or more copies, up to the amount for further utilization being possible. For the sequence specificity, **primers** or DNA fragments having complementary sequences to the target region are included in the reaction mixture. This time we will use genomic DNA extracted from *C.elegans* as DNA template to amplify the specific DNA sequence in wild type worms and mutants.



Polymerase chain reaction (PCR) experiment

∗Materials

(1) Genomic DNA (extracted from worm strain N2 and RB809)

(2) Taq DNA polymerase

(3) 10X Taq buffer

(4) 2.5mM dNTP (2’ -deoxynucleoside 5’ -triphosphate) mix

(5) Forward and backward primer

(6) Sterile deionized water

∗Methods

(1) Mix all the reagents in PCR tube and polymerase should be added as last order.

(2) Set up the reaction program in PCR machine.

(3) Put PCR tubes into PCR machine and strart it.

∗Formula (Example)

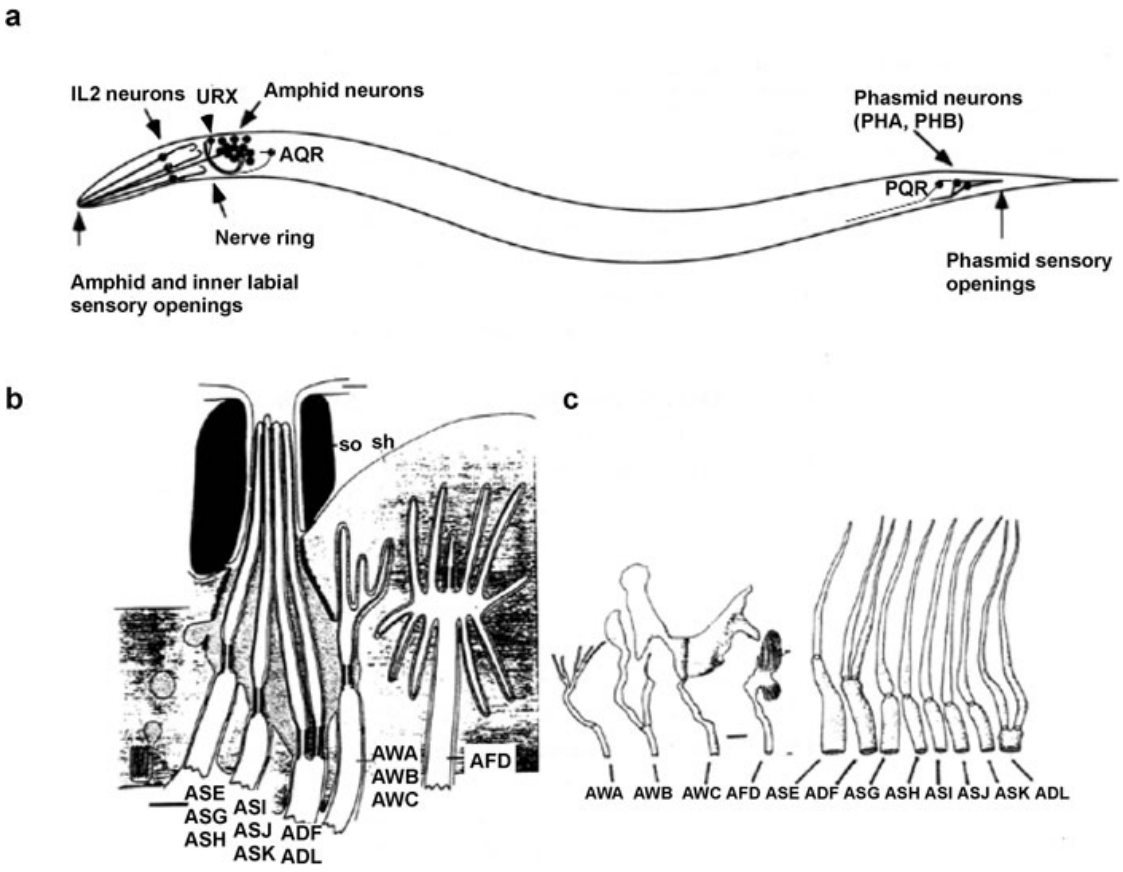
Reagent	**Final concentration**	**Quantity, for 50µl of reaction mixture**
Sterile deionized water	X	37µl
10X *Taq* buffer	1X	5µl
2.5mM dNTP mix	0.2mM of each	4µl
Primer I	0.1-1µM	1µl
Primer II	0.1-1µM	1µl
***Taq* DNA Polymerase**	1.25u/50µl	1µl
Template DNA	10pg-1µg	1µl

∗PCR program (Example, 35 cycles)

95℃	1.5 min
95℃	1 min
Annealing temperature: X℃	2 min
68℃	5 min
68℃	15 min
4℃	hold

(2) Chemosensation in *C.elegans*

C. elegans has a highly developed chemosensory system that enables it to detect a wide variety of volatile (olfactory) and water-soluble (gustatory) cues associated with food, danger, or other animals. Much of its nervous system and more than 5% of its genes are devoted to the recognition of environmental chemicals. Chemosensory cues can elicit chemotaxis, rapid avoidance, changes in overall motility, and entry into and exit from the alternative dauer developmental stage. These behaviors are regulated primarily by the amphid chemosensory organs, which contain eleven pairs of chemosensory neurons. Each amphid sensory neuron expresses a specific set of candidate receptor genes and detects a characteristic set of attractants, repellents, or pheromones. Chemosensory preferences can be modified by sensory adaptation, developmental history, and associative learning, allowing *C. elegans* to integrate context and experience into its behavior. Chemotaxis describes the phenomenon that C. elegans can direct its movement according to certain chemicals in the environment.



Chemotaxis Experiment

∗Materials

(1) Strain: N2, PR802.

(2) NGM plates spread with worm food : *E.Coli* op50 mixed with 3M NaCl.

∗Methods

(1) Pick 10 L4 to young adult stage worms and put them on the center of the NGM plate.

(2) Observe the distribution of worms on NGM plates after 2hours and 1 day.

∗Homework

(1) What kind of gene is mutated in RB809? What is the mammalian analogue? Are there any human diseases associated with this gene? (use the “wormbase” and “google” to find out)

(2) Please describe the gel from the PCR experiment. What happen to the gene covered by the primers in mutant compared to wildtype? What is your conclusion of the experiment (what kind of mutation do you expect)?

(3) Please graphically display the worm behavior by chemosensation. Could you think about a better method describing chemotaxis?

Experiment manual

(1) Polymerase chain reaction (PCR) experiment

∗Worm strains:

(1) Wild type: N2

(2) Mutant: RB809 / ptl-1(ok621)III.

∗Methods:

(1) Mix all the reagents in PCR tube and polymerase should be added as last order.

(2) Add 1ul genomic DNA from N2(wild type) and mutant(RB809):ptl-1(ok621)III.

(3) Set up the reaction program in PCR machine.

(4) Put PCR tubes into PCR machine and strart it.

(5) After PCR program finished, take out the eppendorf tubes from PCR machine.

∗Formula

Reagent	Quantity, for 15µl of reaction mixture
Sterile deionized water	9.3µl
10X *Taq* buffer	1.5µl

2.5mM dNTP mix	1.7µl
Primer I	0.5µl
Primer II	0.5µl
Taq DNA Polymerase	0.5µl
Template DNA	1µl

＊PCR program (from step2 to step4 is in 36 cycles)

Step-1: 95℃	3min
Step-2: 95℃	1.5min
Step-3: 53℃	1.5 min
Step-4: 72℃	3 min
Step-5: 72℃	3 min
Step-6: 4℃	Hold

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(2) Chemotaxis test.

＊Worm strains:

(1)N2(wild type)

(2)PR802(mutant, osm-3(p802)IV)
Phenotype: fails to avoid high osmotic strength solutions of NaCl.

＊NGM plate contained high salt.

Spread pure op50 in half surface of NGM plate, the other half spreaded with op50 mixed with 3M NaCl.

＊Methods:

(1) Prepare NGM plate spread with op50 mixed with 3M NaCl.
(2) Pick and transfer 10 L4~young adult worms to the center area of NGM plates.
(3) After 2 hours and 1 day, observe different strain worms' distribution on the NGM plate.

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(3) Calculate the survival rate of defozen worms after 4 days.

(1) Count the number of dead worm(X) on NGM plate.
(2) Count the number of live worm(Y) on NGM plate.
(3) Survival rate= Y/X+Y.

(4) Observe the result of worm mating

(1) See if there is any new born worm with fluorescence in mating plate.
(2) See if the ratio of male worm increased in F1 after mating.

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