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LAMPIRAN

Lampiran 1. Penyiapan Hewan Uji

```
┌─────────────────────────────────┐
│   Drosophila melanogaster        │
│ genotip PGRP-LBᐃ dan Oregon      │
└─────────────────────────────────┘
                  │
                  │  - dikembangbiakkan
                  │  - dijaga pada suhu 25˚C
                  │
      ┌───────────────────────────┐
      │   Drosophila usia 2-4 hari │
      └───────────────────────────┘
```

Drosophila melanogaster genotip PGRP-LB$^{\Delta}$ dan *Oregon*

- dikembangbiakkan
- dijaga pada suhu 25˚C

Drosophila usia 2-4 hari
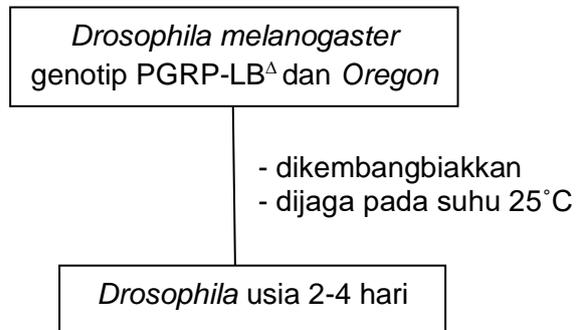
Lampiran 2. Penyiapan sampel

```
                        ┌──────────────────┐
                        │  Sampel Aspirin  │
                        └──────────────────┘
                                 │  Larutkan dengan etanol 96%
                                 ▼
                    ┌───────────────────────┐
                    │  Larutan aspirin 100  │
                    └───────────────────────┘
                       ╱       │        ╲  Encerkan
                      ▼        │         ▼
        ┌──────────────┐       │    ┌──────────────┐
        │Larutan aspirin│      │    │Larutan aspirin│
        │    10 mM     │       │    │    1 mM      │
        └──────────────┘       │    └──────────────┘
               │               │           │  Encerkan
               ▼               ▼           ▼
        ┌──────────┐    ┌──────────┐  ┌──────────┐
        │ Aspirin  │    │ Aspirin  │  │ Aspirin  │
        │  50 µM   │    │  500 µM  │  │   5 µM   │
        └──────────┘    └──────────┘  └──────────┘
```
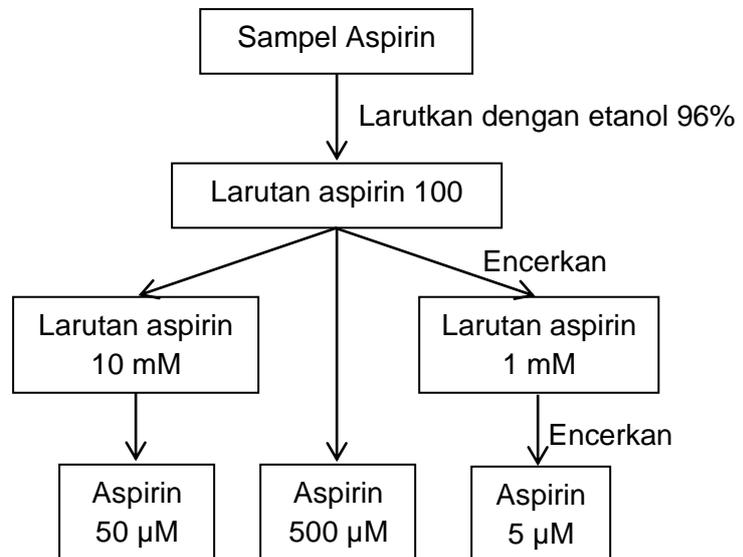
Lampiran 3. Penyiapan Pakan

```
┌─────────────────────────────┐
│  Tepung jagung, gula pasir,  │
│      ragi, agar dan air      │
└─────────────────────────────┘
               │
               │   - dicampur
               │   -dipanaskan hingga
┌─────────────────────────────┐
│   Asam propionat dan metil   │
│           paraben            │
└─────────────────────────────┘
               │
               │   - dipindahkan
               │
┌─────────────────────────────┐
│   Vial pakan *Drosophila*    │
│        *melanogaster*        │
└─────────────────────────────┘
```
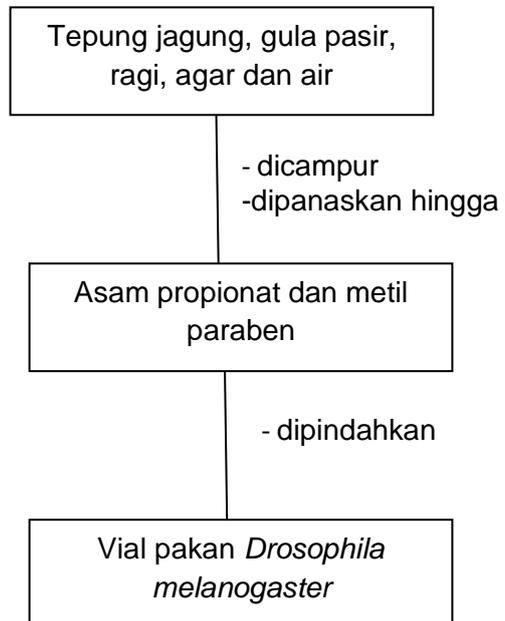
Lampiran 4. Penyiapan sampel RNA

```
┌─────────────────────────┐
│      Lima Drosophila     │
│       melanogaster       │
└─────────────────────────┘
            │
            │  - dimasukkan
            │
┌─────────────────────────┐
│        Treff tube        │
└─────────────────────────┘
            │
            │  - dihancurkan
            │
┌─────────────────────────┐
│        Micropastel       │
└─────────────────────────┘
            │
            │  - diproses
            │
┌─────────────────────────┐
│    RNAqueous™ Total      │
│    RNA Isolation Kit     │
│      (Invitrogen™)       │
└─────────────────────────┘
            │
            │  - dipindahkan
            │  - disimpan pada suhu 80˚C
            │
┌─────────────────────────┐
│        Elution tube      │
└─────────────────────────┘
```
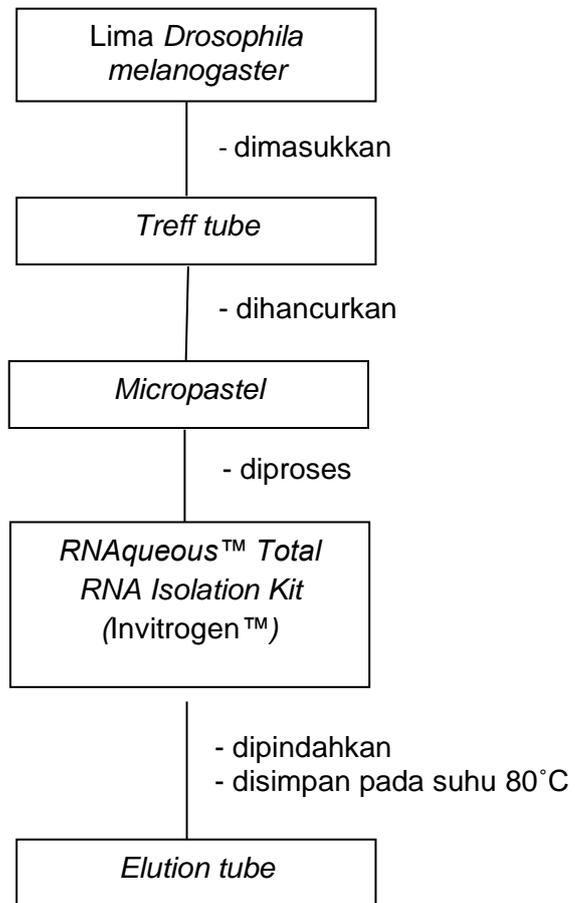
Lampiran 5. Pengujian dengan PCR

```
┌─────────────────────────┐
│   Elution tube berisi    │
│       sampel RNA         │
└─────────────────────────┘
            │  - diproses
┌─────────────────────────┐
│  RNAqueous™ Total RNA    │
│      Isolation Kit       │
│ (Invitrogen™)menggunak   │
│  an 1 set Primer cat dan │
│  atg8a dengan kontrol    │
│      internal rp49       │
└─────────────────────────┘
            │  - dianalisis
┌─────────────────────────┐
│      Thermal cycler      │
└─────────────────────────┘
```
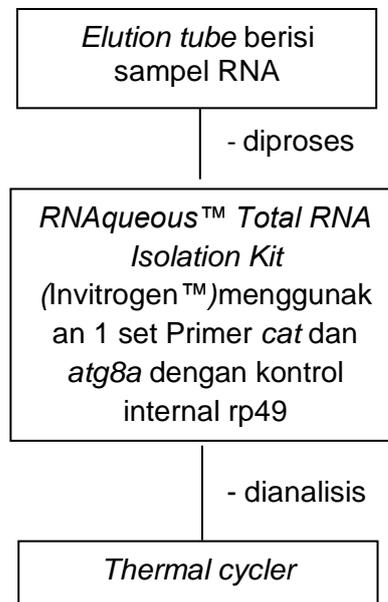
Lampiran 6. Perhitungan Pengenceran Aspirin

Pembuatan larutan aspirin 100 mM (100 x 10^{-3} M)

$$M = \frac{m}{MR} \times \frac{1000}{ml}$$

$$100 \times 10^{-3} = \frac{m}{180,16} \times \frac{1000}{25}$$

$$10^{-3} = \frac{10m}{4.504}$$

$$4,504 = 10\,m$$

$$m = 0,4504 \text{ gram (ad 25 ml EtOH 96\%)}$$

Dibuat pengenceran dengan konsentrasi 10 mM:

$$N_1 \times V_1 = N_2 \times V_2$$

$$100 \times V_1 = 10 \times 5 \text{ ml}$$

$$V_1 = 0.5 \text{ ml}$$

$$V_1 = 500 \text{ µl (larutan aspirin 100 mM, ad 5 ml etOH 96\%)}$$

Dibuat pengenceran dengan konsentrasi 1 mM:

$$N_1 \times V_1 = N_2 \times V_2$$

$$100 \times V_1 = 1 \times 5 \text{ ml}$$

$$V_1 = 0.05 \text{ ml}$$

$$V_1 = 50 \text{ µl (larutan aspirin 100 mM, ad 5 ml etOH 96\%)}$$

Pembuatan pakan Drosophila yang mengandung aspirin dengan konsentrasi 500 µM

$$500 \text{ µM} = 500 \times 10^{-3} \text{ mM}$$

$$N_1 \times V_1 = N_2 \times V_2$$

$$100 \times V_1 = 500 \times 10^{-3} \times 5 \text{ ml}$$

$$V_1 = 25 \times 10^{-3} \text{ ml}$$

$$V_1 = 25 \text{ µl (dari larutan aspirin 100 mM, ad 5 ml pakan)}$$

Pembuatan pakan Drosophila yang mengandung aspirin dengan konsentrasi 50 µM

$50 \ \mu M = 50 \times 10^{-3} \ mM$

$N_1 \times V_1 \qquad = N_2 \times V_2$

$10 \times V_1 \qquad = 50 \times 10^{-3} \times 5 \ ml$

$V_1 \qquad = 25 \times 10^{-3} \ ml$

$V_1 \qquad = 25 \ \mu l$ (dari larutan aspirin 10 mM, ad 5 ml pakan)

Pembuatan pakan Drosophila yang mengandung aspirin dengan konsentrasi 5 µM

$5 \ \mu M = 5 \times 10^{-3} \ mM$

$N_1 \times V_1 \qquad = N_2 \times V_2$

$1 \times V_1 \qquad = 5 \times 10^{-3} \times 5 \ ml$

$V_1 \qquad = 25 \times 10^{-3} \ ml$

$V_1 \qquad = 25 \ \mu l$ (dari larutan aspirin 1 mM, ad 5 ml pakan

Lampiran 7. Data Statistik

7.1 Data Statistik Analisis Ekspresi Gen *cat* pada Kelompok Perlakuan

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	B-?	
Kontrol Pelarut vs. Tanpa Perlakuan	-0.07000	-4.255 to 4.115	No	ns	>0.9999	A	Tanpa Perlakuan
Kontrol Pelarut vs. Aspirin 5 mM	-11.11	-15.30 to -6.925	Yes	***	0.0008	C	Aspirin 5 mM
Kontrol Pelarut vs. Aspirin 50 mM	-3.180	-7.365 to 1.005	No	ns	0.1239	D	Aspirin 50 mM
Kontrol Pelarut vs. Aspirin 500 mM	-1.765	-5.950 to 2.420	No	ns	0.4679	E	Aspirin 500 mM

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Kontrol Pelarut vs. Tanpa Perlakuan	3.640	3.710	-0.07000	1.204	2	2	0.05814	5
Kontrol Pelarut vs. Aspirin 5 mM	3.640	14.75	-11.11	1.204	2	2	9.228	5
Kontrol Pelarut vs. Aspirin 50 mM	3.640	6.820	-3.180	1.204	2	2	2.641	5
Kontrol Pelarut vs. Aspirin 500 mM	3.640	5.405	-1.765	1.204	2	2	1.468	5

7.2 Data Statistik Analisis Ekspresi Gen *atg8a* pada Kelompok Perlakuan

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	B-?	
Kontrol Pelarut vs. Tanpa Perlakuan	-0.1730	-4.751 to 4.405	No	ns	0.9998	A	Tanpa Perlakuan
Kontrol Pelarut vs. Aspirin 5 mM	-14.47	-19.05 to -9.896	Yes	***	0.0003	C	Aspirin 5 mM
Kontrol Pelarut vs. Aspirin 50 mM	-7.098	-11.68 to -2.520	Yes	**	0.0087	D	Aspirin 50 mM
Kontrol Pelarut vs. Aspirin 500 mM	-4.318	-8.896 to 0.2600	No	ns	0.0615	E	Aspirin 500 mM

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Kontrol Pelarut vs. Tanpa Perlakuan	1.877	2.050	-0.1730	1.317	2	2	0.1314	5
Kontrol Pelarut vs. Aspirin 5 mM	1.877	16.35	-14.47	1.317	2	2	10.99	5
Kontrol Pelarut vs. Aspirin 50 mM	1.877	8.975	-7.098	1.317	2	2	5.390	5
Kontrol Pelarut vs. Aspirin 500 mM	1.877	6.195	-4.318	1.317	2	2	3.279	5

Lampiran 8. Gambar Penelitian

Gambar 8 Pembuatan pakan

Gambar 9 Pemisahan lalat jantan & bentina

Gambar 10 Sampel isolasi RNA

Gambar 11 Isolasi RNA

Gambar 12 *Running real time* PCR