|  |  |  |
| --- | --- | --- |
| **DAY ONE** | | |
| Embryos divided | |  |
| Water bath: **70°C no shaking** | |  |
| Rehydrate *5min/step* | 100% MeOH |  |
| 25% PBT |  |
| 50% PBT |  |
| 75% PBT |  |
| 100% PBT |  |
|  |
|  |
|  |
| **1µl of PK added to 2 ml of PBT** | |  |
| **Aliquots of 4% PFA thawed** | |  |
| ***Aliquots of HYB+ and HYB- placed in water bath*** | |  |
| Embryos digested in PK for ***12 minutes*** | |  |
| Brief PBT Rinse | |  |
| PBT for 5 min | |  |
| Post-fix with 4% PFA for 20 min | |  |
| Brief PBT Rinse | |  |
| PBT Washes (5 min/wash) | |  |
|  |
|  |
|  |
|  |
| *Warm HYB- added (5 min)* ***NO SHAKING*** | |  |
| HYB- removed | |  |
| *Warm HYB+ added (4 hr or O/N)* | |  |
|  | |  |

|  |  |
| --- | --- |
| **DAY ONE or TWO** | |
| **Aliquots of HYB+ placed in water bath** (if not done previously) |  |
| HYB+ removed |  |
| HYB+ added |  |
| Probe added |  |
| ***Probe made contact with all embryos*** |  |
| Incubate in water bath O/N |  |

Gene 1: Label and tape color

Gene 2: Label and tape color

Initials and date:

|  |  |  |
| --- | --- | --- |
| **DAY THREE** | | |
| Water bath turned on **70°C, 40rpm** | |  |
| Blocking solutions prepared | |  |
|  |
| ***HYB+ with probe removed and saved*** | |  |
| ***HYB+ with probe stored and Label:*** | |  |
| Solutions prepared:  **\*Place in water bath** | **HYB-\*** |  |
| **25% SSC\*** |  |
| **50% SSC\*** |  |
| **75% SSC\*** |  |
| **2xSSC\*** |  |
| **0.2xSSC\*** |  |
|  |
| 25% PBT |  |
| 50% PBT |  |
| 75% PBT |  |
| 100% PBT |  |
| Decreasing HYB- rinse series  (10 min/step) | HYB- (briefly) |  |
| 25% SSC |  |
| 50% SSC |  |
| 75% SSC |  |
| 2x SSC for 10 min | |  |
| 0.2x SSC for 30 min | |  |
|  |
| **Water bath turned off** | |  |
| Increasing PBT rinse series (10 min/step) | 25% PBT |  |
| 50% PBT |  |
| 75% PBT |  |
| 100% PBT |  |
| Wash with MABT  (5 min/step) | |  |
|  |
| **Blocking solution fully dissolved** | |  |
| Incubate in blocking solution for 4 hours | |  |
| Add 2μL Anti-DIG to blocking solution | |  |
| Incubate in BS w/ Anti-DIG O/N at 4°C | |  |

|  |  |
| --- | --- |
| **DAY FOUR** | |
| NGS thawed |  |
| Add 100µl of NGS to 900µl of MABT |  |
| MABT Washes (30 min/step)  AT LEAST 12 washes |  |
|  |
|  |
|  |
|  |
|  |
|  |
|  |
|  |
|  |
|  |
|  |
| Incubate O/N in 4°C in walk-in |  |

|  |  |  |
| --- | --- | --- |
| **DAY FIVE** | | |
| Make up AP Buffer | |  |
| Pull off MABT | |  |
| Wrap drams in foil | |  |
| 100% AP Buffer for 5 minutes | |  |
|  | |  |
| Incubate in 1mL of AP Buffer/NBT/BCIP for 1 hour. Switch out every hour. | |  |
| Pull off AP Buffer/NBT/BCIP | |  |
| 100% AP Buffer for 5 minutes | |  |
| Increasing PBT dilutions | 25% PBT |  |
| for 5 minutes each | 50% PBT |  |
|  | 75% PBT |  |
|  | 100% PBT |  |
| Terminate reaction by rinsing in PBT until clear (5mL). Switch out every hour. | |  |
| Rinse in PBS (5min/step) | |  |
|  |
| Thaw aliquots of 4% PFA | |  |
| Fix embryos either for 1 hour at RT or O/N in 4°C | |  |
| 100% PBS for 5 minutes | |  |
|  | |  |
| Store embryos in 100% PBS at 4°C | |  |

Exposure time gene 1:

Exposure time gene 2: