

## Individual Run Sheet – Visualizing the Biotinylated Dextran Amine (BDA)

Bird ID: \_\_\_\_\_

Reagent: \_\_\_\_\_

### Bleach Solution (per tray, 18.7 mL)

- 30% Hydrogen peroxide ( $H_2O_2$ ) 700  $\mu$ L
- Methanol ( $CH_3OH$ ) 9 mL
- 1x PBS 9 mL

### Streptavidin Solution (per tray, ~20 mL)

- 0.4% PBS-Tx 20 mL
- Streptavidin (fluorescent)  
or  
Streptavidin – HRP (chromogenic) \_\_\_\_  $\mu$ L

1. Wash sections in 1x PBS for 3x5 minutes. ☐ ☐ ☐

2. Bleach sections for 20 minutes.

1 mL solution per section	Methanol	30% Hydrogen Peroxide	1x PBS
1 section			
20 sections	9 mL	700 $\mu$ L	9 mL

3. Wash sections in 1x PBS for 5x5 minutes. ☐ ☐ ☐ ☐ ☐

4. Incubate sections in **reagent** (\_\_\_\_\_) (dilution 1: \_\_\_\_)  
+ 0.4% PBS-Tx for 1 hour at room temperature on the rotator.

1 mL solution per section	Reagent	0.4% PBS-Tx
1 section	____ $\mu$ l	1 mL
20 sections	____ $\mu$ l	20 mL

5. Wash sections in 1x PBS for 5x5 minutes. ☐ ☐ ☐ ☐ ☐

6. Store the sections at 4°C in either 1x PBS (for short-term storage, <24 hrs) or 1x PBS Azide (for long-term storage, >24 hrs).

## DAB Staining

### Chromogenic

#### Diaminobenzidine peroxidase reaction medium for BROWN reaction product

– 30% Hydrogen peroxide ( $H_2O_2$ )	7.5 $\mu$ l	15 $\mu$ l
– DAB (Sigma)	12.5 mg	25 mg
– 1x PBS	50 mL	100 mL

#### Diaminobenzidine peroxidase reaction medium for BLACK reaction product

– 0.5% Cobalt(II) chloride ( $CoCl_2$ )	1.5 mL	3 mL
– 30% Hydrogen peroxide ( $H_2O_2$ )	7.5 $\mu$ l	15 $\mu$ l
– DAB (Sigma)	12.5 mg	25 mg
– 1x PBS	48.5 mL	97 mL

#### Protocol

1. Prepare a waterbath containing bleach to neutralize spills and to deactivate DAB after experiment is finished.
2. Prepare diaminobenzidine peroxidase reaction medium.
  - a. BDA is detected with a black DAB reaction while CTB is detected with a brown DAB reaction.
3. Incubate the sections in the reaction medium until the reaction is considered complete – this can be from 10 seconds to 2 minutes.
4. Wash sections in 1x PBS for 3x10 minutes.