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Ventral Midbrain Genomic PCR

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

This protocol details ventral midbrain genomic PCR.

Materials

Stainless Steel Brain Matrices, 1.0mm **Stoelting** **Catalog #**51386

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen** **Catalog #**69504

GeneJET PCR Purification Kit **Thermo Fisher Scientific** **Catalog #**K0702

	A	B	C
	Reagent	**Final conc.**	**ul/sample**
	5X KAPA2G Buffer A	1.3X	6.5 µl
	25 mM MgCl2	2.60 mM	2.6 µl
	10 mM KAPA dNTP Mix	0.26 mM	0.65 µl
	Forward primer (10 µM) CTGCAGCTTCGAGAGGAAAG	0.5 µM	0.5 µl
	Flox reverse primer (10 µM) CACTCTGTCCTCAGGCTTTC	0.5 µM	0.5 µl
	KO reverse primer (10 µM) AGGTGGGAATCGGGCTAGAG	0.5 µM	0.5 µl
	50% Glycerol	6.50 %	3.25 µl
	5 U/ µl KAPA2G Fast Hotstart DNA Polymerase	0.5 U/ul	0.1 µl
	DNA (diluted to 25 ng/uL)	150 ng total	6.0 µl
	H2O	to 25 ul	4.4 µl

Brain tissue collection

1 Euthanize mouse via cervical dislocation.

2 Isolate 2 mm coronal midbrain section using stainless steel brain matrix (Stoelting 51386).

2.1 Remove cortex and dorsal midbrain tissue.

2.2 Separate ipsilateral and contralateral ventral midbrain regions.

2.3 Immediately freeze on dry ice and store tissue at -80 °C until DNA extraction.

DNA extraction from frozen brain tissue

3 Use Qiagen DNeasy Blood and Tissue Kit (Cat 69504).

4 Equilibrate ventral midbrain tissue to Room temperature.

> Note
>
> Starting material amount ~ 15 mg of tissue.

5 Cut tissue into small pieces.

5.1 Use a pipette tip to transfer tissue to a sterile 6cm dish.

5.2 Add 180 µL of **Buffer ATL** to the tissue.

5.3 Dice up tissue with sterile scalpel.

5.4 Transfer tissue and buffer to 1.5mL etube.

6 Add 20 µL **Proteinase K** to each sample.

6.1 Mix thoroughly by vortexing.

6.2 Incubate at 56 °C until samples are completely lysed.

> Note
>
> Use thermomixer – check samples after 01:00:00 , may take up to 03:00:00 .

7 Add 4 µL **RNase A** (100 µL) to each sample.

7.1 Mix by vortexing.

7.2 Incubate at Room temperature for 00:02:00 . 2m

8 Mix **Buffer AL** with ethanol 1:1.

8.1 Add 400 µL Buffer AL / ethanol mix to each sample.

8.2 Vortex for 00:00:15 . 15s

9 Transfer samples to DNeasy Mini spin columns (placed in 2mL collection tubes).

9.1 Centrifuge at ≥ 6000 x g for 00:01:00 . 1m

9.2 Discard flow through.

10 Transfer column to new collection tube.

10.1 Add 500 µL **Buffer AW1.**

10.2 Centrifuge at ≥ 6000 x g for 00:01:00 . 1m

10.3 Discard flow through and collection tube.

11 Place the column in a new collection tube – add 500 µL **Buffer AW2.**

11.1 Centrifuge 20000 x g for 00:03:00 to dry the column. 3m

11.2 Carefully remove the column to avoid contamination with residual ethanol.

11.3 If the column touches ethanol flow through, spin again in new collection tube for 00:01:00 . 1m

12 Place the column in clean 1.5mL etube – add 150 µL of elution buffer **(Buffer AE).**

12.1 Incubate at Room temperature for 00:01:00 . 1m

12.2 Centrifuge at ≥ 6000 x g for 00:01:00 to elute DNA. 1m

Genomic PCR

13 DNA concentration measured using a NanoDrop One Spectrophotometer (Thermofisher Scientfiic).

13.1 All samples diluted to 25 µL with **Buffer AE.**

14 Use the Kapa2g Fast HotStart PCR Kit (Roche 07960930001) according to the manufacturer's instructions.

	A	B	C
	Reagent	**Final conc.**	**ul/sample**
	5X KAPA2G Buffer A	1.3X	6.5 µl
	25 mM MgCl2	2.60 mM	2.6 µl
	10 mM KAPA dNTP Mix	0.26 mM	0.65 µl
	Forward primer (10µM)	0.5 µM	0.5 µl
	CTGCAGCTTCGAGAGGAAAG		
	Flox reverse primer (10µM)	0.5 µM	0.5 µl
	CACTCTGTCCTCAGGCTTTC		
	KO reverse primer (10µM)	0.5 µM	0.5 µl
	AGGTGGGAATCGGGCTAGAG		
	50% Glycerol	6.50%	3.25 µl
	5 U/ µl KAPA2G Fast Hotstart DNA Polymerase	0.5 U/ul	0.1 µl
	DNA (diluted to 25ng/uL)	150ng total	6.0 µl
	H2O	to 25 ul	4.4 µl

PCR Cycle:

	A	B	C	D
	Step	**Temp (°C)**	**Time**	**Note**
	1	94.0 °C	5 min.	
	2	94.0 °C	30 sec.	
	3	65.0 °C	15 sec.	-0.5 °C per cycle decrease
	4	68.0 °C	1 sec.	
	5			repeat steps 2-4 for 10 cycles (touchdown)
	6	94.0 °C	30 sec.	
	7	60.0 °C	15 sec.	
	8	72.0 °C	1 sec.	
	9			repeat steps 6-8 for 20 cycles
	10	72.0 °C	5 min.	
	11	4.0 °C	hold	Hold

15 Run samples on 2% Agarose gel at 100V for 35-45 minutes.

	A	B
	Wild type allele	400 bp
	Flox allele	500 bp
	KO allele	270 bp