Developmental Cell *14*

Supplemental Data

piggyBac-Based Mosaic Screen Identifies a Postmitotic Function for Cohesin in Regulating Developmental Axon Pruning

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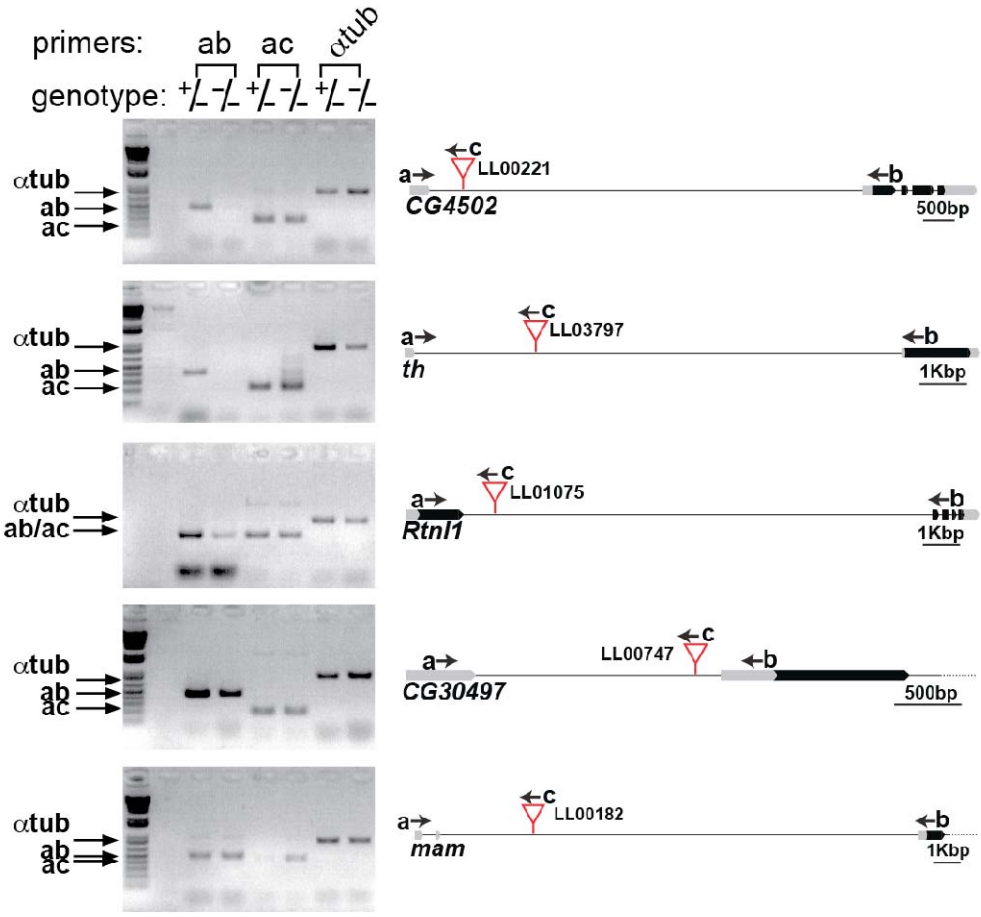


Figure S1. Splicing of Intronic *piggyBac* Insertions into Endogenous Transcripts

RT-PCR of heterozygous (+/-) and homozygous (-/-) flies for five different intronic insertions. Primers were chosen to distinguish between endogenous transcripts (primer a plus primer b: ab) and *piggyBac*-trapped transcripts (primer a plus primer c: ac). In two examples (*CG4502* and *th*) the endogenous transcript was absent in homozygous flies. In

two other examples (*Rtnl1*and *CG30497*) a moderate reduction of the endogenous transcript was observed in homozygous flies. In one example (*mam*) there was no difference in the endogenous transcript between homozygous and heterozygous flies. In all cases a *piggyBac*-trapped transcript could be amplified, usually exhibiting a stronger band in the homozygous flies. To calibrate the quantities of cDNA used in the PCR reaction we used *α-Tubulin* (αtub) and *β-Actin* (not shown) primers.

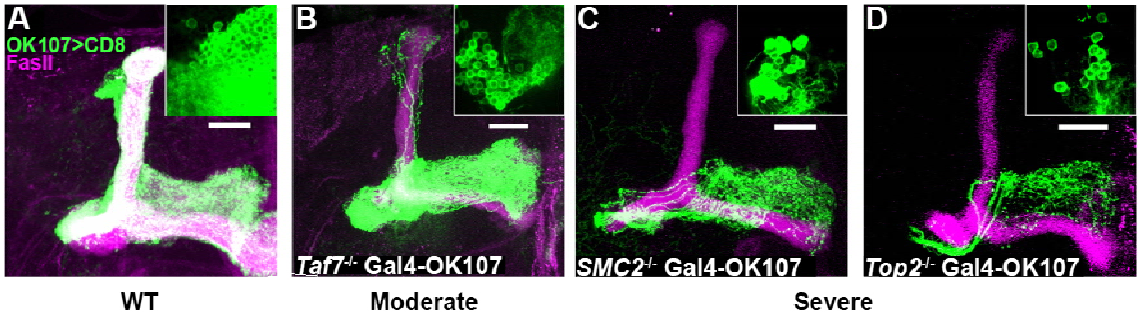


Figure S2. Examples of *piggyBac* Insertions Causing Neuroblast Proliferation Defects

(A-D) Mushroom body MARCM neuroblast clones for the following genotypes: wt (A) *piggyBac* insertions LL01754 (*Taf7*; B), LL01426 (*SMC2*; C) and LL01835 (*Top2*; D). Classification of moderate and severe is according to cell number (inset) and axonal projections, as Table S2: neuroblast clones containing a robust γ lobe or a γ lobe plus some α/β neurons were categorized as having a moderate proliferation defect; neuroblast clones containing a partial γ lobe were categorized as having a sever proliferation defect. Green, Gal4-OK107 driven mCD8::GFP; magenta, anti-FasII. Scale bars, 20μm.

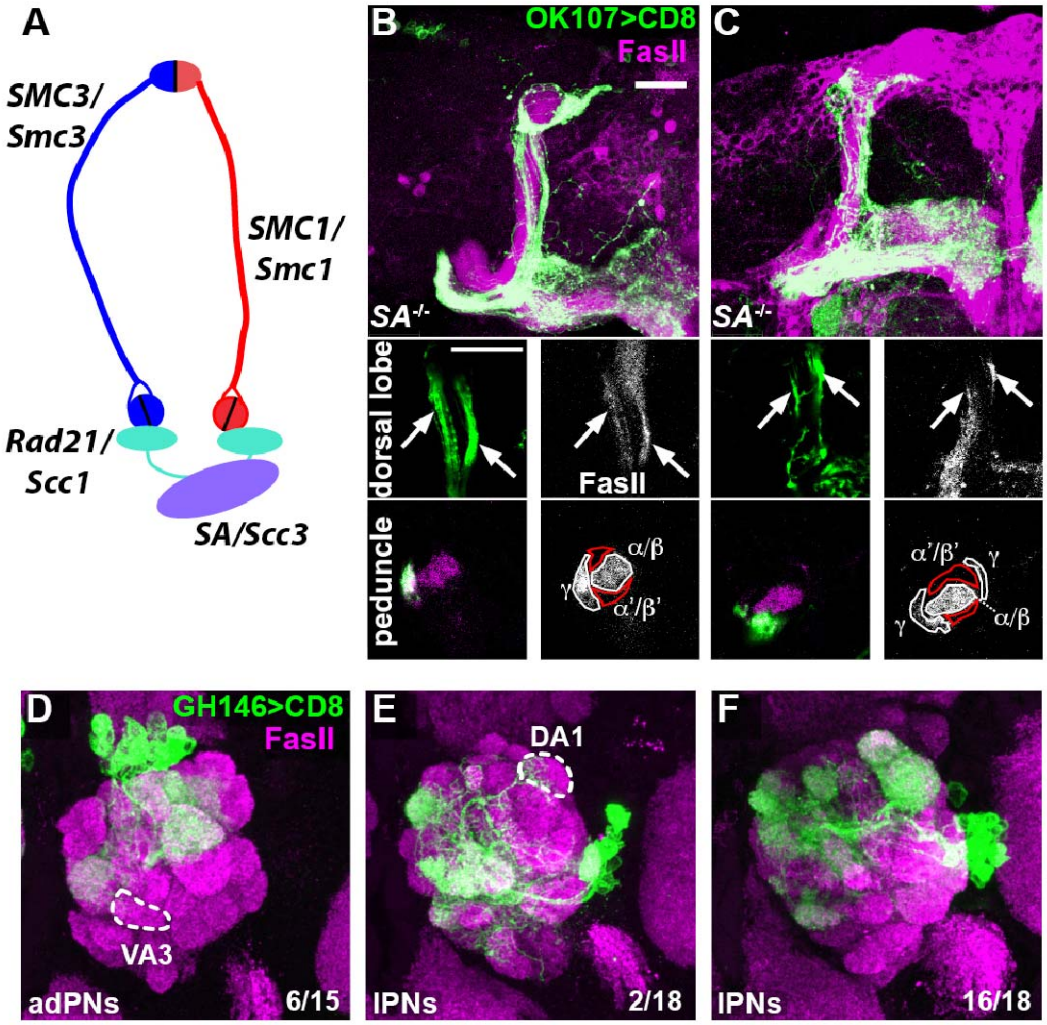


Figure S3. Stromalin (SA) is Required for Axon Pruning and Dendrite Targeting

(A) Schematic representation of the core cohesin complex. Gene names: *Drosophila/S.cerevisiae.*

(B,C) Two examples of $SA^{-/-}$ mushroom body neuroblast clones labeled with Gal4-OK107. Middle panels show single sections of the dorsal lobe with mCD8::GFP and FasII channels separated. Lower panels show single cross sections in the peduncle. Most if not all dorsal projections are unpruned γ neurons based on the fact that they express FasII (middle panels; arrows) and project through the γ specific region in the peduncle (lower panels). Zones in the peduncle were assigned by the virtue of the concentric structure (Crittenden et al., 1998) and FasII labeling.

(D-F) $SA^{-/-}$ PN neuroblast clones labeled with Gal4-GH146. $SA^{-/-}$ adPNs fail to innervate the VA3 glomerulus (dotted outlines in D; 6/15). $SA^{-/-}$ lPN dendrites are shifted to medial areas in the antennal lobe (E, F) and fail to target DA1 occasionally (dotted outlines in E; 2/18). About half of all examined vPNs mistarget into the SOG (data not shown).

Note: Insertion LL01226 in *SA* was lost after the primary screening, preventing further phenotypic characterization.

Green, Gal4-OK107 (B, C) and Gal4-GH146 (D-F) driven mCD8::GFP; magenta, anti-FasII (B, C) or nc82 (D-F), respectively. Scale bars, 20μm.

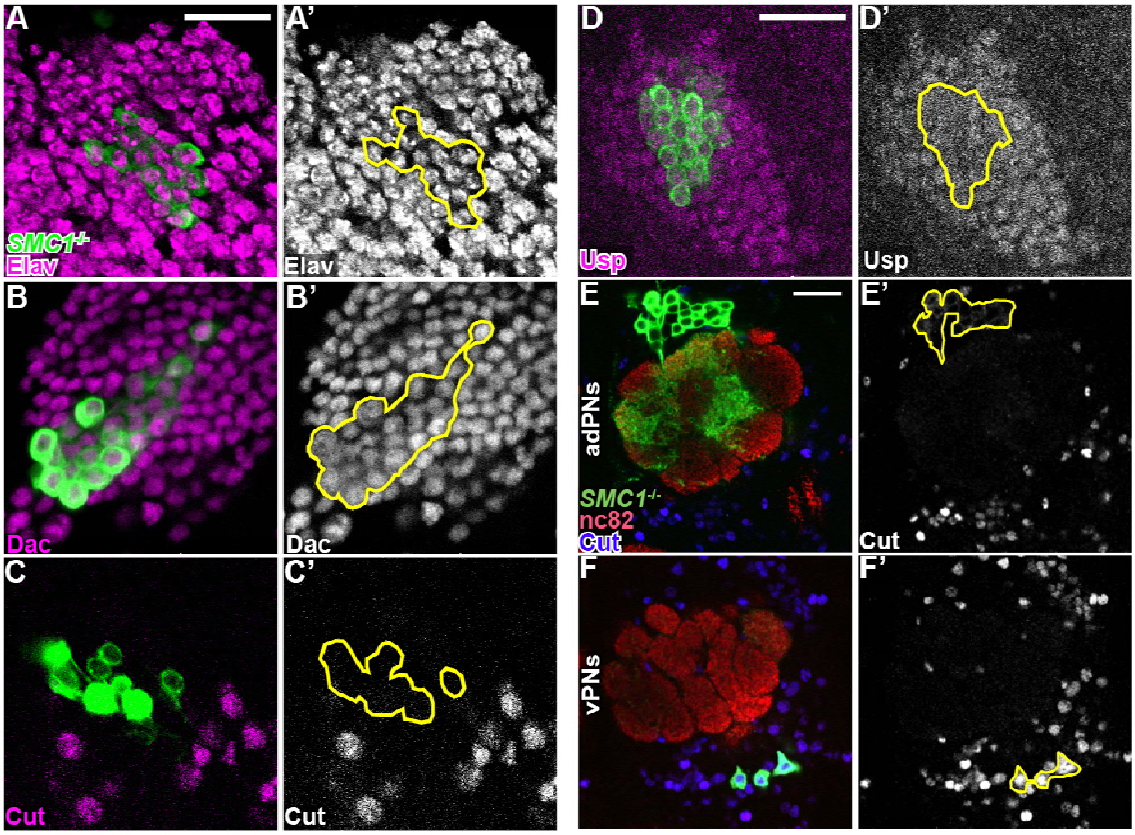


Figure S4. *SMC1* Does Not Effect the Expression of Cut, Elav, and Dac

(A-D) Single confocal sections of *SMC1*$^{-/-}$ mushroom body neuroblast clones at 0h APF labeled by Gal4-201Y. Brains were stained with anti-Elav (A), anti-Dac (B) anti-Cut (C) or anti-Usp (D), shown separately in A'-D' with the borders of the clones outlined by a yellow line. Elav (embryonic lethal, abnormal vision) is an RNA-binding protein widely used as a marker of all postmitotic neurons in *Drosophila* (Robinow et al., 1988); Dac (Dachshund) is a transcription factor expressed in mushroom body neurons (Martini et al., 2000); Cut is a homeodomain transcription factor previously suggested to be negatively regulated by cohesin in the wing disc (Dorsett et al., 2005), and Usp (Ultraspiracle) is the EcR co-receptor (Yao et al., 1993). No change was observed in the expression of Elav, Dac or Usp compared to wt clones (not shown) or neighboring cells. Cut was neither expressed in γ neurons in wt clones (not shown) nor in *SMC1*$^{-/-}$ clones.

(E,F) Single confocal sections of *SMC1*$^{-/-}$ PN neuroblast clones labeled with Gal4-GH146. Adult brains were stained with anti-Cut shown separately in E' and F' with the border of the clones outlined by a yellow line. The levels of Cut remained unchanged in all PN lineages tested: larval born adPNs don't express Cut in wt (not shown; Komiyama and Luo, 2007) or *SMC1*$^{-/-}$ clones (E); all vPNs express Cut in wt (not shown; Komiyama and Luo, 2007) and mutant clones (F); lPNs (not shown) include ~8 Cut-positive cells while the rest are Cut-negative in both wt (Komiyama and Luo, 2007) and mutant clones. Green, Gal4-201Y (A-E) and Gal4-GH146 (E,F) driven mCD8::GFP; red (E,F), nc82. Magenta, blue, red and grayscale are as depicted for individual panels. Scale bars, 20μm.

Supplemental Experimental Procedures

Genotypes:

Genotypes abbreviations: hsFlp is y,w,hsFlp122; CD8 is *UAS*-mCD8::GFP; 40A, G13, 2A and 82B are FRTs on 2L, 2R, 3L and 3R respectively; 201Y is Gal4-201Y, OK107 is Gal4-OK107, GH146 is GH146-Gal4.

Figure 2: (C_2) *hsFlp, CD8 /y,w; G13 /G13, Gal80; OK107/+*. (C_3) *hsFlp, CD8 /y,w; G13, 201Y, CD8, UBA^{S3484}/G13, Gal80; OK107/+*. (C_4) *hsFlp, CD8 /y,w; 40A, G13, cn, bw, pB-LL03617/G13, Gal80; OK107/+*. (D_2) *hsFlp, CD8/y,w; G13, tsr^{N121} /G13, Gal80; OK107/+*. ($D_{3\text{-}4}$) *hsFlp, CD8/y,w; 40A, G13, cn, bw, pB-LL01333 or LL02200/G13, Gal80; OK107/+*. (E_2) *hsFlp, CD8/y,w; 201Y, CD8/+; 2A, 82B/82B, Gal80 (E_3) hsFlp, CD8/y,w; 201Y, CD8/+; trio^{3}, 2A/ Gal80, 2A*. (E_4) *hsFlp, CD8/y,w; 201Y, CD8/+; pB-LL00125, 2A, 82B/Gal80, 2A*.

Figure 3: X and 2nd chromosomes (B-D): *hsFlp, CD8/y,w; 201Y, CD8/+*. 3rd chromosome: (B) *2A, 82B/82B, Gal80*. (C) *2A, 82B, pB-LL01162/82B, Gal80*. (D) *82B, SMC$^{\Delta exc46}$/82B, Gal80*.

Figure 4: (A) *hsFlp, CD8/y,w; 2A, 82B, pB-LL01162/82B, Gal80; OK107/+* (B) *hsFlp, CD8/y,w; UAS-SMC1::HA/+; 2A, 82B, pB-LL01162/82B, Gal80; OK107/+* (C) *hsFlp, CD8/y,w; 201Y, CD8/+; 2A, 82B, pB-LL01162/82B, Gal80* (D) *hsFlp, CD8/y,w; UAS-SMC1::HA/201Y, CD8; 2A, 82B, pB-LL01162/82B, Gal80*.

Figure 5: (C) see Figure 3B (D,F) see Figure 3C (G) *hsFlp, CD8/y,w; 201Y, CD8/ UAS-EcR-B1; 2A, 82B, LL01162/82B, Gal80* (H) *hsFlp, CD8/y,w; EcR^{554}, 201Y/+; 2A, 82B, pB-LL01162/82B, Gal80* (I) *hsFlp, CD8/y,w; 201Y, CD8/ babo^{9}, CD8; 2A, 82B, LL01162/82B, Gal80*.

Figure 6: (A-C) *hsFlp, CD8/yw; GH146, CD8/+; 2A, 82B/ 82B, Gal80* (D-F) *hsFlp, CD8/yw; GH146, CD8/+; 2A, 82B, pB-LL01162/ 82B, Gal80* (G-I) *hsFlp, CD8/yw; GH146, CD8/ UAS-SMC1::HA; 2A, 82B, pB-LL01162/ 82B, Gal80.*

Antibody Staining Conditions:

Rat monoclonal anti-mouse CD8 α subunit, 1:100 (Caltag, Burlingame, CA); mouse monoclonal anti-nc82, 1:30 (gift of E. Buchner, University of Wuerzberg); rabbit polyclonal anti-HA (ab9110), 1:2000 (Abcam, Cambridge, MA); mouse monoclonal anti-Usp, 1:50 (gift of R. Barrio); the remaining antibodies were all obtained from the Developmental Studies Hybridoma Bank: mouse monoclonal anti-FasII (1D4), 1:50; mouse monoclonal anti-EcR-B1 (AD4.4), 1:25; mouse monoclonal anti-Elav (9F8A9), 1:100; mouse monoclonal anti-Dac (mAbdac2-3), 1:30; mouse monoclonal anti-Cut (2B10), 1:20.

Genetic Scheme of *piggyBac* Screen:

The scheme is based on, and most of the fly stocks are from, Hacker et al. (2003). Abbreviation: F2c: *FRT40A, FRTG13, cn,bw.* F3: *FRT2A, FRT82B,* y^+. J10: *piggyBac* transposase on III. J2: *piggyBac* transposase on II. *pB*: *piggyBac.*

Start on X

I. $pB[Ds\,\mathrm{Re}\,d^+];F2c;F3\otimes\frac{y,w}{};F2c;\frac{J10}{Tm3,Sb}$

non Sb male

II. single ♂: $\frac{pB[Ds\,\mathrm{Re}\,d^+]}{};F2c;\frac{F3}{J10}\otimes y,w;F2c$

♂, $DsRed^+$, y^+ (This essentially selects for the F3 chr. therefore no selection against J10 necessary)

III. single ♂ $y,w;\frac{F2c,pB[Ds\,\mathrm{Re}\,d^+]}{F2c};\frac{F3,pB[Ds\,\mathrm{Re}\,d^+]}{+}\otimes y,w;\frac{Pin}{CyO^{cn,bw}}\&\,y,w;\frac{Ly}{Tm6,Tb}$

iPCR single male after 3 days of mating.

Flip females to a new box.
Determine insertion location:
➔ Repetitive, Intergenic, Short sequence or on 4th chromosome → discard
➔ Otherwise → balance appropriately (as shown below)

If On 3rd:

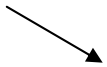
→ Select DsRed+(also y+), Tb♂and ♀.
Stocks on 3rd $\frac{y,w}{};\frac{F2c}{+};\frac{F3[Ds\,\mathrm{Re}\,d^{+}]}{Tm6,Tb}$ ➔ STOCK (also lethality test)

On 2nd:
→ Select DsRed+, CyO → Due to cn,bw – will be white eyed
Stocks on 2nd $\frac{y,w}{};\frac{F2c,pB[Ds\,\mathrm{Re}\,d^{+}]}{CyO^{cn,bw}};\frac{F3/+}{+}$ ➔ STOCK (also lethality test)

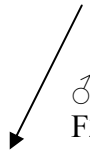
Determine lethality and setup MARCM crosses with appropriate chromosome arm.

Start on 2nd

I. $\text{♀}\, F2c;\frac{J10}{Tm3,Sb} \otimes pB[Ds\,\mathrm{Re}\,d]^{II};F3$



II. Single $\text{♂}^{y,w};\frac{F2c}{pB[Ds\,\mathrm{Re}\,d]^{II}};\frac{F3}{J10} \otimes y,w;F2c$



♂ DsRed^{+}, white eye (by virtue of homozygous F2c, y^{+} - Selects for F3 and against J10).

III. And onward, like scheme on X

inverse PCR (iPCR) protocol for mapping *piggyBac* insertions from a single fly in a 96 well plate:

a) **DNA preparation**:

1.1 Collect and freeze one fly per well in -80º for about 10-15 minutes

1.2 Prepare buffer A:

Component	per reaction (µl)	per plate (µl)
1M **Tris** pH 7.5	10	1000
500 mM **EDTA** pH 8.0	20	2000
4M **NaCl**	2.5	250
10% **SDS**	5	500
Water (ddw)	62.5	6250
Total	100	10000

1.3 Crush flies while adding 100µl buffer A (to crush, what works best is to pickup 100µl of buffer A, then bend the tip in an empty eppendrof tube or in a blank well in the PCR plate, then crush and only lastly add the buffer to squashed fly)

1.4 Incubate 30min @ 65ºc (preferably in a PCR machine)

1.5 Add 100µl 3M KAc - mix well (use tape foil)

1.6 Incubate 10 min on ice

1.7 Spin 30 min @ ≥4000 g @ 10ºc

1.8 Transfer 150µl into new plate excluding crude

1.9 Add 90µl Isopropanol - seal well with tape foil - mix well

1.10 Spin 30 min @ ≥4000 g @ 10ºc

1.11 Replace Isopropanol with 150µl cold 70% EtOH - seal with foil

1.12 Spin 10 min @ ≥4000 g @ 10ºc

1.13 Remove EtOH

1.14 Dry well using speedvac (low to med temp) - if no speedvac available - dry over night (ON)

1.15 Add 50µl double distiled water (ddw) - let dissolve ON or 2h in 37ºc

b) DNA digestion:

2.1 Prepare digestion mix:

Component	per reaction (µl)	per plate (µl)
10X **buffer**	2.5	250
RNase A (100µg/ml)	2	200
TaqIa	0.5	50
BSA 100X	0.25	25
Water (ddw)	4.75	475
Total	10	1000

2.2 Aliquot 10µl per well

2.3 Add 15µl DNA

2.4 Incubate 3.5 h at 65ºc

c) Ligation:

3.1 Prepare ligation mix:

Invitrogen ligase
(for NEB use appropriate buffer volume - it is 10X - and half of ligase)

Component	per reaction (µl)	per plate (µl)
Buffer (5X)	2	200
Water	4	400
Ligase	1	100
Total:	7	700

3.2 Aliquot 7 µl per well
3.3 Add 3 µl digested DNA
3.4 Cover with Tape pad and incubate 30-45 mins RT
3.5 Proceed directly to PCR

d) PCR

4.1 Prepare mix - on Ice

Component	per reaction (µl)	per plate (µl)
dNTPs 10mM	0.4	40
Primer 5F0 10µM	1	100
Primer 5R2 10µM	1	100
5X **buffer**	4	400
Taq (phusion - NEB)	0.1	10
Water (ddw)	11.5	1150
Total	18	1800

4.2 On Ice, aliquot 18 µl per well. Add 2µl ligation product
4.3 Run PCR:

PCR program	
98ºc	30 sec
98ºc	10 sec
70ºc	20 sec
72ºc	30 sec
X35	
72ºc	7 min
4ºc	hold

4.4 Run samples on agarose gel (1.5-2% works best).

e) Exo/AP purification

5.1 Prepare mix on Ice in this order:

Component	per reaction (µl)	per plate (µl)
10X **AP buffer**	0.5	50
Water (ddw)	2.5	2

Incubate 5' on ice, then add other ingredients:

Antarctic phosphatase - NEB (5U/µl)	2	200
ExoI NEB (20U/µl)	0.5	50
Total:	5.5	550

5.2 Aliqote 5µl per well, add 7µl PCR product
5.3 Run Exo/AP program:

37ºc	45 min
70ºc	15 min

5.4 Samples are ready to be sequenced without additional purifications. Ideally, get to sequencing as soon as possible. Use primer **pB5-seq** for sequencing

Important notes:

6.1 In general, this protocol is a fusion of the BGDP and Exelixis protocols

6.2 In all steps be careful not to allow cross contaminations. The most important steps to take caution are the squashing (a3) and in every mixing step (a5, a9). Seals that work well are aluminum seals (available from many companies - we use E&K scientific #T592100) for all steps that need mixing - although more expensive, we find them superior even in PCR and digestions. For the ligation, it is possible to use a tape pad which is virtually packing tape cut at the size of a plate by Qiagen (#19570).

6.3 The temperature when spinning is not that important. If you don't have a cooled plate centrifuge, most likely it will work without cooling.

6.4 Use multi-channel pipettes in all stages.

6.5 Digestion - we use Taq1a by NEB, which is compatible with the 5' end of our *piggyBac* vector (based on pXL-BacII-ECFP, Li *et al.* 2005). If using another transposon, use appropriate enzyme and digestion temp. We make our own RNAse (follow the BGDP protocol for this) but commercial ones should work as well.

6.6 For PCR: we have tried several Taqs (platinum - invitrogen; Taq-Pro - Denville Scientific) with some success. By far, the best results were obtained using phusion Taq from Finnzymes, distributed in the USA by NEB. This allows a robust, single step PCR without the need to perform antherm nested PCR. If using a different taq,, make sure to change the protocol appropriately.

6.7 Primer sequences:

5F0: CGACCGCGTGAGTCAAAATGAC

5R2: TCCAAGCGGCGACTGAGATG

pB5-seq: CGCGCTATTTAGAAAGAGAGAG

Supplemental References

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