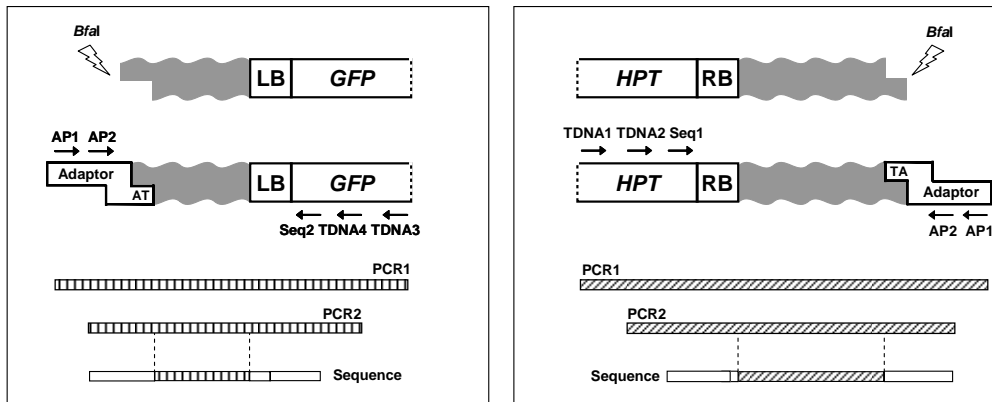




Protocol for *Brachypodium* FST retrieval

[Vain P. et al. \(2008\)](#) Plant Biotechnology Journal, 6:236-245

[Thole V. et al. \(2009\)](#) Nature Protocols, 4:650-661.



Extraction of *Brachypodium* genomic DNA

1. Collect leaf samples from one month-old potted T-DNA insertion *Brachypodium* plants. Samples are either collected individually (100 mg of fresh weight in a sterile 2-ml screw cap microfuge tube) or by batches of 96 samples (50 mg of fresh weight in a dedicated collection microtube of the Qiagen DNeasy 96 Plant kit). Tubes are stored in a -80°C freezer.
2. Disrupt leaf material using either a pestle, mortar and liquid nitrogen (individual samples) or tungsten carbide beads and a Retsch MM300 mixer mill according to the manufacturer's specifications (batch of 96 samples).
3. Extract genomic DNA using either the Qiagen DNeasy Plant Mini Kit (individual samples) or the Qiagen DNeasy 96 Plant kit according to the manufacturer's specifications.
4. Check DNA quality and concentration by loading 3 µl of each DNA sample onto a 1-1.2% (w/v) agarose gel containing ethidium bromide.

Digestion of *Brachypodium* genomic DNA

5. Digest genomic DNA with *Bfal* by combining the following components. Mix components 2-4 and add 10 µl of the mixture to each genomic DNA sample (*i.e.* component 1).

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Component	Amount per tube/well (µl)	Final (in 20 µl)
1 DNA sample	10	up to 100 ng
2 Ultrapure H ₂ O	7.5	
3 NEBuffer 4 (10x)	2	1x
4 <i>Bfal</i>	0.5	2.5 U

6. Incubate samples at 37°C for 3h.
7. Inactivate *Bfal* at 65°C for 20 min.

Ligation of adapter

8. Prepare the *Bfal*-adapter solution by adding 12.5 µl of ADP2 oligonucleotide (200 µM in sterile ultrapure H₂O), 12.5 µl of ADP3 oligonucleotide (200 µM in sterile ultrapure H₂O), 10 µl of NEBuffer 4 (10x) and 64 µl of H₂O in a 1.5 ml microfuge tube. Incubate tube for 2 min in 0.5 l of water at 95°C in a glass beaker placed on a hot plate with a magnetic stirrer. Cool down to 22°C over a period of 45 min.
9. Ligate the *Bfal*-adapter to the digested genomic DNA by combining the following components. Mix components 2-7 and add 5 µl of the mixture to each digested DNA sample (*i.e.* component 1).

Component	Amount per tube/well (µl)	Final (in 25 µl)
1 Digested DNA	20	up to 100 ng
2 Ultrapure H ₂ O	3	
3 NEBuffer 4 (10x)	0.5	1x
4 ATP (100 mM)	0.2	2.5 U
5 BSA (4.5 mg/ml)	0.14	25 µg/ml
6 <i>Bfal</i> -adapter	1	1 µM
7 T4 DNA ligase (6 Weiss U/ µl)*	0.16	1 Weiss U

* 1 cohesive end unit (New England Biolabs) = 0.015 Weiss unit

10. Incubate samples overnight at room temperature (20-25°C).
11. Inactivate T4 DNA ligase at 65°C for 10 min.

PCR amplification of the regions flanking the RB or LB of the T-DNA insert(s)

12. Transfer 7 µl of adapter-ligated genomic DNA from Step 11 to a fresh tube/well.

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13. Mix components 2-7 and add 43 μ l of the mixture to each adapter-ligated DNA sample (*i.e.* component 1). To PCR-amplify the regions flanking the RB of the T-DNA insert, use the AP1 and TDNA1 primers. To amplify the regions flanking the LB of the T-DNA insert, use the AP1 and TDNA3 primers.

Component		Amount per tube/well (μ l)	Final (in 50 μ l)
1	Adapter-ligated DNA	7	up to 30 ng
2	Ultrapure H ₂ O	28.8	
3	Taq DNA polymerase buffer (10x)	5	1x
4	dNTPs (2 mM)	5	250 μ M
5	AP1 primer (10 μ M)	2	0.4 μ M
6	TDNA1 (RB) or TDNA3 (LB) primer (10 μ M)	2	0.4 μ M
7	Taq DNA polymerase (5 U/ μ l)	0.2	1 U

14. PCR-amplify the T-DNA flanking regions using a thermocycler and the following cycle conditions:

Number of cycles	Temperature	Duration
1x	94°C	2 min
30x	94°C	30 sec
	60°C	45 sec
	72°C	2 min 30 sec
1x	72°C	10 min
1x	10°C	pause

15. Transfer 1 μ l of PCR1 reaction to a fresh tube/well.

16. Mix components 2-7 and add 49 μ l of the mixture to each PCR1 product (*i.e.* component 1). To PCR-amplify the regions flanking the RB of the T-DNA insert, use the AP2 and TDNA2 nested primers. To amplify the regions flanking the LB of the T-DNA insert, use the AP2 and TDNA4 nested primers.

Component		Amount per tube/well (μ l)	Final (in 50 μ l)
1	PCR1 product	1	
2	Ultrapure H ₂ O	34.8	
3	Taq DNA polymerase buffer (10x)	5	1x
4	dNTPs (2 mM)	5	250 μ M
5	AP2 primer (10 μ M)	2	0.4 μ M
6	TDNA2 (RB) or TDNA4 (LB) primer (10 μ M)	2	0.4 μ M
7	Taq DNA polymerase (5 U/ μ l)	0.2	1 U

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17. PCR-amplify using a thermocycler and the following cycle conditions:

Number of cycles	Temperature	Duration
1x	94°C	2 min
25x	94°C 62°C 72°C	30 sec 45 sec 2 min 30 sec
1x	72°C	10 min
1x	10°C	pause

18. Check the number and concentration of PCR2 products for each T-DNA line on an agarose gel.

This procedure is further detailed in [Thole V. et al. \(2009\) Nature Protocols, 4:650-661](#).