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|-----------------------------------------------------------------------------------|------------------------------------------------------------|------------------------------|
|  | <b>Operating mode</b>                                      | Ref : MO- VG-SNAP-PCR        |
|                                                                                   | <b>Genotyping by SNAP-PCR for Col-0/WS2 discrimination</b> | Version 1<br>date 01/03/2017 |

## 1. Objective :

**This protocol describe a rapid method for checking of genetic background (Col-0/WS2), in case of contamination suspicion (introgression, crossing or bad identification)**

## 2. References

Necessity to validate lines :

Shao et al, The Plant Cell, Vol. 28: 603–605, March 2016

Bergelson, The Plant Cell, Vol. 28: 606–609, March 2016

Methods :

Drenkard, Plant Physiol, 2000 ,124 : 1483-1492

Bui et al, Plant Methods 2009, 5:1 doi:10.1186/1746-4811-5-1

Edwards, Nucleic acid research, 1991 Mar 25; 19(6): 1349.

Table 1 : Primers for SNAP-PCR (annex A-primers-SNAP-PCRdistinction Col-Ws)

## 3. Diffusion list

BPMP

## 4. Health and safety

## 5. Method principle :

SNAP-PCR amplification on one step extraction DNA

## 6. Materials and Reactifs

- 1 young leaf or in vitro plantlet
- Edward's buffer
- PCR componants

## 7. Operating mode

### I One step DNA extaction :

- Grind (with balls mill or crush directly in extraction buffer) 1 leaf or young in vitro plantlet

|       |                  |              |             |
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|       | Rédacteur        | Vérificateur | Approbateur |
| Nom : | C Tournaire-Roux |              |             |

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- Add 200 µl extraction buffer :  
200 mM Tris-HCl (pH 7.5)  
250 mM NaCl  
25 mM EDTA  
0.5% SDS

- centrifugate 5' 13000 rpm
- Recover supernatant and add 1 vol isopropanol,
- Incubate 5 to 30' in ice, and centrifugate 10' 13000 rpm
- wash pellet with 70 % etahanol
- dissolve pellet in TE
- quantify with nanodrop

## **II. PCR:**

Perform in parallel 2 PCR reactions with :

- a forward primer specific either from Col-0 **or** WS-2
- a reverse primer common for both (Col-0 and WS-2) (table1annex A-primers-SNAP-PCRdistinction Col-Ws2eng )
- 50 ng -75 ng ADN (too much DNA result in amplication in both reactions)
- Reaction volume / 20 µl :

|                              |     |
|------------------------------|-----|
| <b>Go taq 5X buffer (µl)</b> | 4   |
| <b>dNTP 2,5 mM (µl)</b>      | 1,6 |
| <b>Forward primer (5 µM)</b> | 1   |
| <b>Reverse primer (5µM)</b>  | 1   |
| <b>Go-taq</b>                | 0,2 |
| <b>H2O</b>                   | 9,2 |

Program :  
95°C 5'  
95 °c 30" } 30 à 35 cycles  
52°C 30"  
72°C 30"  
72°C 1' 1x

Separate amplification products on 1.2% agorose gel.

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