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Hydroponic Culture of 'Micro-Tom' Tomato

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7 [Abstract] We use 'Micro-Tom' to study tomato fruit ripening and development mechanisms. 8 'Micro-Tom' is suitable for cultivation and experiments due to its small size of 10 to 20 cm in 9 height and short life cycle of 3 months. There is also an abundance of publically available 10 information on 'Micro-Tom' including EST, full-length cDNA clones and transcriptome data. 11 'Micro-Tom' plants are grown in hydroponic culture under fluorescents using Arabidopsis cultural shelves in greenhouses or plant rooms to get data with reproducibility for transcriptome and 1213proteome analyses.

15Materials and Reagents

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1. 'Micro-Tom' (Prof. Ezura and Prof. Mizoguchi of Tsukuba University, Solanum lycopersicum cv. 'Micro-Tom', a model plant in the Solanaceae family). 'Micro-Tom' is a dwarf phenotype cultivar, originally first reported of in 1989, fixed by crossbreeding Florida Basket and Ohio 4013-3 (12th filial generation) (Martí et al., 2006) (Figure 1).

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Figure 1. Solanum lycopersicum cv. 'Micro-Tom'

- 2. Nutrient solution [Ootsuka house 5 (OAT house 5) OATagurio Co. Ltd. 2776] (see
 - Recipes) (http://www.oat-agrio.co.jp/en/product/index.html)
- 2526
- 273. MS (Murashige and Skoog) medium
- 28MS (Murashige and Skoog) mineral salts (Wako Co. Ltd. 392-00991)
- 29Gamborg's B-5 vitamin (Sigma Co. Ltd. G1019)
- 30 Sucrose 2% (Wako Co. Ltd. 193-00025)
- 31Agar 0.8% (Bacto agar, Becton, Dickinson and company, 214010)

32		Adjust pH to 5.7with KOH
33		(Li. 2011 please see bio-protocol)
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36	<u>Equip</u>	<u>nent</u>
37		
38	1.	Rock wool (a kind of mineral wool) (Nitoubou Co. Ltd. A0 25/40, 25x25x40 mm
39		Cat-762-090)
40	2.	Blower (Air pump, 100 V, working pressure 0.012 MPa) (Yasunaga Co. Ltd. LP-30A
41		(Figure 2 A)
42	3.	Three-way tube (metal 6 mm caliber) (NISSEISANGYO Co. Ltd. 25424 (Figure 2B and
43		2C)
44	4.	6mm silicon tube (bore diameter 4mm) (Figure 2B)
45	5.	Air stone (17x17x60 mm) (Figure 2D)
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Figure 2. Parts of hydroponic culture system

- A. Blower, B. 6mm silicon tube, C. Three-way tube, D. Air stone (17x17x60 mm)
- 516. Shallow container (Size accordingly to match the scale of your project. In this case we52are using a container 10cm deep, a good example is Tupperware. We should use53light-proof type of containers, because they protect to occur algae.)
- 547. Perforated polystyrene foam board (2.5 cm thickness, 3.5 cm hole diameter, distance55between holes is approximately 12cm) (Figure 3)



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57		Figure 3. Perforated Polystrylene Foam Board (2.5 cm thickness, 3.5cm hole
58		diameter)
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60	8.	Plant boxes (DUCHEFA Biochemie B.V. Co.Ltd. Steri Vent Low container, 720pcs/BOX
61		Cat. S1682.0048 and Standard Closure lids, 480pcs/BOX Cat. S1681.0032)
62	9.	Sponge (household daily-use type)
63		
64	Pro	<u>ocedure</u>
65		
66	Α.	In greenhouse (Figures 4A and 4B)
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- Transfer 'Micro-Tom' seedlings by removing them with rock wool still attached and placing them into the holes in the polystyrene foam board (Figure 4A).
- 5. Add 1/2 formula nutrient solution (Diluted to half concentration with water) into the container (filling it to about 2/3), and use the blower to send air bubbles into the root zone of the tomato plants (Figure 5). Flowers bloom at approximately 45 days after sowing, and fruits begin to grow larger at approximately 53 days after sowing. Ripening begins at approximately 70 days after sowing (Figure 4B).

84 Note: By using the three-way tube, you can send air bubbles from 1 blower into multiple 85 containers for increased efficiency. It is worth mentioning that improper air bubble supply to root zones will usually result in poor plant growth and ultimately rot symptoms. 86 87 Hydroponic culture does not rot as it is constantly receiving air supply, but needs to be changed about once very 7 days (with a new nutrient solution). Polystyrene foam board 88 89 that is smaller than the container may leave hydroponic culture exposed to light, which can lead to algae growth. As plant growth continues hydroponic culture will slowly be 90 91consumed, which can leave a thin air space between the polystyrene foam board and 92the culture, leading to problems with growth. The foam board needs to be the same size 93 and fit well into the container, preferably with sponge/rock wool being soaked in culture.



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Figure 5. Hydroponic culture system



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Figure 6. 'Micro-tom' plants are grown in hydroponic culture (16 h light/8 h

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dark at 26 °C)

- 101 In addition to growth in greenhouse conditions, we also developed a protocol for hydroponic 102growth in a plant room (6 h light/8 h dark at 26 °C).
- 103 1. Sow sterilized seeds and grow 'Micro-Tom' plants in plant boxes (MS medium containing 104 2% sucrose) for 3 weeks (Figure 7) (Li. 2011 please see bio-protocol).
- 105 2. Extract 3-week-plants from the boxes so as not to hurt their roots (Figure 7).
- 106 3. Gently wind sponge around the stems of extracted seedlings (Figure 7).
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3-week-plants

109 Figure 7. 3-week-plants grown in MS medium are ready to be transferred into 110

hydroponic culture. Sponges are wound stems of seedlings and placed into holes of 111 polystyrene foam board.

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- 113 4. Transfer seedlings with sponge attached into the holes of polystyrene foam board 114 (Figure 5).
- 1155. Send air bubbles to hydroponic culture using a blower in the same protocol as above (in 116 greenhouse). Roots grow within the hydroponic culture after 2 to 3 weeks (Figure 8).
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Figure 8. Roots of 'Micro-Tom' in hydroponic culture after 2~3 weeks

120 Note: Plant density per container is an important consideration, as higher density can lead to

- 121 increased mildew and other problems. For a container of about 30 x 50 cm size, consider
- 122 growing 15 plants or less to avoid diseases and other issues (Figure 5 and 6).

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124 **Recipes**

- 125
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 KNO₃ 808 mg/L
- 128 MgSO₄/7H₂O 492 mg/L
- 129 Ca(NO₃)/4H₂O 944 mg/L
- 130 NH4H2PO4 152 mg/L
- 131 Microelement 50 mg/L
- 132 Mn 0.77 ppm
- 133 B 0.32 ppm
- 134 Fe 2.85 ppm
- 135 Cu 0.020 ppm
- 136Zn 0.040 ppm137Mo 0.020 ppm
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