Wave line

information sheet

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Wave constructs and lines, as well as pNIGEL vector series were published:

Rapid, combinatorial analysis of membrane compartments in intact plants with a multicolor marker set.
Geldner N, Dénervaud-Tendon V, Hyman DL, Mayer U, Stierhof YD, Chory J.
Plant J. 2009 Jul;59(1):169-78. Epub 2009 Feb 26.
PMID: 19309456

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Table Wave lines:

All the Wave markers below are available as EYFP, mCherry, mCerulean and mTFP1 fusions (yellow, red, blue and bluegreen). Shorthand for the FPs is: **Y** (EYFP), **R** (mCherry), **C** (mCerulean), **T** (mTFP1). When referring to lines or constructs, please use the following nomenclature: Wave[number][fluorophore letter]. For example **Wave129Y** for the EYFP fusion to Wave line 129 (RabA1g).

Wave number	***name***	***name ref.***	***At number***	***U-clone***	***FM4-64 co-localisation***	***BFA sensitivity***	***Assignment localisation***	***Loc. Ref.***	***remarks***
1	tag only	n.a.	n.a.	pUNI51 vector	n.a.	n.a.	Cytosol / nucleus	n.a.	
2	RabF2b (ARA7)	[1]	At4g19640	U09899	Partial	Low	late endosome/ pre-vacuolar compartment	[2]	Extensive experimental evidence for localization to multi-vesicular bodies and function in trafficking to vacuole
3	Rab C1	[1]	At1g43890	U09677	Partial	Intermediate	Post-Golgi / endosomal		No experimental data found
5	RabG3f	[1]	At3g18820	U10069	Partial	Very low	late endosome / vacuole	[2, 3]	Homologs identified in vacuolar proteome
6	NIP1;1	[4]	At4g19030	U11812	Non-significant	Very low	Endoplasmic Reticulum/ / plasma membrane	[5]	Marker also observed to localise to plasma membrane, possibly upon prolonged immersion
7	RabF2a (Rha1)	[1]	AT5g45130	U12647	Partial	Low	late endosome/ pre-vacuolar compartment	[2]	Extensive experimental evidence for localization to multi-vesicular bodies and function in trafficking to vacuole
9	VAMP711	[6]	AT4g32150	U14984	Non-significant	Very low	vacuole	[3, 7]	Consistent with localisation in protoplasts and identifiaciton in vacuolar proteome

11	Rab G3c	[1]	At3g16100	U23198	Non-significant	Very low	late endosome / vacuole	[2, 3]	Homologs identified in vacuolar proteome
13	VTI12	[6]	At1g26670	U60796	strong	Intermediate	trans-Golgi network / early endosome	[7, 8]	pUNI clone truncated, fusion product contains only aa 126-222. Localisation consistent with that reported for full-length protein.
18	Got1p homolog	TAIR annot.	At3g03180	U63080	Non-significant	Very low	Golgi	[9]	Localisation to Golgi, as described in S. cerevisiae, no experimental data found in plants
22	SYP32	11	At3g24350	U20852	Non-significant	Very low	Golgi	[7, 10]	Consistent with localisation in protoplasts, possibly different from SYP31
24	Rab A5d	[1]	At2g31680	U22946	Partial	High	Endosomal/ Recycling endosome	[11]	
25	Rab D1	[1]	At3g11730	U50900	Partial	Intermediate	Post-Golgi / endosomal		Similar to Wave29 and 33
27	Rab E1d	[1]	At5g03520	U09494	Partial	Intermediate	Post-Golgi / endosomal	[12]	Different from reported Golgi localisation by transient expression in tobacco cells
29	Rab D2a	[1]	At1g02130	U13716	Partial	Intermediate	Golgi / endosomal	[2]	Broad localisation, clearly extending beyond Golgi into endosomal compartments, see text
33	Rab D2b	[1]	At5g47200	U10162	Partial	Intermediate	Golgi / endosomal	[2]	Broad localisation, clearly extending beyond Golgi into endosomal compartments, see text
34	Rab A1e	[1]	At4g18430	U63334	Partial	High	Endosomal/ Recycling endosome	[11]	Also strong cell plate localisation, data not shown

127	MEMB12	[6]	At5g50440	U50585	Non-significant	Very low	Golgi	[7, 13]	Consistent with reported localisation of MEMB11 to Golgi in tobacco cells
129	Rab A1g	[1]	At3g15060	U51331	partial	High	Endosomal/ Recycling endosome	[11]	Also strong cell plate localisation, data not shown
131	NPSN12	[6]	At1g48240	U60291	n.a.	n.a.	plasma membrane	[14]	Different from reported NPSN11 localisation to the cell plate.
138	PIP1;4	[4]	At4g00430	U16966	n.a.	n.a.	plasma membrane	[4]	Consistent with reported PM localisation of PIP1;1

1. Rutherford, S. & Moore, I. The Arabidopsis Rab GTPase family: another enigma variation. *Curr Opin Plant Biol* **5**, 518-528 (2002).
2. Nielsen, E., Cheung, A.Y. & Ueda, T. The regulatory RAB and ARF GTPases for vesicular trafficking. *Plant Physiol* **147**, 1516-1526 (2008).
3. Carter, C. et al. The vegetative vacuole proteome of Arabidopsis thaliana reveals predicted and unexpected proteins. *Plant Cell* **16**, 3285-3303 (2004).
4. Boursiac, Y. et al. Early effects of salinity on water transport in Arabidopsis roots. Molecular and cellular features of aquaporin expression. *Plant Physiol* **139**, 790-805 (2005).
5. Choi, W.G. & Roberts, D.M. Arabidopsis NIP2;1, a major intrinsic protein transporter of lactic acid induced by anoxic stress. *J Biol Chem* **282**, 24209-24218 (2007).
6. Sanderfoot, A.A., Assaad, F.F. & Raikhel, N.V. The Arabidopsis genome. An abundance of soluble N-ethylmaleimide-sensitive factor adaptor protein receptors. *Plant Physiol* **124**, 1558-1569 (2000).
7. Uemura, T. et al. Systematic analysis of SNARE molecules in Arabidopsis: dissection of the post-Golgi network in plant cells. *Cell Struct Funct* **29**, 49-65 (2004).
8. Sanderfoot, A.A., Kovaleva, V., Bassham, D.C. & Raikhel, N.V. Interactions between syntaxins identify at least five SNARE complexes within the Golgi/prevacuolar system of the Arabidopsis cell. *Mol Biol Cell* **12**, 3733-3743 (2001).
9. Conchon, S., Cao, X., Barlowe, C. & Pelham, H.R. Got1p and Sft2p: membrane proteins involved in traffic to the Golgi complex. *EMBO J* **18**, 3934-3946 (1999).
10. Rancour, D.M., Dickey, C.E., Park, S. & Bednarek, S.Y. Characterization of AtCDC48. Evidence for multiple membrane fusion mechanisms at the plane of cell division in plants. *Plant Physiol* **130**, 1241-1253 (2002).
11. Chow, C.M., Neto, H., Foucart, C. & Moore, I. Rab-A2 and Rab-A3 GTPases define a trans-golgi endosomal membrane domain in Arabidopsis that contributes substantially to the cell plate. *Plant Cell* **20**, 101-123 (2008).
12. Zheng, H. et al. A Rab-E GTPase mutant acts downstream of the Rab-D subclass in biosynthetic membrane traffic to the plasma membrane in tobacco leaf epidermis. *Plant Cell* **17**, 2020-2036 (2005).
13. Chatre, L., Brandizzi, F., Hocquellet, A., Hawes, C. & Moreau, P. Sec22 and Memb11 are v-SNAREs of the anterograde endoplasmic reticulum-Golgi pathway in tobacco leaf epidermal cells. *Plant Physiol* **139**, 1244-1254 (2005).
14. Zheng, H. et al. NPSN11 is a cell plate-associated SNARE protein that interacts with the syntaxin KNOLLE. *Plant Physiol* **129**, 530-539 (2002).

Verification of Wave lines:

In order to confirm the identity of the different Wave lines, plants were checked for the presence of the correct insert by PCR amplification and sequencing.

Primers used for amplification from genomic DNA preps:

Primers for EYFP and mCerulean containing lines:

Sense: 5'-CTGGGGCACAAGCTGGAGTACA-3'

Antisense: 5'-GACAGTGGGAGTGGCACCTTCC-3'

Primer for internal sequencing of PCR product:

5'- CACAATTCTAGTCGACGGCCCA-3'

Primers for mCherry containing lines:

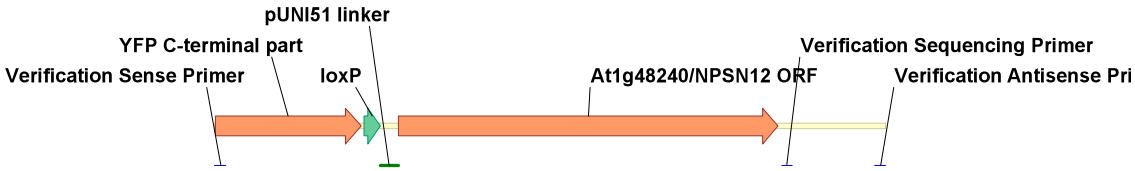
Sense: 5'-CAGAGGCTGAAGCTGAAGGACG-3'

Antisense (same as above): 5'-GACAGTGGGAGTGGCACCTTCC-3'

Primer for internal sequencing of PCR product (same as above):

5'- CACAATTCTAGTCGACGGCCCA-3'

Example of Amplification product:



Example PCR-product for verification
1407 bp

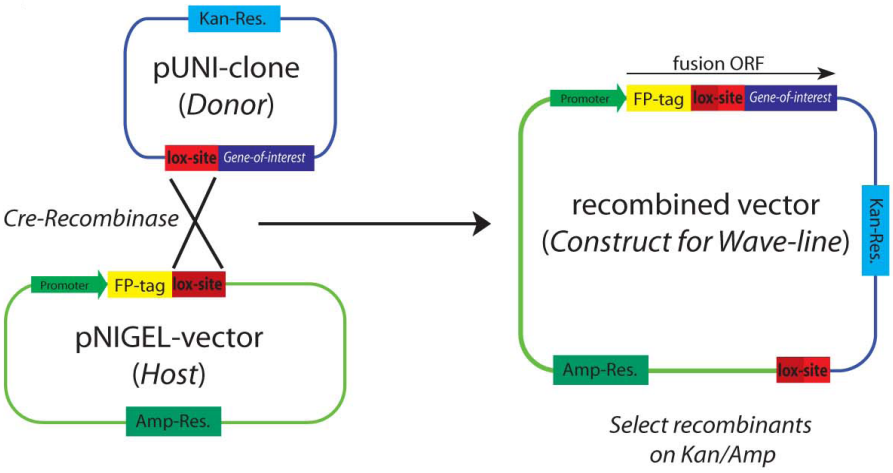
this sequence file can be downloaded from our homepage:

http://www.unil.ch/dbmv/page52928_en.html

Generation of new fusion constructs using pNIGEL vectors:

pNIGEL vectors were designed to allow generation of fusion constructs with the pUNI library of Arabidopsis ORF clones, available from ABRC. Below is a description of the cloning procedure, recombination protocol and a list of all available pNIGEL vectors.

Cartoon of CRE/lox cloning using pNIGEL vectors and pUNI clones from ABRC:

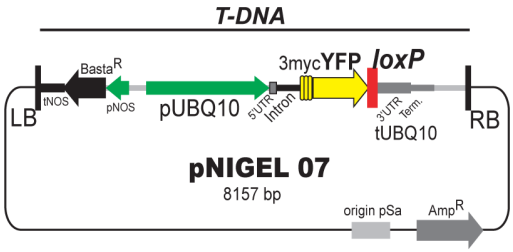


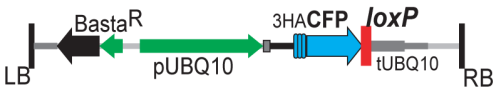
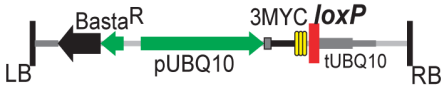
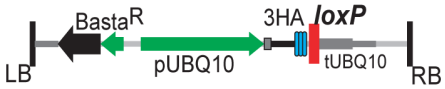
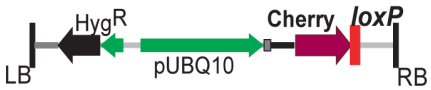
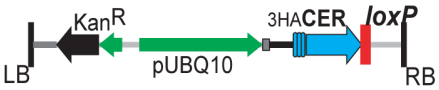
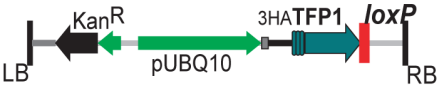
Protocol for CRE/lox Recombination cloning

CRE/lox recombination cloning is done based on the protocol in [1]. 500 ng host vector DNA (pNIGEL vectors) are used with an equal amount of the ORF-bearing pUNI vectors and incubated with 1 unit of Cre recombinase for 30 min in supplier's buffer (NEB). The reaction is transformed into chemically competent DH5alpha cells after heat inactivation of the enzyme for 10 min at 70°C. Transformants were selected on double-selection Ampicillin/Kanamycin plates. Generally, only a few colonies were recovered per reaction/transformation, but those were always found to contain the fusion product upon sequence confirmation.

1. Liu, Q., Li, M.Z., Leibham, D., Cortez, D. & Elledge, S.J. The univector plasmid-fusion system, a method for rapid construction of recombinant DNA without restriction enzymes. *Curr Biol* **8**, 1300-1309 (1998).

Overview of pNIGEL vectors:



Vector name	Resistance	Fluorophore	Epitope tag
 **pNIGEL 09** 8121 bp	BASTA[1]	ECFP[4]	3 times haemaglutinin
 **pNIGEL 11** 7434 bp	BASTA[1]	none	3 times myc
 **pNIGEL 13** 7398 bp	BASTA[1]	none	3 times haemaglutinin
 **pNIGEL 15** 7977 bp	BASTA[1]	mCherry[5]	none
 **pNIGEL 16** 7977 bp	BASTA[1]	mOrange[5]	none
 **pNIGEL 17** 7842 bp	HYGROMYCIN[2]	mCherry[5]	none
 **pNIGEL 18** 7581 bp	KANAMYCIN[3]	mCerulean[6]	3 times haemaglutinin
 **pNIGEL 19** 7570 bp	KANAMYCIN[3]	mTFP1[7]	3 times haemaglutinin

1 *pat* gene conferring resistance to Basta (phophinotricine)
2 *aph*III genegene conferring resistance to Hygromycin
3 *Npt*II gene conferring resistance to Kanamycin
4 enhanced CFP from Clontech
5 as described in Shaner et al., Nature Biotechnology, 2007
6 as described in Rizzo et l., Nature Biotechnology, 2004
7 as described in Ai et al., Biochemistry, 2007

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