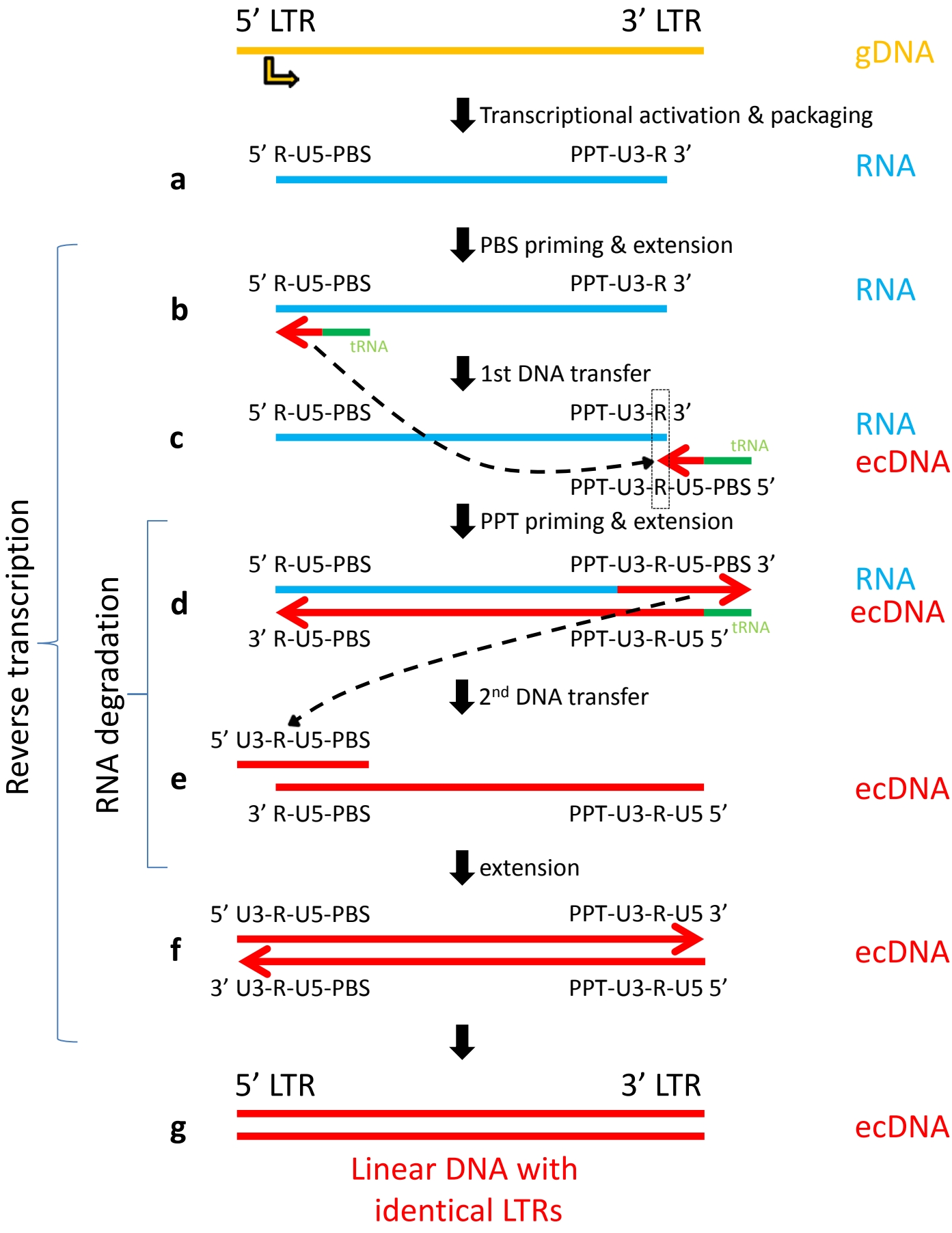
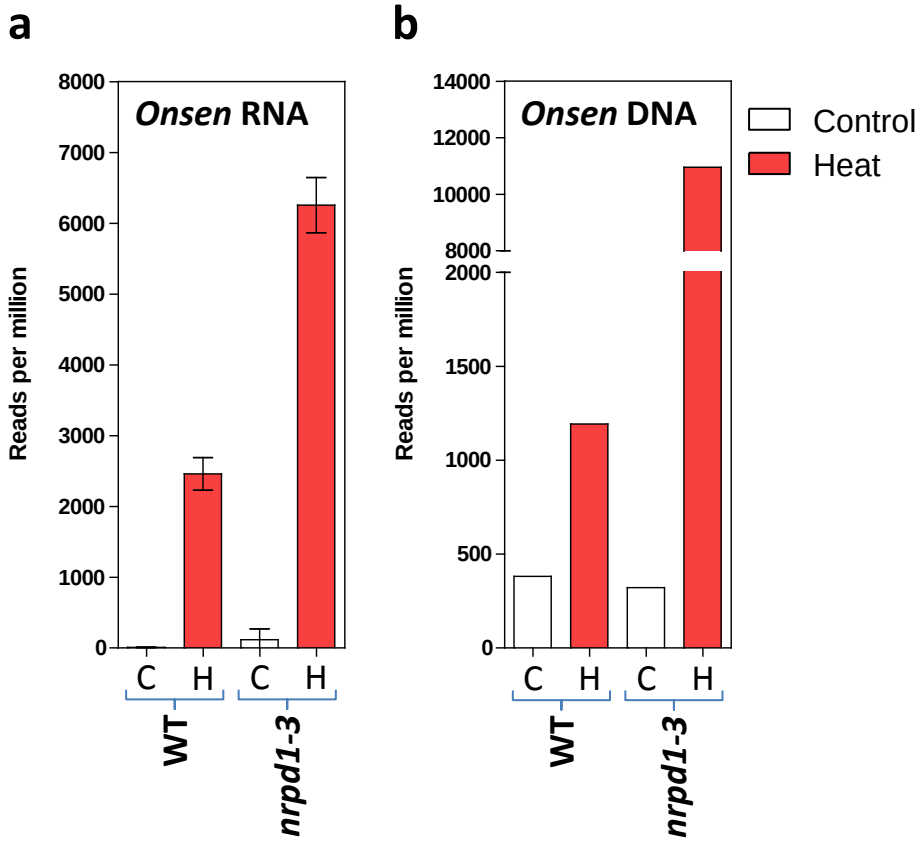
Supplementary Fig. 1



# Supplementary Fig. 1

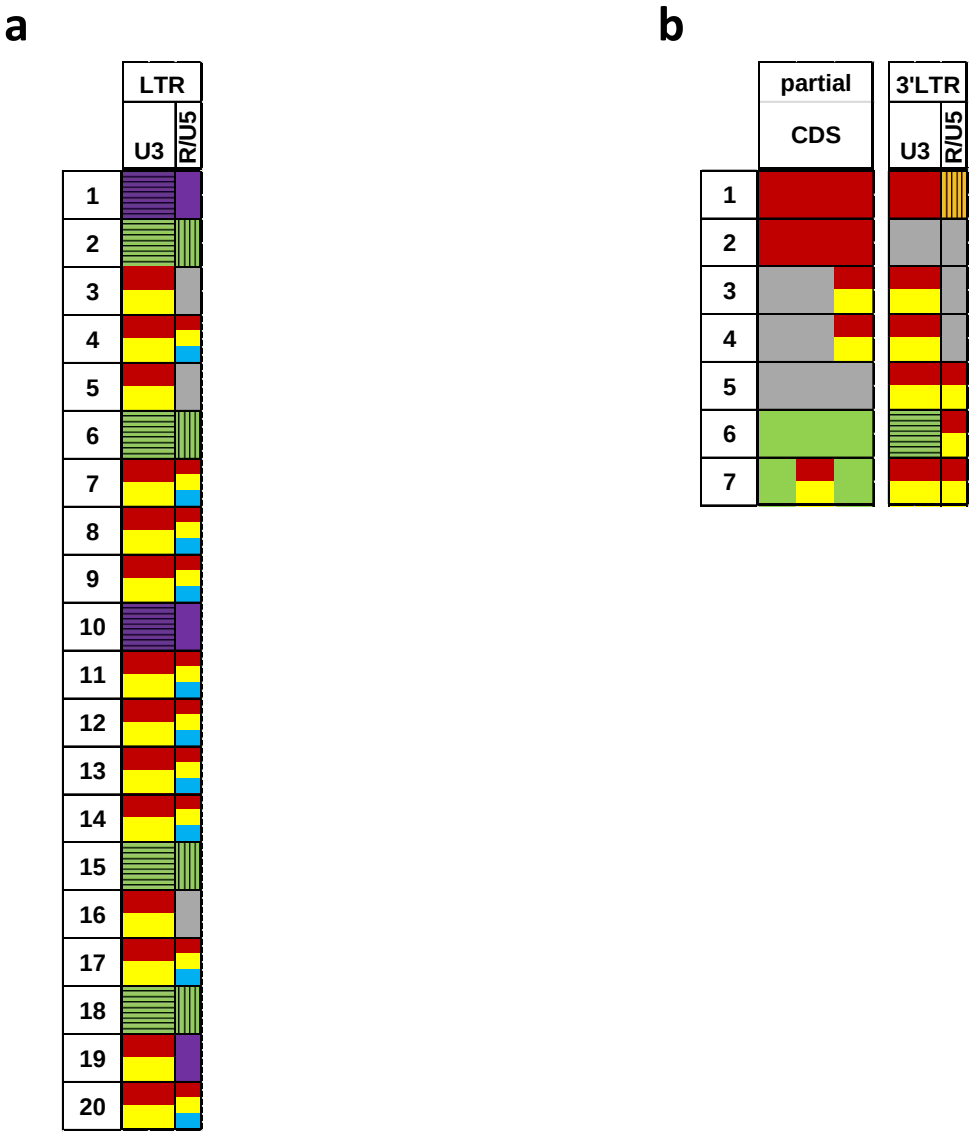
**Supplementary Fig. 1**: Schematic modelled representation of reverse transcription steps of LTR retrotransposons. LTR - long terminal repeat. R - repeated region, U5 - unique 5' region, U3 - unique 3' region, PBS - primer binding site, PPT - polypurine track, ecDNA - extra-chromosomal DNA. (**a**) The LTR retrotransposon is transcriptionally activated and the RNA genome (starting and ending in the LTR R region) is packaged with reverse transcriptase, RNAseH and integrase into GAG-derived virus-like particles. (**b**) Host tRNA primed at PBS and (-)-strand cDNA is synthesized by reverse transcriptase. (**c**) (-)-strand cDNA-tRNA transfer using sequence homologies in the R region. (**d**) Reverse transcriptase extends (-)-strand cDNA until the beginning of the transcript, after RNAseH degradation of the RNA, except at the PPT, where synthesis of (+)-strand cDNA is primed and synthetized using (-)-strand cDNA-tRNA as a template. (**e**) (+)-strand cDNA transfers to 5' area of (-)-strand cDNA. (**f**) Reverse transcriptase performs final extensions. (**g**) The resulting ecDNA molecule is a double-stranded, linear molecule encoding the entire retrotransposon with two identical LTRs.

Supplementary Fig. 2



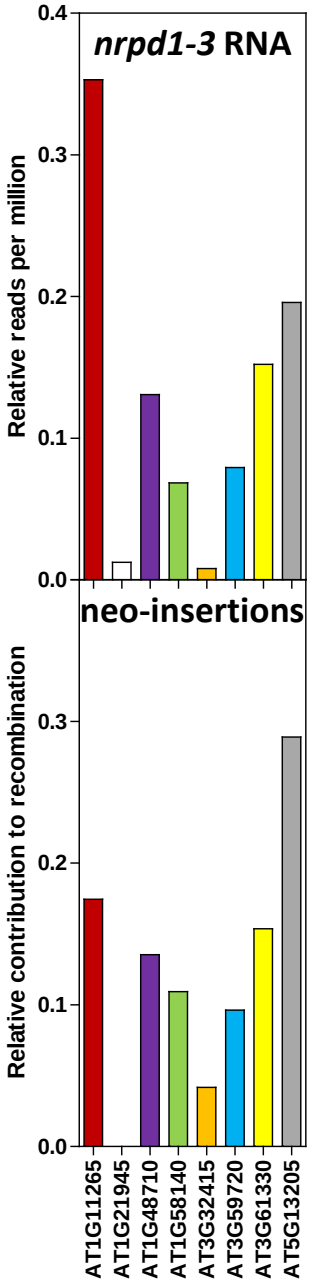

**Supplementary Fig. 2**: Heat stress induced increase of reads mapping to *Onsen* in the NGS transcriptome and whole-genome sequencing. Total NGS reads mapping to *Onsen* were counted and normalized to library size in wild-type and *nrpd1-3* plants under control (white bars) and heat stress (red bars) conditions. (**a**) RNA-seq analysis. The increased reads in heat samples originated from transcriptionally activated *Onsen*. Data represent means ± SEM for n=2 independent biological replicates. (**b**) Whole-genome sequencing analysis. The increased reads in heat samples originated from activated *Onsen* producing ecDNA.

# Supplementary Fig. 3



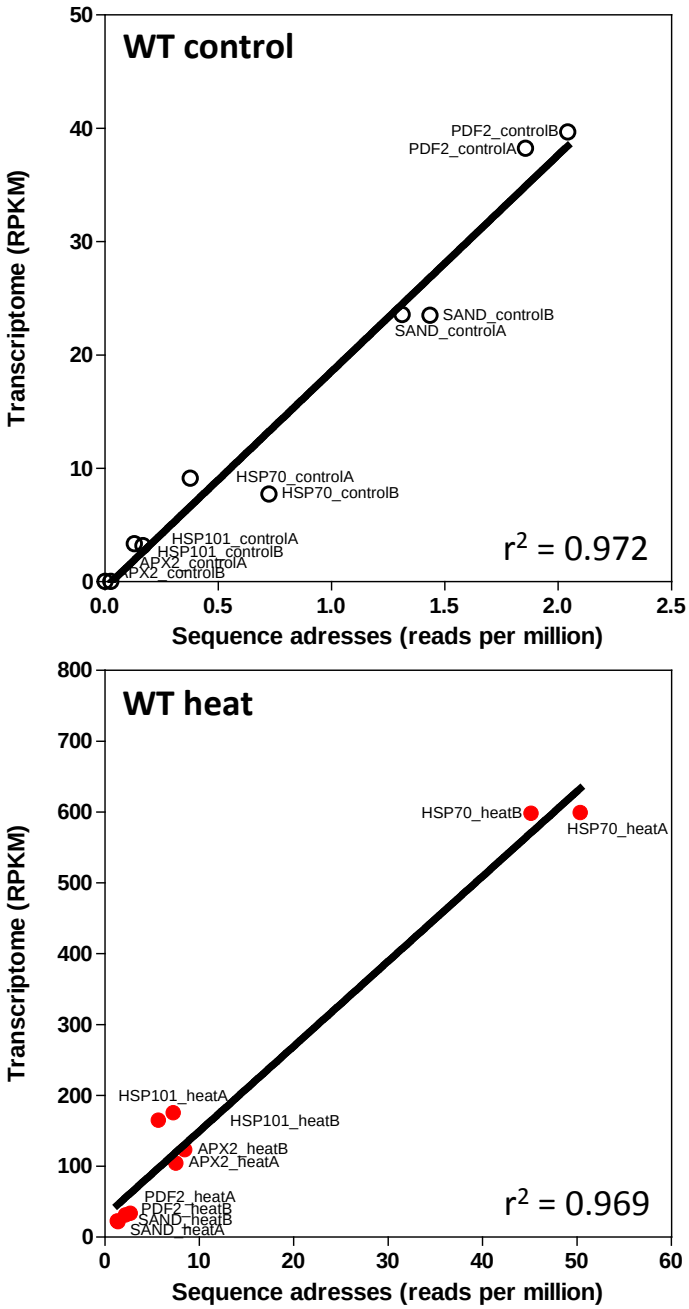

**Supplementary Fig. 3**: Independent plant lines showing evidence of *Onsen* intra-family recombination. (**a**) Origin of reconstructed LTRs from new chromosomal *Onsen* insertions recovered in progeny of an independent plant (*nrpd1* plant 5L2) with 20 new insertions [8]. LTRs domains are marked as U3 and R/U5. Informative SNPs and indels were used to infer the parental origin of LTRs. Several colours are used in the same area when these polymorphisms are shared between more than one member. Colour code as in Fig. 1a. (**b**) First cloned and Sanger-sequenced new chromosomal *Onsen* insertions from progeny of independent heat stressed *nrpd1-3* mutant plants (initial experiments). Informative SNPs and indels were used to determine the parental origin of new inserted retrotransposon copies. The sequenced area was between positions 3701 and 4957, when aligned to AT1G11265/'red'. Retrotransposon domains and colour code as in Fig. 1a.

Supplementary Fig. 4




**Supplementary Fig. 4**: Relative contributions of family members to the *Onsen* mRNA pool and to the 32 cloned and Sanger-sequenced new insertions. Mean relative transcript levels (upper panel) were calculated from Fig. 1b; the relative contribution to neo-insertions (lower panel) was computed with a sum of scores as described in Methods. Colour code as in Fig. 1a.

Supplementary Fig. 5




**Supplementary Fig. 5**: Validation of the sequence addresses strategy to estimate transcript levels. In both control (upper panel) and heat stress (lower panel) samples, standard determination of transcript levels (RPKM) was calculated from transcriptome data of wild-type samples and correlated with the normalized read count of addresses across case examples of housekeeping and heat responsive genes.

# Supplementary Table 1

Non-parentally originated polymorphism events observed in cloned and Sanger-sequenced *Onsen* new chromosomal copies. 'Position' refers to alignment on AT1G11265/'red' as a reference.

| New copy *Onsen* | Event | Position (alignment) |
|---|---|---|
| #2 | G to T | 3729 - ORF |
| #8 | Deletion+SNPs | 456 to 503 - ORF |
| #13 | G to A | 3598 - ORF |
| #17 | T to C | 148 and 4664 - LTR |
| #18 | T to C | 3121 - ORF |
| #18 | Insertion A | 4910 - ORF |
| #26 | T to C | 3322 - ORF |
| #26 | C to T | 4211 - ORF |
| #30 | A to T | 1598 - ORF |
| #30 | G to A | 1874 - ORF |

# Supplementary Table 2

List of primers used.

For fig. 3, cloning and Sanger-sequencing of *Onsen* new insertions in *nrpd1* 2L plant

| | Insertion - Position | Primer Forward | Sequence (5'-3') | Primer Reverse | Sequence (5'-3') |
|---|---|---|---|---|---|
| **1** | exon AT1G17430 | NI_1F | GTGGAGTGGGATTTACACAGCAGCA | NI_1R | AACGAACAAACAATTTACGGGACCAGA |
| **2** | exon AT1G58050 | NI_2F | CTAAAGGCACCCGCAGCATCTC | NI_2R | TTGTCAAGCATGGTGGAAGATTGG |
| **3** | exon AT3G47760 | NI_3F | TCCTTCTCCATATGGCTTGCAAAGA | NI_3R | TCACATTGGAAGTGAGATGAGCCCTA |
| **4** | exon AT4G29110 | NI_4F | ATAACCAACCTCCGCCGTCGAT | NI_4R | CGAATCCGTCACCCTGAGCTGT |
| **5** | exon AT1G61470 | NI_5F | CCAGCAGCTTTTGGGAAGGTTTG | NI_5R | TTGCTGATGCTGCTGCTGCTCT |
| **6** | exon AT1G12140 | NI_6F | ACCGAAGTAGTTCTTGTCGAGCCTGA | NI_6R | TGTTTCCATCGTCAAAAACGCACA |
| **7** | 3'UTR AT2G22330 | NI_7F | GGTGAAGTGAGGCGACGGTGAC | NI_7R | TTGTCGCGACCCAATCATTGAC |
| **8** | exon AT4G34530 | NI_8F | TGGCTTATCTGTTTCTTCTGTGTGTGC | NI_8R | GCCTGTGATCTTGTCGCATCCA |
| **9** | exon AT4G31690 | NI_9F | ACCACTCTCTTCGCCTTCTTCATGTC | NI_9R | GAGCACATCGAGGGAAAACACGA |
| **10** | exon AT2G36570 | NI_10F | CCTTCCCTCTCCTTACGAGGACCA | NI_10R | CCGGATCGTTAAAACCCGGGTAAA |
| **11** | exon AT1G11370 | NI_11F | TCAAACAGTTGAATGAGTATGCAAAAGATGA | NI_11R | ACCACGGTGAATGAAGCGGTTG |
| **12** | exon AT2G14285 | NI_12F | GCGCAGACCCTCAAGCATCATC | NI_12R | TGAATGAAGCTTGGTTTGTAATTGAACGTC |
| **13** | exon AT3G23060 | NI_13F | CGTTCCAGAAACGTGGGAGAAAA | NI_13R | TGGTACGACTTCCTGAAAAGTTGATCC |
| **14** | exon AT5G39310 | NI_14F | TCCACCATTGATGTCACCGTAGAATG | NI_14R | TCCCAAAAATATCGACAAGCCCAAA |
| **15** | exon AT3G04010 | NI_15F | TTTCAGATCACTACCCAATATCTAAAAAC | NI_15R | TGAATTATCGACGAAAACAGAGTGC |
| **16** | 5'UTR AT4G35770 | NI_16F | GTCCGAGTCAGCAAGTTATCCTCTT | NI_16R | TTCTCGTCGTTGATTGCTAGCTTAC |
| **17** | exon AT5G22250 | NI_17F | CCCATCCTCAACGAAATACAAATC | NI_17R | GGATCGATTTCGAGCGGAAC |
| **18** | exon AT5G13940 | NI_18F | TCTCATTCAAACAAAAACTAAAAGTGGAA | NI_18R | TGCAATAACCGGCTTGTTGACTC |
| **19** | exon AT2G16960 | NI_19F | GGGGTTCAATACGACCAAAATATCG | NI_19R | TCCGGGAGTACCTGTTAGTGTAGCC |
| **20** | intron AT2G35640 | NI_20F | TTAGGATTTGTAGCTCCGATGATGG | NI_20R | CGACCAAAATGCGGGTTTAATTACT |
| **21** | upstream AT1G51970 | NI_21F | GTTTCCCAAATTCTTGACCAAACC | NI_21R | TGTGTACATATAAACATAAACGGTAAGTCG |
| **22** | exon AT3G53590 | NI_22F | GAACCAAACAGAACACCTATGTGGA | NI_22R | AAAACGTGTTTGTAAGCAACTCAGC |
| **23** | intron AT1G05320 | NI_23F | GAAAAATACCGGAAAAGAAACAGATT | NI_23R | CAATATGGAAAACTAACGGCGATT |
| **24** | 3'UTR AT1G01530 | NI_24F | CTCATATTCCACAAGAAAATGATATAAAGC | NI_24R | CCAACACTGCCGTTTGGAT |
| **25** | Upstream AT4G34600 | NI_25F | TCGATGTATATTCATGCCGATTAAA | NI_25R | TGAAGGAATAAAGCATACGTCGAT |
| **26** | intron AT2G32520 | NI_26F | CTGAAGCAGAAACGCCATTG | NI_26R | TCTCGCTTATCAGTGCGATCA |
| **27** | 5'UTR AT4G16830 | NI_27F | GGTAGGGGACACTGGACAGG | NI_27R | CCTCAGCATCATCATCCAACA |
| **28** | Upstream AT1G61300 | NI_28F | GCCATGGACTCTGCTTTTAGC | NI_28R | TCTGGCTTGGCTTCTGTTTG |
| **29** | exon AT1G13510 | NI_29F | GCTCGGTACGAAGCTCTGTG | NI_29R | TTCAGGGTATGACTTTGAATCTTGA |
| **30** | intron AT5G15380 | NI_30F | TGAATTTGTGATCTTTGCCATCTT | NI_30R | ACAGAAAGGCTGAATCCCAATC |
| **31** | exon AT2G42000 | NI_31F | CCCAGCAGATGCTTCTCTCA | NI_31R | CAGGATGCTTTGTCACGACTG |
| **32** | exon AT4G31710 | NI_32F | TGATTTCTCCCAATGCAACG | NI_32R | GCATCGTAAGCCCTCAATCC |

| **Sanguer-sequencing *Onsen* internal primers** | |
|---|---|
| **ONSENQ1** | ACTACCTTCATTCTCCGGTTC |
| **ONSENQ2** | CCTCTTGATGAGTTTTCACCTC |
| **ONSENQ3** | TTTCGAATGTTGAGGACAAAC |
| **ONSENQ4** | CCCACAACTCTTTTGGTAGTCT |
| **ONSENQ5** | TCCCATGTGTCATTCTTTTGTA |
| **ONSENQ6** | CTTGTTTTACTTCAATTCCGAGA |
| **ONSENQ7** | CTTAACCAAATAGCATGGCATA |
| **ONSENQ8** | ACTTGATTTTGCTACTCCAAGC |
| **ONSENQ9** | GCAATAGCTTTGGCGAAGAA |
| **ONSENQ10** | GCTAGATGATGTGAGAATCATGGA |
| **ONSENQ11** | GGAGCTTGATGAATCGGTGA |
| **ONSENQ12** | AAGCCCATGTTGAGAAGGAGA |
| **ONSENQ13** | CAACTCCAAAGGCTACAAGCTC |
| **ONSENQ14** | GCCCCAAGAGCTTGGAATAC |

For Supplementary Fig. 3b, initial partial cloning of *Onsen* new insertions in independent *nrpd1-3* plants from transposon display

| **Primer Forward** | **Sequence (5'-3')** |
|---|---|
| ONS3643F | TGACGGACATTGGATTGATG |

# Supplementary Table 3

Sequence polymorphisms in the form of 70-bp sequence 'addresses' used for assigning NGS reads to specific genes or each *Onsen* member. The assignment of NGS reads was made by perfect string matching.

> AT2G28390/SAND_adress1
AAAGTCAATTAGAGTCAATGAATTTATCTCAACCTAGCGAAGTCTCTGATGGTAGCCACACCGAATTTA
> AT1G13320/PDF2_adress1
CTATCATTGCTCGTGCTCTTGGAGAGGAGAGGACAAGAAAAGAGTTGATTCCATTTCTTAGTGAGAACA
> AT1G74310/HSP101_adress1
TAGCTGGTGCTTTGATCTCTGATCCCACCGGTATATTTCCTCAAGCAATCTCTAGTGCCGGTGGCGAGA
> AT3G12580/HSP70_adress1
ACGATCAAGGCAACCGCACCACTCCTTCCTACGTTGCTTTCACTGACAGCGAGCGTCTCATCGGGGATG
> AT3G09640/APX2_adress1
GCTAGCCCATGATGCCAACAATGGTCTTGATATTGCCGTTAGGCTTCTTGACCCTATCAAGGAGCTGTT
>AT1G11265/red_adress1
GTTATTAATGGCTAGCTACAAGAAAGATGAACAAAAAGAGAATCATAAGTGGTACCTCGATAGTGGTGCA
>AT1G21945/white_adress1
AATTGTGGGAAGTTTGGACATTAGGCTTCTGAATGTAAAGCTCCTAGCAACAAAAAATTTGAGGAGAAGG
>AT1G48710/violet_adress1
GCTTTCCAAAGGAGTCAAGTTCAAGAGCACAAAAATCGTTGGAGCTAATACATACCGATGTGTGTGGTCC
>AT1G58140/green_adress1
TCGAAGATGGAGGTAAAAGGTAAAGGAAACATTCTTATTCGATTGAAGAATGGAGATCATCAATTTATTT
>AT3G32415/orange_adress1
GAAGACATGTTATTAATGGCTAGTTACAAGAAAGATGAACAAGAAAAGAATCATAAGTGGTACCTCGATA
>AT3G59720/blue_adress1
CTACAATTGCGGGAAGTTTGGACATTATGCTTCTGAATGTAAAGCTCCTAGCAACAAAAAATTTAAGGAG
>AT3G61330/yellow_adress1
GTTATTAATGGCTAGCTACAAGAAAGATGAACAAAAAGAGAATCATAAGTGGTACCTCGATAGTGGTGCA
>AT5G13205/grey_adress1
GTGCAAGTAATCACATGTGCGGGAGAAAAAGTACGTTCGCGGAGCTTGATGAATCGGTGAGAGGAAATGT
>AT1G11265/red_adress2
AATTGGAGTATGTGAAGACACATGATCAAGTAGCCGATTTTTTTACCAAGCCTCTCAAGCGTGAAAACTT
>AT1G21945/white_adress2
ATTGACACACGCTATCACTACATTAGAGAGTGTGTTAGCAAAAAGGACGTGCAATTGGAGTATGTGAAGA
>AT1G48710/violet_adress2
ACAAGAGGAACCAACGAAGATCTTTGTGGATAACAAGTCGGCAATAGCTTTGGCGAAGAACCCGGTCTTC
>AT1G58140/green_adress2
TACATAGTCAAAGGTGAAGAAGACAAAGTCTTGAGACTAAAAAAGGCGCTTTATGGATTAAAACAAGCCC
>AT3G32415/orange_adress2
ACGCTATCACTACATTAGAGAGTGTGTTAGCAAGAAGAACGTGCAATTGGAGTATGTGAAGACACATGAT
>AT3G59720/blue_adress2
TTAGAGAGTGTGTTAGCAAGAAGGACGTGCAATTAGAGTATGTGAAGACACATGATCAAGTAGCCGATAT
>AT3G61330/yellow_adress2
AATTGGAGTATGTGAAGACACATGATCAAGTAGCCGATTTTTTTACCAAGCCTCTCAAGCGTGAAAACTT
>AT5G13205/grey_adress2
ATCAAGTAGCCGATATTTTTACCAAGCCTCTCAAGGGTGAAAACTTTATCAAGATGAGGAGTTTGCTTGG

# Supplementary Table 3

>AT1G11265/red_adress3
AAGCAAGATTGGTTGCAAAAGGTTATAGTCAAAGAGTCGGAATTGACTATGACGAGGTATTTGCTCCCGT
>AT1G21945/white_adress3
GTGGAAGATACATCAAATGGATTTCAAGTTGGCCTTCTTAAATGGAGATTTTGAAGAAGAAGTTTACATT
>AT1G48710/violet_adress3
AAGATACAAAGCAAGATTGGTTGCAAAAGGTTATATTCAAAGAGCCGGAATTGACTATGACGAGGTATTT
>AT1G58140/green_adress3
ATAGTCAAAGGTGAAGAAGACAAAGTCTTGAGACTAAAAAAGGCGCTTTATGGATTAAAACAAGCCCCAA
>AT3G32415/orange_adress3
GAAACGGTTAGACTAATCATTTCACTAGCGGCTCAAAACAAGTGGACGATACATCAAATGGATGTCAAGT
>AT3G59720/blue_adress3
GGTTATAGTCAAAGAGCCGGAATTGACTATGACGAGATATTTGCTCCCGTTGCTCGTCTAGAAACGGTTA
>AT3G61330/yellow_adress3
AAGCAAGATTGGTTGCAAAAGGTTATAGTCAAAGAGTCGGAATTGACTATGACGAGGTATTTGCTCCCGT
>AT5G13205/grey_adress3
TGCAAAAGGTTATAGTCAAAGAGCCGGAATTGACTATCACGAGGTATTTGCTCCCGTTGCTCGTCTAGAA

Since AT1G11265/'red' and AT3G61330/'yellow' have high sequence similarity, their reads were first pooled together using the same three addresses for both, but subsequently divided to each member using one extra specific address which allowed us to distinguish the count between them. This address was:

>AT1G11265/red_discriminative
AACTACGTTGAAGAAAAAATTCAAGAAGAAGACATGTTATTAATGGCTAGCTACAAGAAAGATGAACAAA