

Figure S1 Laggards and bridges in meiotic (A) and mitotic (B-D) cells of allohexaploids. Cells were prepared for FISH as described in the methods and centromeres labeled with fluorescein (green, *At*) or Texas Red (red, *Aa*). Arrows point to bridges or laggards between dividing cells. A: F9 12-3-5-2, B: F8 2-8-5-3, C: F8 2-8-5-3, D: F8 2-8-5-3.

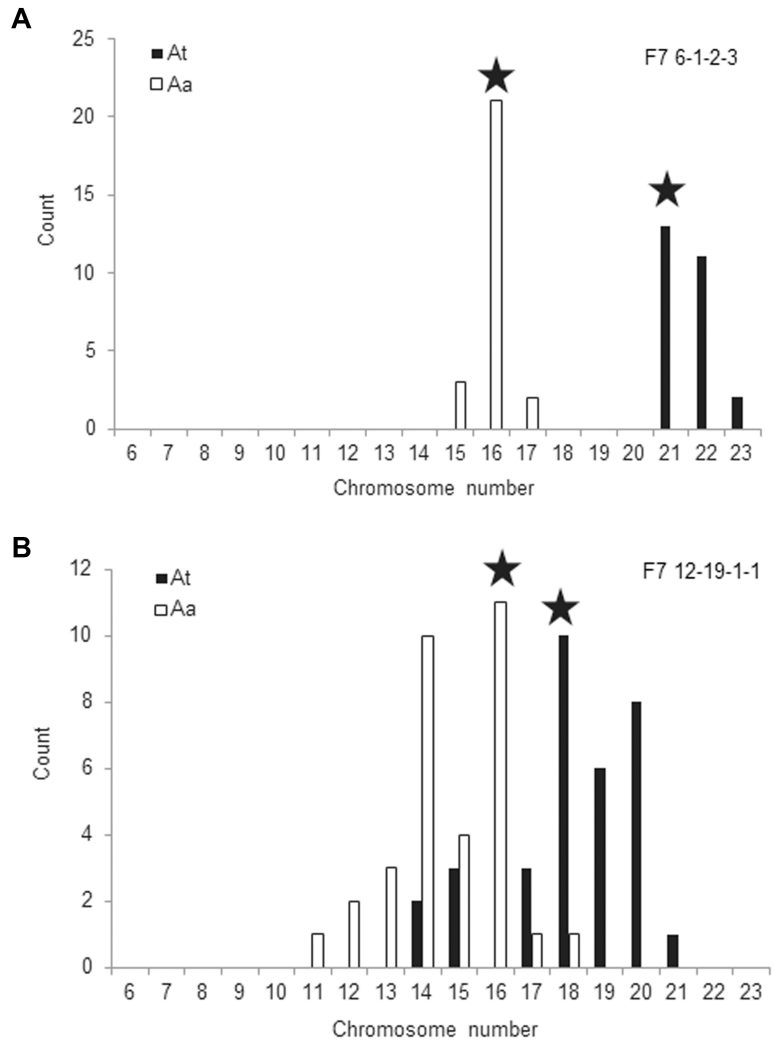


Figure S2 Somatic mosaicism in F7 *Arabidopsis* allohexaploids. Two examples illustrating the adjusted euploid number in two individuals (12-19-1-1 and 6-1-2-3) from separate sibling lines. N= 33 analyzed cells (A), N= 26 analyzed cells (B). The modal chromosome numbers (“adjusted euploid” values) are denoted by stars for each parental genome. Original euploid chromosome numbers (additive from the progenitors) were 20/16.

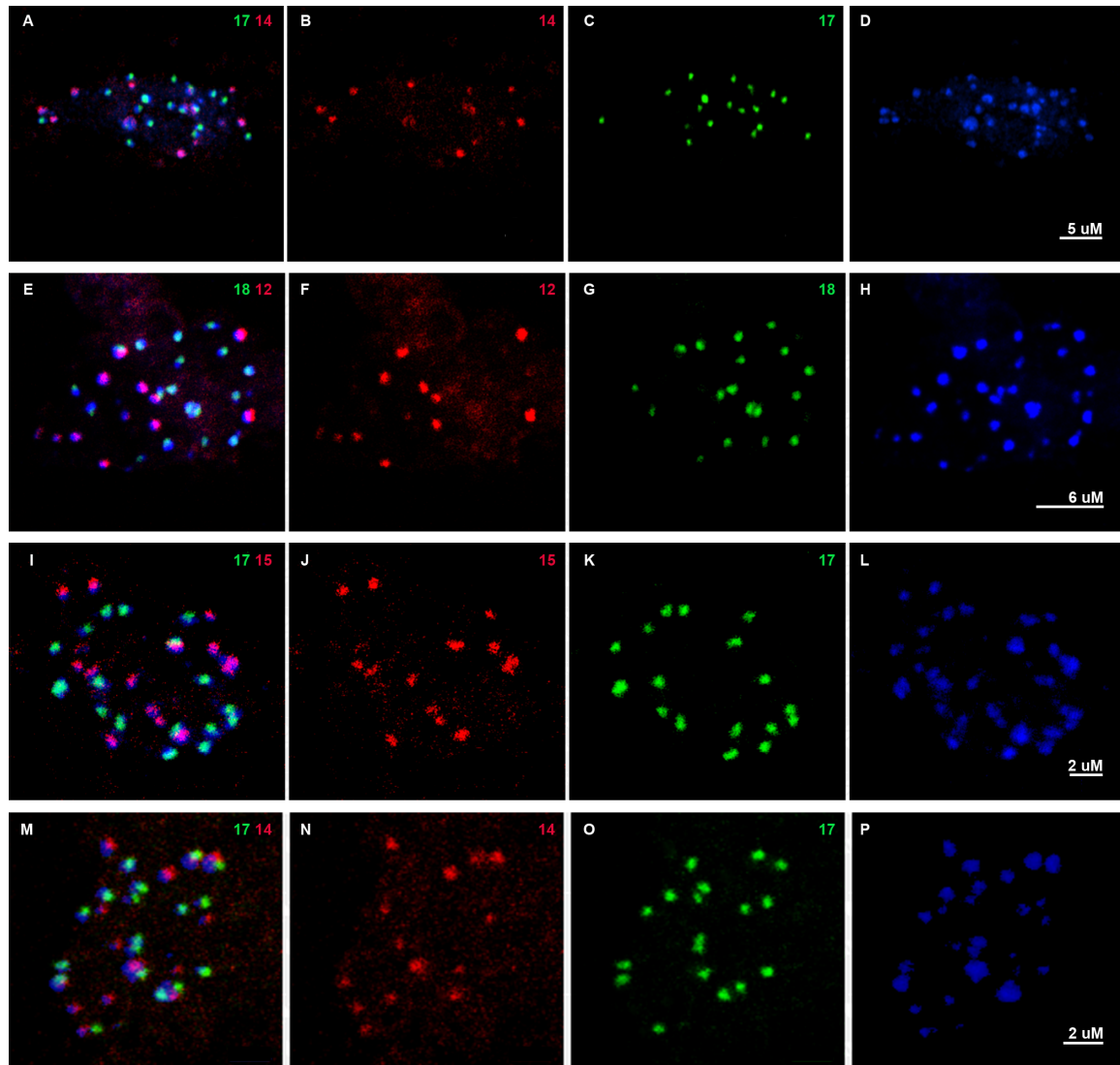


Figure S3 Mitotic chromosomes in root tip cells of allohexaploids. FISH was performed as described in Fig. 3. Composites as well as separate Texas Red, Fluorescein, and DAPI channels are shown for 4 examples A-D: F6 6-1-8, E-H: F7 6-1-9. I-L: F7 19-1-9-1. M-P F7 19-1-9-1.. Numbers in top right corner of each panel indicate the chromosome number of that particular cell (green: *At* chromosomes; red: *Aa* chromosomes). In panel O the *At* chromosome number maybe 17 or 18, but either case indicates hyperploidy.

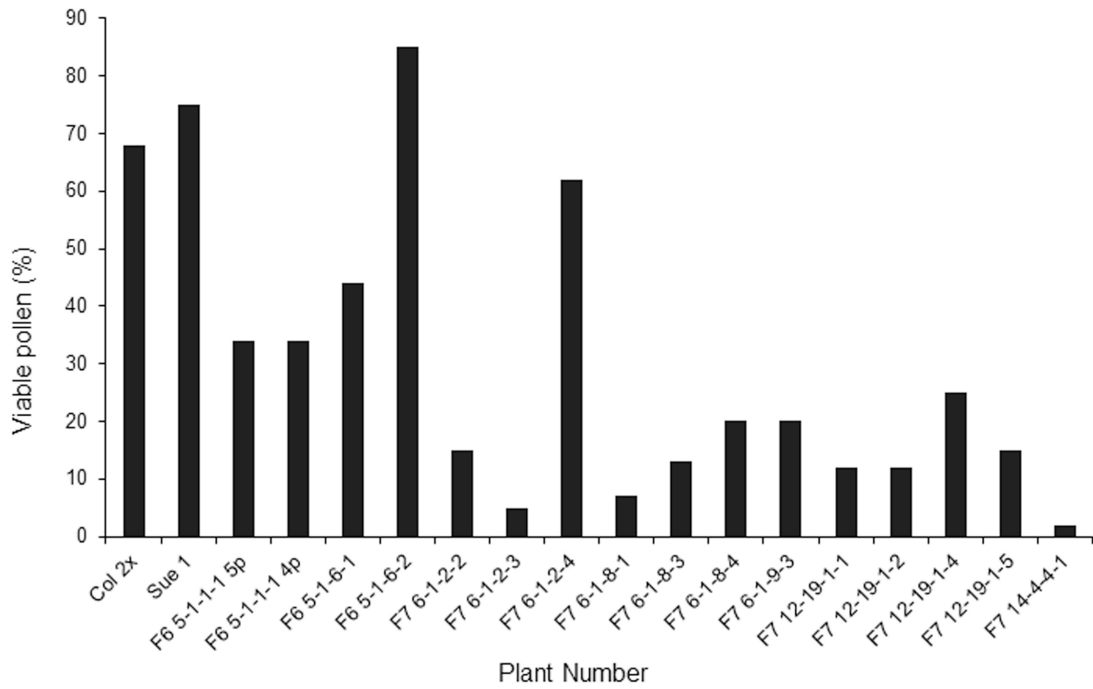


Figure S4 Pollen viability in allohexaploids and progenitors. Live/dead staining of pollen was performed using acetocarmine. The average percent viability for the parent species was $71 \pm 2.5\%$, (SE, N=2) while the overall average for allohexaploids was $25 \pm 5.6\%$ (SE, N=16), which was statistically significantly different ($p=0.012$, $t=2.84$, $df=16$).



Figure S5 Phenotypic variation in F6 and F7 allohexaploids. A) F6 5-1-1-1 individual with 5 petals. B) Rosette of F7 6-1-2-3. C) F7 14-4-4-1. D) F7 12-19-1-1 with close-up of rosette. E) F7 6-1-8-4. F) 19-1-10-1 with close-up of rosette. Size bar = 2cm.

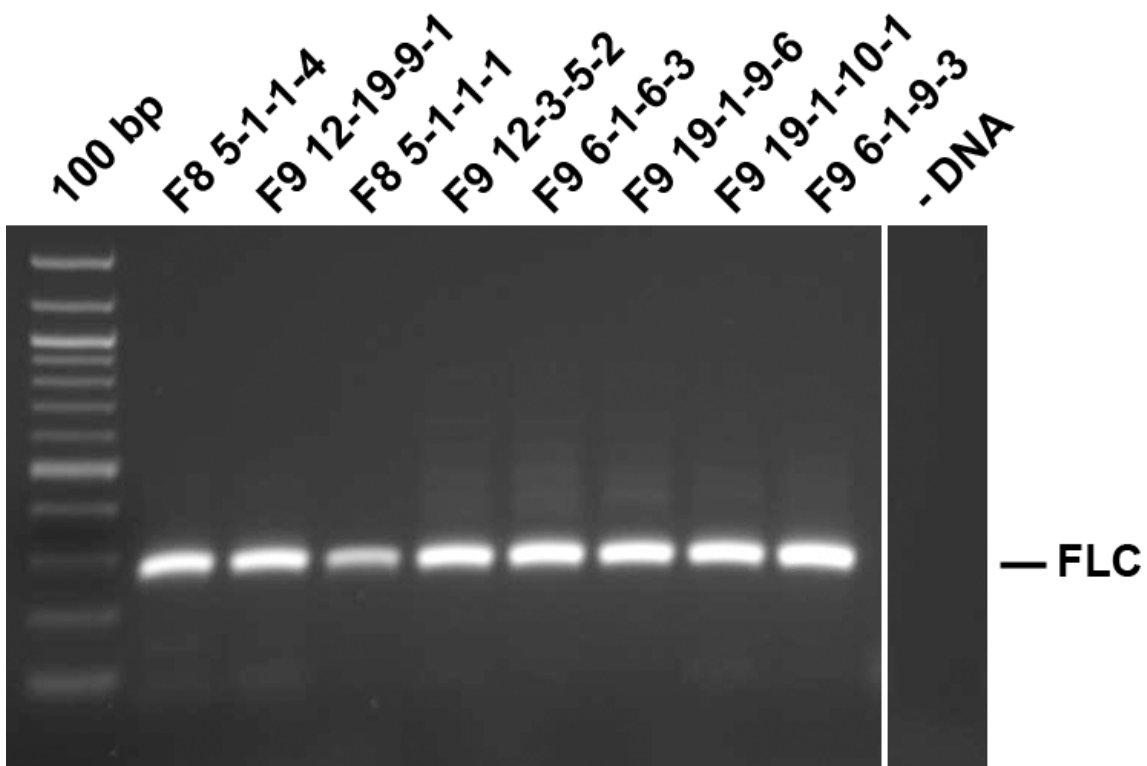


Figure S6 Steady state expression of *FLC* in early and late flowering allohexaploids. RNA was isolated from two late flowering (5-1-1-4; 12-19-9-1) and six early flowering allohexaploid individuals, DNase-treated, and reverse transcribed. Intron-spanning primers for *FLC* were used in RT-PCR (809 bp for genomic DNA, 266 bp for cDNA). Loading control experiments to ascertain that similar amounts of cDNA were loaded were performed with actin and tubulin (data not shown). PCR products were digested with *Clal* which uses a polymorphic site that distinguishes between *FLC* alleles derived either from *A. thaliana* or from *A. arenosa/suecica*. *Clal* digest showed that both types of alleles were expressed (data not shown). The data suggest that *FLC* expression in these non-vernalized plants is not the primary cause for the difference in flowering time.

Table S1 Frequency of Aneuploidy in Allohexaploids over 3 Generations Based on Expected Chromosome Numbers*.

Generation	Plant Number	Cells analyzed	Aneuploid cells (%)	Cells with	Cells with	At	Aa	At	Aa
				expected At chromosome number (%)	expected Aa chromosome number (%)	hyperploid** chromosomes (%)	hyperploid** chromosomes (%)	hypoploid chromosomes (%)	hypoploid chromosomes (%)
F3	2-1	10	100	0	30	20	20	80	50
	2-5	10	90	20	40	0	10	80	50
	2-8	11	100	0	27	0	27	100	45
	5-1	10	90	10	30	0	0	90	70
	5-2	10	90	10	40	10	0	80	60
	5-3	14	100	0	93	0	0	100	7
	6-1	11	91	18	45	9	18	73	36
	6-5	11	100	0	64	0	9	100	27
	6-10	11	91	27	9	0	27	73	64
	12-3	12	92	25	30	0	17	75	50
	12-11	10	90	20	50	0	10	80	40
	12-19	11	91	9	55	0	0	91	45
	14-2	10	100	0	10	0	10	100	80
	14-4	10	100	30	40	10	30	60	30
	14-9	21	95	10	24	5	0	86	76
	19-1	10	100	0	30	0	0	100	70
	19-3	11	100	0	18	0	0	100	82
	19-5	11	100	18	18	18	9	64	73
	Average			96	11	36	4	10	85

Generation	Plant Number	Cells analyzed	Aneuploid cells (%)	Cells with expected At chromosome number (%)	Cells with expected Aa chromosome number (%)	At hyperploid** chromosomes (%)	Aa hyperploid** chromosomes (%)	At hypoploid chromosomes (%)	Aa hypoploid chromosomes (%)	
F6	2-8-3	1	100	0	100	0	0	100	100	
	2-8-5	9	44	78	78	0	0	33	22	
	5-1-1-1 5	25	96	25	72	0	0	96	68	
	5-1-1-1 4	25	100	25	88	0	0	96	12	
	5-1-6-1	26	100	26	35	0	0	65	58	
	5-1-6-2	6	100	6	33	0	0	100	50	
	5-1-6-4	13	100	13	77	0	8	100	15	
	6-1-2	8	50	63	75	13	13	25	13	
	6-1-6	7	71	29	71	0	0	71	43	
	6-1-8	7	100	0	86	100	0	0	14	
	6-1-9	4	50	50	100	50	0	0	0	
	12-19-1	10	100	0	100	0	0	20	0	
	12-19-9	1	0	100	100	0	0	0	0	
	14-4-3	2	100	0	100	0	0	100	0	
	14-4-4	7	100	0	0	0	0	100	100	
	19-1-4	7	14	86	100	0	0	14	0	
	19-1-9	6	100	0	100	0	0	100	0	
	19-1-10	7	86	14	100	71	0	14	0	
	Average			78	29	79	13	1	58	27

Generation	Plant Number	Cells analyzed	Aneuploid cells (%)	Cells with expected At chromosome number (%)	Cells with expected Aa chromosome number (%)	At hyperploid** chromosomes (%)	Aa hyperploid** chromosomes (%)	At hypoploid chromosomes (%)	Aa hypoploid chromosomes (%)
F7	6-1-2-1	7	100	7	14	0	86	100	0
	6-1-2-2	24	100	13	4	88	0	0	96
	6-1-2-3	26	100	0	81	100	8	0	12
	6-1-2-4	6	50	50	83	0	0	50	17
	6-1-6-2	11	18	82	100	0	0	18	0
	6-1-6-4	4	100	0	75	75	0	25	25
	6-1-8-1	38	89	38	50	3	0	74	50
	6-1-8-2	9	100	11	11	11	11	78	78
	6-1-8-3	13	92	15	46	62	0	23	54
	6-1-8-4	28	93	7	68	71	0	18	32
	6-1-9-1	28	96	7	18	0	0	93	89
	6-1-9-3	13	92	31	38	15	8	54	54
	6-1-9-4	11	100	0	55	0	0	100	73
	12-1-9-4	38	95	8	16	0	0	92	84
	12-3-5-1	9	89	9	45	0	0	89	44
	12-19-1-1	33	82	24	33	3	6	73	61

Generation	Plant Number	Cells analyzed	Aneuploid cells (%)	Cells with expected At chromosome number (%)	Cells with expected Aa chromosome number (%)	Cells with At hyperploid** chromosomes (%)	Cells with Aa hyperploid** chromosomes (%)	Cells with At hypoploid chromosomes (%)	Cells with Aa hypoploid chromosomes (%)
F7	12-19-1-2	27	89	26	63	0	4	74	33
	12-19-1-4	15	93	7	87	0	0	93	87
	12-19-1-5	11	100	0	9	0	0	100	91
	12-19-9-1	7	29	86	86	14	0	0	14
	14-3-3-1	17	100	17	12	0	6	94	82
	14-4-4-1	25	100	25	4	0	0	100	96
	19-1-9-1	35	100	35	11	0	6	100	83
	19-1-9-2	9	100	9	22	0	0	100	78
	19-1-9-3	26	100	27	22	0	4	96	70
	19-1-9-4	28	82	18	43	0	0	79	57
	19-1-9-5	20	100	20	30	0	0	100	70
	19-1-9-6	20	100	20	10	0	5	100	85
	19-1-9-7	17	100	17	6	0	6	76	88
	19-1-9-9	15	100	15	33	0	0	93	67
	19-1-10-1	40	70	40	60	40	5	15	35
	19-1-10-2	16	62	16	50	0	0	63	50
	Average			89	24	34	3	2	80

* Expected chromosome number is: At - 20; Aa - 16

** Hyperploid chromosomes are those that exceed the expected number and hypoploid chromosomes are those that are fewer than the expected number

Table S2 Frequency of Aneuploidy in Allohexaploids over 3 Generations Based on Modal Chromosome Numbers.

Generation	Plant number	Cells analyzed	Modal chromosome		Aneuploid (%)
			numbers*		
			At	Aa	
F3	2-1	10	18	16	90
	2-5	10	18	16	90
	2-8	11	19	16	91
	5-1	10	17	16	100
	5-2	10	17	14	80
	5-3	14	19	16	64
	6-1	11	19	16	73
	6-5	11	16	16	73
	6-10	11	16	13	91
	12-3	12	17	16	75
	12-11	10	19	16	70
	12-19	11	19	16	64
	14-2	10	16	15	80
	14-4	10	20	16	100
	14-9	21	17	15	86
	19-1	10	19	15	80
	19-3	11	19	14	91
	19-5	11	19	16	82
		Average			
F6	2-8-3	1	19	16	0
	2-8-5	9	20	16	56
	5-1-1-1 5p **	26	17	16	36
	5-1-1-1 4p**	25	17	16	16
	5-1-6-1	26	16	16	77
	5-1-6-2	6	13	14	100
	5-1-6-4	13	15	16	31
	6-1-2	8	20	16	50
	6-1-6	7	20	16	100
	6-1-8	8	23	16	38
	6-1-9	4	20	16	50
	12-19-1	10	19	16	20
	12-19-9	1	20	16	0

14-4-3	2	18	16	0
14-4-4	7	17	15	71
19-1-4	7	20	16	43
19-1-9	6	19	16	17
19-1-10	7	21	16	43
Average				41

Generation	Plant number	Cells analyzed	Modal chromosome numbers		Aneuploid (%)
			At	Aa	
F7	6-1-2-1	7	18	17	57
	6-1-2-2	24	21	15	29
	6-1-2-3	26	21	16	58
	6-1-2-4	6	20	16	50
	6-1-6-2	11	20	16	18
	6-1-6-4	4	21	16	50
	6-1-8-1	38	16	16	84
	6-1-8-2	9	18	11	89
	6-1-8-3	13	21	16	85
	6-1-8-4	28	23	16	46
	6-1-9-1	28	18	14	93
	6-1-9-3	13	20	16	92
	6-1-9-4	11	19	15	82
	12-1-9-4	38	17	14	95
	12-3-5-1	9	19	16	78
	12-19-1-1	33	18	16	91
	12-19-1-2	27	17	16	81
	12-19-1-4	15	19	16	27
	12-19-1-5	11	19	15	27
	12-19-9-1	7	20	16	29
	14-3-3-1	17	16	14	88
	14-4-4-1	25	16	15	76
	19-1-9-1	35	16	14	92
	19-1-9-2	9	14	12	67
	19-1-9-3	26	16	15	77
	19-1-9-4	28	16	16	82
	19-1-9-5	20	16	16	80

19-1-9-6	20	16	12	90
19-1-9-7	17	18	15	76
19-1-9-9	15	17	16	73
19-1-10-1	40	20	16	70
19-1-10-2	16	20	16	69
Average				69

* Modal chromosome numbers were determined as the most common chromosome number for *At* and *Aa* individually for each plant.

** 4p and 5p denotes cells from different floral clusters of the same plant that either produced 5-petaled or 4-petaled flowers.

Table S3 Complete FISH Data Set

Generation	Plant Number	Cell Number	At	Aa	Total Number
F3	2-1	1	18	15	33
		2	22	21	43
		3	17	14	31
		4	14	13	27
		5	22	20	42
		6	19	12	31
		7	16	16	32
		8	18	15	33
		9	18	16	34
		10	17	16	33
	2-5	1	18	18	36
		2	19	14	33
		3	18	15	33
		4	18	15	33
		5	17	13	30
		6	20	16	36
		7	18	16	34
		8	19	16	35
		9	20	15	35
		10	16	16	32
	2-8	1	19	17	36
		2	18	13	31
		3	19	16	35
		4	15	15	30
		5	19	15	34
		6	17	16	33
		7	16	13	29
		8	17	18	35
		9	16	18	34
		10	11	13	24
		11	18	16	34
	5-1	1	17	15	32
		2	17	12	29
		3	18	16	34
		4	16	14	30
		5	17	12	29

	6	20	16	36
	7	19	14	33
	8	16	11	27
	9	18	15	33
	10	19	16	35
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	2	17	12	29
	3	17	14	31
	4	23	16	39
	5	20	16	36
	6	13	14	27
	7	18	14	32
	8	17	16	33
	9	16	14	30
	10	17	14	31
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	2	19	16	35
	3	19	16	35
	4	18	16	34
	5	17	16	33
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	7	19	16	35
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	10	17	16	33
	11	19	16	35
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	7	18	12	30
	8	19	15	34
	9	19	17	36
	10	18	16	34

	11	20	17	37
6-5	1	14	14	28
	2	18	16	34
	3	15	17	32
	4	15	16	31
	5	13	16	29
	6	17	15	32
	7	16	16	32
	8	17	16	33
	9	15	15	30
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	11	16	16	32
6-10	1	20	13	33
	2	19	14	33
	3	19	17	36
	4	20	16	36
	5	16	12	28
	6	18	15	33
	7	15	17	32
	8	20	17	37
	9	16	15	31
	10	16	13	29
	11	19	13	32
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	10	17	17	34
	11	17	16	33
	12	20	15	35
12-11	1	18	18	36
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	3	20	15	35
	4	20	16	36

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	8	17	15	32
	9	18	14	32
	10	17	16	33
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	10	18	15	33
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	2	19	16	35

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	7	20	14	34
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	4	19	12	31
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	7	15	15	30
	8	15	13	28
	9	19	14	33
	10	19	16	35

		11	17	15	32
	19-5	1	21	25	46
		2	19	16	35
		3	16	15	31
		4	19	16	35
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		10	17	15	32
		11	21	15	36
F6	2-8-3	1	19	16	35
	2-8-5	1	17	14	31
		2	19	16	35
		3	20	16	36
		4	20	16	36
		5	20	16	36
		6	20	16	36
		7	20	15	35
		8	20	16	36
		9	18	16	34
	5-1-1-1 5p	1	17	15	32
		2	17	16	33
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		6	17	16	33
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	24	19	15	34
	25	14	10	24
	26	13	10	23
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	4	13	14	27
	5	14	16	30

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7	15	12	27	
8	16	15	31	
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10	14	14	28	
11	16	12	28	
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18	14	13	27	
19	14	12	26	
20	16	14	30	
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22	15	17	32	
23	16	16	32	
24	16	16	32	
25	16	16	32	
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	2	16	14	30
	3	13	17	30
	4	13	12	25
	5	14	16	30
	6	14	14	28
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5-1-6-4	1	14	14	28
	2	15	16	31
	3	14	14	28
	4	15	17	32
	5	15	16	31
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	7	15	16	31
	8	15	16	31
	9	16	16	32
	10	15	16	31
	11	15	16	31
	12	15	16	31

	13	15	16	31
6-1-2	1	21	15	36
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	3	19	16	35
	4	20	16	36
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	4	23	16	39
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