Table S1. List of probes and their chromosomal positions, including barcode[1], BAC clones[1], and 5S and 35S rDNA[1,2], tested and used for comparative FISH mapping among *V. unguiculata, V. lasiocarpa* and *V. longifolia*, organized from the terminal region of the short arm to the terminal region of the long arm. These probe sets were used to characterize the *V. lasiocarpa* karyotype and to identify chromosomal rearrangements and homoeology with *V. unguiculata* and *V. longifolia*. Probes positions on *V. longifolia* chromosomes are based on Dias et al. [27]. No BACs probes were hybridized to the chromosomes of this species. Hashtags (#) on *Vl*2, *Vla*3, *Vla*4, *Vla*6, *Vl*6 and *Vla*9 indicate changes in chromosome arm orientation compared to *V. unguiculata*.

Chr	Probe/chromosomal position (arm)		
	V. unguiculata	***V. lasiocarpa***[a]	***V. longifolia***[a]
1	35S rDNA/subterminal (S) Red signal/interstitial (S) Green signal/interstitial (L) H004H23*/subterminal (L) Green signal/subterminal (L)	Green signal/subterminal (S) H004H23*/subterminal (S) Green signal/interstitial (S) M50F11*/ interstitial (L) Green signal/subterminal (L)	Green signal/subterminal (S) Green signal/interstitial (S) Green signal/subterminal (L)
2	35S rDNA/subterminal (S) Red signal/interstitial (S) Green signal/interstitial (L) Red signal/subterminal (L)	Red signal/interstitial (S) Green signal/interstitial (L) Red signal/subterminal (L)	Red signal/subterminal (L)# 35S rDNA/proximal (L) Green signal/interstitial (S) Red signal/subterminal (S)
3	Green signal/subterminal (S) Green signal/interstitial (S) 142D9**/ interstitial (L) Red signal/interstitial (L) H050P11*/ interstitial (L) Red signal/interstitial (L) 199D13**/interstitial (L) Red signal/subterminal (L)	Green signal/subterminal (L)# Green signal/interstitial (L) 142D9**/interstitial (L) Red signal/interstitial (L) Red signal/interstitial (S) 199D13**/interstitial (S) Red signal/interstitial (S) H050P11*/ subterminal (S)	Green signal/subterminal (S) Green signal/interstitial (S) Red signal/interstitial (L) Red signal/interstitial (L) Red signal/subterminal (L)
4	Green signal/subterminal (S) Green signal/interstitial (S) Red signal/interstitial (L) Red signal/interstitial (L)	Green signal/interstitial (L)# Red signal/interstitial (L) Green signal/interstitial (S) Red signal/interstitial (S)	Green signal/subterminal (S) Red signal/interstitial (S) Green signal/interstitial (L) Red signal/interstitial (L)
5	Green signal/subterminal (S) Green signal/interstitial (S) M50F11*/ interstitial (L) Green signal/subterminal (L)		Red signal/interstitial (S) Green signal/interstitial (L) Green signal/ interstitial (L)
6	Red signal/interstitial (S) 35S rDNA/subterminal (L)	Red signal/subterminal (L)#	35S rDNA/subterminal (S)# Red signal/subterminal (L)
7	H088A15*/subterminal (S) Red signal/interstitial (L) Red signal/interstitial (L)		Red signal/proximal (L) Red signal/subterminal (L)
8	Green signal/ interstitial (S) Red signal/interstitial (L)	Green signal/ interstitial (S) Red signal/interstitial (L)	Green signal/ interstitial (S) Red signal/interstitial (L)

9	Red signal/interstitial (S) 35S rDNA /proximal (L) Red signal/interstitial (L) H10M18*/subterminal (L)	Red signal/interstitial (L)# Red signal/interstitial (L) H10M18*/subterminal (S)	Red signal/interstitial (S) Red signal/proximal (L)
10	5S rDNA/ interstitial (S) Green signal/ interstitial (L) Green signal/ interstitial (L) 35S rDNA/subterminal (L)	35S rDNA/proximal (L) 5S rDNA/subterminal (L) Green signal/ interstitial (L) Green signal/ interstitial (L)	5S rDNA/proximal (L) Green signal/ interstitial (L) Green signal/ interstitial (L)
11	Green signal/subterminal (S) 35S rDNA/interstitial (S) 5S rDNA/interstitial (S) Red signal/ interstitial (L) Red signal/subterminal (L)	Green signal/subterminal (S) Red signal/ interstitial (L) Red signal/ interstitial (L)	Green signal/subterminal (S) Red signal/ interstitial (L) Red signal/subterminal (L)
*Vla*7/5		Red signal/ interstitial (S) Red signal/ interstitial (L) H088A15*/interstitial (L) Red signal/ interstitial (L) Green signal/ interstitial (L) Green signal/subterminal (L)	

[1] Barcode, BACs and rDNA marks of *V. unguiculata* followed de Oliveira Bustamante et al. [26].

[2] rDNA marks of *V. longifolia* followed Dias et al. [27]

[a] Chromosomes of *V. lasiocarpa* and *V. longifolia* are numbered according to their homoeology with *V. unguiculata*.

* BACs from *V. unguiculata* chromosomes.

** BACs from *Phaseolus vulgaris* chromosomes.

L = long arm; S = short arm.

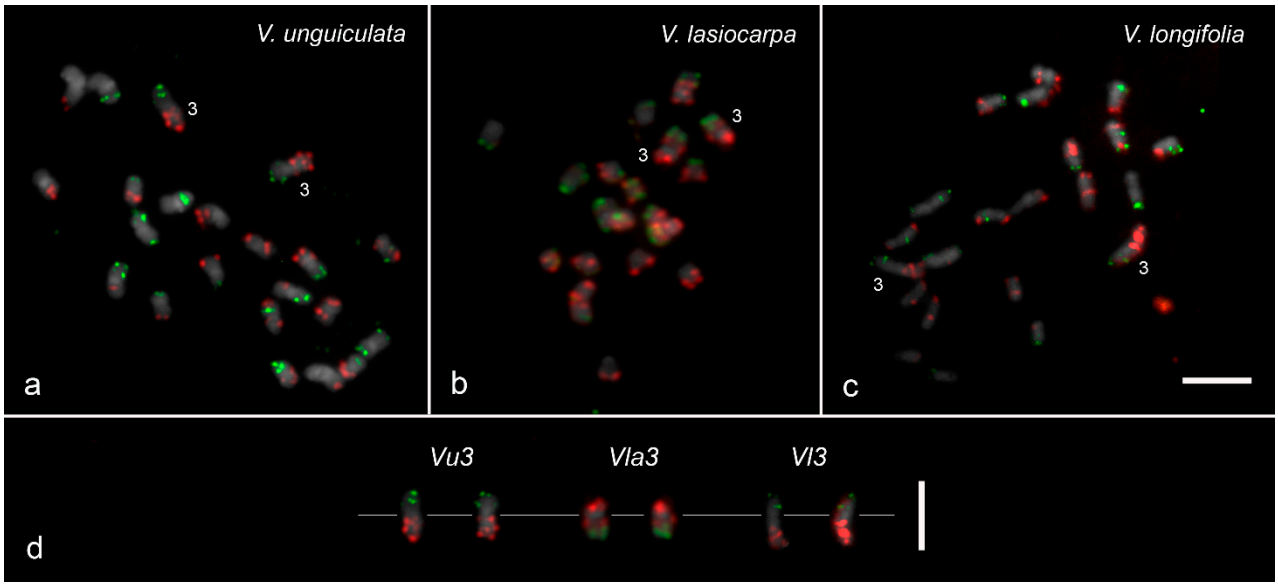


Figure S1. Oligobarcode probes (red and green) hybridized to metaphase chromosomes of *Vigna unguiculata* (**a**, *Vu*, *2n* = 22), *V. lasiocarpa* (**b**, *Vla*, *2n* = 20), and *V. longifolia* (**c**, *Vl*, *2n* = 22). (**d**) Karyogram showing the barcode pattern on chromosome 3 of the three species, with the centromeres aligned along a thin white line. All chromosomes were counter-stained with DAPI (pseudocolored in gray). Bars in (**c**) and (**d**) = 5 μm.