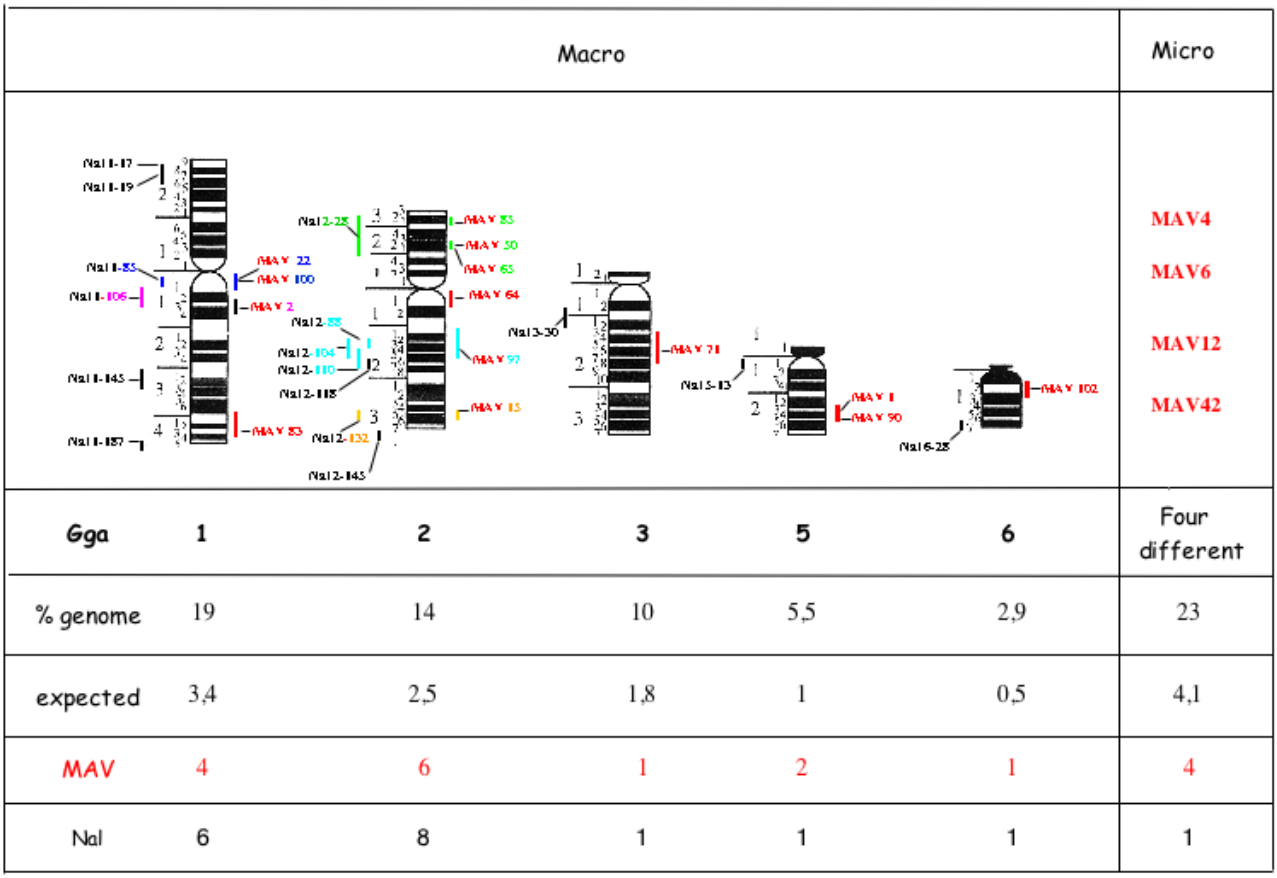

Gga	1	2	3	5	6	Four different
% genome	19	14	10	5,5	2,9	23
expected	3,4	2,5	1,8	1	0,5	4,1
MAV	4	6	1	2	1	4
Nal	6	8	1	1	1	1

Figure 3
Distribution of positive BACs on chicken chromosomes.

Isolation of polyadenylated RNA from normal kidneys and nephroblastomas

Frozen tissues were homogenized with a polytron and 0.5 g of powder was resuspended in 9 ml guanidine thiocyanate buffer for purification of total RNA as previously described [21]. Final RNA pellets were resuspended in 400 µl sterile distilled water and the concentration of each sample was determined by densitometry. To isolate polyadenylated RNA species, each sample (1 mg total RNA in 500 µl water) was mixed with 55 µl Oligitex Suspension (Qiagen) and incubated for 3 min at 70 min in a water bath. After 10 min at room temperature the Oligotex:mRNA complex was pelleted by 2 min centrifugation at 14000–18000 g and the supernatant carefully removed. The pellet was further treated as recommended by the supplier and the polyadenylated RNA fraction was collected in a final volume of 50 µl.

Labelling of polyadenylated RNA preparations

To prepare labelled RNA probes, 500 ng of each polyA-RNA preparation were mixed with 500 ng oligo dT, incubated for 10 min at 70°C and chilled on ice for 5 min. Samples were then mixed with 5 µl of 10 × PCR buffer, 5 µl of MgCl2 25 mM, 5 µl DTT 0.1 M, 2.5 µl mixture of dTTP, dATP, dGTP(10 mM each), 2.5 µl of ddTTP(1 mM), 5 µl of ^{32}P-dCTP, and incubated for 5 min at 25°C. After addition of 1 µl of reverse transcriptase (Invitrogen) (200 U/ µl), the mix was incubated for 10 more min at 25°C and for 50 min at 42°C. The reaction was stoped by incubation at 70°C for 15 min. Each labelled preparation was purified by chromatography through a column of Sephadex G50.

Hybridization of BAC DNA filters

The blots were rinsed with 6 × SSC, and prehybridized at 68°C for 18 hours. After hybridization with labeled probe in the presence of COT I DNA the blots were washed with 2 × SSC, 0.1%SDS at 56°C for 1 hour and with: 0.1 × SSC, 0.1%SDS at 65°C for 1 more hour. Autoradiography of the dried blot was performed at -80°C.