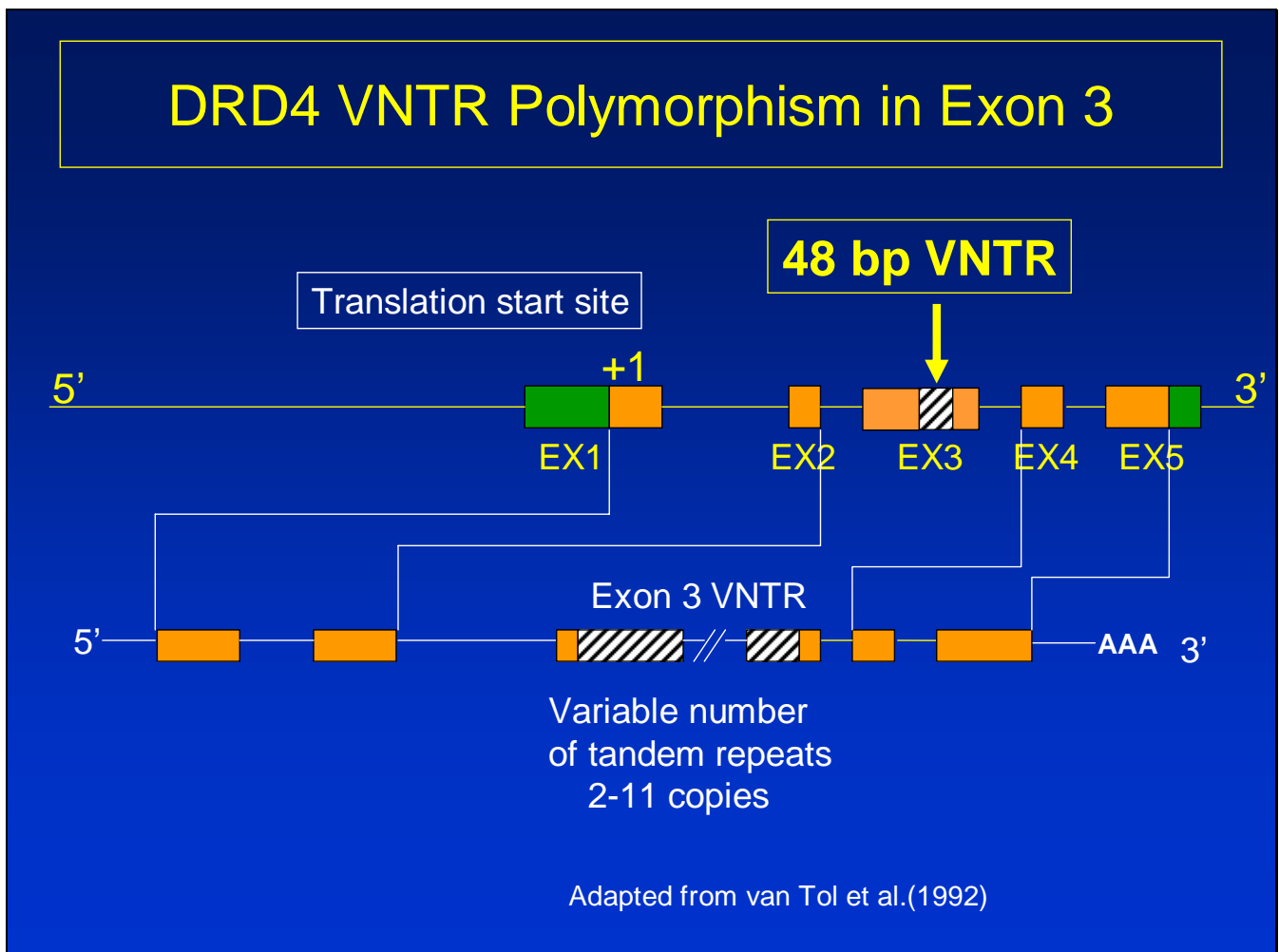


DRD4 Exon 3 48 bp VNTR

The dopamine D4 receptor (DRD4). The D4 receptor gene, which maps to 11p15.5, contains a 48 bp Variable Number Tandem Repeat (VNTR) polymorphism in the third exon (van Tol et al., 1992), which results in considerable heterogeneity (ten allelic products comprised of from 2-11 repeats). The 48-bp repeat is thought to reside in the third cytoplasmic loop of the receptor protein and this variation has been shown to affect the function of the D4 receptor in vivo: With respect to ^3H -spiperone binding, the seven repeat variant receptor was not affected by NaCl concentration, whereas the smaller repeat variants were (Van Tol et al., 1992). The most common alleles consist of four repeats (4R) and seven repeats (7R). It was suggested that there may also be variation in G-protein interactions among the different forms of the receptors.



The assay we use (Anchordoquy et al, 2003) is a modification of the method of Sander *et al.* (1997). The primer sequences are from Lichter et al., 1993:

Forward: 5'-HEX-AGG ACC CTC ATG GCC TTG-3',
Reverse: 5'-GCG ACT ACG TGG TCT ACT CG-3'.

DRD4 PCR Master Mix for 20 μ L reactions

(18 μ L Master mix + 2 μ L DNA)

Component	1	100	Concentration of component in:		
	Tube vol (μ L)	Tubes vol (μ L)	Stock	Master Mix	PCR
Water	9.6	960			
DMSO	2.0	200	100%	10.9 %	10%
10x buffer	2.0	200	10 x	0.109 x	1 x
MgCl ₂	1.6	160	25 mM	2.18mM	2.00 mM
dNTP+deazaGTP	2.0	200	2 mM (ea)	218 μ M	200 μ M (ea)
Forward	0.5	50	10 μ M	270 μ M	245 μ M
Reverse	0.5	50	10 μ M	270 μ M	245 μ M
AmpliTaq Gold®	0.2	20	5 Units/ μ L	1 Unit	1.0 Units
Total volume (μ L)	18.4	1840			

dNTPs + 7-deaza-2-deoxy GTP

Component	volume (μ L)	Concentration (mM)	
		Stock	Final
dATP	40	100	2
dTTP	40	100	2
dCTP	40	100	2
dGTP	20	100	1
deazaGTP	200	10	1
Water	1660		

DRD4 PCR Setup

Mastermix	18 μ L
DNA	1-2 μ L (20 ng or less)
Water	0-1 μ L
Total volume	20 μ L

DRD4 Touchdown PCR Cycling

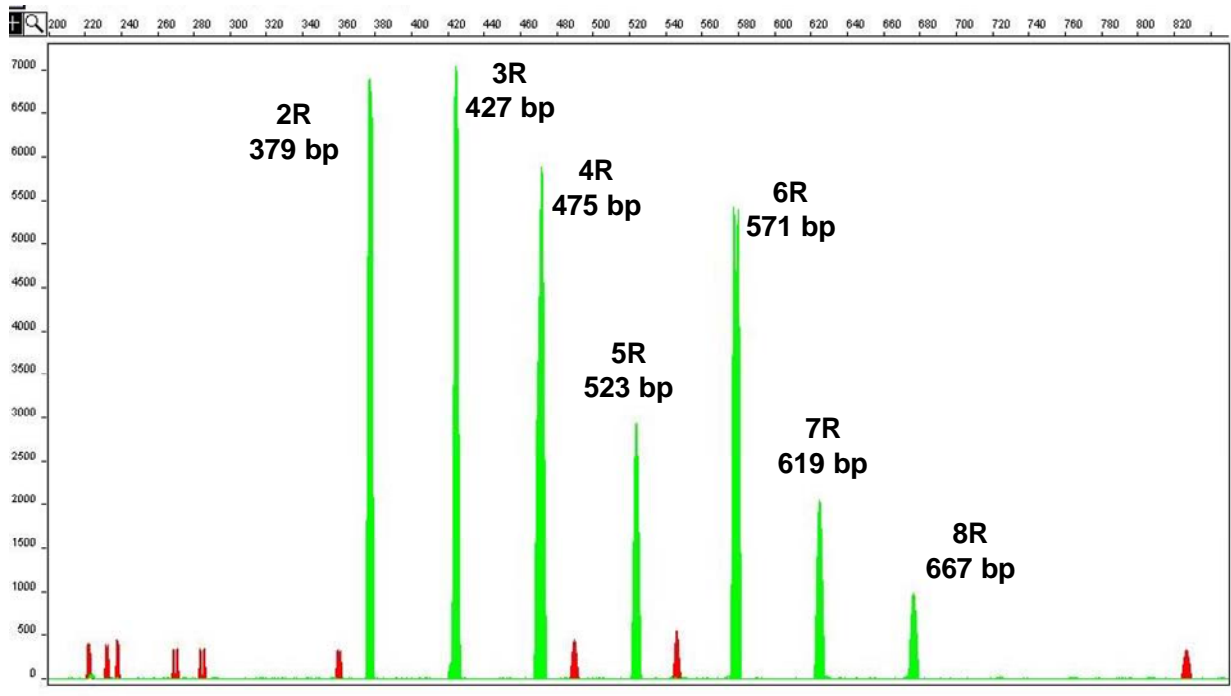
1x	95 °C	10 min			
2x	94 °C	30 sec	65 °C	30 sec	72 °C 60 sec
2x	94 °C	30 sec	63 °C	30 sec	72 °C 60 sec
2x	94 °C	30 sec	61 °C	30 sec	72 °C 60 sec
2x	94 °C	30 sec	59 °C	30 sec	72 °C 60 sec
2x	94 °C	30 sec	57 °C	30 sec	72 °C 60 sec
30x	94 °C	30 sec	55 °C	30 sec	72 °C 60 sec
1x	72 °C	30 min			
	4 °C	hold			

DRD4 Electrophoresis

2 µL PCR product
20 µL Hi-Di formamide
0.5 µL Genescan 2500 Rox

Samples are analyzed on an ABI PRISM® 3100 Genetic Analyzer using standard company protocols without modification

DRD4 Exon-3 48 bp VNTR



DRD4. The figure above is reproduced from a run from an ABI PRISM® 3100 Genetic Analyzer. The amplicons are labelled with both the number of tandem repeats and the size in base pairs. The sizes given are those calculated from the DNA sequence, but in actual runs the size of the amplicon sizes calculated by the software are usually 3-4 bp greater than expected. The figure above shows seven alleles that have approximately equal peak heights. In practice; however, the peak heights of the 7R and 8R alleles are generally found to be quite a bit smaller. The red peaks are size standards (Genescan ROX 2500).

The table below lists the frequencies of the ten possible alleles in approximately 1000 subjects taken from the National Youth Survey Family Study.

Amplicon Size	#VNTR repeats	Frequency
379	2	.09
427	3	.04
475	4	.65
523	5	.02
571	6	.01
619	7	.18
667	8	.01
715	9	<.01
763	10	<.01
811	11	<.01

Notes:

For consistent results with this primer set the use of 10% DMSO and 7-deaza-2-deoxy GTP (Roche Applied Science, Indianapolis, IN) is essential.

Use a very good grade of DMSO. We use Sigma's Hybra-Max® grade or that supplied with New England Biolab's Phusion™ buffers.

We use touchdown PCR (Don et al, 1992) routinely as a simple short cut. It cuts down on the need to optimize annealing conditions for multiple primer sets when you want to do several loci in the same thermocycler.

The amplicons are large (370 to 811 bp). That requires the ROX 2500 size standard. ABI does not recommend it, but there really is no other choice. With the "large fragment enabler" added to GeneScan, it works.

Citation: When reporting results for this locus, please cite *Anchordoquy et al, 2003* as the analytical method used for genotyping.

References:

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