Section IV. Descriptive Information

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Section IV. Descriptive Information

A. Product Description

VLN™ Kings and Menthol Kings are conventional cigarettes. The products will be manufactured at the Company's plant in Mocksville, NC. The relevant plant information is listed below:

Facility Name: NASCO Products, LLC,

Facility Address: 321 Farmington Rd, Mocksville, NC 27028

Facility Phone Number: 1-336-940-3769

Plant Manager: (b) (6)

Plant Manger Phone Number: (b) (6)

Plant Manager E-mail Address: (b) (6)

A detailed description of the manufacturing process and location is found in this section.

Figure IV.A-1. *Diagram of VLN™ Cigarette*, shows a diagram of the a VLN™ cigarette. The products use a dual filter. The mouth end is a cellulose acetate filter and the section closest to the tobacco is a paper filter. Figure IV.A-2. *Filter Diagram*. shows a diagram of the filter. The various parts of the product are identified in the product Formulation Description below. The VLN™ Menthol King has a (b) (4)

Figure IV.A-1. Diagram of VLN™ Cigarette

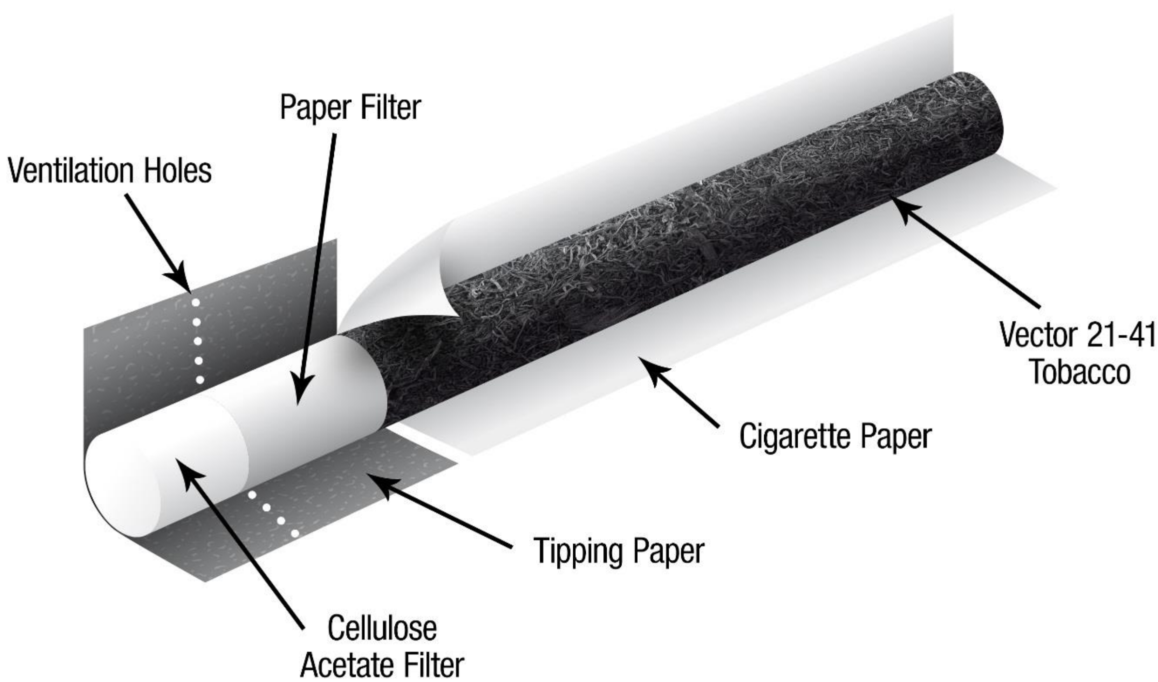
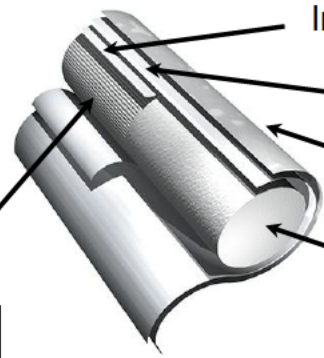


Figure IV.A-2. Filter Diagram.



B. Product Formulation

1. Listing of Design Features

This is a conventional fire safe compliant cigarette product. The design features are listed in the Table IV.B-1. *Product Target Design Features*, below.

Table IV.B-1. Product Target Design Features

Design Feature	**VLN™ King**			**VLN™ Menthol King**		
	Target Specification	**Upper Limit**	**Lower Limit**	**Target Specification**	**Upper Limit**	**Lower Limit**
Cigarette Weight (mg/cig)	960	1010	860	970	1020	870
Cigarette Length (mm/cig)	83	84	82	83	84	82
Cigarette Diameter (mm/cig)	7.9	8.0	7.8	7.9	8.0	7.8
Ventilation (%)	13	20	6	13	20	6
Cigarette Pressure Drop (mm H_2O)	120	130	110	120	130	110
Filter Weight (mg)	195	205	185	203	213	193
Filter Length (mm)	24.5	24.7	24.3	24.5	24.7	24.3
Resistance to Draw (full filter)	325	367	288	325	367	288

Design Feature	VLN™ King			VLN™ Menthol King		
	Target Specification	Upper Limit	Lower Limit	Target Specification	Upper Limit	Lower Limit
CA Filter Denier per filament (g/9000m)	3.9	-	-	3.9	-	-
CA Filter Total Denier (g/9000m)	30,000	-	-	30,000	-	-
CA Filter Density (mg/mm^3 per tip)	0.107	-	-	0.107	-	-
Plug wrap Porosity (CU)	23840	28665	19320	23840	28665	19320
Filter Efficiency (%)	60.7	65	56	63.3	68	58
Cigarette Draw Resistance (mm H_20)	91.5	103	83	91.5	103	83
Tobacco Filler Mass (mg/cig)	686	723	600	688	736	600
Tobacco Rod Density (mg/cm^3)	0.24	0.25	0.22	0.24	0.25	0.22
Tobacco Rod Length (mm/cig)	58	59	57	58	59	57
Tobacco Cut Width (mm)	0.8	0.875	0.725	0.8	0.875	0.725
Tobacco Oven Volatiles (%)	14	15	13	14	15	13
Tipping Paper Length (mm/cig)	30	32	28	30	32	28
Tipping Paper Porosity (CU)	100	118	82	100	118	82
Cigarette Paper Length (mm)	58	58.5	57.5	58	58.5	57.5
Cigarette Paper Base Paper Basis Weight (g/m^2)	27.5	-	-	27.5	-	-
Cigarette Paper Base Paper Porosity (CU)	80	-	-	80	-	-
Cigarette Paper Band Diffusion (cm/s)	0.045	-	-	0.045	-	-
Cigarette Paper Band Width (mm)	6	-	-	6	-	-

Design Feature	VLN™ King			VLN™ Menthol King		
	Target Specification	Upper Limit	Lower Limit	Target Specification	Upper Limit	Lower Limit
Cigarette Paper FSC Banded Space (mm)	18	-	-	18	-	-
Burn Rate[1] (mm/min)	6.16	-	-	6.20	-	-

2. Listing of Materials

The materials used to manufacture the VLN™ products are listed in Table IV.B-2. *Listing of ingredients used to produce VLN™ cigarettes.*, below. The same materials are used to make both VLN™ products, except that (b) (4) mentholated product.

Table IV.B-2. Listing of ingredients used to produce VLN™ cigarettes.

	VLN™ King			VLN™ Menthol King		
Material	**Material Identification *(Internal #)***	**Supplier**	**Weight per cigarette (mg)**	**Material Identification *(Internal #)***	**Supplier**	**Weight per cigarette (mg)**
Tobacco Blend	(b) (4)		686	(b) (4)		688
Tipping Paper			29			29
Tipping Adhesive			1.5			1.5
Cigarette Paper			47			47
Side Seam Adhesive			1			1
Filter Plug			195			203
			960			**970**

- (b) (4) .

[1] (b) (4)

(b) (4)

3. Confidential Listing of Ingredients

The amounts of chemicals in various part types and flavor mixtures are determined by taking the supplier disclosed percentages and multiplying by the target specification weight of the component or material. In some cases, for example in the filter, the supplier discloses the percentage of each component it uses to make the filter. Each component supplier to the filter supplier then discloses the amount of chemicals in their material. Multiplication of these values results in numbers with significant figures that are probably not representative of the real product. These numbers with significant digits are representative of the theoretical amount of the chemical based off of target levels. Significant digits are provided to make the total weights of the components add up to target weights.

i. *Nicotine in Filler Specification*

Tobacco is an agricultural commodity. It is well known that the following variables can affect nicotine production in the plant:

- The tobacco type and varieties
- The soil types found in each field and geographic region

- Climate conditions associated with different latitudes
- The particular weather events faced by each tobacco field
- Management practices, including fertilizers and soil amendments
- The presence (or absence) of any insect infestation
- The specific stage of maturity at harvest
- Curing and weather conditions.

VLN™ tobacco is subject to these agricultural variables and this affects the amount of nicotine in the plant. VLN™ cigarettes are made with a blend of Vector 21-41 very low alkaloid tobacco. The components of the blend derived from Vector 21-41 tobacco leaf are approximately (b) (4). The blend also contains flavorings and humectants. The same tobacco blend, (b) (4), is used in SPECTRUM and VLN™ cigarettes. Analysis of the (b) (4) blend has been conducted since 2011. (b) (4) (b) (4) (b) (4) (b) (4) (b) (4). Table IV.B-3. *Summary of Nicotine levels in* (b) (4) *blend over the period 2011 to 2018.* shows a summary of the nicotine results. (b) (4) (b) (4) (b) (4). Table IV.B-4. *Nicotine in* (b) (4) *Blend by Case/batch.* is a listing of the results of these analyses. (b) (4) (b) (4). The results are shown in Table IV.B-5. *Comparison of Results - Enthalpy Analytical vs. Global Laboratories*.. There was essentially no difference in the results.

Table IV.B-3. Summary of Nicotine levels in (b) (4).

		Nicotine "As Is" (mg/g)			Nicotine "Dry Weight" (mg/g)		
Year	Laboratory	Average	Low	High	Average	Low	High
(b) (4)							
	Overall Average	(b) (4)					
	Range						

* (b) (4) [redacted].

** (b) (4) [redacted].

Table IV.B-4. Nicotine in (b) (4).

		Nicotine "As Is" Basis				Nicotine "Dry Weight" Basis				
Year	Batch/Case	Average	Low	High	N	Average	Low	High	N	Reference
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
	2018 Enthalpy Average	(b) (4)				(b) (4)				
	Range		(b) (4)	(b) (4)			(b) (4)	(b) (4)		

		Nicotine "As Is" Basis				Nicotine "Dry Weight" Basis				
Year	**Batch/Case**	**Average**	**Low**	**High**	**N**	**Average**	**Low**	**High**	**N**	**Reference**
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
	2018 Global Average	(b) (4)				(b) (4)				(Global Laboratory Services 2018 3-14-2018) [pg 75]
	Range		(b) (4)8	(b) (4)			(b) (4)	(b) (4)		
2017	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]

		Nicotine "As Is" Basis				Nicotine "Dry Weight" Basis				
Year	**Batch/Case**	**Average**	**Low**	**High**	**N**	**Average**	**Low**	**High**	**N**	**Reference**
2017	(b) (4) [redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4) [redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4) [redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
2017	(b) (4) [redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2017 10-26-2017) [pg 75]
	2017 Global Average	(b) (4)				(b) (4)				
	Range		(b) (4)	(b) (4)			(b) (4)	(b) (4)		
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
2015	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2015 11-10-2015) [pg 75]
	2015 Global Average	(b) (4)				(b) (4)				
	Range		(b) (4) **1**	(b) (4)			(b) (4)	(b) (4) **8**		
2012	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	-	-	(Arista Laboratories 2012 ProjCode 12137) [pg 74]
2012	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Arista Laboratories 2012 ProjCode 12137) [pg 74]
2012	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Arista Laboratories 2012 ProjCode 12137) [pg 74]
2012	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Arista Laboratories 2012 ProjCode 12137) [pg 74]
	2012 Arista Average	(b) (4)								
	Range		(b) (4)	(b) (4)						
2011	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	-	-	-	(Arista Laboratories 2011a ProjCode 11112) [pg 74]
2011	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	-	-	-	-	(Arista Laboratories 2011a ProjCode 11112) [pg 74]
2011	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	-	-	-	-	(Arista Laboratories 2011a ProjCode 11112) [pg 74]

		Nicotine "As Is" Basis				Nicotine "Dry Weight" Basis				
Year	**Batch/Case**	**Average**	**Low**	**High**	**N**	**Average**	**Low**	**High**	**N**	**Reference**
2011	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	-	-	-	-	(Arista Laboratories 2011a ProjCode 11112) [pg 74]
	2011 Arista Average	(b) (4)								
	Range		(b) (4)	(b) (4)						

Table IV.B-5. Comparison of Results - Enthalpy Analytical vs. Global Laboratories.

		Nicotine "As Is" Basis				Nicotine "Dry Weight" Basis				
Year	**Batch/Case**	**Average**	**Low**	**High**	**N**	**Average**	**Low**	**High**	**N**	**Reference**
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	3	(Enthalpy Analytical Project Code 0918-512B) [pg 75]
	2018(b) (4) **Average**	(b) (4)				(b) (4)				
	Range		(b) (4)	(b) (4)			(b) (4)	**0.55**		
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
2018	(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(Global Laboratory Services 2018 3-14-2018) [pg 75]
	2018 Global Average	(b) (4)				(b) (4)				
	Range		(b) (4)	(b) (4)			(b) (4)	(b) (4)		

As mentioned above, the (b) (4) blend is a mixture of (b) (4) (b) (4). (b) (4) (b) (4). Analysis has been performed on these components of the blend (Table IV.B-6. *Blend Component Analysis*).

Table IV.B-6. Blend Component Analysis

Year	Designation	Type	Nicotine (mg/g) "As Is"	Reference
2018	(b) (4)			(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 3-1-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]

2018	(b) (4)			(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2018				(Global Laboratory Services 2018 2-15-2018) [pg 75]
2015				(Global Laboratory 2015 11-10-2015) [pg 75]
2015	(b) (4)	(b) (4)	Below Quantitation Limit	(Global Laboratory 2015 11-10-2015) [pg 75]
2013	(b) (4)			(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013				(Arista Laboratories 2013 ProjCode 13291) [pg 74]
2013[2]				(Global Laboratory Services 06-2013) [pg 75]
2013				(Global Laboratory Services 06-2013) [pg 75]
2013				(Global Laboratory Services 06-2013) [pg 75]

[2] These (b) (4)

2013	(b) (4)	(Global Laboratory Services 06-2013) [pg 75]
2013		(Global Laboratory Services 06-2013) [pg 75]
2012		(Arista Laboratories 2012 ProjCode 12137) [pg 74]
2012		(Arista Laboratories 2012 ProjCode 12137) [pg 74]
2012		(Arista Laboratories 2012 ProjCode 12137) [pg 74]
2012		(Arista Laboratories 2012 ProjCode 12137) [pg 74]
2011		(Arista Laboratories 2011a ProjCode 11112) [pg 74]
2011		(Arista Laboratories 2011a ProjCode 11112) [pg 74]
2011		(Arista Laboratories 2011a ProjCode 11112) [pg 74]
2011		(Arista Laboratories 2011a ProjCode 11112) [pg 74]
2011		(Arista Laboratories 2011a ProjCode 11112) [pg 74]
2011		(Arista Laboratories 2011a ProjCode 11112) [pg 74]

(b) (4)

(b) (4)

ii. *Tobacco*

The amount of un-flavored tobacco in the VLN™ product (mg/product) is listed in Table IV.B-7. *Total amount of tobacco in the product*. below (without flavor ingredients). (b) (4) (b) (4). The regular product is made to a target weight of (b) (4) mg and the menthol product is made to a target weight of (b) (4) mg. The menthol product has 8 mg of menthol solution added to the filter. (b) (4) (b) (4) (b) (4). Table IV.B-8. *Tobacco leaf parts used in the products (un-flavored)*. shows the individual VLN™ tobacco leaf parts used in each VLN™ product. (b) (4) (b) (4).

Table IV.B-7. Total amount of tobacco in the products

Tobacco Type (curing method)	**VLN™ King** (mg/product)	**VLN™ Menthol King** (mg/product)
Vector 21-41 (b) (4))	(b) (4)	

Table IV.B-8. Tobacco leaf parts used in the products (un-flavored).

Tobacco Type	**% in Blend**	**VLN™ King** Target mg/cigarette	**VLN™ Menthol King** Target mg/cigarette
(b) (4)			
Total	(b) (4)		

The VLN™ tobacco is produced from the very low alkaloid variety identified as Vector 21-41. This variety was developed by using antisense RNA to specifically suppress expression of a gene encoding quinolate phosporibosyltransferase, an enzyme involved in nicotine biosynthesis in tobacco roots (Xie 2004) [pg 77]. Vector 21-41 does not express any novel agronomic trait, such as insect resistance or herbicide tolerance. The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has prepared an environmental assessment of tobacco line Vector 21-41 and granted a petition to designate this product as non-regulated (USDA 2002) [pg 77]. Except for the use of the tobacco variety Vector 21-41 and some measures taken to maintain segregation of the VLN™ tobacco, the production and processing are the same for VLN™ tobacco as for conventional tobacco. The processes are shown in Figure IV.B-1. *Flowchart for Processing of Tobacco into Filler Blend Components*, and Figure IV.B-2. *Flowchart for Production of Filler Blend*. The VLN™ tobacco is grown in the U.S. by 22nd Century under direct contracts with growers. (b) (4) (b) (4) (b) (4).

iii. (b) (4)

The Vector 21-41 (b) (4)s are prepared at (b) (4). following the procedures outlined in Figure IV.B-1. *Flowchart for Processing of Tobacco into Filler Blend Components*. The casing process consists of (b) (4) (b) (4) (b) (4) (b) (4) [redacted]. Table IV.B-9. [redacted] *Component*, lists the weights of the materials in the [redacted]. [redacted]. [redacted]. The same [redacted]s are used to making the regular and menthol version of the VLN™ products.

Table IV.B-9. [redacted] Components

Material	Supplier	VLN™ King mg/cig	VLN™ Menthol King mg/cig
[redacted]			
	Total	[redacted]	

The [redacted] is supplied by [redacted]. [redacted]. The formula for the casing is as shown in Table IV.B-10. *Ingredients in* [redacted], below. The [redacted] and thus the [redacted] are used for the regular and menthol versions of the VLN™ product.

Table IV.B-10. Ingredients in [redacted]

Material /Chemical Name	**Supplier**	**Function**	**Purity**	**CAS#**	**VLN™ King (mg/cig)**	**VLN™ Menthol King (mg/cig)**
[redacted]						
				TOTAL	**33**	**33**

The ingredients in the (b) (4) [redacted] provided by Tobacco Technology, Inc. are listed in Table IV.B-11. Ingredients in (b) (4) [redacted]., below.

Table IV.B-11. Ingredients in Kentucky Burley Concentrate.

Ingredient	CAS #	Function	Purity	% of (b) (4) [redacted]	VLN™ King (mg/cig)	VLN™ Menthol King (mg/cig)
(b) (4) [redacted]						

Ingredient	CAS #	Function	Purity	% (b) (4)	VLN™ King (mg/cig)	VLN™ Menthol King (mg/cig)
(b) (4)						
			Total	(b) (4)		

iv. *Burley Stem*

Commercial cigarettes in the U.S. typically contain proportions of “stem.” Stem is actually processed pieces of tobacco leaf midribs removed during the production of tobacco strips. As stem content influences sensorial characteristics and yields, it has been included in the (b) (4) blend. The (b) (4)

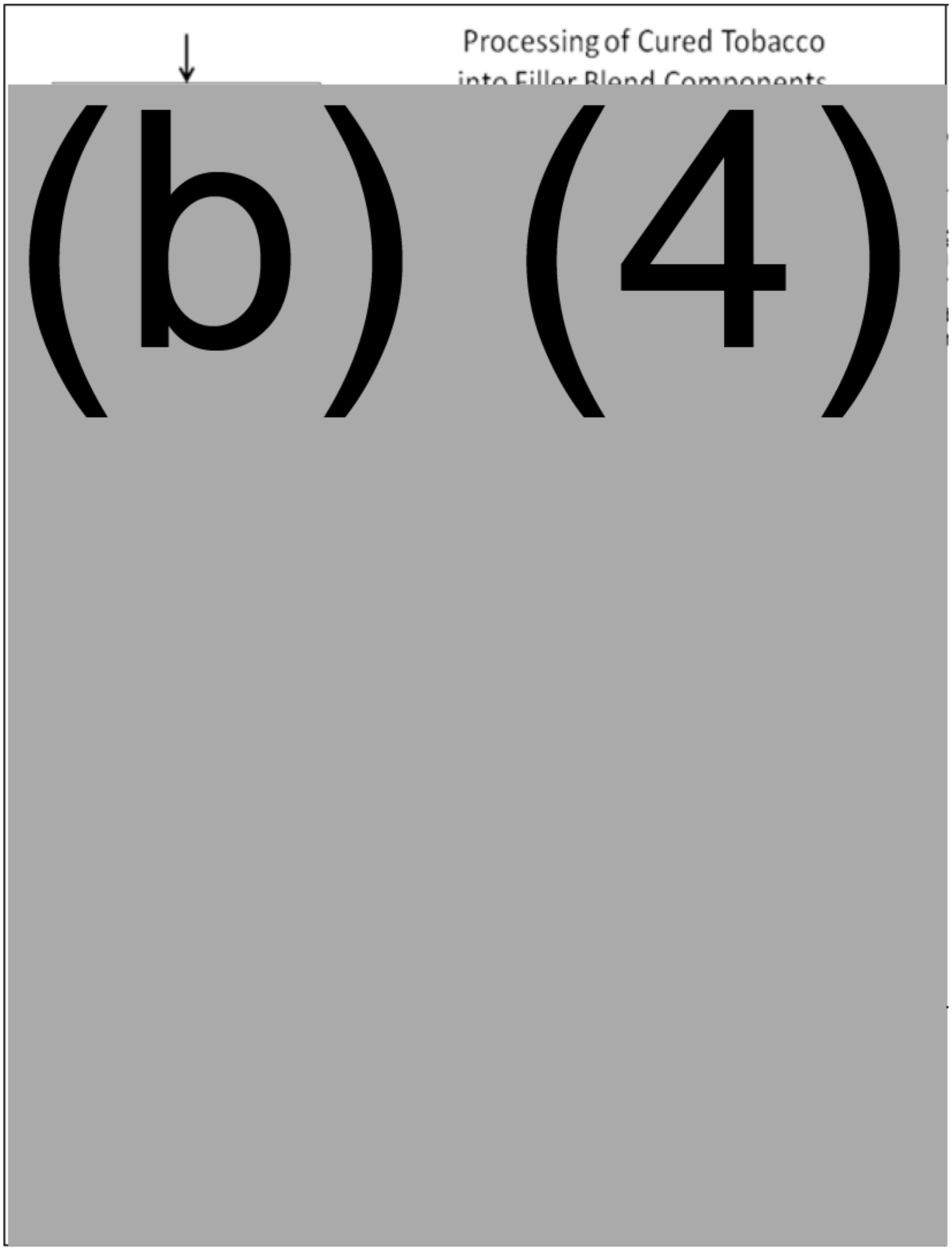
(b) (4)

(b) (4)

(b) (4)

(b) (4) The cartons are labeled with the type of (b) (4). Figure IV.B-1. *Flowchart for Processing of Tobacco into Filler Blend Components,* shows the process for making the (b) (4).

Figure IV.B-1. Flowchart for Processing of Tobacco into Filler Blend Components



v. (b) (4)

As shown in Figure IV.B-2. *Flowchart for Production of Filler Blend*, a(b) (4) is applied to the tobacco ((b) (4)). (b) (4) (b) (4) (b) (4). The ingredients in the (b) (4) are shown in Table IV.B-12. *Ingredients in* (b) (4) [redacted]. [redacted] (b) (4) [redacted] (b) (4)

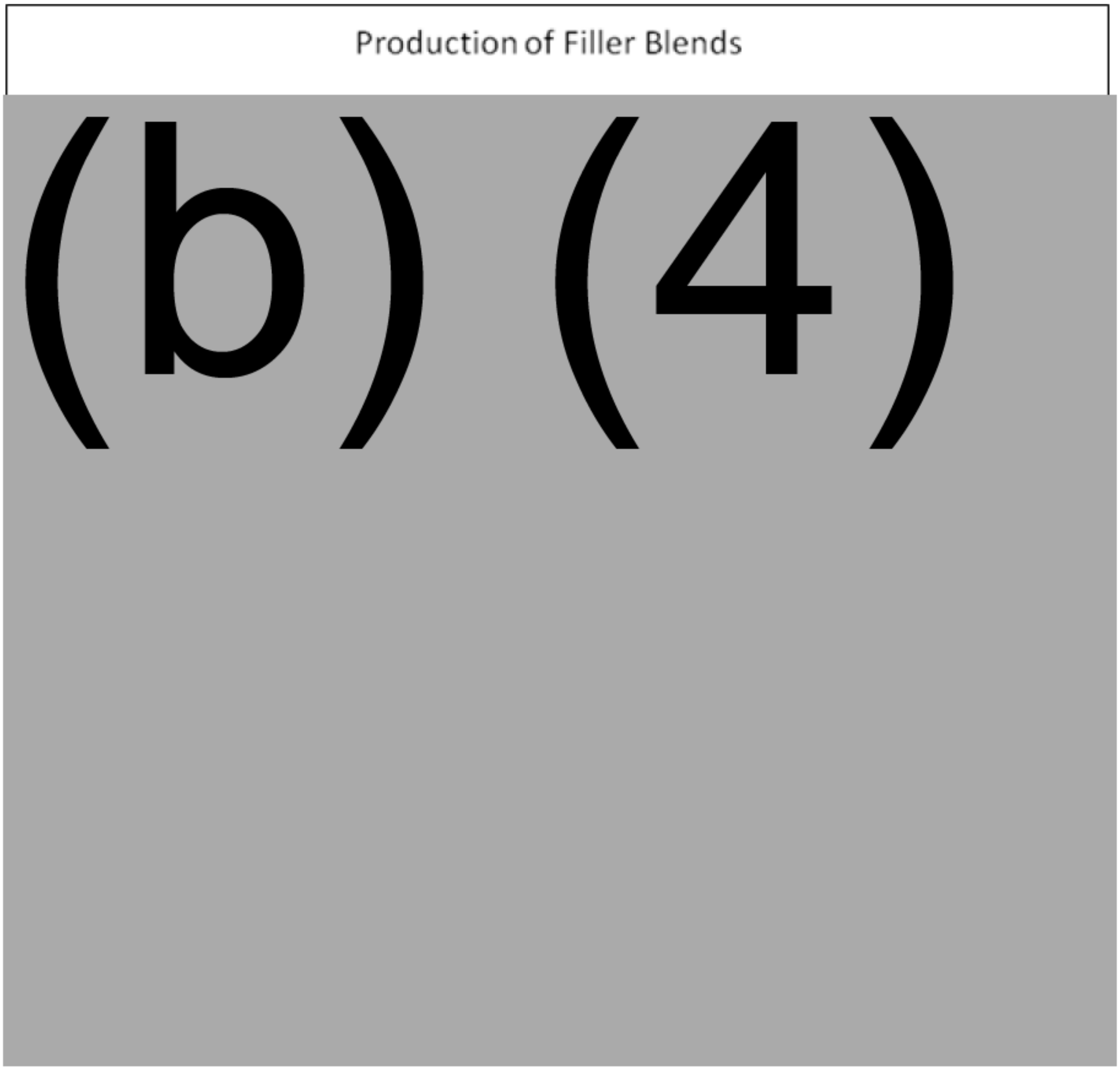
Table IV.B-12. Ingredients in (b) (4)

Ingredient	**CAS Number**	**Function**	**Purity**	**Weight %**	**VLN™ King (mg/cig)**	**VLN™ Menthol King (mg/cig)**

(b) (4)

Ingredient	CAS Number	Function	Purity	Weight %	VLN™ King (mg/cig)	VLN™ Menthol King (mg/cig)
(b) (4)						
			Total	(b) (4)		

Figure IV.B-2. Flowchart for Production of Filler Blend



vi. *Top Flavor*

The top flavor used is provided directly to the (b) (4) by (b) (4) (b) (4) [redacted]. The ingredients in the top flavor are shown in the Table IV.B-13. *Ingredients in Top Flavor*.

Table IV.B-13. Ingredients in Top Flavor

Chemical Name	CAS Number	Function	Purity	Weight %	VLN™ King (mg/cig)	VLN™ Menthol King (mg/cig)

(b) (4)

Chemical Name	CAS Number	Function	Purity	Weight %	VLN™ King (mg/cig)	VLN™ Menthol King (mg/cig)
(b) (4)						
			Total	(b) (4)		

(b) (4) is a (b) (4). The ingredients in (b) (4) listed in Table IV.B-14.

Ingredients in (b) (4)

Table IV.B-14. Ingredients in (b) (4)

Chemical	CAS Number	Function	Weight %	VLN™ King (mg/cig)	VLN™ Menthol King (mg/cig)
(b) (4)					
		Total	(b) (4)		

vii. *Ingredients in Cigarette Material Components*

The VLN^{TM} cigarettes are manufactured on conventional cigarette manufacturing machines ("makers"). Figure IV.B-3. *Conventional Cigarette Diagram*, shows a diagram of the

VLN™ product. Figure IV.B-4. *Cigarette Making Flowchart*, shows the VLN™ cigarette making process.

Figure IV.B-3. Conventional Cigarette Diagram

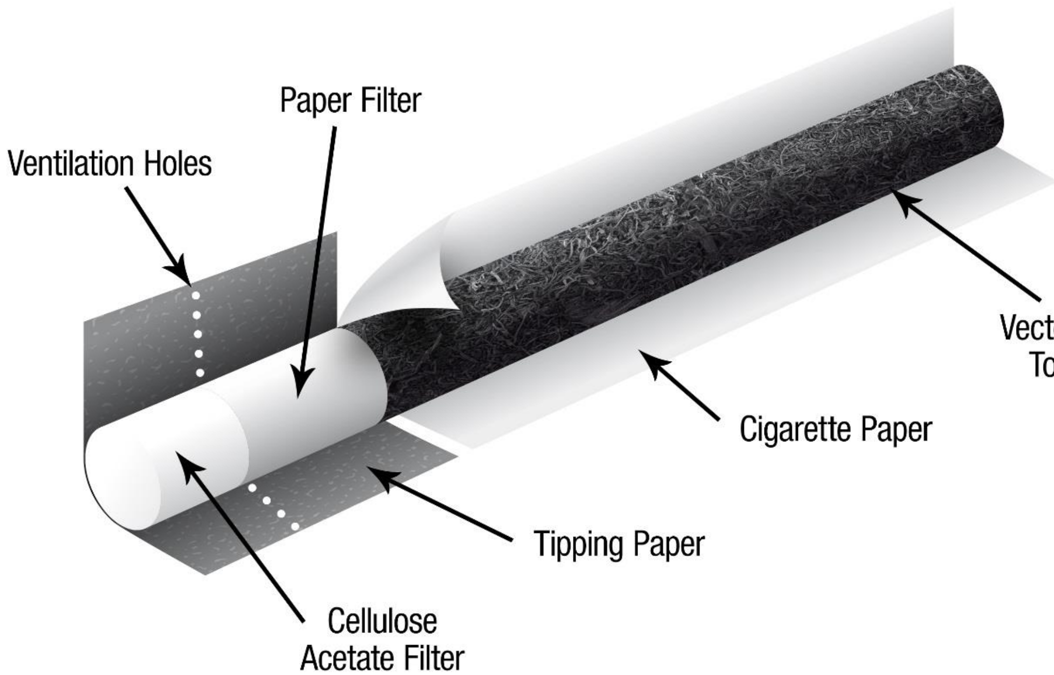
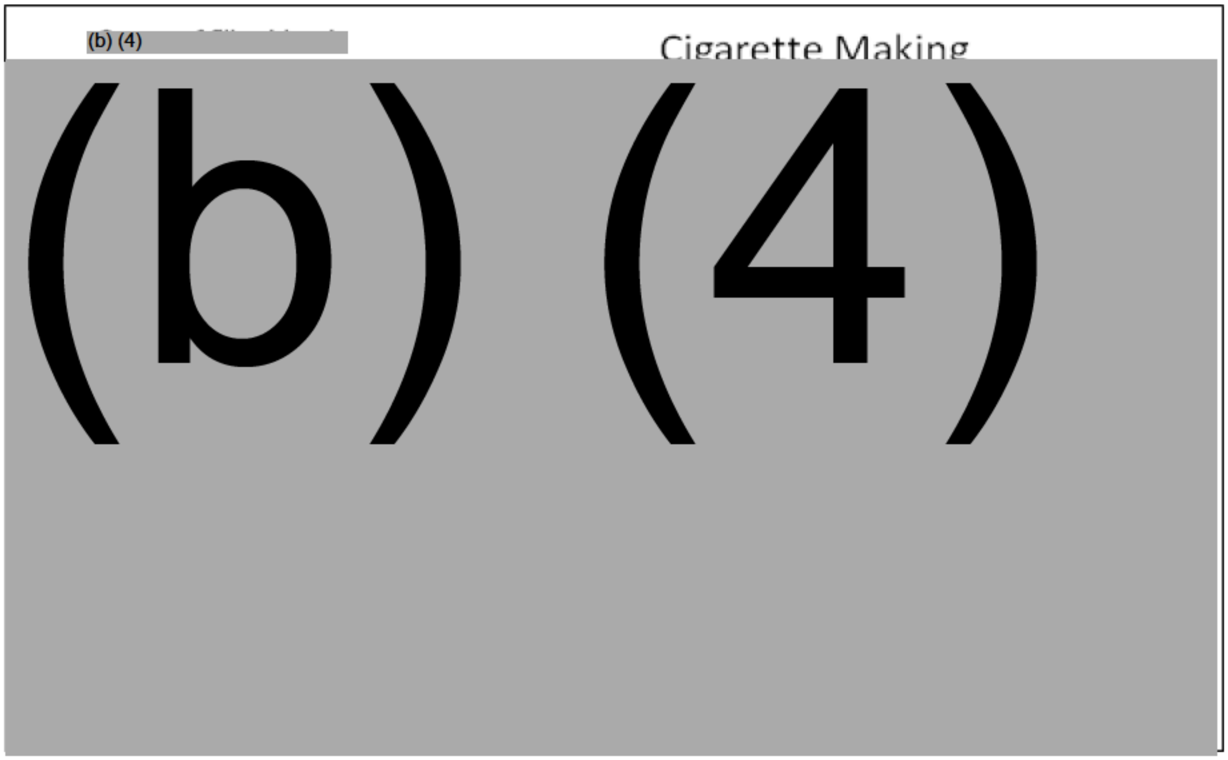


Figure IV.B-4. Cigarette Making Flowchart



VLN™ King and Menthol King cigarettes are made with the same low ignition propensity (LIP) cigarette paper. The paper is composed primarily of (b) (4). The cigarette paper has a porosity of (b) (4). The base paper, (b) (4), is produced by (b) (4) (b) (4) (b) (4) (b) (4). (b) (4). The ingredients in the cigarette paper are listed in Table IV.B-15. *Ingredients in SWM 10-986 IP Cigarette Paper*.

Table IV.B-15. Ingredients in (b) (4) IP Cigarette Paper

Chemical Name	CAS Number	Weight %	VLN™ King mg/cig	VLN™ Menthol King mg/cig
(b) (4)				

Chemical Name	CAS Number	Weight %	VLN™ King mg/cig	VLN™ Menthol King mg/cig
(b) (4)				
	Total	(b) (4)		

(a) Side Seam Adhesive

(b) (4) . It is manufactured by (b) (4) . The ingredients used in (b) (4) are shown in Table IV.B-16. *Ingredients in Adhesive* (b) (4).

Table IV.B-16. Ingredients in Adhesive (b) (4).

Chemical Name	CAS Number	Weight %	VLN™ King mg/cig	VLN™ Menthol King mg/cig
(b) (4)				
	Total	(b) (4)		

The (b) (4) is a proprietary mixture and contains the ingredients listed in Table IV.B-17. *Ingredients in* (b) (4)

Table IV.B-17. Ingredients in (b) (4)

Chemical Name	CAS #	Weight %	VLN™ King mg/cig	VLN™ Menthol King mg/cig

(b) (4)

Total	(b) (4)	(b) (4)	(b) (4)

(b) Tipping Paper

(b) (4). It is manufactured by (b) (4). The tipping paper is (b) (4) (b) (4) The specifications for the tipping paper are shown in Table IV.B-18. *Tipping Paper Specifications* (Specifications for the SPECTRUM tipping paper are also shown.).

Table IV.B-18. Tipping Paper Specifications.

	SPECTRUM	**VLN™**
Manufacturer Code	(b) (4)	(b) (4)
XXII Code		
Base Paper		
Base Paper Basis Weight		
Bobbin Width		
Bobbin Length		
Laser Perforation		
Perforation to edge distance		
Lip Release		
Silver Line		
Black Logo		

Tipping Paper Diagram	(b) (4)

The ingredients in the tipping paper are listed in Table IV.B-19., *Ingredients in Tipping Paper* [redacted].

Table IV.B-19. Ingredients in Tipping Paper (b) (4) .

Sub-Part	Chemical Name/Component	CAS Number	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

(b) (4)

Total (b) (4)

(c) Tipping Adhesive

[redacted] (b) (4) [redacted]

(b) (4) [redacted]. It is manufactured by (b) (4) [redacted]. The ingredients in the adhesive are listed in Table IV.B-20, *Ingredients in Tipping Adhesive* (b) (4) [redacted].

Table IV.B-20. Ingredients in Tipping Adhesive (b) (4) [redacted]

		VLN™ King		VLN™ Menthol King	
Ingredient	CAS Number	Weight %	mg/cig	Weight %	mg/cig
(b) (4) [redacted]					
	Total	(b) (4) [redacted]			

(d) Filter Plug

The filter plug is manufactured by (b) (4) [redacted]. Figure IV.A-2. *Filter Diagram.*, shows a diagram of the filter. [redacted] (b) (4) [redacted]

(b) (4) [redacted]

(b) (4) [redacted]. The chemicals in each component are listed below. (b) (4) [redacted]

[redacted] (b) (4) [redacted]

(b) (4) [redacted]. Because of the addition of (b) (4) [redacted] to the VLN^{TM} Menthol King, the percentages are slightly different. The components in the filter plug are shown in Table IV.B-21. *Filter Plug Materials.*

Table IV.B-21. Filter Plug Materials

Trade Name	Supplier	Supplier Material Name	ZX-A-470 VLN™ King %	ZX-A-475 VLN™ Menthol King %
Tow	(b) (4)			
Plug Wrap				
Inner Plug Wrap				
(b) (4) Paper				
Plasticizer				
Adhesive				
Adhesive				
Adhesive				
Adhesive				
Flavor				

1) Tow

The tow is purchased from (b) (4). The ingredients are listed in Table IV.B-, *Ingredients in Tow*.

Table IV.B-22. Ingredients in Tow

			VLN™ King		VLN™ Menthol King	
Ingredient	**Chemical**	**CAS Number**	**Weight %**	**mg/cig**	**Weight %**	**mg/cig**
(b) (4)						
		Total	(b) (4)			

2) Porous Plug Wrap

There are two plug wraps on the filter. There is a porous plug wrap that goes over the complete filter and there is a non-porous inner plug wrap that goes over the paper part of the filter. The porous plug wrap is manufactured by (b) (4). The ingredients in the plug wrap are listed in Table IV.B-23, *Ingredients in the Porous Plug Wrap*.

Table IV.B-23. Ingredients in the Porous Plug Wrap

			VLN™ King		VLN™ Menthol King	
Ingredient	Chemical	CAS Number	Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

3) Inner Plug Wrap

The inner plug wrap is produced by (b) (4) The ingredients are listed in Table IV.B-24, *Ingredients in Inner Plug Wrap*.

Table IV.B-24. Ingredients in Inner Plug Wrap

			VLN™ King		VLN™ Menthol King	
Ingredient	Chemical	CAS Number	Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

4) (b) (4) [redacted] Paper Filter

There is a (b) (4) paper filter that is oriented to the tobacco end of the VLN™ product. The paper is made by (b) (4) Table IV.B-25, *Ingredients in* (b) (4) *Paper Filter*, lists the ingredients in the paper filter.

Table IV.B-25. Ingredients (b) (4) Paper Filter.

Ingredient	Chemical	CAS Number	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

5) Menthol

(b) (4) during its manufacture. (b) (4)

(b) (4).

(b) (4). Table IV.B-26, *Ingredients in* (b) (4) lists the ingredients in the (b) (4) provided by (b) (4).

Table IV.B-26. Ingredients in (b) (4)

Ingredient	Chemical	CAS Number	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
				Total	(b) (4)	

6) (b) (4) Adhesive (b) (4)

(b) (4) Adhesive (b) (4) from (b) (4) is used in the filter. Table IV.B-27, *Ingredients in* (b) (4) *Adhesive* (b) (4), lists the ingredients in the adhesive.

Table IV.B-27. Ingredients in (b) (4) Adhesive (b) (4)

Ingredient	Common Name	CAS #	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						

Ingredient	Common Name	CAS #	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

7) (b) (4) Adhesive (b) (4)

(b) (4) Adhesive (b) (4) from (b) (4) is used in the filter. Table IV.B-28, *Ingredients in (b) (4) Adhesive* (b) (4) lists the ingredients in the adhesive.

Table IV.B-28. Ingredients in (b) (4) Adhesive (b) (4)

Ingredient	Common Name	CAS #	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

8) (b) (4) Adhesive (b) (4)

(b) (4) Adhesive (b) (4) from (b) (4) l is used in the filter Table IV.B-29, *Ingredients in* (b) (4) (b) (4) *Adhesive* (b) (4), lists the ingredients in the adhesive

Table IV.B-29. Ingredients in (b) (4) Adhesive (b) (4)

Ingredient	Common Name	CAS #	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

9) (b) (4) Adhesive (b) (4)

(b) (4) Adhesive (b) (4) from (b) (4) is used in the filter. Table IV.B-30, *Ingredients in* (b) (4) *Adhesive* (b) (4), lists the ingredients in the adhesive.

Table IV.B-30. Ingredients in (b) (4) Adhesive (b) (4)

Ingredient	Common Name	CAS #	VLN™ King		VLN™ Menthol King	
			Weight %	mg/cig	Weight %	mg/cig
(b) (4)						
		Total	(b) (4)			

4. List of Packaging Materials

The VLN™ cigarettes are packaged in the same manner as conventional commercial cigarettes, with 20 cigarettes per pack and 10 packs per carton. The VLN™ cigarettes are packed in flip-top hard packs. All packaging components are provided by suppliers of packaging components for commercial cigarettes. The VLN™ cigarettes are packaged by an automated machine. Trays of finished cigarettes are mounted on a feeder hopper. The feeder first forms sets of 20 cigarettes and checks that each cigarette is well filled with an array of 20 plungers. The packer also rejects bundles if the overall dimensions of the bundle are not within pack tolerances.

Bundles of 20 cigarettes are wrapped in paper-backed foil. A cardboard inner frame is punched from a continuous roll and formed around the foil-wrapped pack. The pack is then formed around the bundle and glued.

The completed packs travel along a conveyer to the pack wrapper. Each pack is wrapped with a heat-shrink film. Wrapped packs then travel along a conveyer to the carton filler. Sets of 10 wrapped packs are assembled by the carton filler and the carton is folded around them and glued.

Cartons are packed 30 cartons/case, and labels identifying the VLN™ product and lot are applied to each case. Figure IV.B-5. Cigarette Packaging Flowchart

Cigarette Packaging Flowchart, and Figure IV.B-6. Labeling and Packing Flowchart

, *Labeling and Packing Flowchart*, below show the VLN™ cigarette and carton packing process.

Figure IV.B-5. Cigarette Packaging Flowchart

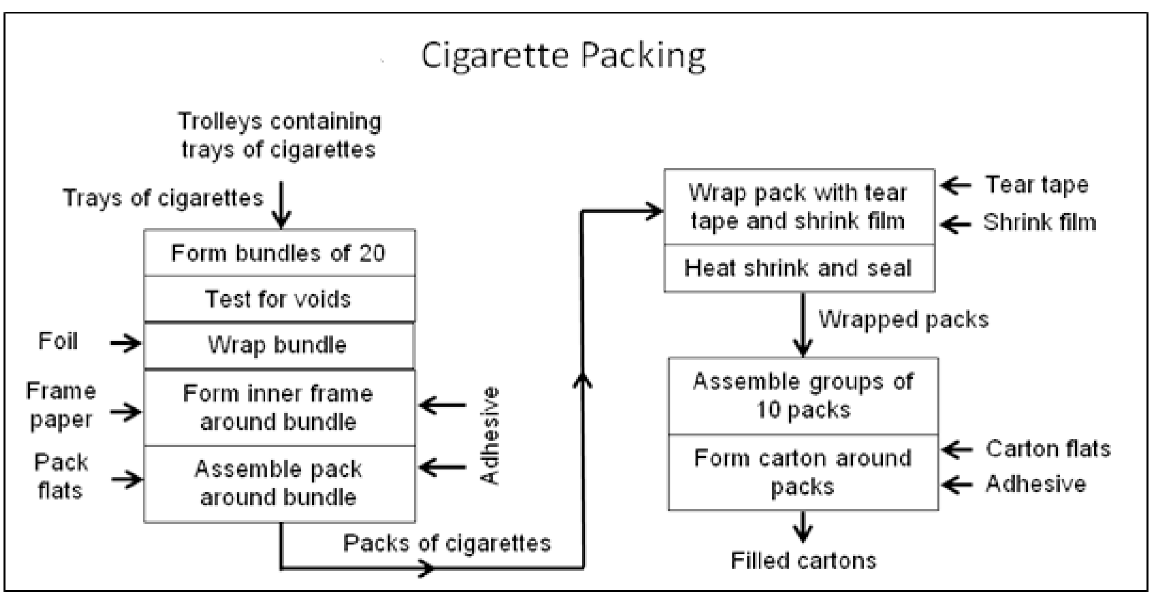


Figure IV.B-6. Labeling and Packing Flowchart

Labelling and packaging

Filled cartons

⇩

Filled cartons moving on the conveyer belt
30 Filled Cartons loaded into a case
Case is sealed using a hot glue, date-stamped & labeled

⇩

Cases

The materials used in the VLN™ packaging are listed in Table IV.B-31, *Materials Used in Packaging* below. The same base materials are used for the VLN™ pack and carton. Different inks are used to differentiate the menthol and regular products.

Table IV.B-31. Materials Used in Packaging

Part Name	Identifying Number	Supplier	VLN™ King (g/pack)	VLN™ Menthol King (g/pack)
Foil (aluminized paper)	(b) (4)			
Inner frame (cardboard)				
Tear-Tape				
Cellophane				
Hinge-Lid Label (cardboard)				
Carton (cardboard)				
Packaging Glue				
Case				

Part Name	Identifying Number	Supplier	VLN™ King (g/pack)	VLN™ Menthol King (g/pack)
(corrugated cardboard)	(b) (4)			

C. Confidential Manufacturing Information

VLN™ is a conventional cigarette. The product will be manufactured at the Company's plant in Mocksville, NC. The relevant plant information is listed below:

Facility Name: NASCO Products, LLC,
Facility Address: 321 Farmington Rd, Mocksville, NC 27028
Facility Phone Number: 1-336-940-3769
Plant Manager: (b) (6)
Plant Manger Phone Number: (b) (6)

1. Production and Facilities

22nd Century's wholly-owned subsidiary, NASCO Products LLC operates an MSA-listed tobacco products manufacturing facility at 321 Farmington Road, Mocksville, North Carolina 27028. The 61,500 square foot facility houses the following equipment:

- (b) (4)
-

Production steps for manufacturing cigarettes include:

(b) (4)

(b) (4)

Production steps for packing cigars and cigarettes include:

(b) (4)

2. Managerial Oversight and Employee Training

Managerial oversight is provided by the department managers and ultimately by the NASCO Operations Manager. Reporting to 22nd Century's Chief Executive Officer, the NASCO Operations Manager is responsible for the entire operation of the manufacturing facility. Reporting to the NASCO Operations Manager are the following:

(b) (4)

(b) (4)

Within the first week of employment, all new employees begin training in their duties and responsibilities. For production employees assigned to operate NASCO's maker units and/or packing units, the training topics include:

(b) (4)

(b) (4)

(b) (4).

Quality Team Members undergo a similar training regimen. (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4) a.

3. Processes and Controls for Product Design and Changes

(b) (4)

(b) (4)

(b) (4)

4. Identifying Suppliers

(b) (4)

5. Validation and Verification to Specifications

i. Acceptance Criteria for Incoming Materials

(b) (4)

(b) (4)

(b) (4)

(b) (4)

ii. *Acceptance Criteria for Parts and Finished Product During Assembly Process*

Table IV.C-2 Finished Product Acceptance Criteria, notes the Acceptance Criteria for the finished product

Table IV.C-2 Finished Product Acceptance Criteria

Design Feature	**VLN™ King**			**VLN™ Menthol King**		
	Target Specification	**Upper Limit**	**Lower Limit**	**Target Specification**	**Upper Limit**	**Lower Limit**
Cigarette Weight (mg/cig)	(b) (4)					
Cigarette Circumference (mm/cig)						
Ventilation (%)						
Cigarette Pressure Drop Open (mm H_2O)						

(b) (4)

(b) (4)

6. Frequency of Product Specification Validation

(b) (4)

(b) (4)

7. Diagnostic and Measuring Device Validation

(b) (4)

(b) (4)

8. Testing procedures carried out before the product is released to market:

(b) (4)

9. Handling of complaints, nonconforming products and processes, and corrective and preventative actions:

(b) (4)

(b) (4)

D. Conditions for Using the Product

1. Narrative

VLN™ cigarettes are intended for use by current smokers who wish to reduce their exposure to nicotine from cigarette smoke. VLN™ cigarettes will be used in the same manner as conventional nicotine content cigarettes.

2. Description of Length of Time to Consume

These VLN™ products perform just like conventional cigarettes. The time to consume each VLN™ cigarette will be based on the smoking intensity. That is, the time to consume will depend on how intensely the consumer smokes the product. The static burn rate for the product is about 7 mm per minute (Enthalpy Analytical 2018, Project Code 0818-525) [pg 75]. The puff count under ISO conditions for VLN™ is 5.76 as compared to 8.31[3] for the top 100 brands in the U.S. suggesting that VLN™ may burn faster than conventional cigarettes. In the abuse liability study (Altasciences. 2018.) [pg 74], the subjects were allowed to smoke the VLN™ product *ad libitum* over 4 hours. Table IV.D-1 shows the number of cigarettes consumed and the duration of smoking. The subjects smoked about the same number of VLN™ cigarettes as usual brand (8.2 cigarettes vs. 7.8), but their mean smoking time was faster (4.7 minutes vs. 6.0). In Part B of the abuse liability study, the subjects were allowed to smoke one cigarette *ad libitum.* Table IV.D-2 shows the smoking topography results. The number of puffs was less for VLN™ than usual brand (9.5 vs.

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[3] See Section VIII.B.1 Scientific Studies and Analyses – Product Analysis - Nicotine in Tobacco.

12.6). Puff duration was not affected. Thus, it appears the VLN™ smokers will smoke their cigarette slightly faster than they would their usual brand.

Table IV.D-1. Summary of product use Part A (Product A = VLN™, Product B = Usual Brand, Product C = 4 mg Nicotine gum).

		Study Product A (N=66)	Study Product B (N=65)	Study Product C (N=65)
Number of Product Used	n	66	65	65
	Mean (SD)	8.2 (4.34)	7.8 (2.61)	3.5 (1.36)
	Median	7.0	8.0	4.0
	Q1, Q3	5.0, 11.0	6.0, 10.0	2.0, 5.0
	Min, Max	2, 18	3, 13	1, 6
Duration of Product Used (mins)	n	410	443	187
	Mean (SD)	4.7 (1.71)	6.0 (2.07)	16.3 (11.45)
	Median	4.0	6.0	12.0
	Q1, Q3	4.0, 6.0	4.0, 7.0	7.0, 23.0
	Min, Max	1, 14	0, 12	0, 56

Table IV.D-2. Summary of product use Part B (Product A = VLN™, Product B = Usual Brand, Product C = 4 mg Nicotine gum).

		Study Product A (N=57)	Study Product B (N=56)	Study Product C (N=58)
Total Number of Inhalations per Subject	n	57	56	
	Mean (SD)	9.5 (3.42)	12.6 (4.29)	
	Median	9.5	12.0	
	Q1, Q3	7.0, 12.0	10.0, 16.0	
	Min, Max	2, 17	3, 22	
Duration of Inhalations (sec)	n	534	691	
	Mean (SD)	2.1 (1.07)	1.9 (0.95)	
	Median	2.0	2.0	
	Q1, Q3	1.0, 3.0	1.0, 2.0	
	Min, Max	1, 8	1, 5	
Average Duration of Inhalations per Subject (sec)	n	57	56	
	Mean (SD)	2.0 (1.04)	1.9 (0.93)	
	Median	2.0	2.0	
	Q1, Q3	1.0, 3.0	1.0, 2.0	
	Min, Max	1, 8	1, 5	
Total Duration of Inhalations per Subject (sec)	n	57	56	
	Mean (SD)	21.3 (6.77)	26.2 (9.62)	
	Median	21.0	25.0	
	Q1, Q3	17.0, 24.0	21.0, 29.0	
	Min, Max	4, 46	7, 60	
Duration of Gum Product Use (min)	n			58
	Mean (SD)			7.1 (3.52)
	Median			9.0
	Q1, Q3			4.0, 10.0
	Min, Max			0, 11

3. Specific Instructions on How to Use and Store the Product

Since this VLN™ product performs essentially the same as a conventional cigarette, no unique instructions for use are needed. The consumer will use the VLN™ product the same as their usual brand. The VLN™ cigarettes are stable for at least 1 year at room temperature and do not require any unique storage conditions or directions.

4. Specific Instructions on How to Avoid Using the Product in a Way that Reduces the Benefit or Increases the Risk

The only two ways possible for the consumer to defeat the design element of low nicotine is to either compensate by smoking differently (i.e. different puff profile) or smoking more. The clinical studies show that consumers do not compensate for the lower nicotine levels in VLN™ cigarettes by smoking more VLN™ cigarettes. They also do not alter their puff profile. Total puff volume generally decreases. Because of the extremely low levels of nicotine in the VLN™ product (95% less nicotine than conventional cigarettes), the consumer would need to smoke at least 20 times more VLN™ cigarettes to get the same level of nicotine per day as compared to conventional cigarettes. For example, in a pack a day smoker this would be equal to the need to smoke 20 packs or two cartons per day, or 400 cigarettes per day, for such smoker to obtain the same level of nicotine. The 2013-2014 NHANES data reports that the maximum number of cigarettes smoked per day in the last 30 days was 90. (Center for Disease Control and Prevention 2015 [pg 74]) Since it is essentially physically impossible to smoke 400 cigarettes per day and the independent clinical studies show that smokers of VLN™ cigarettes do not engage in compensatory smoking, no specific instructions are necessary to guide the smoker on how to use the VLN™ product.

No specific instructions were given in the clinical studies on how to use the VLN™ product (other than smoke it as a smoker would normally smoke the smoker's usual brand). The clinical results demonstrate that the smoker understands the benefit of the VLN™ product without needing specific instructions. No specific instructions are needed for the consumer on how to avoid using the VLN™ product.

E. How Consumers Actually Use the Product

VLN™ cigarettes are intended for smokers who desire to reduce their nicotine consumption. The VLN™ cigarettes contain at least 95% less nicotine than conventional cigarettes on a nicotine content and smoke basis. The VLN™ product is intended to be smoked just like a conventional cigarette. As with conventional cigarettes, there are no specific instructions for use or storage. The VLN™ product is essentially the same as a conventional cigarette but contains less nicotine in the tobacco.

The VLN™ products have design characteristics that are consistent with conventional cigarettes[4]. Market-leading cigarette brands were analyzed for physical properties (Table IV.E-3). VLN™ falls within the range of the existing market-leading brands[5]. The market-leading brands were also analyzed for TNCO (Table IV.E-4). The TNCO for VLN™ is similar to the market-leading brands (except for nicotine), however the number of puffs in the VLN™ cigarettes are less than the market-leading brands[6]. When consumers smoked VLN™ Kings *ad labitum* as part of the

[4] See Section IV.B.1. Descriptive Information - Product Formulation - List of Design Features
[5] See Section VIII.B.2.iii Scientific Studies and Analyses – Product Analysis - Nicotine in Smoke and HPHC Analysis - HPHC Analysis on Market Leaders
[6] The range for puff count for the top 100 brands was 5.3 to 12.3. VLN™ falls within this range.

Abuse Liability Study,[7] their number of cigarettes consumed was the same as usual brand, but the duration of smoking was less (4.7 minutes ± 1.7 vs. 6.0 ± 2.1) than with usual brand. Their number of inhalations was less (9.5 ± 3.4 vs. 12.6 ± 4.3), but the duration of inhalation was not affected (2.1 seconds ± 1.0 vs. 1.9 ± 1.0) (see Table IV.E-1 and Table IV.E-2). Thus, it appears that consumers might smoke the same number of VLN™ cigarettes as their usual brand but they may smoke VLN™ slightly faster than their usual brand.

Table IV.E-1. Descriptive Statistics of Product Use Behavior During Part A.

	VLN™ Cigarette N=66	**Own-Brand Cigarette N=65**
Number of Cigarettes Consumed		
Mean (SD)	8.2 (4.34)	7.8 (2.61)
Median	7.0	8.0
Min, Max	2, 18	3, 13
Time Spent per Cigarettes (minutes)		
Mean (SD)	4.7 (1.71)	6.0 (2.07)
Median	4.0	6.0
Min, Max	1, 14	0, 12

Table IV.E-2. Descriptive Statistics of Product Use during Part B (Uncontrolled Use).

	VLN™ Cigarette N=57	**Own-Brand Cigarette N=56**
Number of Inhalations per Subject		
Mean (SD)	9.5 (3.42)	12.6 (4.29)
Median	9.5	12.0
Min, Max	2, 17	3, 22
Average Duration of Inhalations per Puff (sec)		
Mean (SD)	2.0 (1.04)	1.9 (0.93)
Median	2.0	2.0
Min, Max	1, 8	1, 5

Table IV.E-3. Measured physical properties of king size market leaders and VLN™.

Product	**Cigarette Length (mm)**	**Cigarette Weight (mg)**	**Cigarette Tobacco Weight (mg)**	**Cigarette Circumference (mm)**	**Tip Ventilation (%)**	**Pressure Drop (Open) (mm H_20)**	**Static Burn (min)**
Camel Blue King	82.9	932	678	24.6	33.6	109	3.30
Marlboro Gold King	82.8	912	648	24.8	28.8	122	5.99
Marlboro Menthol Gold King	82.9	889	635	24.9	34.4	99.8	6.14
Marlboro Red King	78.9	894	698	24.8	10.8	110	4.85
Marlboro Special Blend King	82.8	911	644	24.8	32.6	115	4.63
Newport Menthol Green King	79.8	895	671	24.7	2.20	133	6.54
VLN™ King	82.8	936	663	24.9	13	119	6.2

Product	Cigarette Length (mm)	Cigarette Weight (mg)	Cigarette Tobacco Weight (mg)	Cigarette Circumference (mm)	Tip Ventilation (%)	Pressure Drop (Open) (mm H_2O)	Static Burn (min)
VLN™ Menthol King	82.8	945	661	24.9	13	120	6.2
Market Leader Range	78.9-82.9	889-911[8]	635-698	24.7-24.9	2.2-34.4	99.8-133	3.3-6.54

Table IV.E-4. TNCO of market leaders and VLN™ under ISO conditions.

ISO Smoking Conditions		VLN™ King	VLN™ Menthol King	Camel Blue King	Marlboro Gold King	Marlboro Menthol Gold King	Marlboro Red King	Marlboro Special Blend Gold King	Newport Menthol Green King
Constituent	**Unit**								
Carbon Monoxide	(mg/cig)	11.8 (0.6)	12.3 (0.7)	10.9 (0.7)	10.2 (0.6)	9.90 (0.54)	13.3 (0.90)	9.92 (0.64)	15.1 (1.1)
Nicotine	(mg/cig)	0.0246 (0.0015)	0.0257 (0.0012)	0.837 (0.028)	0.670 (0.026)	0.741 (0.036)	0.956 (0.055)	0.675 (0.039)	1.08 (0.05)
Tar	(mg/cig)	6.98 (0.4)	7.37 (0.39)	9.84 (0.53)	8.97 (0.55)	9.44 (0.55)	14.0 (1.0)	8.87 (0.55)	15.4 (1.0)
Water	(mg/cig)	0.466 (0.146)	0.490 (0.114)	0.997 (0.159)	0.751 (0.145)	0.779 (0.182)	1.96 (0.37)	0.747 (0.131)	3.17 (0.76)
Puffs	(#/cig)	5.76 (0.17)	5.85 (0.17)	8.14 (0.21)	7.29 (0.21)	7.48 (0.25)	7.30 (0.29)	7.43 (0.25)	7.32 (0.31)
Date of testing		5/30/2018	5/30/2018	9/5/18	9/5/18	9/5/18	9/5/18	9/5/18	9/5/18
Laboratory		Enthalpy Analytical	Enthalpy Analytical	Enthalpy Analytical	Enthalpy Analytical	Enthalpy Analytical	Enthalpy Analytical	Enthalpy Analytical	Enthalpy Analytical
Publication/ Report No.		(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75]	(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75]	(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75] 2018 Proj Code 0718-022) [pg 75]	(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75] 2018 Proj Code 0718-022) [pg 75]	(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75] 2018 Proj Code 0718-022) [pg 75]	(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75] 2018 Proj Code 0718-022) [pg 75]	(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75] 2018 Proj Code 0718-022) [pg 75]	(Enthalpy Analytical 2018 ProjCode 0318-026) [pg 75] 2018 Proj Code 0718-022) [pg 75]

Research studies with the VLN™ products show that daily smokers gradually reduce their cigarette consumption by as much as 50% over 20 weeks of use (Hatsukami *et al.* 2018 [pg **76**];

[8] The filter in VLN™ is a dual filter that weighs more than the single filter in the market leading brands.

Donny *et al.* 2015 [pg 75]; 22nd Century Group. 2011 [74]; Hatsukami *et al.* 2017 [pg 76]). Non-daily smokers also reduce their cigarette consumption (Shiffman *et al.* 2018) [pg 77] after extended use of VLNC cigarettes. Upon switching to VLNC cigarettes, smokers will experience withdrawal symptoms (Donny and Jones 2009) [pg 75]. Upon the first use of the VLNC product, smokers may slightly modify their smoking topography by increasing the puff volume or flow rate as they try to understand the product and its lack of nicotine delivery and throat hit (MacQueen *et al.* 2012 [pg 76]; Mercincavage *et al.* 2016 [pg 76]). Within a few cigarettes to a few days, smoking topography returns to normal (Donny and Jones 2009) [pg 75]. There is no compensation (CO boost) while using the product (Donny *et al.* 2015 [pg 75]; Denlinger-Apte *et al.* 2017 [pg 74]; Hatsukami *et al.* 2017 [pg 76]; Pacek *et al.* 2016 [pg 77]; Hatsukami *et al.* 2013 [pg 76]; Cassidy *et al.* 2018 [pg 74]; Hatsukami *et al.* 2018 [pg 76]). In studies where the subjects gradually reduced their nicotine content by switching to progressively lower levels of nicotine, there appeared to be some compensation (Hatsukami *et al.* 2018 [pg 76]; Mercincavage *et al.* 2017 [pg 76]). This is not relevant to VLN™ since only one level of nicotine in tobacco will be offered. People who use the product eventually become less dependent on the nicotine in cigarettes (Hatsukami *et al.* 2010 [pg 75]; Benowitz *et al.* 2012 [pg 74]; Donny *et al.* 2015 [pg 75]; Hammond and O'Connor 2014 [pg 75]) and may transition to abstinence (Donny *et al.* 2015 [pg 75]). Studies indicate that there are more quit attempts (Donny *et al.* 2015 [pg 75], Walker *et al.* 2012 [pg 77]), smoke free days (Hatsukami *et al.* 2018 [pg 76]) and cessation Hatsukami *et al.* 2010 [pg 75]). Dual use of VLNC cigarettes with NRT increases abstinence in people motivated to quit (Becker et al. 2008 [pg 74]; McRobbie *et al.* 2016 [pg 76]; Walker *et al.* 2012 [pg 77]; Vector Tobacco Inc. 2006 [pg 77]; Hatsukami *et al.* 2017 [pg 76]; Hatsukami *et al.* 2013 [pg 76]; McRobbie *et al.* 2016 [pg 76]).

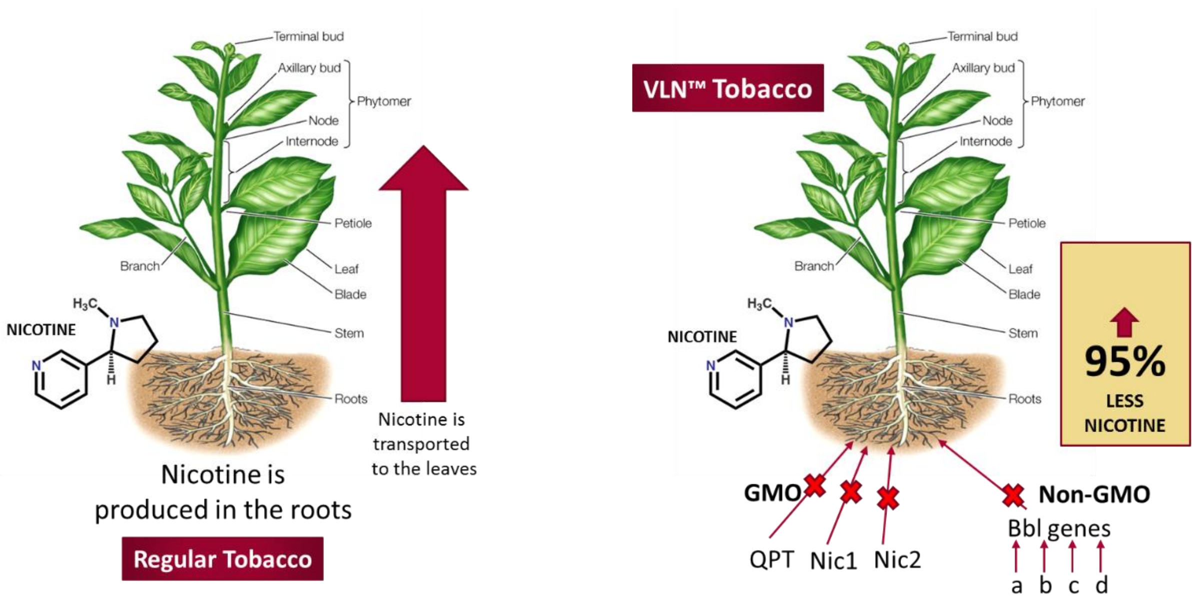
There is limited evidence that suggests VLNC cigarettes with NRT increases smoking abstinence in smokers not motivated to quit trial Benowitz *et al.* 2012 [pg 74]; Walker *et al.* 2015 [pg 77]; Shiffman *et al.* 2018 [pg 77]).

Overall, these results suggest that on an individual basis, the smoker may smoke the VLN™ cigarette slightly faster than their usual brand. Initially, the smoker may experience withdrawal symptoms. Consumption of VLN™ (number of cigarettes per day) may gradually decline. Co-use of NRT may help alleviate some of the negative effects of withdrawal and leads to abstinence.

F. Overview of Vector 21-41 Tobacco Variety

In conventional tobacco plants, nicotine is produced in the roots and transported to the leaves (Figure IV.F-1. Pictogram of Nicotine Production in Conventional and VLN™ Tobacco.). In VLN™ the production of nicotine is inhibited, and minimal nicotine is transported to the leaves. The current tobacco used in VLN™ tobacco is Vector 21-41 Burley. The tobacco has been genetically engineered (GMO in the figure) to alter the nicotine production in the plant. The Company has other tobacco lines that are not genetically modified under development (Non-GMO).

Figure IV.F-1. Pictogram of Nicotine Production in Conventional and VLN™ Tobacco.



1. Summary of Technology

Vector 21–41 tobacco has been genetically engineered to express a quinolinic acid phosphoribosyltransferase (QPTase) in the reverse, or antisense position, which disrupts the normal expression of QPTase, a key enzyme in the biosynthetic pathway leading to the production of nicotine and related alkaloids. The effect of this genetic change is to reduce the nicotine levels of nicotine, nor-nicotine, and total alkaloids in the leaves of Vector 21–41 tobacco. The tobacco also contains the *nptII* marker gene derived from the bacterium Escherichia coli. The *nptII* gene encodes the enzyme neomycin phosphotransferase type II (*NPTII*) and was used as a selectable marker in the initial laboratory stages of plant cell selection. Expression of the added genes is controlled in part by gene sequences from the plant pathogen *Agrobacterium tumefaciens*. The A. tumefaciens method was used to transfer the added genes into the parental recipient Burley 21–LA tobacco variety. The Animal and Plant Health Inspection Service de-regulated Vector 21-41 in 2002. The effect of this determination is that Vector's 21–41 tobacco is no longer considered a regulated article under APHIS 'regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the tobacco or its progeny.

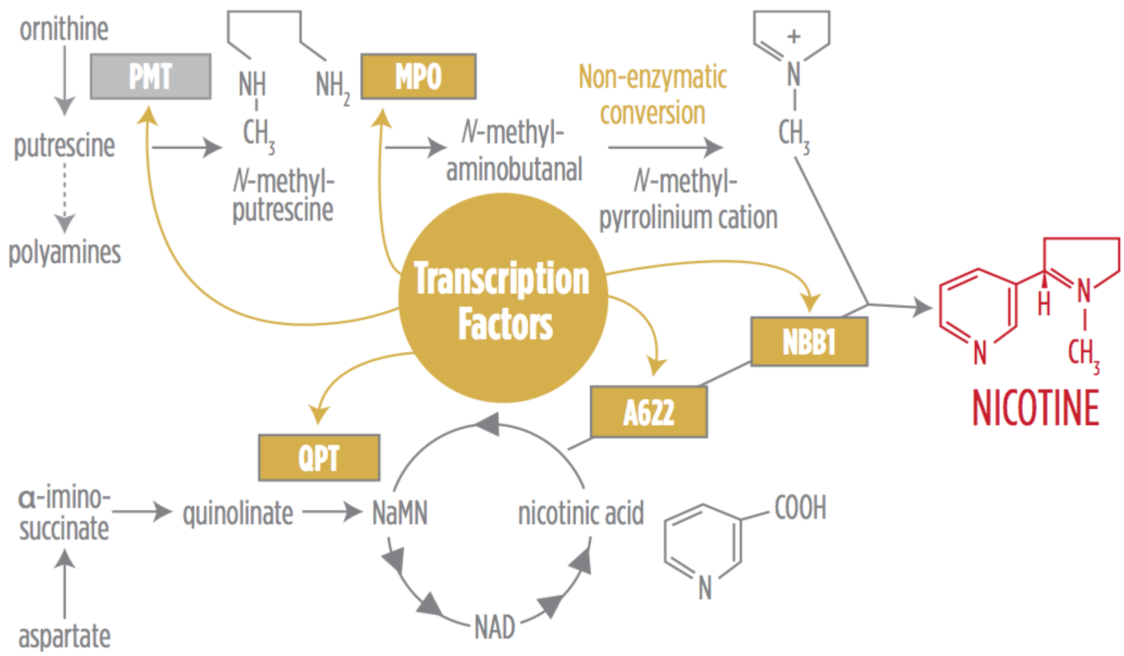
2. History

Legg and Collins (1969) [pg 76] incorporated *nic1* and *nic2* genes from low alkaloid Cuban cigar cultivars into cultivar Burley 21 by hybridizing the two cultivars followed by a series of twelve back-crosses to produce LA (low alkaloid) Burley 21. LA Burley 21 is not cultivated for use in commercial cigarettes. Collins, Legg, and Kasperbauer 1974 [pg 74] compared Burley 21 and Burley 21 LA lines and found that they did not differ in days to flower, number of leaves, leaf size, and plant height. Legg, Colons, and Litton (1970) [pg 76] registered the Burley 21 LA germplasm. Other low alkaloid varieties were similarly produced by crossing the *nic1 nic2* loci into other tobacco cultivars. These low alkaloid varieties have 10-20% of the alkaloid content of the varieties from which they were derived. Vector 21-41 was developed by further reducing the synthesis of nicotine in the roots of the tobacco plant by specific suppression of the enzyme quinolinate phosphoribosyltransferase (QPTase) (Xie et al. 2004) [pg 77]. Vector 21-41 produced from this variety has 5% or less of the nicotine content of typical varieties used for cigarette tobacco but is otherwise similar to conventional tobacco.

3. Nicotine Biosynthesis

Nicotine is the predominant alkaloid found in tobacco. During the domestication of tobacco, varieties producing high levels of nicotine were typically selected. Commercial varieties generally produce nicotine at levels between 2 and 4 percent of dry weight (20,000 to 40,000 ppm). Studies have been carried out to establish the nicotine biosynthetic pathway) by administering labeled compounds, studying tissue cultured plants and recently using molecular techniques. Nicotine is composed of pyridine and N-methylpyrrolidine rings linked together and is produced by the condensation of two metabolites, nicotinic acid and N-methylpyrroline, that are synthesized by two separate pathways. The pathway is shown in Figure IV.F-2. *Nicotine Biosynthetic Pathway in Tobacco*.. Nicotinic acid is synthesized in the pyridine-nucleotide cycle, whereas N-methylpyrroline is synthesized from ornithine or arginine via putrescine.

Figure IV.F-2. Nicotine Biosynthetic Pathway in Tobacco.



Quinolinic acid phosphoribosyltransferase (QPTase), which converts quinolinic acid to nicotinic acid mononucleotide (NAMN), serves as the entry-point enzyme into the pyridine-nucleotide cycle that supplies nicotinic acid (Wagner and Wagner, 1985 [77]). The pyridine-nucleotide cycle produces nicotinamide adenine dinucleotide (NAD) and nicotinic acid and is a part of primary metabolism, but also produces components for the secondary metabolism such as nicotine synthesis in the root of tobacco. The *NtQPT1* gene that encodes QPTase was isolated as part of a screen for genes expressed specifically in roots of tobacco. Using an RNA blot, it was confirmed that *NtQPT1* is expressed almost exclusively in the roots (Xie *et al.*, 2004 [pg 77]).

The other precursor for nicotine is the *N*-methylpyrrolinium cation. Putrescine *N*-methyltransferase (PMTase) catalyzes the formation of *N*-methylputrescine which is then oxidatively de-aminated to 4-aminobutanol by diamine oxidase (DAO). 4-aminobutanol spontaneously cyclizes to the N-methylpyrrolinium cation, a direct precursor of nicotine (Feth *et al.*, 1986 [pg 75]).

4. Modified Nicotine Synthesis: *NtQPT* antisense construct

An antisense strategy was used to reduce expression of the endogenous *NtQPTl* gene. Antisense RNA is complementary to the mRNA transcribed by the endogenous gene. Expression of an antisense transcript in the same cell expressing sense transcripts of the endogenous gene can down-regulate the production of the gene product encoded by the endogenous gene. Antisense suppression of gene expression requires a high degree of sequence complementarities between the antisense and sense transcripts.

The full-length *NtQPT1* cDNA was cloned in the antisense orientation behind the 2.0 kb *NtQPT1* promoter. The 2.0 kb *NtQPT1* promoter directs transgene expression at high levels in the same tissues under the same conditions as the endogenous *NtQPT1* gene.

The antisense transcript was terminated by the nos termination/polyadenylation sequences. This gene cassette (*NtQPT1* promoter - *NtQPTl* cDNA antisense – *nos* terminator) was introduced into tobacco via Agrobacterium transformation as described below.

5. Agrobacterium Transformation

The vector system, pBin19, used to transform genes in tobacco is a binary system based on the Ti plasmid of *Agrobacterium tumefaciens*. pBin19 has been sequenced and is in the GenBank database, accession number U09365. Although some of the DNA sequences used in the transformation process were derived from the plant pathogen *A. tumefaciens*, the genes responsible for crown gall disease were first removed; therefore, the recipient plant does not have crown gall disease. Figure IV.F-3. *Plamid PYTY32.*, illustrates the plasmid vector, pYTY32. Plasmid pYTY32 was produced by cloning the *NtQPTl* antisense expression cassette into plasmid pBin19.

In addition to the *NtQPT1* antisense gene, the *nptII* gene from the *Escherichia coli* transposon Tn5 was introduced into the transgenic tobacco for use as a selectable marker. The gene encodes neomycin phosphotransferase that confers resistance to the common aminoglycoside antibiotic kanamycin. Expression of the *nptII* gene is under the control of the *nos* promoter and the *nos* terminator.

The sizes, in kb, of the genetic elements of pBin19 are shown in Figure IV.F-3. In addition, the direction of transcription of promoter elements and the direction of the sense orientation of the structural genes within the T-DNA are also shown in Figure IV.F-3. Details of the genetic elements present in PYTY32 and their origin are listed in Table IV.F-1. *Genetic Elements Present in PYTY32*.

Figure IV.F-3. Plamid PYTY32.

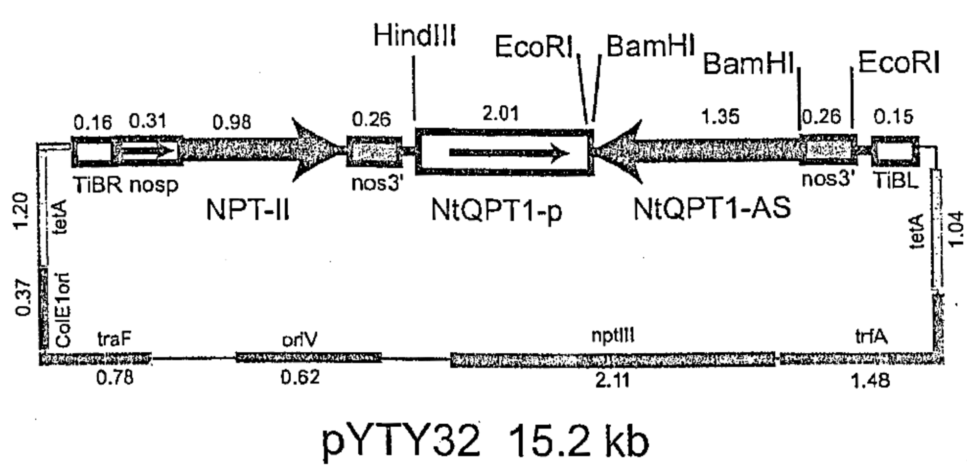


Table IV.F-1. Genetic Elements Present in PYTY32

T-DNA SEQUENCES

Genetic element	Origin	Size (kb)	Function
T-Border-Right (TiBR)	*A. tumefaciens*	0.16	Right border of T-DNA
Nos Promoter (nosp)	*A. tumefaciens*	0.31	Transcribe the npt-II gene
Nos Terminator (nos3')	*A. tumefaciens*	0.26	Terminate transcription
Neomycin phosphotransferase (npt-II)	*E. coli* Tn5	0.98	Kanamycin resistance/ Plant selectable marker
NtQPT1 Promoter (NtQPT1-p)	*N. tabacum*	2.01	Transcribe NtQPT1 antisense
NtQPT1 cDNA Antisense (NtQPT1-AS)	*N. tabacum*	1.35	Suppress QPTase expression
T-Border-Left (TiBL)	*A. tumefaciens*	0.15	Left border of T-DNA

pBin19 SEQUENCES OUTSIDE OF T-DNA

Genetic element	Origin	Size (kb)	Function
tetA	*P. aeruginosa*	2.24	None. T-DNA disrupts gene
trfA	*E. coli* pRK2	1.48	Plasmid transfer/ conjugation
nptIII	*S. faecalis*	2.11	Kanamycin resistance/ Bacteria selectable marker
oriV	*E. coli* pRK2	0.62	Wide host range origin of replication
traF	*E. coli* pRP4	0.78	Plasmid transfer/ Conjugation
ColE1 Ori	*E. coli* pcolE1	0.37	Origin of replication

pYTY32 was transformed into the disarmed *A. tumefaciens* strain LBA4404. LBA4404 carries a Ti-plasmid that has had the T-DNA region completely removed. It provides the *vir* gene products in trans for plant transformation.

Tobacco leaf disks were transformed, and transgenic plants regenerated. Transformed plant cells, callus, seedlings and plantlets were selected on medium containing kanamycin at 300 µg/ml.

Independent transformants were allowed to self. Progeny of the selfed-plants were screened for segregation of the transgene. T1 progeny segregating 3:1, indicating transformation at a single locus were selfed and subjected to further analysis. Alkaloid levels in homozygous and heterozygous T2 progeny were measured. Homozygous T2 progeny of transformant #41 had the lowest nicotine levels and were renamed Vector 21-41. (Xie *et al.*, 2004 [pg 77])

6. Characterization of Vector 21-41

Nicotine is synthesized in the roots of tobacco plants and translocated to the leaves, where it accumulates. Analyses of leaf from Vector 21-41 and the non-genetically engineered variety from which it was derived did not show any differences in alkaloid precursors in the leaves.

An independent laboratory quantified the principal biosynthetic intermediates of the nicotine biosynthesis pathway in samples of tobacco produced from the tobacco varieties Vector 21-41 and LA Burley 21 grown, harvested, and cured under identical conditions, as well as filler from a reference cigarette.

The biosynthetic precursors tested were divided into two groups: 1) principal polyamine intermediates (i.e., putrescine, *N*-methylputrescine, spermidine, and spermine); and 2) quinolinic acid and nicotinic acid. These specific compounds were selected based upon their importance in the biosynthetic production of nicotine, and their probability for analytical recovery. Three minor tobacco alkaloids (anabasine, anatabine and myosmine) were also analyzed for their presence in each of the processed tobacco samples.

Polyamine levels are considered to be semi-quantitative; mean levels reported for each processed tobacco type are provided in Table IV.F-2. *Polyamine Results (Mean (SD))*.. Vector 21-41 had lower or comparable levels of polyamine intermediates tested when compared to its parent, LA Burley 21. Both Vector 21-41 and LA Burley 21 tobacco contain higher levels of putrescine and spermidine than the Kentucky 2R4F Reference Cigarette filler, which is comprised of flue-cured and Oriental tobacco varieties, and reconstituted tobacco, as well as burley tobacco.

Table IV.F-2. Polyamine Results (Mean (SD)).

Tobacco Type	Putrescine* (µg/g)	N-Methylputrescine* (µg/g)	Spermidine* (µg/g)	Spermine* (µg/g)
Burley 21LA	23.3 (0.66)	<LOQ	10.2 (1.64)	N/A
Vector 21-41	19.1 (0.23)	<LOQ	10.2 (1.17)	N/A
KY Reference	7.72	<LOQ	3.16	N/A

* Semi-quantitative
<LOQ: Below limit of quantification
N/A: Not Applicable

Summary results of mean nicotinic and quinolinic acid levels recovered are provided in Table IV.F-3. *Nicotinic and Quinolinic Acid Results; (Mean (SD))*.. The levels of nicotinic acid are similar in Vector 21-41 and its non-transgenic parent, LA Burley 21 and are about one-third of the level in the Kentucky 2R4F reference cigarette filler. Quinolinic acid levels were below the limit of quantitation across all three processed tobacco types.

Table IV.F-3. Nicotinic and Quinolinic Acid Results; (Mean (SD)).

Tobacco Type	Nicotinic Acid (µg/g)	Quinolinic Acid (µg/g)
Burley 21LA	27.7 (0.8)	<LOQ
Vector 21-41	32.7 (2.6)	<LOQ
KY Reference	95.4 (1.1)	<LOQ

<LOQ: Below limit of quantification

Minor alkaloids were analyzed. The mean results for the minor alkaloids are summarized in Table IV.F-4. *Minor Alkaloid Results; (Mean (SD)).*. Vector 21-41 tobacco contains levels of minor alkaloids comparable to those in its non-transgenic parent, LA Burley 21. The levels of anabasine and anatabine are substantially lower than those in the Kentucky 2R4F reference cigarette filler.

Table IV.F-4. Minor Alkaloid Results; (Mean (SD).

Tobacco Type	Anabasine (µg/g)	Anatabine (µg/g)	Myosmine (µg/g)
Burley 21LA	<LOQ	15.8 (1.7)	N/A
Vector 21-41	<LOQ	29.0 (5.0)	N/A
KY Reference	86.6 (5.1)	495 (33)	10.2 (0.7)

<LOQ: Below limit of quantification
N/A: Not Applicable

7. USDA Deregulation of Vector 21-41

Vector 21-41 does not express any novel agronomic trait such as insect resistance or herbicide tolerance. The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has prepared an environmental assessment of tobacco line Vector 21-41 and granted a petition to designate this product as non-regulated (USDA 2002 [pg 77]).

The only new protein possibly expected in Vector 21-41 is neomycin phosphtransferase encoded by *nptII*. The *nptII* gene was the first selectable marker for plant transformation in products approved for human consumption. The biosafety of the use of kanamycin resistance as a selectable marker in transgenic plants has been reviewed extensively. The FDA evaluated the potential for horizontal transfer of the *nptII* gene to soil or intestinal (human and animal) microorganisms. They concluded that the probability of gene transfer was substantially less than the occurrence of kanamycin-resistance arising by mutation. Similarly, horizontal gene transfer to the epithelial cells lining the intestinal lumen was not observed. The *nptII* gene product has no significant homology with proteins listed as food allergens or toxins and is instable to proteolytic enzymes, heat, and stomach acid conditions. For these reasons, FDA had no allergenicity or toxicity concerns regarding npt II.

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