



DuPont Qualicon

Guidelines for Pharmaceutical/GMP Applications: Manual Assignment of Taxonomic Identification

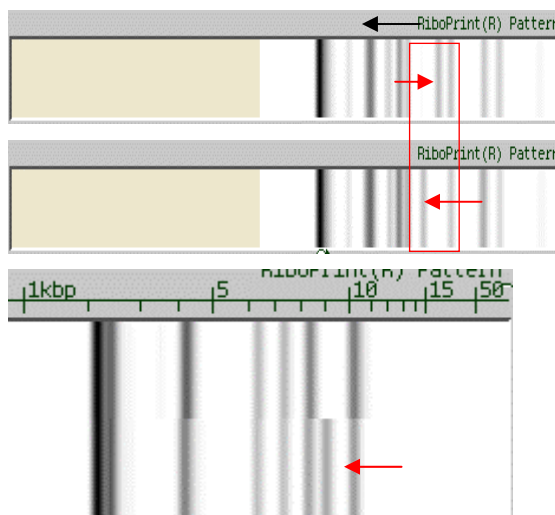
Overview

The Nearest Neighbor features of the current software can be useful in assigning manual identifications. These choices permit you to create manual DuPont Identifications that provide classical taxonomy for organisms not currently assigned an automatic identification by the system. The following guidelines should help you assign such names in a reasonable and documentable manner.

Guidelines

Manual DUP identifications should be assigned **only** if at least one of the following seven major guidelines applies when comparing the sample pattern to DUP or CID "nearest neighbor" patterns.

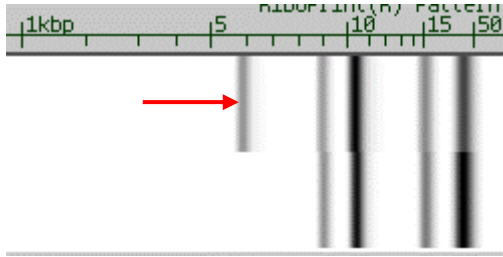
1. **For patterns with at least six bands which range throughout the 700 bps-48 kbs MW scale (at least one band <5 kb): No more than two bands are different in position and relative intensity.**



Six or more bands
Two band difference.
**Assign Manual DuPont ID
or Manual Custom ID**

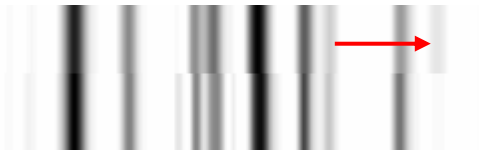
six bands or more;
One band difference.
**Assign Manual DuPont ID
or Manual Custom ID**

2. **For patterns with at least four bands: No more than one band is different.**



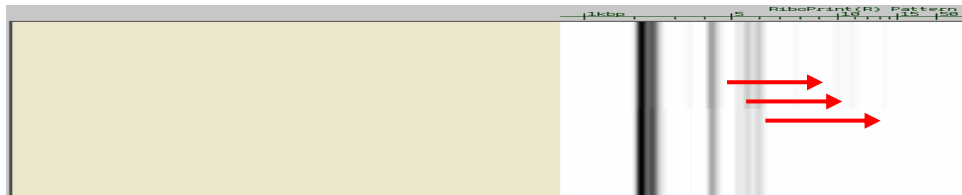
Four or more bands;
One band difference.
**Assign Manual DuPont ID
or Manual Custom ID**

3. **For patterns with at least four bands: patterns are identical except for the presence/absence or position of a single high molecular weight band.**



At least four bands;
One High Molecular Weight
band difference.
**Assign Manual DuPont ID or
Manual Custom ID**

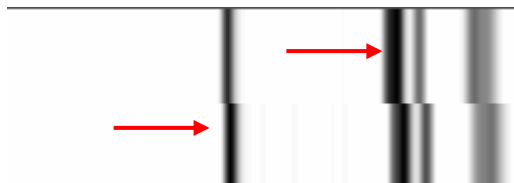
4. **For patterns with at least four bands: patterns are identical except for the presence/absence of very light minor bands (ghost bands) resulting from light sample load, heavy sample load, and/or the presence of high in-lane background. You must verify the absence of the minor bands in the raw image lane. (Use the Batch Information Window).**





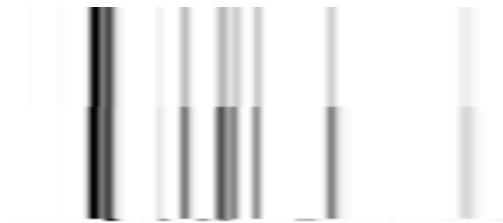
Light bands do not appear in raw image lane

- Patterns are similar, but one is shifted with respect to the other.



Patterns nearly identical but slightly **shifted**.
Assign Manual DuPont ID or Manual Custom ID

- Band positions are identical, but intensities differ, particularly for high molecular weight fragments.



Patterns nearly identical but **intensities** differ.
Assign Manual DuPont ID or Manual Custom ID

- Any of the above guidelines may be used in combination.

Combining guideline 1 & sub-guideline C would allow for a difference in intensity or positioning of up to 3 bands in cases where one of the three bands is present in the high molecular weight region (50Kb).

*Doublets should be considered two distinct bands for the purpose of this document

Note: Some Genus/species exhibit greater diversity than others do, so that there can be exceptions to the above rules.

Supporting Guidelines

Three additional “sub-guidelines” may either **increase the confidence** of any manual DuPont or Custom ID assignment or in some cases **provide exception** to the major rules.

- A. A **high similarity index** or high degree of **visual similarity** exists between the sample and DUP/CID Nearest Neighbor.
- B. There is a high level of **taxonomic agreement** between the surrounding Nearest Neighbors
- C. **Additional supporting information** from alternative testing methods such as sequencing, biochemical, fatty acid, or cultural tests supports the choice.

Adding Manual Identifications to the CID Database

Once you have assigned a Manual DUP or Custom Identification to a pattern using the above guidelines, you should generally add a custom identification pattern to the CID Database. However, this should only be done if the pattern has at least one additional member in its RiboGroup; this shows good reproducibility. Creating a CID will ultimately eliminate the need to repeatedly assign manual identifications to same patterns as they occur in the future.

How to Assign Manual DuPont Identifications

The following examples indicate how you might choose to assign manual Identifications based on real nearest neighbor comparisons.

All examples are shown in the Sample Information window.

Note that in most cases the pattern with the highest the highest similarity measure will also give the best neighbor match. For many examples, multiple guidelines apply. This should increase your confidence about the accuracy of the manual identification.

These examples demonstrate Guideline 1.

- **At least **six bands** which range throughout the MW scale**
- **At least one band < 5 kb**
- **No more than **two** bands are different in position and relative intensity**
- **In each example the DuPont Manual ID is shown as the first item**

Sample Protocol: VCA		
Type/	Number/	Sim./ Label
		RiboPrint(R) Pattern
DuPont Manual ID {DUP 12103}	{0.70}	{Klebsiella pneumoniae ss. pneumoniae}
Custom Neighbor CID 41	0.55	Bacillus megaterium
Custom Neighbor CID 54	0.53	Bacillus megaterium
Custom Neighbor CID 124	0.53	Bacillus pumilus
Custom Neighbor CID 35	0.52	Streptococcus pyogenes ATCC 19615
Custom Neighbor CID 37	0.51	Bacillus pumilus

Sample Protocol: VCA		
Type/	Number/	Sim./ Label
		RiboPrint(R) Pattern
DuPont Manual ID {DUP 10115}	{0.78}	{Burkholderia gladioli}
Custom Neighbor CID 136	0.62	Stenotrophomonas maltophilia
Custom Neighbor CID 34	0.61	Stenotrophomonas maltophilia
Custom Neighbor CID 126	0.61	Stenotrophomonas maltophilia
Custom Neighbor CID 86	0.60	Stenotrophomonas maltophilia
Custom Neighbor CID 147	0.54	Bacillus circulans

Manual DuPont Identification Assignment Guidelines









Sample Protocol: VCA			
Type/	Number/	Sim./	Label
DuPont Manual ID {DUP 6018} {0.67} {Paenibacillus macerans}			RiboPrint(R) Pattern
Custom Neighbor	CID 48	0.59	Stenotrophomonas maltophilia
Custom Neighbor	CID 104	0.55	Stenotrophomonas maltophilia
Custom Neighbor	CID 34	0.49	Stenotrophomonas maltophilia
Custom Neighbor	CID 119	0.46	Paenibacillus polymyxa
Custom Neighbor	CID 142	0.45	Bacillus circulans

Sample Protocol: VCA			
Type/	Number/	Sim./	Label
DuPont Manual ID {DUP 4149} {0.79} {Staphylococcus hominis}			RiboPrint(R) Pattern
Custom Neighbor	MASTER 86	0.78	Staphylococcus hominis
Custom Neighbor	MASTER 47	0.76	Staphylococcus hominis
Custom Neighbor	MASTER 50	0.72	Staphylococcus hominis
Custom Neighbor	MASTER 94	0.72	Staphylococcus hominis
Custom Neighbor	MASTER 102	0.71	Staphylococcus hominis

Manual DuPont Identification Assignment Guidelines

Sample Protocol: VCA		RiboPrint(R) Pattern	
Type/	Number/	Sim./	Label
DuPont Manual ID	{DUP 11034}	{0.80}	{Bacteroides vulgatus}
Custom Neighbor	MASTIER 813	0.57	Bacteroides fragilis group
Custom Neighbor	MASTIER 708	0.47	Bacteroides fragilis
Custom Neighbor	MASTIER 612	0.46	Bacteroides fragilis
Custom Neighbor	MASTIER 824	0.45	Bacteroides fragilis
Custom Neighbor	MASTIER 677	0.43	Klebsiella pneumoniae

Sample Protocol: VCA		RiboPrint(R) Pattern	
Type/	Number/	Sim./	Label
DuPont Manual ID	{DUP 11046}	{0.66}	{Burkholderia cepacia}
Custom Neighbor	MASTIER 308	0.61	Enterobacter species
Custom Neighbor	MASTIER 159	0.59	Streptococcus pneumoniae (presumptive)
Custom Neighbor	MASTIER 265	0.51	Bordetella species (presumptive)
Custom Neighbor	MASTIER 204	0.50	Lactobacillus kunkeei ATCC 700308
Custom Neighbor	MASTIER 64	0.50	Plesiomonas shigelloides ATCC 1903

Sample Protocol: VCA					
Type/	Number/	Sim./	Label	RiboPrint(R)	Pattern
DuPont Manual ID	{DUP 6819}	{0.83}	{Bacillus mycoides}		
Custom Neighbor	MASTER 244	0.70	Listeria monocytogenes (presumptive)		
Custom Neighbor	MASTER 123	0.70	Bacillus cereus		
Custom Neighbor	MASTER 111	0.69	Bacillus cereus		
Custom Neighbor	MASTER 67	0.67	Bacillus megaterium		
Custom Neighbor	MASTER 77	0.65	Bacillus cereus		

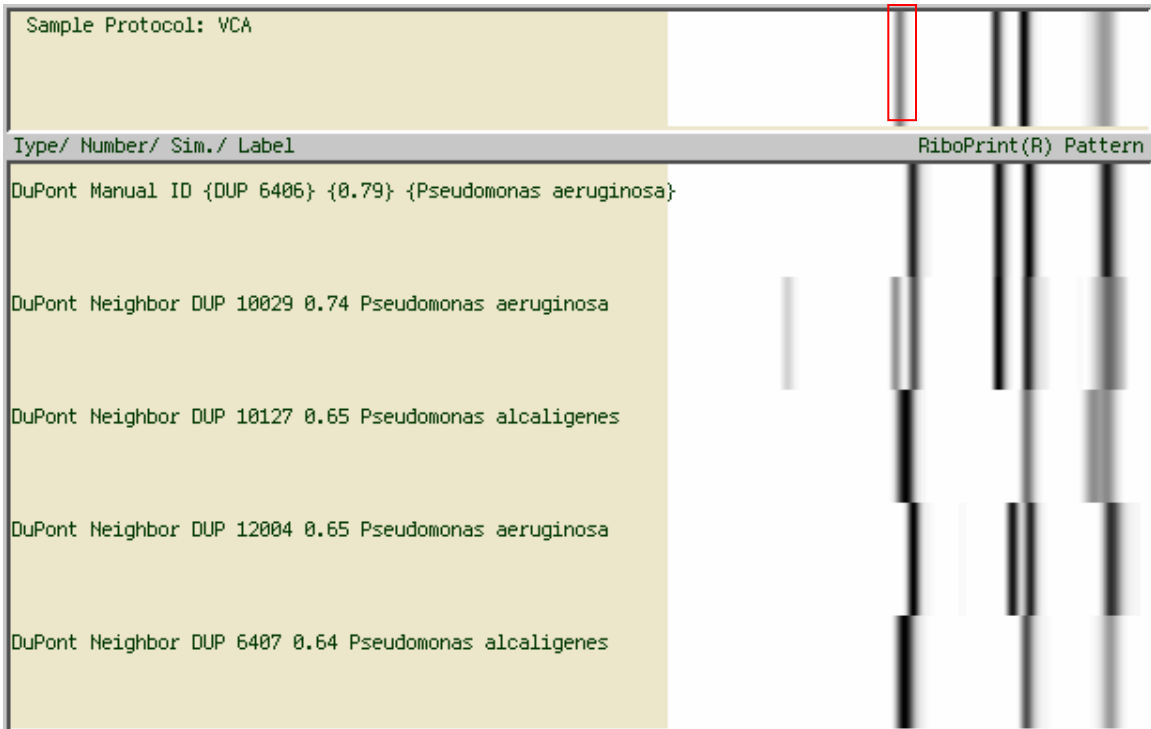
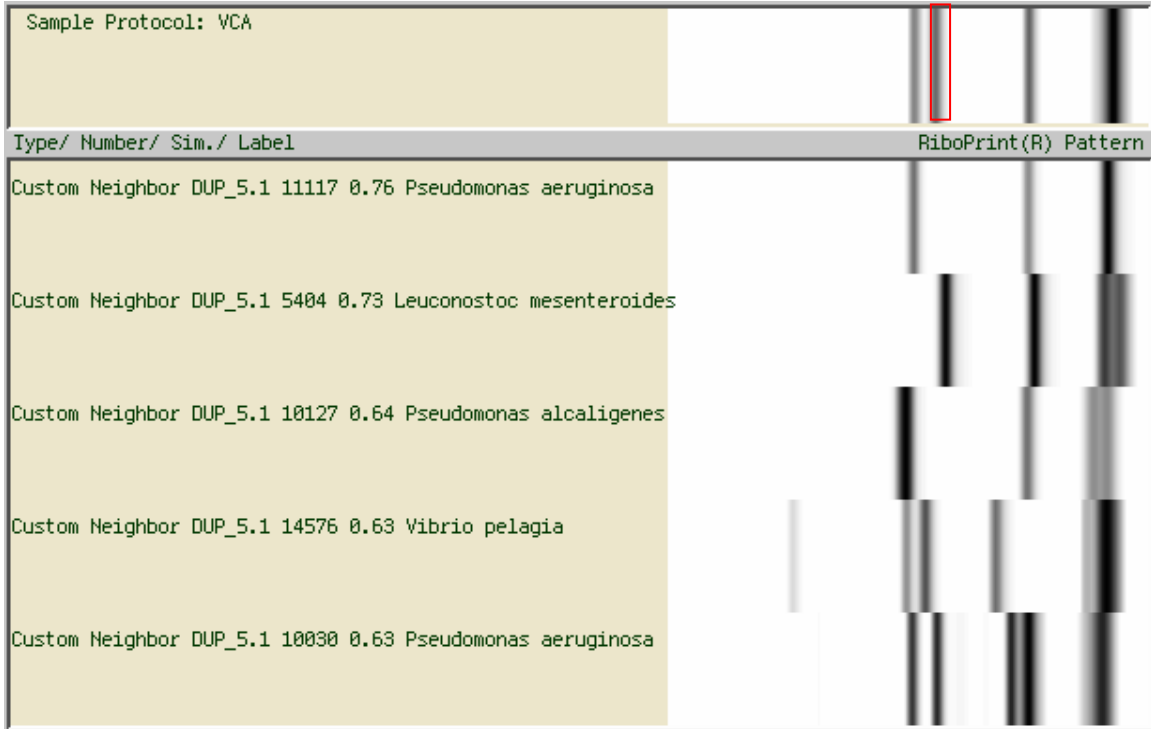
Sample Protocol: VCA					
Type/	Number/	Sim./	Label	RiboPrint(R)	Pattern
DuPont Manual ID	{DUP 4113}	{0.84}	{Staphylococcus epidermidis}		
Custom Neighbor	CID 95	0.74	Staphylococcus epidermidis		
Custom Neighbor	CID 67	0.67	Staphylococcus epidermidis		
Custom Neighbor	CID 53	0.67	Staphylococcus epidermidis		

Sample Protocol: VCA					
Type/	Number/	Sim./	Label	RiboPrint(R)	Pattern
DuPont Manual ID	{DUP 6067}	{0.83}	{Bacillus pumilus}		
Custom Neighbor	CID 57	0.75	Bacillus pumilus		
Custom Neighbor	CID 124	0.72	Bacillus pumilus		
Custom Neighbor	CID 26	0.69	Bacillus pumilus		
Custom Neighbor	CID 116	0.67	Bacillus pumilus		
Custom Neighbor	CID 73	0.65	Bacillus pumilus		

Sample Protocol: VCA					
Type/	Number/	Sim./	Label	RiboPrint(R)	Pattern
DuPont Manual ID	{DUP 4195}	{0.82}	{Staphylococcus saprophyticus}		
Custom Neighbor	MASTER 99	0.69	Staphylococcus haemolyticus		
Custom Neighbor	MASTER 81	0.59	Staphylococcus aureus		
Custom Neighbor	MASTER 83	0.58	Staphylococcus haemolyticus		
Custom Neighbor	MASTER 737	0.58	Staphylococcus aureus		
Custom Neighbor	MASTER 721	0.57	Staphylococcus aureus		

These examples demonstrate Guideline 2.

- **At least four bands**
- **No more than one band is different**



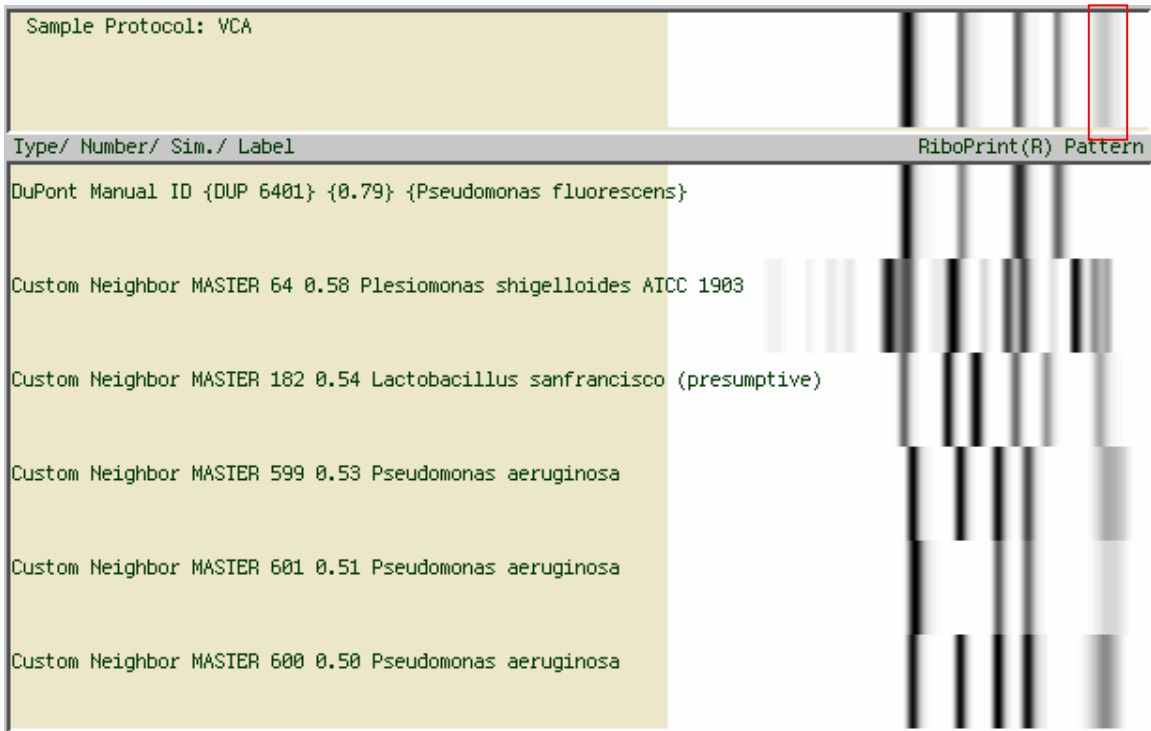
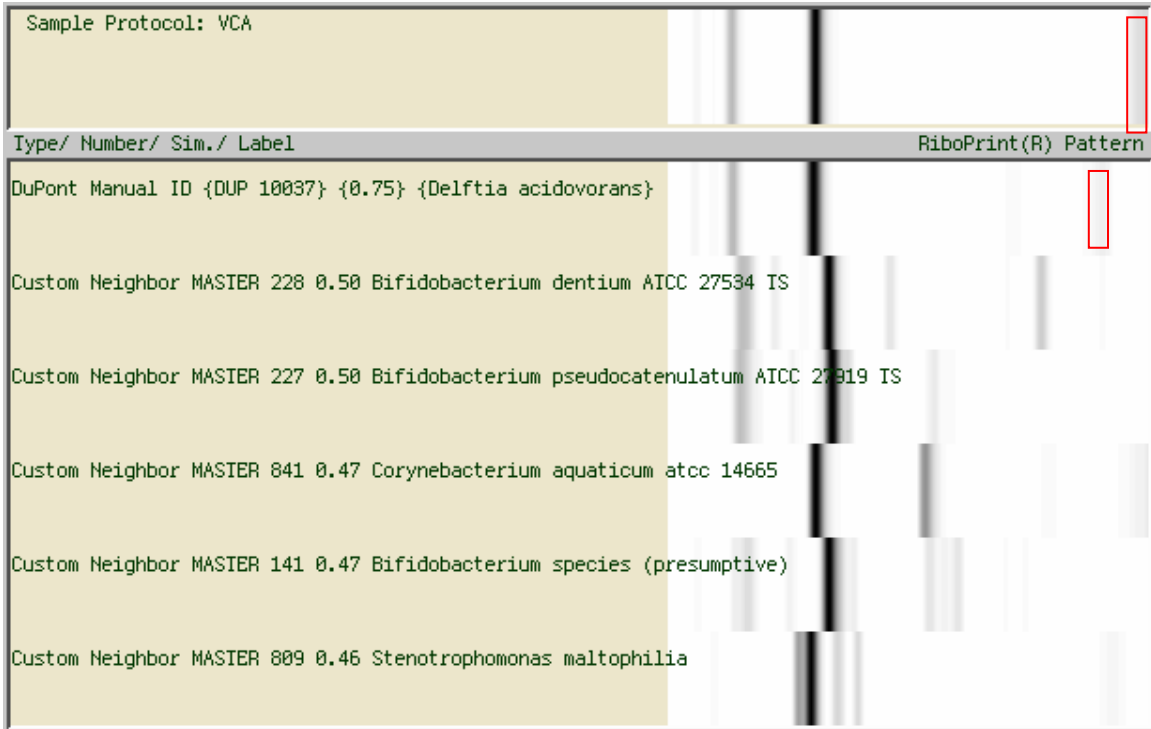
These examples demonstrate Guideline 3.

- **At least four bands**
- **Patterns identical except for the presence/absence or position of a single high molecular weight band.**

Type/ Number/ Sim./ Label	RiboPrint(R) Pattern
Sample Protocol: VCA	
DuPont Manual ID {DUP 10114} {0.80} {Burkholderia cepacia}	
Custom Neighbor CID 20 0.65 Burkholderia cepacia	
Custom Neighbor CID 140 0.56 Burkholderia cepacia	
Custom Neighbor CID 66 0.45 Staphylococcus xylosus	
Custom Neighbor CID 119 0.44 Paenibacillus polymyxa	
Custom Neighbor CID 98 0.42 Staphylococcus aureus	

Sample Protocol: VCA		
Type/	Number/	Sim./ Label
		RiboPrint(R) Pattern
DuPont Manual ID {DUP 10115}	{0.78}	{Burkholderia gladioli}
Custom Neighbor CID 136	0.62	Stenotrophomonas maltophilia
Custom Neighbor CID 34	0.61	Stenotrophomonas maltophilia
Custom Neighbor CID 126	0.61	Stenotrophomonas maltophilia
Custom Neighbor CID 86	0.60	Stenotrophomonas maltophilia
Custom Neighbor CID 147	0.54	Bacillus circulans

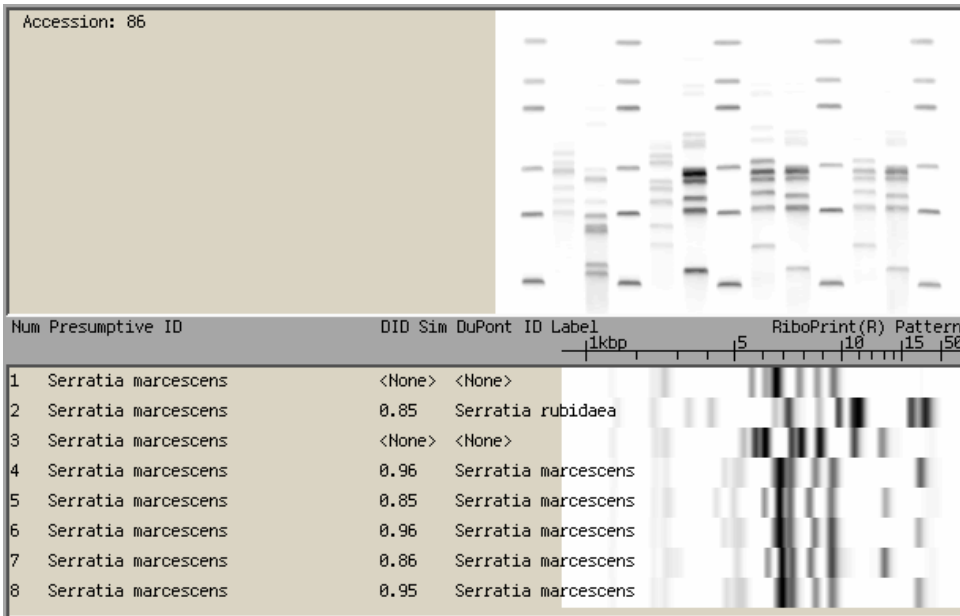
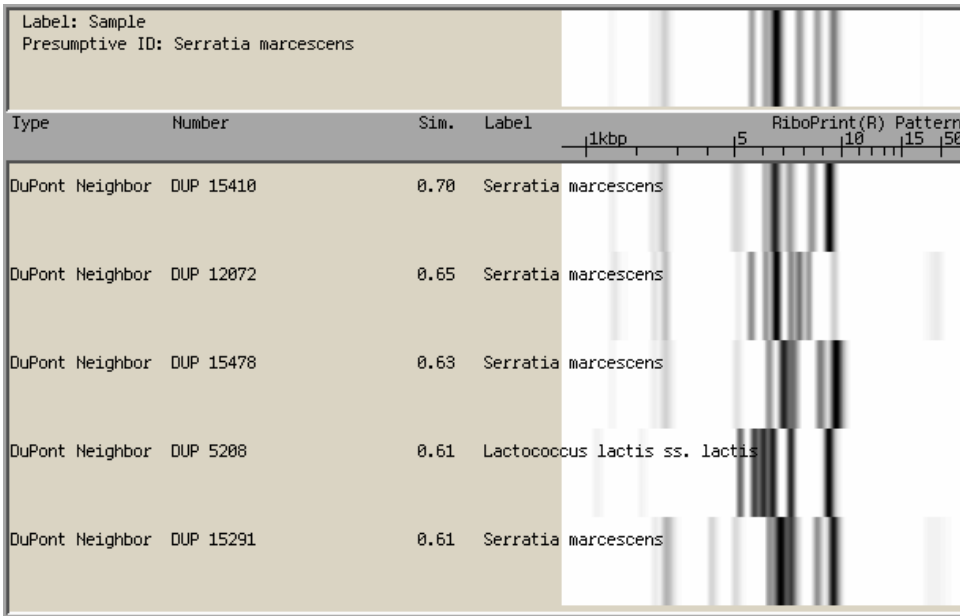
Sample Protocol: VCA		
Type/	Number/	Sim./ Label
		RiboPrint(R) Pattern
DuPont Manual ID {DUP 4149}	{0.79}	{Staphylococcus hominis}
Custom Neighbor MASTER 86	0.78	Staphylococcus hominis
Custom Neighbor MASTER 47	0.76	Staphylococcus hominis
Custom Neighbor MASTER 50	0.72	Staphylococcus hominis
Custom Neighbor MASTER 94	0.72	Staphylococcus hominis
Custom Neighbor MASTER 102	0.71	Staphylococcus hominis



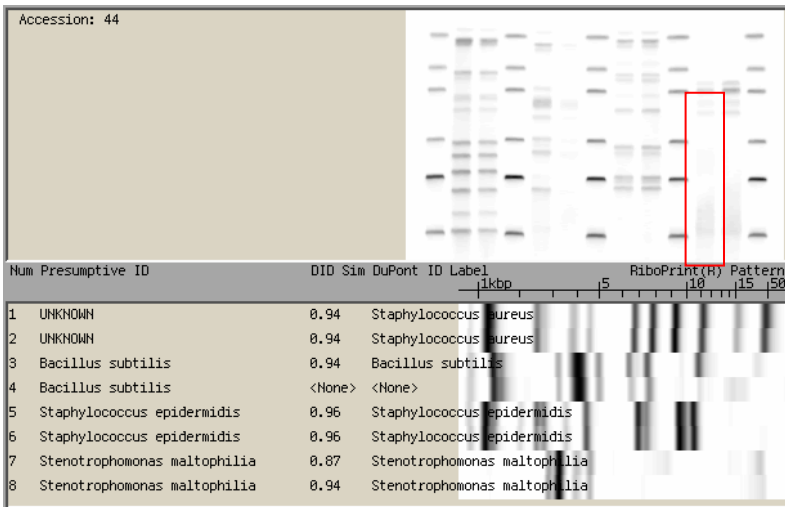
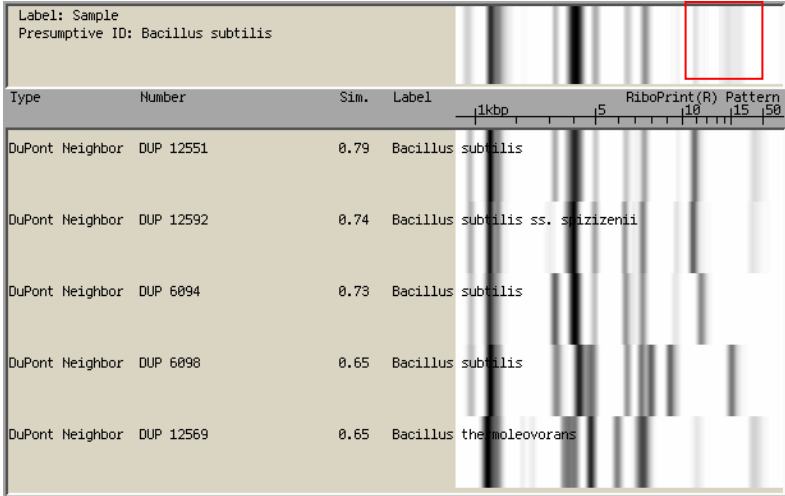
These examples demonstrate Guideline 4.

- **At least four bands**
- **Patterns identical except for the presence/absence of very light minor bands**

- **Verify the absence of the minor bands in the raw image lane**
- **In each example the DuPont Manual ID is shown as the first item**

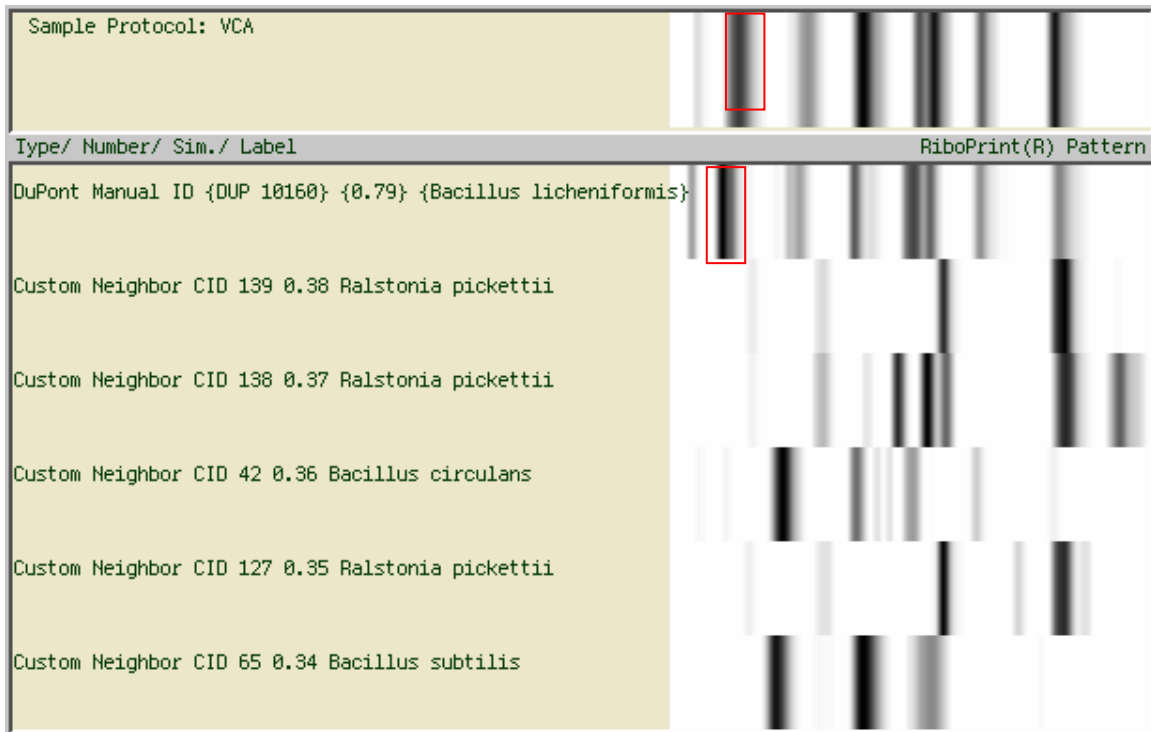


Manual DuPont Identification Assignment Guidelines



This example demonstrates Guideline 5.

Patterns are similar, but one is shifted with respect to the other



These examples demonstrate Guideline 6.

- **Band positions are identical,**
- **Intensities differ, particularly for high molecular weight fragments**

Manual DuPont Identification Assignment Guidelines

Sample Protocol: VCA			
Type/	Number/	Sim./	Label
DuPont Manual ID	{DUP 11034}	{0.80}	{Bacteroides vulgatus}
Custom Neighbor	MASTER 813	0.57	Bacteroides fragilis group
Custom Neighbor	MASTER 708	0.47	Bacteroides fragilis
Custom Neighbor	MASTER 612	0.46	Bacteroides fragilis
Custom Neighbor	MASTER 824	0.45	Bacteroides fragilis
Custom Neighbor	MASTER 677	0.43	Klebsiella pneumoniae

Sample Protocol: VCA			
Type/	Number/	Sim./	Label
DuPont Manual ID	{DUP 6019}	{0.83}	{Bacillus mycoides}
Custom Neighbor	MASTER 244	0.70	Listeria monocytogenes (presumptive)
Custom Neighbor	MASTER 123	0.70	Bacillus cereus
Custom Neighbor	MASTER 111	0.69	Bacillus cereus
Custom Neighbor	MASTER 67	0.67	Bacillus megaterium
Custom Neighbor	MASTER 77	0.65	Bacillus cereus

Sample Protocol: VCA	
Type/ Number/ Sim./ Label	RiboPrint(R) Pattern
DuPont Manual ID {DUP 10160} {0.79} {Bacillus licheniformis}	
Custom Neighbor CID 139 0.38 Ralstonia pickettii	
Custom Neighbor CID 138 0.37 Ralstonia pickettii	
Custom Neighbor CID 42 0.36 Bacillus circulans	
Custom Neighbor CID 127 0.35 Ralstonia pickettii	
Custom Neighbor CID 65 0.34 Bacillus subtilis	

Here are some examples of the supporting guidelines.
Sub-guideline B is based on a high level of taxonomic agreement between the surrounding Nearest Neighbors.

Sample Protocol: VCA	
Type/ Number/ Sim./ Label	RiboPrint(R) Pattern
DuPont Manual ID {DUP 4149} {0.79} {Staphylococcus hominis}	
Custom Neighbor MASTER 86 0.78 Staphylococcus hominis	
Custom Neighbor MASTER 47 0.76 Staphylococcus hominis	
Custom Neighbor MASTER 50 0.72 Staphylococcus hominis	
Custom Neighbor MASTER 94 0.72 Staphylococcus hominis	
Custom Neighbor MASTER 102 0.71 Staphylococcus hominis	






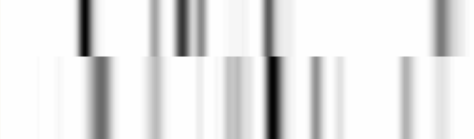
Sample Protocol: VCA	
Type/ Number/ Sim./ Label	RiboPrint(R) Pattern
DuPont Manual ID {DUP 4146} {0.85} {Staphylococcus haemolyticus}	
Custom Neighbor CID 22 0.83 Staphylococcus haemolyticus	
Custom Neighbor CID 92 0.80 Staphylococcus haemolyticus	
Custom Neighbor CID 60 0.78 Staphylococcus haemolyticus	
Custom Neighbor CID 38 0.78 Staphylococcus haemolyticus	
Custom Neighbor CID 68 0.72 Staphylococcus haemolyticus	

Finally, here are some examples that demonstrate Guideline 7.

This example demonstrates Guidelines 1 and 3 as well as sub-guideline B.

Sample Protocol: VCA	
Type/ Number/ Sim./ Label	RiboPrint(R) Pattern
DuPont Manual ID {DUP 4149} {0.79} {Staphylococcus hominis}	
Custom Neighbor MASTER 86 0.78 Staphylococcus hominis	
Custom Neighbor MASTER 47 0.76 Staphylococcus hominis	
Custom Neighbor MASTER 50 0.72 Staphylococcus hominis	
Custom Neighbor MASTER 94 0.72 Staphylococcus hominis	
Custom Neighbor MASTER 102 0.71 Staphylococcus hominis	

This example demonstrates Guidelines 1, 4 and 6.

Sample Protocol: VCA	
Type/ Number/ Sim./ Label	RiboPrint(R) Pattern
DuPont Manual ID {DUP 11034} {0.80} {Bacteroides vulgatus}	
Custom Neighbor MASTER 813 0.57 Bacteroides fragilis group	
Custom Neighbor MASTER 708 0.47 Bacteroides fragilis	
Custom Neighbor MASTER 612 0.46 Bacteroides fragilis	
Custom Neighbor MASTER 824 0.45 Bacteroides fragilis	
Custom Neighbor MASTER 677 0.43 Klebsiella pneumoniae	



DuPont Qualicon

Qualicon, Inc.
Bedford Building, 3531 Silverside Road
Wilmington, DE 19810 USA
Phone: 800-863-6842 or 302-695-5300 Fax: 302-695-5301

Qualicon Europe, Ltd.
Drakes Court, 302 Alcester Road
Wythall, Birmingham B47 6JR UK
Phone: +44 (0) 1564 821129 Fax: +44 (0) 1564 821192

DuPont de Nemours (France) SA
European Research & Development Centre
24 rue du Moulin, F-68740, Nambenheim, France
Phone: +33 (0) 3 89 83 27 30 Fax: +33 (0) 3 89 83 27 27

DuPont Singapore, Ltd.
One Maritime Square, 07-01
World Trade Centre, Singapore 099253
Phone: +65 273 2244 Fax: +65 271 0494

Visit our web site at www.qualicon.com

©Copyright 2000 Qualicon, Inc., a subsidiary of E. I. du Pont de Nemours and Company, Wilmington, DE
Printed in the U. S. A.

All rights reserved worldwide. No part of this publication may be reproduced or transmitted in any form, or by any information storage and retrieval system, without the prior agreement and written permission of Qualicon, Inc..

Not approved for clinical diagnosis. Please read the Limitation of Warranty and Liability before using the RiboPrinter® Microbial Characterization System.

1C-136-101