Genetic Diversity Among Accessions of Cucurbita pepo Resistant to Phytophthora Crown Rot

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Abstract. Phytophthora capsici, the causal agent of Phytophthora crown rot in squash (Cucurbita pepo L.), is an economically important pathogen worldwide. Currently, no C. pepo cultivars immune to the pathogen are commercially available, but sources of resistance to Phytophthora crown rot have been identified in a set of 16 C. pepo plant introductions (PIs). Knowledge of the genetic relationships among these accessions and their relatedness to economically important morphotypes of C. pepo would inform breeders’ best strategies for introgressing resistance; however, this information is currently lacking. The goal of the current study was to determine genetic diversity among the resistant accessions and their genetic relatedness to susceptible morphotypes of subspecies pepo (Zucchini and Pumpkin) and texana (Acorn, Straightneck, and Crookneck) using 39 SSR markers. The markers revealed 132 alleles averaging 4.40 alleles per locus and had a mean polymorphic information content (PIC) and genetic diversity of 0.44 and 0.49, respectively. CMTP235 had the highest PIC and genetic diversity of 0.80 and 0.82, respectively. Hierarchical clustering by UPGMA and principal coordinate analysis (PCOA) revealed grouping into two major C. pepo subspecies, texana and pepo, with all the resistant accessions grouping in the latter. In order of increasing genetic distance (GD), the resistant accessions were least distant to Zucchini (GD = 0.34), followed by Pumpkin (GD = 0.40), Crookneck (GD = 0.56), Acorn (GD = 0.60), and Straightneck (GD = 0.61) morphotypes. Mean GD among the resistant accessions was 0.31 and was highest between PIs 615142 and 615132 (0.61). Based on genetic similarity, PIs 174185 and 181761 (disease severity 0.31 and was highest between PIs 615142 and 615132 (0.61). Based on genetic similarity, PIs 174185 and 181761 (disease severity 0.31 and was highest between PIs 615142 and 615132 (0.61). Based on genetic similarity, PIs 174185 and 181761 (disease severity 0.31 and was highest between PIs 615142 and 615132 (0.61).

Materials and Methods

Plant material and DNA extraction. Seeds of 16 C. pepo PIs previously identified as resistant to Phytophthora crown rot (Supplemental Table 1) (Padley et al., 2008) were obtained from the germplasm collection of the U.S. Department of Agriculture, Agricultural Research Service. These accessions have a mean disease score (DS) between 1.3 and 2.5 on a scale of 0 to 5 (Padley et al., 2008). Squash breeders can use this germplasm to transfer resistance into elite morphotypes of C. pepo. However, information on the genetic basis of crown rot resistance in these accessions would inform breeders of best strategies for accumulating diverse disease resistance alleles through gene pyramiding and is currently lacking. In addition, information on the genetic diversity among the resistant accessions and their relatedness to susceptible morphotypes of C. pepo is known. Although no hybridization barriers exist among various subspecies of C. pepo, vast segregation in fruit phenotypes resulting from such crosses requires numerous backcrosses to recover elite parent genetic background (Loy, 2011). Therefore, even for intersubspecific trait transfer, breeders should consider selecting parents with the least intergenotype genetic distance (Gong et al., 2013; Paris, 2016). Measurement of genetic diversity and elucidation of phylogenetic relationships among C. pepo genotypes has been accomplished using a variety of marker systems including allozymes (Decker, 1985) and DNA markers [random amplified polymorphic DNA, amplified fragment length polymorphism, sequence-related amplified polymorphism, intersimple sequence repeats, high-frequency oligonucleotide-targeting active gene and simple sequence repeats (SSR)] (Decker-Walters et al., 2002; Ferriol et al., 2003; Formisano et al., 2012; Gong et al., 2012; Paris et al., 2003, 2015). Among these, SSR markers are preferred because of their abundance in the genome, reproducibility, high level of polymorphism and codominance (Hodel et al., 2016; Powell et al., 1996). Moreover, a high level of synteny among Cucurbita species allows transferability of SSR markers across the genus for various applications (Gong et al., 2008a, 2013; Kazmińska et al., 2017; Sim et al., 2015; Stift et al., 2004).

The goal of the current study was to determine the genetic diversity among a set of 16 C. pepo accessions previously identified as resistant to Phytophthora crown rot (Padley et al., 2008) using SSR markers developed for Cucurbita (Gong et al., 2008b). Further, genetic similarity between these accessions and susceptible morphotypes of C. pepo (Zucchini, Acorn, Straightneck, Crookneck, and Pumpkin) was determined.

Squash (Cucurbita spp.) is an economically important crop of Cucurbitaceae family valued at $230 million annually in the United States (U.S. Department of Agriculture–National Agricultural Statistics Service, 2017). Among the five cultivated species of Cucurbita genus, C. pepo L. is the most popular and consists of four subspecies: texana (Scheele) Filov (also called ovifera (L.) Deck); pepo; fraterna (L. H. Bailey) Lira, Andres, and Nee; and gumiла Teppner (Gong et al., 2012; Paris et al., 2003; Robinson and Decker-Walters, 1997; Teppner, 2000). Among these, texana and pepo are the most cultivated and are further classified into eight edible morphotypes (cultivar groups) (Paris, 2001). Crookneck, Straightneck, Acorn, and Scallop morphotypes are in subsp. texana, whereas Cucuzza, Vegetable Marrow, Zucchini, and Pumpkin belong to subsp. pepo (Paris, 1989; Paris et al., 2012). Cultivation of C. pepo is significantly limited by chronic losses to Phytophthora capsici Leonian, the causal agent of crown rot, foliar blight, and fruit rot in many vegetable crops (Hausbeck and Lamour, 2004). Currently, there are no commercial C. pepo cultivars immune to P. capsici, but sources of resistance to Phytophthora crown rot have been identified in C. moschata Duchesne (Chavez and Kabelka, 2010) and noncultivated species of squash [C. lundelliana Bailey and C. okeechoboeensis (Small) Bailey] (Padley et al., 2009). However, transfer of resistance into C. pepo is difficult because of wide genetic distances between the species that require resource-intensive techniques, such as embryo rescue and backcrossing (Rakha et al., 2012; Zhang et al., 2012).

In an effort to identify sources of resistance within C. pepo, Padley et al. (2008) inoculated 115 PIs from 24 countries with three virulent isolates of P. capsici, but none was immune to the pathogen. However, 16 of the accessions showed moderate to high resistance to Phytophthora crown rot [disease severity between 1.3 and 2.5 on a scale of 0 (no symptoms) to 5 (plant death)] (Padley et al., 2008). Squash breeders can use this germplasm to transfer resistance into elite morphotypes of C. pepo. However, information on the genetic basis of crown rot resistance in these accessions would inform breeders of best strategies for accumulating diverse disease resistance alleles through gene pyramiding and is currently lacking. In addition, information on the genetic diversity among the resistant accessions and their relatedness to susceptible morphotypes of C. pepo is known. Although no hybridization barriers exist among various subspecies of C. pepo, vast segregation in fruit phenotypes resulting from such crosses requires numerous backcrosses to recover elite parent genetic background (Loy, 2011). Therefore, even for intersubspecific trait transfer, breeders should consider selecting parents with the least intergenotype genetic distance (Gong et al., 2013; Paris, 2016).

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Godiva), and Straightneck (Early Prolific) morphotypes were included to study their relationship with the resistant accessions. For each genotype, five seeds were germinated in a greenhouse maintained at 22 to 32 °C using cells (5.98 × 3.68 × 4.69 cm) filled with Fafard 3B soil, which was amended with Osmocote Classic fertilizer (1.38 g/kg N, 1.38 g/kg P, 1.38 g/kg K). At the two true-leaf stage, three leaf punches from three individuals of each accession were collected, bulked, and immediately frozen in liquid nitrogen. DNA was extracted using the E.N.Z.A. kit (Omega Biotek, Norcross, GA) according to manufacturer’s instructions.

SSR amplification and allele scoring.

Thirty-nine previously published SSR primer pairs distributed across 19 linkage groups of C. pepo were used for genetic diversity analysis (Supplemental Table 2) (Gong et al., 2008b). For each primer pair, polymerase chain reaction was performed in a 15-μL reaction containing 40 ng of template DNA, 0.32 μM of a fluorescently (either 6-FAM, VIC, or PET; Supplemental Table 2) labeled M13 forward primer (GCCTCCCTCGGCCA) (Blacket et al., 2012), 0.04 μM of M13-tagged forward primer, 0.4 μM unlabeled reverse primer, and 1·PROMEGA Colorless GoTaq mastermix (Promega, Madison, WI). Amplification was performed in 96-well plates on a Simplicity thermal cycler (Applied Biosystems, Foster City, CA) using an initial 3 min denaturation at 95 °C, followed by 35 cycles of 15 s at 95 °C, 20 s at 52 °C, and 30 s at 72 °C. The amplification was followed by a final extension step of 10 min at 72 °C. Depending on band intensity for each primer pair on an agarose gel (1% w/v), products were diluted appropriately for capillary electrophoresis. Amplification products for three primer pairs, each labeled with a different fluorescent dye, were multiplexed and combined with a GeneScan-500 ROX internal-lane size standard and Hi-Di Formamide before analysis on a 3730 96-capillary DNA Analyzer (Applied Biosystems) at the Gene Expression and Genotyping Core facility, Interdisciplinary Center for Biotechnology Research, University of Florida. GeneMarker software (SoftGenetics, State College, PA) was used for allele calling and size estimation.

**Table 1. Genetic parameters revealed by 30 simple sequence repeat (SSR) markers on 16 Cucurbita pepo accessions resistant to Phytophthora crown rot and eight susceptible cultivars.**

| SSR        | Number of alleles expected | Number of alleles observed | Allele size range (bp) | Gene diversity (Hd) | Heterozygosity (Ho) | PIC  |
|------------|-----------------------------|----------------------------|------------------------|---------------------|---------------------|------|
| CMTp239    | 3                           | 2                          | 99–114                 | 0.04                | 0.04                | 0.04 |
| CMTp127    | 3                           | 2                          | 107–113                | 0.08                | 0.00                | 0.08 |
| CMTp21     | 4                           | 3                          | 179–203                | 0.12                | 0.04                | 0.12 |
| CMTp39     | 4                           | 5                          | 118–130                | 0.30                | 0.17                | 0.28 |
| CMTp84     | 2                           | 2                          | 144–150                | 0.33                | 0.00                | 0.28 |
| CMTp141    | 3                           | 6                          | 108–132                | 0.34                | 0.22                | 0.33 |
| CMTp53     | 3                           | 3                          | 117–125                | 0.36                | 0.00                | 0.32 |
| CMTp133    | 3                           | 5                          | 105–121                | 0.40                | 0.13                | 0.39 |
| CMTp109    | 5                           | 3                          | 100–122                | 0.42                | 0.09                | 0.38 |
| CMTp62     | 5                           | 5                          | 118–172                | 0.45                | 0.19                | 0.41 |
| CMTp205    | 6                           | 5                          | 116–142                | 0.45                | 0.29                | 0.41 |
| CMTp177    | 5                           | 3                          | 189–215                | 0.47                | 0.29                | 0.38 |
| CMTp68     | 4                           | 4                          | 160–186                | 0.47                | 0.21                | 0.42 |
| CMTp36     | 3                           | 3                          | 143–158                | 0.47                | 0.00                | 0.43 |
| CMTp229    | 4                           | 2                          | 97–99                  | 0.49                | 0.00                | 0.37 |
| CMTp224    | 5                           | 4                          | 147–156                | 0.54                | 0.17                | 0.50 |
| CMTp206    | 6                           | 4                          | 114–123                | 0.54                | 0.14                | 0.48 |
| CMTp264    | 3                           | 3                          | 100–130                | 0.54                | 0.83                | 0.45 |
| CMTp176    | 4                           | 5                          | 100–118                | 0.55                | 0.04                | 0.51 |
| CMTp265    | 3                           | 4                          | 78–98                  | 0.55                | 0.56                | 0.45 |
| CMTp231    | 9                           | 5                          | 133–197                | 0.56                | 0.50                | 0.52 |
| CMTp37     | 9                           | 6                          | 111–197                | 0.60                | 0.13                | 0.57 |
| CMTp249    | 4                           | 6                          | 136–162                | 0.61                | 0.41                | 0.56 |
| CMTp178    | 4                           | 4                          | 125–149                | 0.63                | 0.30                | 0.55 |
| CMTp245    | 5                           | 4                          | 108–132                | 0.65                | 0.48                | 0.57 |
| CMTp202    | 4                           | 9                          | 124–174                | 0.67                | 0.27                | 0.64 |
| CMTp26     | 5                           | 8                          | 111–197                | 0.69                | 0.17                | 0.65 |
| CMTp129    | 5                           | 4                          | 119–151                | 0.70                | 0.42                | 0.65 |
| CMTp201    | 8                           | 4                          | 89–111                 | 0.75                | 0.43                | 0.70 |
| CMTp235    | 6                           | 8                          | 124–154                | 0.82                | 0.48                | 0.80 |
| Mean       | 4.56                        | 4.40                       | —                      | 0.49                | 0.23                | 0.44 |

*Number of alleles reported in Gong et al. (2008b).
PIC = polymorphic information content.

**Fig. 1.** UPGMA dendrogram showing clustering of 16 Cucurbita pepo accessions resistant to Phytophthora crown rot (Padley et al., 2008) and 8 susceptible cultivars (with asterisk). Tree scale represents branch length. Only bootstrap values above 45% are shown.
then converted into a binary format (1,0 for presence or absence of alleles) and genetic similarity calculated using Dice coefficient of similarity, \( S_{D} \) (Dice, 1945). Genetic similarity matrix was used to construct a dendrogram by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) hierarchical clustering, replicated with 1000 bootstraps on FreeTree program (Hampl et al., 2001), and visualized on the web-based interactive Tree of Life (iTOL) tool (Letunic and Bork, 2016). Genetic distances were obtained by subtracting genetic similarity values from 1 (1 – \( S_{D} \)) (Nei and Li, 1979). PCOA (Gower, 1966) was calculated and displayed with factorial analysis option in Darwin Software (v6.0) (Perrier and Jacquemoud-Collet, 2006).

**Results and Discussion**

**SSR analysis.** Out of the initial 39 SSR markers tested, a set of 9 markers were dropped during analysis and included six that were monomorphic and three that had missing data in more than five accessions. The remaining 30 markers revealed 132 alleles, which ranged in size from 78 bp (marker CMTp265) to 215 bp (marker CMTp177) (Table 1). The number of alleles per locus ranged from two to nine alleles, with 86.7% of the markers having more than three alleles (Table 1). The mean number of alleles per locus was 4.4 and was comparable to that observed among 88 Cucurbita accessions by Gong et al. (2013) (4.3 alleles/locus, 74 SSR markers). Other studies in Cucurbita have reported wide variation in mean number of alleles per locus, for instance, Gong et al. (2012) (3.0 alleles/locus; 104 accessions; 134 SSR markers), Gong et al. (2008b) (3.3 alleles/locus; 12 accessions; 405 SSR markers), Verdone et al. (2018) (3.6 alleles/locus; 30 accessions; 8 SSR markers), Formisano et al. (2012) (3.8 alleles/locus; 23 accessions; 30 SSR markers), Murovec (2015) (6.06 alleles/locus; 51 accessions; 18 SSR markers), Barzegar et al. (2013) (6.71 alleles/locus; 26 accessions; 14 SSR markers), and Sim et al. (2015) (7.41 alleles/locus; 160 accessions; 29 SSR markers).

Variation in mean number of alleles per locus across studies can be explained by differences in the number and geographic distribution of accessions used and the number and level of polymorphism of SSR markers assayed (Murovec, 2015).

Variation in discrimination power of the markers was evident as shown by the wide range in gene diversity (\( H_e \); 0.04–0.82) and polymorphic information content (PIC; 0.04–0.80) (Table 1). Among the markers assayed, discriminating power was highest in CMTp235 (\( H_e = 0.82; \) PIC = 0.80), and lowest in CMTp239 (\( H_e = 0.04; \) PIC = 0.04) (Table 1).

A similar observation was made by Murovec (2015), who found marker CMTp239 (\( H_e = 0.028; \) PIC = 0.027) to have the least discriminating power among 18 SSR markers assayed.

**Dendrogram and PCOA.** The UPGMA dendrogram separated the genotypes into two distinct clusters (Fig. 1). All five cultivars of C. pepo subsp. texana grouped in Cluster 1 (bootstrap value = 96), and further separated into two subclusters, one consisting of Straightneck (Early Prolific) and Crookneck (Early Golden) morphotypes and the other Acorn (Table Queen, Bush Delicata, and Honey Bear) (Fig. 1). The close association between Straightneck and Crookneck morphotypes is supported by evidence that the former was derived from the latter through...
selection (Gong et al., 2012; Paris, 2001), a relationship corroborated in previous phylogenetic studies (Decker, 1985; Ferriol et al., 2003; Gong et al., 2012; Paris, 2001). Within the Acorn subcluster, Honey Bear and Table Queen cultivars, which have typical turbinate fruits, associated more closely, but farther from Delicata, which has oblong fruit shape (Loy, 2011). In Cluster 2 (bootstrap value = 89), Zucchini (‘Black Beauty’) and Pumpkin (‘Beppo’ and ‘Lady Godiva’) morphotypes of C. pepo subsp. pepo grouped together with the 16 resistant accessions. The pumpkin cultivars, used in the seed oil and snack industry (Lelley et al., 2009), formed a tight subcluster (bootstrap value = 99). Zucchini morphotype associated more closely with accessions from South Africa (PI 299574) and Spain (PI 512709). The lower section of cluster 2 (Fig. 1) was occupied by resistant accessions originating from Germany (PIs 266925 and 209783) and the Mediterranean Basin (PIs 167053, 169417, 169450, 174185, 179267, 181761, 181944, and 288240). The Mexican accession, PI 615132, assumed an outlying position in the second cluster and generated unique alleles in 10 of the polymorphic loci examined (data not shown).

A scatter plot based on PCOA supported UPGMA clustering and revealed a clear delineation between the two C. pepo subsp. species. All the resistant accessions grouped in subsp. pepo (Fig. 2). The PCOA also confirmed subclustering according to fruit morphotypes within subsp. texana. The first three PCOA coordinates explained 58% of the variability contained in the genetic distance matrix.

Genetic distance. Genetic distance (GD) among all genotypes ranged from 0.12 to 0.68 (Table 2). Among cultivars of subsp. texana, the average GD was 0.41, which was largest between Acorn and Crookneck morphotypes (0.52), but lowest between Straightneck and Crookneck (0.33) (Table 3). Similarly, the average GD among cultivars of subsp. pepo was 0.39 and was largest between Zucchini and Pumpkin morphotypes (0.42) but lowest between the two Pumpkin cultivars (0.17). As expected, the average GD between subsp. texana and subsp. pepo (intersubspecific) (0.56) was higher than that observed among different morphotypes within each subspecies (intrasubspecific) (0.42–0.52) (Table 3). Previous studies also support a general trend in which GD between subspecies is larger than that among morphotypes within the same subspecies. For example, Gong et al. (2012) reported larger GD between subsp. texana and pepo (0.67), than among morphotypes within the same subspecies (0.02–0.41). Similarly, Paris et al. (2003) observed larger genetic separation between subsp. texana and pepo (average GD = 0.22) than among morphotypes within the same subspecies (0.09–0.11).

Overall, there was low average GD among the 16 resistant accesses (0.31), with the least GD between PIs 179267 and 209783 (0.12), and largest between PIs
Table 3. Average genetic distances between 16 Cucurbita pepo accessions resistant to Phytophthora crown rot and susceptible morphotypes of subsp. texana (Acorn, Straightneck, and Crookneck) and pepo (Pumpkin and Zucchini).

| Morphotype   | Resistant accessions | Acorn | Zucchini | Pumpkin | Crookneck |
|--------------|----------------------|-------|----------|---------|-----------|
| Acorn        | 0.60                 | 0.57  | 0.42     | 0.51    | 0.53      |
| Zucchini     | 0.34                 | 0.57  | 0.57     | 0.33    |           |
| Pumpkin      | 0.40                 | 0.59  | 0.63     | 0.33    |           |
| Crookneck    | 0.56                 | 0.37  | 0.63     |         |           |
| Straightneck | 0.61                 | 0.52  | 0.63     | 0.53    |           |

Overall, data (dendrogram, PCOA, and GD) presented here support a closer genetic relationship among the resistant accessions and morphotypes of subsp. pepo than those of subsp. texana. Further, considering intergenotype GDs, PIs 181761 and 174185 are the best sources of resistance for subsp. pepo and texana, respectively. However, PI 615132 may harbor unique resistance alleles that could augment resistance to Phytophthora crown rot in breeding programs. The results of the current study will be useful for future breeding and genetic studies.

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Supplemental Table 1. Mean disease severity (DS) score and country of origin of 16 Cucurbita pepo accessions resistant (mean disease score < 2.5) to Phytophthora crown rot.

| Accession | Country      | DS |
|-----------|--------------|----|
| PI 181761 | Lebanon      | 1.3|
| PI 615132 | Mexico       | 1.3|
| PI 174185 | Turkey       | 1.4|
| PI 615142 | Kazakhstan   | 1.4|
| PI 169417 | Turkey       | 1.6|
| PI 266925 | Germany      | 1.8|
| PI 209783 | Germany      | 1.9|
| PI 512709 | Spain        | 1.9|
| PI 169450 | Turkey       | 2.1|
| PI 181944 | Syria        | 2.1|
| PI 167053 | Turkey       | 2.3|
| PI 169476 | Turkey       | 2.3|
| PI 179267 | Turkey       | 2.3|
| PI 181878 | Syria        | 2.3|
| PI 288240 | Egypt        | 2.3|
| PI 295754 | South Africa | 2.5|

*Mean disease severity score determined by Padley et al. (2008) on a scale of 0 to 5.

Supplemental Table 2. Thirty-nine simple sequence repeat primers used in this study (Gong et al., 2008b).

| Marker name | Forward primer | Reverse primer | Expected size (bp) | Linkage group | M13-primer fluorescent label |
|-------------|----------------|----------------|--------------------|---------------|-----------------------------|
| CMTp21      | TGTCCAATTCATTCATTCATCTTCAT | GGATTCCACCACCATTTTGAGA | 201 | LGp7 | 6-FAM |
| CMTp26      | GTCTTTGTCTTGGGTTGGTT | AAAACAGTGTGTGGTGGTGGT | 187 | LGp10a | VIC |
| CMTp36      | GAAAGGCGCTGCATGCGAGAG | TTTCATCCCGGATATATTG | 151 | LGp12 | PET |
| CMTp37      | GTCTGTGCTTGGGTTGGTTTC | AGAAACAGTGTGGTGTTG | 186 | LGp10a | 6-FAM |
| CMTp38      | AGGATTCAGGTTAGTGTGCTG | AGAGGTTCTCCCTCTCTCT | 160 | LGp8 | VIC |
| CMTp39      | GGCGGAGAAGGAAAGCAAT | TTTTTCCTCCCTTCCATC | 132 | LGp9 | PET |
| CMTp43      | ACAAACACTACAAGCTTTC | TTTGGAATCAGTTCCAGTTC | 118 | LGp19 | PET |
| CMTp46      | GAAGGTTCTGCTGAGAAGACT | CAAAGACTCTCCAGGACCTAT | 118 | LGp6 | VIC |
| CMTp42      | GACCGGCTGACTGAGGAAAA | TCGGATAGAAAAGATGAGAAG | 107 | LGp6 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
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| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |
| CMTp47      | ACCCACTTCATTCATTTGACCC | ATTAGGGCGACCTGAAGA | 189 | LGp13 | 6-FAM |